

**Research into process validation in pharmaceutical companies,
with specific reference to Roche, South Africa**

by

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Abstract

Each manufacturing facility needs to develop, based on scientific rationale, detailed customized programs for validation. This necessity arises from the fact that the guidelines set for the pharmaceutical industry by regulatory authorities are rather broad. It is also not practical to adopt standards for process validation since every strategy must be tailored to the actual circumstances of every manufacturing site.

The aim of this research study was therefore to develop validation approaches capable of ensuring that pharmaceutical products are manufactured free from microbiological and decomposition products. Furthermore, the study has focused on exploring the adequate methods through which the manufacturing/cleaning processes are kept under a state of control, therefore preventing the risk of product contamination (e.g., with cleaning agents) and cross-contamination (e.g., with previously manufactured drug residues) within a given manufacturing group.

A cleaning validation program was formulated and implemented in the pharmaceutical manufacturing plant of Roche, South Africa. The approach for validation was proposed after a risk analysis. The various aspects of validating the cleaning procedures were challenged with regards to selecting the appropriate sampling methods, acceptance criteria and suitable analytical techniques. The cleaning validation approach proposed was exemplified with studies for two sulfonamide drugs formulated in combination with their potentiators, namely Fansidar® and Co-trimoxazole.

For Fansidar® tablets an RP-HPLC method was developed and validated in conjunction with a swab sampling method. The validated method was used to assess the level of cleanliness (LOC) of the equipment chain used in the manufacturing process. The results obtained were situated below the calculated LOC values deemed to be safe ($4.99 \mu\text{g}/\text{cm}^2$ for PYR and $19.14 \mu\text{g}/\text{cm}^2$ for SUL). A sufficient LOC was therefore achieved with the designed cleaning procedures.

For Co-trimoxazole syrup a capillary electrophoresis MEKC method was developed for assaying swab samples containing active pharmaceutical ingredients, their decomposition products and detergent at trace levels. The method was validated for the following optimum experimental conditions established through method development: 30 mM phosphate electrolyte, pH 7.8,

containing 10 mM SDS and an applied voltage of 22 kV. All the components were resolved within 14 minutes.

A qualification approach for a steam sterilizer was elaborated and exemplified for the trypticase soy broth. The sterilization process was designed and validated such as to obtain a satisfactory sterility assurance level without physically or chemically altering the media during its sterilization.

These validation approaches have set practical examples for the future validation work within the manufacturing facility under examination. Although the validation strategy follows several standard steps, exact prescriptions for validation cannot be offered because of the multitude of variables involved in every process. As long as the strategy for validation is based on logical, systematic approach and all the activities undertaken are based on a scientific rationale the outcome results of the validation will be meaningful.

Declaration

I, Mădălina Brîndușa Boca hereby declare that this thesis entitled:

“Research into process validation in pharmaceutical companies, with specific reference to Roche, South Africa”

which I herewith submit to the University of Pretoria for the Degree of Philosophiae Doctor in Anatomy, is my own original work and has never been submitted for any academic award to any other tertiary institution for any degree.

Date

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Table of contents

ABSTRACT	II
DECLARATION	IV
ACKNOWLEDGEMENTS	V
LIST OF PUBLICATIONS	VI
TABLE OF CONTENTS	VII
LIST OF TABLES	XI
LIST OF FIGURES	XIII
LIST OF ABBREVIATIONS AND SYMBOLS	XV
CHAPTER 1: INTRODUCTION	1
1.1 PROCESS VALIDATION AND QUALITY ASPECTS	1
1.1.1 <i>The first concerns for quality and the definition of the concept</i>	<i>1</i>
1.1.2 <i>Early contamination issues for drug and medical devices.....</i>	<i>3</i>
1.1.3 <i>The development of the validation concept</i>	<i>4</i>
1.1.4 <i>Why validate a process?</i>	<i>6</i>
1.1.5 <i>What needs validation?</i>	<i>8</i>
1.1.6 <i>Process validation steps as determined by the product “life-cycle”</i>	<i>10</i>
1.1.7 <i>Validation approaches</i>	<i>12</i>
1.1.8 <i>The South African context.....</i>	<i>13</i>
1.1.9 <i>Current validation trends</i>	<i>14</i>
1.2 STUDY LAYOUT	15
1.2.1 <i>Motivation</i>	<i>15</i>
1.2.2 <i>Aim of study.....</i>	<i>15</i>
1.2.3 <i>Objectives.....</i>	<i>16</i>
1.2.4 <i>Hypotheses.....</i>	<i>16</i>
1.2.5 <i>Null hypotheses</i>	<i>16</i>
CHAPTER 2: CLEANING VALIDATION – REVIEW OF LITERATURE.....	18
2.1 DEFINITION OF TERMS	18
2.2 REASONS AND BENEFITS FOR CLEANING VALIDATION	20
2.3 OBJECTIVES FOR CLEANING VALIDATION	21
2.4 REGULATORY ASPECTS AND REVIEW OF OFFICIAL DOCUMENTS	22
2.5 FIELD OF APPLICABILITY FOR CLEANING VALIDATION	24
2.5.1 <i>Cleaning validation for galenical manufacturing facilities.....</i>	<i>24</i>
2.5.2 <i>Cleaning validation for biotechnological manufacturing facilities.....</i>	<i>25</i>
2.5.3 <i>Cleaning validation for API manufacturing facilities</i>	<i>25</i>
2.5.4 <i>Cleaning validation for research facilities</i>	<i>26</i>
2.5.5 <i>Cleaning validation in other industries</i>	<i>27</i>
2.6 GENERAL CONSIDERATIONS FOR A CLEANING VALIDATION PROGRAM	27
2.7 PREREQUISITE CONDITIONS FOR CLEANING VALIDATION	30
2.8 APPROACH FOR CLEANING VALIDATION	30
2.8.1 <i>Product grouping</i>	<i>31</i>
2.8.2 <i>Equipment grouping</i>	<i>33</i>
2.9 POINTS TO CONSIDER FOR CLEANING VALIDATION	34
2.9.1 <i>Sources of contamination</i>	<i>34</i>
2.9.2 <i>Non-uniformity of contamination</i>	<i>35</i>
2.9.3 <i>Critical points</i>	<i>37</i>
2.9.4 <i>Cleaning mechanisms</i>	<i>37</i>
2.9.5 <i>Criteria for the selection of cleaning agents and methods.....</i>	<i>39</i>
2.9.6 <i>Requirements for cleaning procedures.....</i>	<i>41</i>
2.9.7 <i>Cleaning procedure - development and validation.....</i>	<i>45</i>
2.9.8 <i>Equipment storage and holding times</i>	<i>47</i>
2.10 SETTING ACCEPTANCE LIMITS FOR RESIDUE CLEANING	48
2.10.1 <i>Limits based on the pharmacological potency of a drug product.....</i>	<i>51</i>

2.10.2	<i>Percentage of toxic doses</i>	53
2.10.3	<i>No more than X ppm</i>	54
2.10.4	<i>Limits based on analytical limitations</i>	54
2.10.5	<i>Visual acceptance limits</i>	55
2.10.6	<i>Limits for microbiological contamination</i>	56
2.11	SAMPLING METHODS	58
2.11.1	<i>Swab sampling</i>	59
2.11.2	<i>Sampling of rinse solution</i>	61
2.11.3	<i>Placebo sampling</i>	68
2.11.4	<i>Product sampling</i>	68
2.11.5	<i>Contact sampling</i>	69
2.11.6	<i>Direct surface monitoring</i>	69
2.11.7	<i>Visual examination</i>	69
2.12	DOCUMENTING A CLEANING VALIDATION PROGRAM	69
2.12.1	<i>The cleaning validation master plan</i>	70
2.12.2	<i>The operating procedure describing the cleaning</i>	70
2.12.3	<i>Cleaning records</i>	72
2.12.4	<i>The cleaning validation plan/ protocol</i>	72
2.12.5	<i>The cleaning validation report</i>	73
2.13	CHANGE CONTROL	74
2.14	REVALIDATION OF CLEANING PROCEDURES	74
2.15	MAINTAINING THE CLEANING PROCEDURES IN A VALIDATED STATE	76
2.15.1	<i>Operator variability and an effective and continuous training program</i>	77
2.15.2	<i>Equipment aging, repair and maintenance program</i>	77
2.15.3	<i>Potential non-representative results and monitoring program</i>	78
2.15.4	<i>Changes to the product, equipment, process, or batch size: the expansion program</i>	79
2.16	CONCLUSION	79
CHAPTER 3: ANALYTICAL METHODS USED FOR CLEANING VALIDATION - REVIEW OF LITERATURE		81
3.1	GENERAL CONSIDERATIONS	81
3.2	REQUIREMENTS FOR OPERATIONAL PARAMETERS OF ANALYTICAL METHODS	82
3.2.1	<i>Method accuracy</i>	82
3.2.2	<i>Method specificity and selectivity</i>	83
3.2.3	<i>Precision and reproducibility</i>	83
3.2.4	<i>Linearity of response and range</i>	84
3.2.5	<i>Limits of sensitivity</i>	85
3.2.6	<i>Robustness and ruggedness</i>	86
3.3	ANALYTICAL REQUIREMENTS FOR PERCENT SAMPLE RECOVERY AND SAMPLE STABILITY	86
3.3.1	<i>Recovery rate from swab material</i>	87
3.3.2	<i>Recovery rate from coupon materials</i>	88
3.3.3	<i>Recovery rate from rinsates</i>	90
3.3.4	<i>Sample stability</i>	91
3.4	CRITERIA FOR THE SELECTION OF ANALYTICAL METHODS	91
3.5	SPECIFIC VS. NONSPECIFIC METHODS	91
3.5.1	<i>Specific analysis methods</i>	93
3.5.2	<i>Nonspecific analysis methods</i>	101
3.6	DATA ANALYSIS FOR ESTIMATING CONTAMINATION	107
3.7	CONCLUSION	107
CHAPTER 4: A VALIDATED HPLC METHOD FOR DETERMINING RESIDUES OF A DUAL ACTIVE INGREDIENT ANTIMALARIAL DRUG ON MANUFACTURING EQUIPMENT SURFACES		109
4.1	INTRODUCTION	109
4.2	MATERIALS AND METHODS	112
4.2.1	<i>Equipment</i>	112
4.2.2	<i>Chemicals and reagents</i>	112

4.2.3	<i>Chromatographic conditions</i>	112
4.2.4	<i>Preparation of calibration solutions</i>	113
4.2.5	<i>Sample preparation</i>	113
4.3	RESULTS AND DISCUSSION	114
4.3.1	<i>Method optimization</i>	114
4.3.2	<i>Selectivity</i>	114
4.3.3	<i>Method range</i>	117
4.3.4	<i>Linearity</i>	117
4.3.5	<i>Precision (by repeatability)</i>	118
4.3.6	<i>Precision (by reproducibility)</i>	119
4.3.7	<i>Accuracy (by recovery)</i>	119
4.3.8	<i>Establishing limits for the cross-contamination level permitted on clean equipment</i>	120
4.3.9	<i>Determination of recovery rate of contaminants from stainless steel</i>	121
4.3.10	<i>Sample stability</i>	123
4.3.11	<i>Assay of swab samples collected from the equipment train</i>	123
4.3.12	<i>The place of the analytical method within a cleaning validation strategy</i>	124
4.4	CONCLUSION	125
CHAPTER 5: ASSESSMENT OF MEKC SUITABILITY FOR RESIDUE DRUG MONITORING ON PHARMACEUTICAL MANUFACTURING EQUIPMENT		126
5.1	INTRODUCTION	126
5.2	MATERIALS AND METHODS	131
5.2.1	<i>Equipment</i>	131
5.2.2	<i>Chemicals and reagents</i>	131
5.2.3	<i>Background electrolyte, standard solutions and sample preparation</i>	132
5.3	RESULTS AND DISCUSSION	133
5.3.1	<i>MEKC method development and optimisation</i>	133
5.3.2	<i>MEKC method validation</i>	141
5.3.3	<i>Establishing limits for the contamination and cross-contamination level permitted on cleaned equipment</i>	143
5.3.4	<i>Determination of recovery rate of contaminants from stainless steel and plexiglas surfaces</i>	145
5.4	CONCLUSION	147
CHAPTER 6: DESIGN OF THE CLEANING VALIDATION PROGRAM FOR THE PHARMACEUTICAL MANUFACTURING PLANT OF ROCHE SA - WORKING MODEL		148
6.1	PLACING THE CLEANING VALIDATION PROGRAM FOR ROCHE SA WITHIN THE BROADER VALIDATION FRAMES	149
6.2	DETAILING THE CLEANING VALIDATION PROGRAM	152
6.2.1	<i>The documentation structure elaborated for cleaning validation</i>	153
6.2.2	<i>Validation strategy</i>	153
6.2.3	<i>Assigning responsibilities for cleaning validation</i>	154
6.2.4	<i>Identification of production equipment, cleaning procedures and products existent in the manufacturing facility</i>	158
6.2.5	<i>Performing the risk assessment analysis</i>	159
6.2.6	<i>Outlining the key testing principles for cleaning validation</i>	159
6.2.7	<i>Tackling a cleaning validation project – Fansidar tablets</i>	165
6.3	CONCLUSION	171
CHAPTER 7: AN OVERVIEW ON THE VALIDATION APPROACH FOR MOIST HEAT STERILIZATION		174
7.1	INTRODUCTION	174
7.2	THE STERILITY CONCEPT AND STERILIZATION PRINCIPLES	174
7.3	STERILIZATION BY SATURATED STEAM IN THE AUTOCLAVE	175
7.4	TYPES OF CYCLES USED IN MOIST HEAT STERILIZATION	176
7.5	DEVELOPMENT OF STERILIZATION CYCLES	179
7.6	MEASURING THE EFFICIENCY OF THE STERILIZATION PROCESS	181

7.7	QUALIFYING AN AUTOCLAVE AND VALIDATING THE STERILIZATION PROCESS	185
7.8	THE QUALIFICATION - VALIDATION PROCEDURE.....	186
7.8.1	<i>Commissioning or pre-installation qualification (IQ) phase.....</i>	187
7.8.2	<i>Installation qualification (IQ) – operational qualification (OQ) phase</i>	188
7.8.3	<i>Performance qualification (PQ) phase.....</i>	190
7.9	QUALIFICATION - VALIDATION REPORT	192
7.10	WHEN REQUALIFICATION - REVALIDATION ACTIVITIES ARE REQUIRED	193
7.11	CHANGE CONTROL	193
7.12	ESTIMATION OF PROBABILITY OF NON-STERILITY OF A LOAD DURING THE VALIDATION OF STEAM STERILIZATION PROCESS	194
7.12.1	<i>Introduction.....</i>	194
7.12.2	<i>Materials and Methods</i>	194
7.12.3	<i>Results</i>	196
7.12.4	<i>Discussion</i>	202
7.13	CONCLUSION	204
CHAPTER 8: FINAL CONCLUSIONS.....		205
REFERENCES		209
SUMMARY		226
APPENDICES		229
Appendix A	<i>An overview of the validation approach for moist heat sterilization, Part I</i>	
Appendix B	<i>An overview of the validation approach for moist heat sterilization, Part II</i>	
Appendix C	<i>A validated HLC method for determining residues of a dual active ingredient anti - malarial drug on manufacturing equipment surfaces</i>	
Appendix D	<i>Cleaning validation plan – working template</i>	
Appendix E	<i>Cleaning validation report – working template</i>	
Appendix F	<i>Sampling record – working form</i>	
	<i>Results of microbiological testing– working form</i>	
	<i>Results of chemical testing– working form</i>	
	<i>Data interpretation for chemical testing– working form</i>	

List of tables

Table 2.1	Comparison of advantages of using swab and rinse samples
Table 2.2	Comparison of disadvantages of using swab and rinse samples
Table 4.1	Parameters of linearity for pyrimethamine and sulfadoxine
Table 4.2	Intra- assay precision (1 day) for PYR and SUL
Table 4.3	Inter-assay precision (three days, one instrument, one operator) for PYR and SUL
Table 4.4	Accuracy (by recovery) for PYR and SUL
Table 4.5	Maximum permitted levels of cross-contamination with PYR and SUL obtained through two different calculation methods
Table 4.6	Recovery of PYR and SUL from stainless steel coupons
Table 4.7	Sample stability for PYR and SUL (samples kept at 4°C in the dark for seven days)
Table 4.8	Results obtained for the determination of PYR and SUL in actual swab samples collected from the manufacturing equipment chain
Table 5.1	Parameters of linearity for the Co-trimoxazole mixture compounds
Table 5.2	Intra- assay precision (one day) for Co-trimoxazole mixture compounds
Table 5.3	Inter-assay precision (three days, one instrument, one operator) for Co-trimoxazole mixture compounds
Table 5.4	Accuracy by recovery for Co-trimoxazole mixture compounds
Table 5.5	Recovery of SMZ, TMP and NP9 from stainless steel and plexiglass coupons
Table 6.1	Responsibilities for cleaning validation
Table 6.2	Matrix of equipment, cleaning procedures and products (solids manufacture and packaging operations)
Table 6.3	Physical characteristics of the products manufactured
Table 6.4	Final selection of cleaning procedures/ equipment/ products for cleaning validation
Table 6.5	Sampling plan for cleaning validation of Fansidar tablets
Table 7.1	Combinations of temperature and time calculated to provide F0-values of 8- and 12- minute equivalents based on reference spores of <i>Bacillus stearothermophilus</i> with a D121 of 1.5 minutes and a z-value of 10°C
Table 7.2	Temperature data interpretation for a heat distribution study during exposure phase

Table 7.3	Temperature data interpretation for a heat penetration study during exposure phase
Table 7.4	Accumulated F0-value interpretation for heat penetration study
Table 7.5	Sterility assurance levels (SALs) attained for each run

List of figures

- Figure 2.1 Allowable contamination limits that take into account the potency of the drug active
- Figure 4.1 Chemical structures of sulfadoxine, pyrimethamine and sodium xylene sulfonate
- Figure 4.2 Absorption spectra of PYR (2 µg/ml), SUL (2 µg/ml) and SXS (12.5 µg/ml) in mobile phase
- Figure 4.3 Typical chromatograms for: mobile phase (a), an extracted swab blank (b), the chromatogram obtained for the 100% level (c), an SXS solution (d), a detergent solution (e) and a combined sample containing detergent, swab and the drug actives (f)
- Figure 5.1 Chemical structures of trimethoprim, 3,4,5 – trimethoxybenzoic acid, sulfamethoxazole, sulfanilamide, sulfanilic acid and nonoxynol – 9
- Figure 5.2 Metabolic pathways of tetrahydrofolic acid and inhibitive actions of SMZ and TMP over the dihydropteroate reductase and dihydrofolate reductase
- Figure 5.3 Electrophoretic mobilities of solutes obtained as a function of pH in the range 6.6 – 8.2. BGE: 25 mM phosphate containing 20 mM SDS. Operating conditions: 20 kV; 25°C
- Figure 5.4 Electrophoretic mobilities of solutes obtained as a function of voltage in the range 18 kV – 28 kV. BGE: 25 mM phosphate pH 7.8 containing 20 mM SDS. Operating conditions: 25°C; injection: 4 s sample followed by 2s BGE
- Figure 5.5 Electrophoretic mobilities of solutes obtained at various BGE concentrations ranging from 10 mM to 50 mM. BGE pH 7.8 contains 10 mM SDS. The calculated ionic strength of BGE solutions is also given on the X axis. Operating conditions: 25°C; 22 kV; injection: 4 s sample followed by 2s BGE.
- Figure 5.6 Electrophoretic mobilities of solutes obtained at various ionic strengths. BGE concentration ranges from 10 mM to 50 mM. Phosphate BGE pH 7.8 containing 10 mM SDS. Operating conditions: 25°C; 22 kV; injection: 4 s sample followed by 2s BGE
- Figure 5.7 Electropherogram obtained with the optimized migration conditions (30 mM phosphate BGE pH 7.8 containing 10 mM SDS, applied voltage of 22 kV and 23°C). Injection: 4 s sample followed by 2s BGE
- Figure 6.1 Place of cleaning validation steps within the broader validation program

- Figure 6.2 Structure of documentation used for cleaning validation
- Figure 6.3 Workflow of manufacturing process for Fansidar tablets
- Figure 6.4 Flowchart expressing the relationships between the elements of the cleaning validation program.
- Figure 7.1 The temperature profile for a saturated steam-vented cycle
- Figure 7.2 The temperature profile for a saturated-steam with forced air removal cycle
- Figure 7.3 Representation of the autoclave chamber (overhead view)
- Figure 7.4 Representation of the middle portion of the temperature profiles in the chamber recorded during the second run

List of abbreviations and symbols

AAS	Atomic absorption spectrometry
ADI	Acceptable daily intake
ANSI	American National Standard Institute
API	Active pharmaceutical ingredient
ATP	Adenosin Triphosphate
AMP	Adenosin Monophosphate
BGE	Background electrolyte
BI	Biological indicator
BP	British Pharmacopoeia
CE	Capillary electrophoresis
CFU	Colony – forming unit
cGMP	Current Good Manufacturing Practices
CIA	Capillary ion analysis
CIP	Clean- in- place
CV	Cleaning validation
CVMP	Cleaning validation master plan
CVP	Cleaning validation plan
CVR	Cleaning validation report
CZE	Capillary zone electrophoresis
DAD	Diode array detector
DCA	Dynamic contact angle
DES	Diethylstilbestrol
DQ	Design Qualification
DRS	Diffuse Reflectance Spectroscopy
ELISA	Enzyme-linked immunosorbent assay
ELSD	Evaporative light scattering detector
EMA	European Medicines Agency
EP	European Pharmacopoeia
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
GC	Gas chromatography

GMP	Good Manufacturing Practices
HPLC	High performance liquid chromatography
H ⁺	Hydrogen ion
IC	Ion chromatography
ICP	Inductively coupled plasma
IMS	Ion Mobility Spectrometry
IP	Intermediate precision
IQ	Installation qualification
kV	Kilovolt
LAL	Limulus Amoebocyte Lysate
LD ₅₀	Lethal dose for 50% of an animal population
LOC	Level of contamination
LOD	Limit of detection
LOQ	Limit of quantification
LTD	Lowest therapeutic dose
MAESA	Multifunctional active-excitation spectral analyzer
MAR	Maximum allowable residue
MCC	Medicines Control Council
MEKC	Micellar electrokinetic chromatography
ml	milliliter
MSDS	Material safety data sheet
NADP ⁺	Nicotinamide adenine dinucleotide phosphate
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
ND	Non-detectable
NDA	New drug approval
ng	nanogram
NOAEL	No observed adverse effect level
OQ	Operational qualification
PAR	Proven acceptable ranges
pH	Concentration of hydrogen ions
pH*	Apparent pH, measured in organic solvents
PIC	Pharmaceutical Inspection Convention
PLC	Programmable logic controller
PMA	Pharmaceutical Manufacturing Association

ppb	Parts per billion
ppm	Parts per million
PQ	Performance qualification
PYR	Pyrimethamine
QA	Quality assurance
QC	Quality control
R&D	Research and development
r^2	Correlation coefficient
SAL	Sterility assurance level
SOP	Standard Operating Procedure
SUL	Sulfadoxine
SXS	Sodium xylene sulphonate
TLC	Thin layer chromatography
ToF-SIMS	Time of flight – secondary ion mass spectrometry
TQM	Total Quality Management
TSA	Trypticase Soy Agar
TSB	Trypticase soy broth
USP	United States Pharmacopoeia
UV-VIS	Ultraviolet – visible
WFI	Water for injection
WIP	Wash-in-place

Chapter 1: Introduction

1.1 Process validation and quality aspects

1.1.1 The first concerns for quality and the definition of the concept

The philosophy of quality control can be traced back to the medieval Europe where craftsmen began organizing into unions in the late 13th century. Inspection committees enforced strict rules for product and service quality. Inspection marks were placed on goods serving as a proof of quality for customers until the Industrial Revolution in the early 19th century. Quality became a critical issue in the United States, during the World War II, when extensive quality checks were being performed on every unit of product received from factories in order to detect unsafe military equipment. In the 1960s the automotive and aerospace industries have joined the strives for quality.

The concept of quality control in areas related to the food and drug safety goes back to 1906 when the Food and Drug Administration (FDA) was formed in the United States as a response to the book “Jungle” of Upton Sinclair depicting the poor conditions of food markets in Chicago. The mission of the agency was initially preventing the adulteration of food products.

In 1937, 108 people - mainly children died because of poisoning with an elixir containing sulfanilamide formulated by using as solvent diethyl glycol, a highly toxic chemical analogue of antifreeze. Through the Food, Drug and Cosmetic act (FDA, 1938) the FDA was charged with the responsibility to promote public health and ensure that companies supplying food, drugs and cosmetics can prove the safety of their products.

The term of quality has no specific meaning unless related to a specific function or object. The Concise Oxford Dictionary (Fowler and Fowler, 1964) defines quality as “degree of excellence”.

The following paragraphs are aimed to provide definitions for quality and quality related terms, within the manufacturing context, as presented by various sources:

Gilmore defines quality as “the degree to which a specific product satisfies the wants of a specific consumer” (Gilmore, 1974).

According to Crosby (1979) quality means “conformance to requirements”.

Juran defines quality as “fitness for use”. He stresses a balance between product features and products free from deficiencies. As used by Juran, the word product refers to the output of any process, and that includes goods as well as services (Juran and Gryna, 1988).

The Roche Pharma Manufacturing Standards glossary (Lothar and Juergen, 2006) defines quality as the “degree to which a set of inherent characteristics of a product, system or service fulfils requirements”.

A quality system as defined by (PIC/S, 2004a) is “the sum of all that is necessary to implement an organisation’s quality policy and meet quality objectives. It includes organization structure, responsibilities, procedures, systems, processes and resources”.

Nally (1984) has proposed the following definitions related to quality assurance and quality control:

Quality control is “the regulatory process through which the industry measures actual quality performance, compares it with standards, and acts on the difference”. It covers all measures taken, including the setting of specifications, sampling, testing, analytical clearance to ensure that raw materials, intermediates, packaging materials and finished pharmaceutical products conform with established limits for identity, strength, purity and other characteristics.

Quality assurance is “the activity of providing, to all concerned, the evidence needed to establish confidence that the quality function is being performed adequately”.

Sometimes the terms quality assurance and quality control are used interchangeably to refer to ways of ensuring the quality of a product. Whilst quality control refers to the testing techniques and activities used to fulfill quality requirements, quality assurance refers to the planned and

systematic activities through which adequate confidence is obtained about a product's quality characteristics.

It is evident from all the above definitions that the quality of a drug product is a sum of all factors which contribute directly or indirectly to the safety, effectiveness and acceptability of the product.

1.1.2 Early contamination issues for drug and medical devices

In the early 1960s FDA became aware of the potential health hazard associated with the cross-contamination of drugs. In one case it was reported that young males were developing female characteristics after taking a vitamin preparation. The FDA inspection revealed manufacturing practices suspected of causing cross-contamination with diethylstilbestrol (DES) (Weber, Grossman *et al.*, 1963). Current knowledge now suggests that DES is an endocrine disrupting chemical and is a synthetic non-steroidal compound with estrogenic activity. However, it was widely used from the late 1940s to 1970 for women with high-risk pregnancies (Herbst, Ulfelder *et al.*, 1999).

Evidence was also found by FDA that certain drug preparations were contaminated by penicillin. The cross-contamination was occurring through product dust while handling penicillin and other drug materials in the same manufacturing and packaging areas (Carter, 1977).

Unfortunately in both cases it was difficult to know what to look for in the manufacturing facilities until the patients had been injured and manifested symptoms that might be associated with the hormone or the antibiotic.

Another example of poor quality control is where a manufacturer had to recall insulin syringes because of complaints that the needles were clogged. Upon investigation it was revealed that silicone oil was used as lubricant for their manufacture. Although the method used to remove the silicone oil was not effective, the visual inspection of the needles did not detect any contamination problems (Chantelau and Berger, 1985).

Because of the sporadic detection of lack of quality control or perhaps a lack of knowledge regarding quality control procedures, the FDA started to develop the philosophy of quality control and how it is to be administrated.

In 1962 the FDA has issued the first set of Good Manufacturing Practices (GMP) for the Pharmaceutical and Medical Device Manufacturers as a means of assessing the quality procedures and practices of a company.

In the early stages of regulating quality control, the FDA was tempted to look only at the final product rather than the process itself. This was reflected by extensive sampling from finished batches of drugs followed by testing in FDA laboratories. However, testing only the finished product was not an assurance that the product was manufactured according to quality standards. Although no real proof of non-sterility could be found by FDA, following complaints for a parenteral drug, the sterility tests performed on the final product could not ensure that the lot from which the sample was taken was sterile (Broker, 1980).

After 1970s, FDA has discovered contamination problems caused by insufficient cleaning or lack of maintenance of equipment (Carter, 1977). In 1988 a batch of finished drug product called Cholesterylamine Resin USP was recalled because the bulk pharmaceutical chemical used to produce the product was contaminated with low levels of degradation products from the production of agricultural pesticides, although no pesticide was produced in the manufacturing site. The cross-contamination was believed to be due to the reuse of recovered solvent kept in contaminated drums (FDA, 1993).

Such incidents and concerns regarding the quality of the final product led to the evaluation of manufacturing processes and the development of the validation concept.

1.1.3 The development of the validation concept

Requirements for process validation, but without the formal recognition of the concept have been developed since 1963, with the current Good Manufacturing Practices (cGMP) regulations for Finished Pharmaceuticals. The FDA was able to consider a product as adulterated if “the methods used in, or the facilities or controls used for its manufacture, processing, packing or holding do

not conform to or are not operated or administered in conformity with current good manufacturing practices to assure that such drug meets the requirements of the act as to safety and has the identity and strength, and meets the quality and purity characteristics, which it purports or is represented to possess” (Federal Register, 1963).

In 1976 FDA proposed a new set of cGMP. The “c” was indicating that the regulations are in constant evolution and what is acceptable today may not be accepted tomorrow.

The earliest reference with regards to the syntax “process validation” goes back to 1977 in a paper entitled “A system approach to validation” (Shatto, 1977). Although, the term validation was by that time known by FDA and even defined by the cGMP regulations for drug products (Federal Register, 1978), the document omitted to explain what was meant by process validation and how does it fit into cGMP.

Only in 1987 some clear guidelines were set. The FDA Guidelines on general principles of process validation (FDA, 1987) offered the first explicit discussion on the subject and for the first time clarified and defined terms used in process validation. According to the document, “process validation is establishing documented evidence which provides a high degree of assurance that a specific process (such as the manufacture of pharmaceutical dosage forms) will consistently produce a product meeting its predetermined specifications and quality characteristics”.

Since the 1987 FDA’s document, other definitions for process validation were developed and used by various authors and regulatory bodies. However, these definitions incorporate the same elements as listed by the FDA definition. Examples are given below:

- Carlberg (1995) defines process validation as ”full detailed documentation that all processes and procedures are functioning in the manner they were designed for”.
- Byers (1981) refers to process validation as “a scientifically designed program to prove that a process consistently does what it is designed (intended) to do“.
- Gold (1996) has presented an extended discussion on validation stating that “validation is necessary since testing of a product to assess its quality is not enough.”

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- According to Chapman (1984) process validation represents “establishing documented evidence that a process does what it purports to do, in other words, that the procedure produces a quality product in such a way that its safety and efficacy are ensured”.
 - EMEA (1999) defines process validation as “the means of ensuring and providing evidence that processes are capable of consistently producing a finished product of the required quality”.
 - According to PIC/S (2004) process validation is “the means of ensuring and providing evidence that processes are capable of repeatedly and reliably producing a finished product of the required quality”.

A current definition of validation is given by the 21 CFR Part 820.3 (2005). “Validation means confirmation by examination and provision of objective evidence that the particular requirements for a specified intended use can be consistently fulfilled”. Also, according to the same document, “Process validation means establishing by objective evidence that a process consistently produces a result or product meeting its predetermined specifications”.

Also, the latest validation guidelines elaborated by WHO (2005) define validation as the “action of providing evidence and documenting that any process, procedure or method actually leads to the expected results”.

It is to be observed that for the pharmaceutical industry validation has gained over time new dimensions and has become the tool through which assurance is obtained that the same quality results can be expected repeatedly from a process provided that its most important variables are under a state of control.

1.1.4 Why validate a process?

The three most important reasons for validation are quality assurance, compliance and economics. These three factors will now be discussed.

1.1.4.1 Quality assurance requirements

Agalloco has stated (1995) that validation should focus on confirmation of product quality attributes and the processes which affect the quality. This statement holds true as the quality of a pharmaceutical product is the first biggest concern of a manufacturer.

The quality of a medicine is associated with its safety and efficacy. Quality should be therefore built into a product by following good manufacturing practices, before the product is tested.

According to Delahunty (1982) quality should not be inspected or tested into the final product. The routine quality control testing does not assure product quality as the proper functioning of various factors involved in the manufacturing process cannot be verified at that point in time. There are also limitations connected to the statistical sampling and a certain level of uncertainty whether the sample collected is truly representative for the entire product batch tested.

The importance of quality assurance within the validation concept has been stressed by the Pharmaceutical Manufacturers Association (1985) and FDA (1987). According to the FDA's document (1987) the basic principles of quality may be stated as follows:

- Quality, safety, and effectiveness must be designed and built into the product;
- Quality cannot be inspected or tested into the finished product; and
- Each step of the manufacturing process must be controlled to maximize the probability that the finished product meets all quality and design specifications.

1.1.4.2 Compliance requirements

The industry's regulatory bodies are seeking to create norms based on which the consistency of various processes can be measured. It is compelling for every manufacturer to study the various manufacturing processes and find the critical process parameters that are likely to influence the quality of the final product.

Process validation is currently required by cGMP regulations promulgated by the FDA. Section 211.110 of these regulations states that it is necessary "to monitor the output and to validate the performance of those manufacturing processes that may be responsible for causing variability in

the characteristics of in-process material and the drug product” (Code of Federal Regulations, 2005).

The Note for Guidance on process validation (EMA, 1999) sets out the data to be included in a marketing authorization application in terms of the validation of the manufacture of medicinal products. In 2001 new guidelines for process validation came into force in Europe (EU Guide to GMP, 2001).

The latest update on regulations for validation have been published by WHO (2005). The document is intended as a guideline for both manufacturers and inspectors.

1.1.4.3 Economic requirements

From an economical point of view a company can save money and time by validating its processes. When a process is validated there is certainty that it will produce its expected results and therefore it will require less monitoring, sampling and testing.

Process validation should be also performed because it makes good engineering sense and it is in accordance with good business judgment. Performing validation would lead to indirect cost saving resulting from the reduction of number of batches rejected /reworked or recalled, less complaints and therefore more technically and economically sound products (Loftus, 1993).

Agalloco (1986) has highlighted the business aspects and listed the numerous benefits of process validation to the manufacturer.

1.1.5 **What needs validation?**

Although agencies like the FDA and researchers have formulated the above-mentioned reasons for validation, and worldwide regulatory authorities have emphasized that process validation is a general concept which should be applied and adjusted to the specific structure and needs of each company, the question that arises is: what needs to be done and by when?

There is some confusion as to what constitutes process validation as interpretations vary between the authorities and the textbooks (Helle, Mannerman & Yliruusi, 2003). Pharmaceutical process

validation has been understood in two different ways – either as the total validation activity in a pharmaceutical manufacturing site from process development, qualification of the equipment and ancillary systems to the final validation of three consequent batches of the final product; or as the final production-scale validation of a pharmaceutical preparation only.

There is no absolute way to perform any validation. The extent to which a company performs the validation is determined by quality, compliance and safety factors and is also influenced by time and economic constraints.

The following is a list of variables that could influence the process outcome and therefore the quality of a product:

- equipment type and configuration;
- adjacent computerized systems;
- process parameters;
- environmental monitoring systems;
- raw materials and packaging materials;
- water, air and gas systems;
- sterilization processes;
- equipment cleaning methods;
- laboratory analysis methods and instruments;
- in-process control instruments.

The above list, which is not exclusive, contains both systems and processes. Often manufacturers refer to the "qualification of systems/ equipment" and "validation of processes". The general idea is that all the critical variables influencing the quality of the final product should be monitored and assurance should be obtained through testing that the product will be of consistent quality and safety. With such a volume of work involved it is to be understood that validation requires a joint effort and expertise from various departments. The validation tasks involve input of personnel from process development, engineering, manufacturing, quality assurance and control departments.

1.1.6 Process validation steps as determined by the product “life-cycle”

Validation, as a dynamic process, follows a timeline, usually called life-cycle, stretching from the initial design of a product through ongoing commercial operations. The paragraphs below identify in chronologic order the steps involved in process validation.

1.1.6.1 Process development

The development stages of the process include pre-requisite activities for process validation which are related to:

- Description of process and product and definition of critical process parameters;
- Specification of proven acceptable ranges (PAR) of parameters.

1.1.6.2 Process validation and equipment qualification

The validation phase itself can be split into pre-validation and an actual execution stages.

The pre-validation stage should include the establishment of a Master Plan, a change control system, master production documents and related standard operating procedures (SOP's), qualification activities for equipment and supporting systems together with the generation of approved qualification/ validation plans. Safety issues and personnel training must be also addressed at this stage.

Qualification is a subset of activities that plays a key role during pre-validation, when the proper design, installation and qualification of equipment and supporting systems ensures the late success in process validation.

Qualification, as defined by the Annex 15 (EU Guide to GMP, 2001) “represents the identification of equipment attributes related to the performance of a particular function or functions and allocation of certain limits or restrictions to those attributes”.

Qualification is regarded by the SA GMP Guide (PMA SA, 2004) as an integral part of process validation. The FDA (1987) has also defined qualification as “establishing documented evidence

which provides a high degree of assurance that a specific piece of equipment/ hardware will consistently work to its pre-determined specifications and quality attributes”.

Qualification activities are subdivided into design qualification (DQ), installation qualification (IQ), operational qualification (OQ) and performance qualification (PQ).

During the DQ phase evidence is gathered that the design of the systems complies with the cGMP requirements. DQ documentation includes approved user requirement specifications, system drawings, construction and assembly schemes. During IQ evidence is gathered and recorded that the equipment as installed complies with the manufacturer’s recommendations. The aim of performing OQ is to gather evidence that the equipment, as installed, performs as intended throughout the anticipated operating ranges. PQ verifies the system performances and is conducted under actual running conditions.

The general qualification approach was debated and exemplified by various authors (Chapman, 1993; King, 1998).

The execution stages of validation include the manufacturing of the product under the pre-defined conditions, data collection and interpretation as well as environmental monitoring activities. In parallel with process validation, cleaning validation should also take place. The validation report should contain comments about the outcome of the validation activities and should record all the deviations from the initial plan together with comments on their effect on validation.

1.1.6.3 Maintenance of the validated state

Maintaining the validated/ qualified status of relevant processes and equipment is as important as the validation itself. Monitoring activities include testing of product, utilities (e.g., water, gas, air) and environment. Through trend analysis should be shown that all processes are stable and under control. Deviations, failures, complaints and recalls should be reviewed as well throughout the life-cycle of a product.

1.1.7 Validation approaches

Various validation approaches are being used by the manufacturers depending on circumstances related to the characteristics and history of a manufacturing process and product. Normally it would be to validate a process prior to the distribution of the finished product (prospective validation). When this is not possible it is necessary to validate the process during routine production (concurrent validation). Processes that have been in use for some time without suffering significant changes may be validated retrospectively.

1.1.7.1 Prospective process validation

The FDA considers prospective validation as “validation conducted prior to the distribution of either a new product, or product made under a revised manufacturing process, where the revisions may affect the product’s characteristics” (FDA, 1987). The main objective of this approach is to minimize the risk of errors in processes for products newly reaching the market (Chao, Forbes *et. al.*, 1993). This approach is also recommended for existing processes that have undergone changes.

1.1.7.2 Concurrent process validation

Concurrent validation can be seen as a special case of prospective validation and refers to “the establishing of documented evidence that a process does what it purports to do based on information generated during actual implementation of the process” (FDA, 1987). However, this situation can lead to questions regarding disposition of the batches that have been released for sale.

Therefore, concurrent validation should mainly be applied through extensive testing and monitoring in cases when changes are made to existing processes, for site transfer validations and line extensions. The decision to perform concurrent validation rather than prospective validation must be scientifically justified.

1.1.7.3 Retrospective process validation

Retrospective process validation consists of a retrospective review and evaluation of historical data of batches already produced. The FDA accepts retrospective validation only for well-established and controlled processes that have not exhibited recent critical changes in raw materials characteristics, product compositions, environmental controls, manufacturing processes and equipment, operating procedures, microbial controls and analytical methodologies and specifications (Agalloco, 1983). The steps involved in retrospective validation still require the preparation of a protocol, the reporting of results leading to conclusions and recommendations.

1.1.8 The South African context

In South Africa, the Medicines Control Council (MCC) is the pharmaceutical manufacturing industry's regulatory body established since 1965 through "The medicines and related substances control", Act No. 101 (van der Merwe, Cowper & White-Guai., 2001). In the circular 14/1983 (MCC, 1983) the concept of process validation was firstly introduced and states the following:

"It was recently resolved by the Medicines Control Council that process and system validation in pharmaceutical processing be accepted in principle as an essential component of quality assurance (QA) and therefore essential to ensure uniform quality on a dosage unit and batch to batch basis. In addition, it was resolved that a list of processes, which should receive priority in terms of safety, be compiled by the Inspectorate, together with a time schedule whereby the respective systems and processes should be validated" (MCC, 1983).

An early edition of the SA Guide to GMP (PMA SA, 1996) defines the term "to validate" as "to provide documented evidence that an item of equipment, process, system or method is in a state of control (i.e. that all assignable causes of variation have been eliminated) and is able to consistently deliver specified results".

In the circular 7/98, the subject of process validation was discussed again by the MCC (MCC, 1998). The MCC circular argues issues such as when should validation/re-validation be performed, to what extent and what does it involve. Minimum requirements for compiling validation protocols and reports are defined.

The more recent SA GMP Guide (PMA SA, 2004) states that “validation is an integral part of current good manufacturing practice; it is therefore, also an element of the quality assurance program associated with a particular product or process”. It is also stated that “a process is in a state of control when all the assignable causes of variation have been eliminated, only chance causes of variation are present. Such a process has thus been demonstrated to be capable of consistently delivering specified results, i.e. the process has been validated”.

1.1.9 Current validation trends

From its beginnings, around 30 years ago, process validation has come a long way gaining more acceptability from the industry side and understanding based on in-depth knowledge from regulatory bodies.

Early approaches consisting in vast amounts of work targeting to control the multitude of variables of pharmaceutical processes have been simplified. Nowadays the risk-benefit analysis and statistical analysis play increasing roles in selecting and monitoring of critical process variables and prioritizing the work for validation.

Currently, the classical approach for process validation based on the lifecycle of a product is still used by lots of pharmaceutical manufacturing companies. The “trial and error” approaches or validating “one factor at a time” are most familiar to the industry. New technologies such as the use of online monitoring and experimental design studies have started to be used in order to aid process optimization, reduce variability and assist with drug formulation and validation of manufacturing processes. Such approaches help to reach the root cause of deviations and variables and reduce their number (Anderson and Anderson, 1999; Faure, York & Rowe, 2001; Rekhi, Nellore *et. al.*, 1999).

Genentech has applied the power of experimental designs during the developmental stages of Avastin[®] in order to gain a thorough understanding of its manufacturing process and identify the critical process parameters (Bush, 2005).

Experimental design studies were also used for the validation of a multilayer tablet manufacturing process (Goutte, Guemguem *et al.*, 2002). Such studies have allowed the evaluation of process ruggedness at pilot scale and the assessment of specifications used for full production.

In a broad sense the validation could be seen as the application of Total Quality Management (TQM) principles within the Good Manufacturing Practices environment. Therefore, process validation needs to become part of the strategic quality management and performance improvement. Its benefits to patients as well as manufacturers have been recognized and from now on, the challenge is to find the right level of cost-effective validation without any risks to consumers (Helle, Mannermaa & Yliruusi, 2003).

1.2 Study layout

1.2.1 Motivation

At present, regulatory bodies set general guidelines without offering clear guidance with regards to validation and qualification. Although there is consensus reached with regards to why validation is vital for the industry, there is not enough information available with regards to how to perform validations. Also pharmaceutical companies perceive validation as a burden rather than a benefit as it involves high costs and resources to be allocated and therefore do not foresee immediate advantages of performing the validation.

Roche, South Africa has initiated a factory upgrade, aiming to set up a state of the art manufacturing facility, complying with good manufacturing practices and addressing the most stringent national and international regulatory requirements for process validation. In this endeavor, the systematization and close coordination of the validation activities in all sectors was crucial for the success of the project. Risk based analysis for all the critical processes are required as well as sound strategies and programs for validation.

1.2.2 Aim of study

The main aim of this research work is to assist the pharmaceutical industry, in particular Roche Products, with elaborating strategies and practical ways of validating their various processes.

1.2.3 Objectives

Being a very vast concept, not all the aspects of process validation can be covered in a single study. This dissertation will deal mainly with working models for:

- i) Cleaning validation in a galenical manufacturing plant. These aspects will be exemplified with the validation of the manual cleaning procedures applied to the manufacturing and packaging equipment used for an anti – malarial drug.
- ii) Development and validation of an analytical method suitable for a routine-type working environment that is capable to assay in a single run the multiple components present in a cleaning validation sample.
- iii) Approach development and validation for moist steam sterilization and qualification of a steam sterilizer.

1.2.4 Hypotheses

- i) Manual cleaning procedures applied to the production equipment can be designed and documented such that they can lead to reproducible cleaning results therefore making such procedures validatable.
- ii) Complex cleaning validation samples containing compounds with very different physio – chemical characteristics can be determined with a suitable analytical method in a single run.
- iii) Performing the validation of the moist steam sterilization process offers the guarantee of sterility and integrity for any batch of product undergoing the sterilization.

1.2.5 Null hypotheses

- i) Manual cleaning procedures applied to the production equipment cannot be reproducible, rugged enough and therefore not able of being validated.

- ii) Complex cleaning validation samples containing compounds with very different physio – chemical characteristics can not be determined in a single run, but rather via diversified analytical methods.
- iii) Performing the validation of the moist steam sterilization process is not a guarantee of sterility and integrity for any batch of product undergoing the sterilization.

Chapter 2: Cleaning Validation – review of literature

Cleaning validation is a key element within the process validation context, contributing to the verdict of release/reject for a pharmaceutical product.

In the past, contamination of a product by material from another product was unobserved due to limited detection possibilities of trace residues levels. Also the specifications for the finished product did not require tests for such residues, found in traces in the bulk product. Moreover, drug products were less potent and the carry-over from one product into the next one was of little practical consequence (Fourman and Mullen, 1993).

Cleaning validation was initially developed for safety purposes such as to reduce toxicological hazards associated with between-product cross-contamination. The importance of cleaning validation has been amplified over time, especially with the introduction of an increasing number of potent or toxic substances for medicinal and therapeutic use.

2.1 Definition of terms

The following paragraphs are intended to present definitions of the cleaning-related terminology discussed in this chapter.

Cleaning is represented by actions taken to eliminate a product, which could induce a minor hazard if present at trace level in another product (Laban, Cauwet *et. al.*, 1997).

Cleanability describes the extent to which a surface can be cleaned so that it can be operated safely and produce a product of acceptable quality (Valvis and Champion, 1999).

Decontamination, according to ASPEC Guidelines (1993) represents an “action taken to eliminate a product which could induce a major or critical hazard if present at trace levels in another product”. Decontamination represents the action of separating and eliminating stains usually invisible on a surface. These stains or contaminants are of chemical, microbiological or

particulate nature. Through decontamination is achieved ultra-cleanliness (Laban, Cauwet *et. al.*, 1997).

Sanitization is defined in the Technical Report of PDA (PDA, 1998) the action to “make physically clean and to remove and destroy, to the maximum degree that is practical agents injurious to health”.

Disinfection means, according to PDA (1998), “to adequately treat equipment, containers or utensils by a process that is effective in destroying vegetative cells or microorganisms of public health significance, and in substantially reducing numbers of other undesirable microorganisms”. The key difference between sanitization and disinfection is the degree of microbial reduction achieved. A sanitizing agent typically provides a bacterial reduction of 3 log percent on a pre-cleaned surface. A disinfectant can provide 100 percent of bacteria, target viruses and fungi.

Many authors and regulatory bodies have proposed definitions for cleaning validation. All definitions convey typical indicators such as: “documented evidence”, “consistent, effective cleaning”, “below acceptable levels of safety”. Examples of such definitions are given below.

In the first article written on cleaning validation, Harder (1984) states that a validated cleaning procedure is one “whose effectiveness has been proven by a documented program providing a high degree of assurance that a specific cleaning procedure, when performed appropriately, will consistently clean a particular piece of equipment to a predetermined level of cleanliness”.

Cleaning validation as defined by Jenkins, Vanderwielen *et. al.*, (1996) is the process of assuring that cleaning procedures effectively remove residue from manufacturing equipment/ facilities below a predetermined level.

According to Voss and Masterson (2000) “cleaning validation is documented proof that one can consistently and effectively clean a system or equipment item”.

A cleaning process it is said to be validated if one can show in a scientific and documented manner that the different steps of this process are capable of reproducibly removing residual contamination from a surface to a pre-determined level (Laban, Cauwet *et. al.*, 1997).

FDA (1993) defines cleaning validation as “establishing documented evidence which provides a high degree of assurance that a cleaning procedure consistently removes residues to pre-determined acceptable levels”.

In the PIC guideline (PIC/S, 2004) cleaning validation is defined as “establishing documented evidence that an approved cleaning procedure will provide equipment which is suitable for processing of pharmaceutical products or active pharmaceutical ingredients”.

In summary, cleaning validation means documented proof that a piece of equipment or tool can be reproducibly and effectively cleaned, based on a documented cleaning procedure, that the level of contamination (LOC) can be reduced through cleaning below a calculated target level or by a certain ratio. This means that sampling and analysis are carried out to assure that the LOC on the cleaned surface is lower than a pre-established, justified target limit.

2.2 Reasons and benefits for cleaning validation

Ensuring the quality, purity and safety of a given product requires for manufacturers to demonstrate that the cleaning procedures used will consistently minimize the probability of product adulteration (McCormick and Cullen, 1993). Therefore the need for safe products dictates all the other reasons arising for cleaning validation.

At customer level, product contamination is associated with serious health hazards that often have resulted in products being recalled from the market, therefore the manufacturer losing the consumer's credibility.

From a manufacturer point of view the main benefits attributed to cleaning validation are ensuring compliance with GMP requirements and international regulations (Jenkins, Vanderwielen *et. al.*, 1996). Also, certifying through validation the effectiveness of a cleaning procedure means that the analytical monitoring may be reduced to a minimum during the production routine phase (HPFBI, 2000).

Although in the initial phases the validation of cleaning procedures may add additional costs to the manufacturing process, in the long run it will save time and money associated with repeated

sampling and testing from batch to batch. Geigert, Klinke *et. al.*, (1994) have emphasized the importance of cleaning validation, as “making good business sense”.

Other reasons are safety of the cleaning process itself, as opposed to the safety of the manufactured product and environmental impact (LeBlanc, Danforth & Smith, 1996).

2.3 Objectives for cleaning validation

In many manufacturing facilities the processing equipment is non-dedicated, meaning that same equipment is used for manufacturing a range of products (more explanations regarding dedicated and non-dedicated equipment are provided under heading 2.9.6.1). A product can be contaminated by preceding batches of the same product, by other products previously manufactured with the same equipment, by cleaning agents, microorganisms or by other foreign materials. Therefore, the general goal of cleaning and sanitizing the pharmaceutical manufacturing equipment, is to ensure that the products manufactured or the cleaning process related contaminants do not leave residues that will adulterate the quality of the following manufactured product.

Typically, cleaning validation draws the line between product contact surfaces and non-product contact surfaces. Therefore, every company needs to develop a policy and objectives for cleaning validation that clearly define the boundaries of what requires cleaning validation and what doesn't.

FDA (1993) has noted that many companies have failed to develop such objectives and focused on extensive sampling and testing programs but without really evaluating the effectiveness of the steps used to clean the equipment. The FDA Guide states that the main rationale for requiring clean equipment is to prevent contamination and adulteration of the product (FDA, 1993). Therefore, the FDA requires cleaning validation to support the removal of drug residue, cleaning agent or sanitizer residue and microbiological residue.

The objective of cleaning validation, as formulated by PDA (1998) is to ensure that the cleaning procedures applied to manufacturing equipment consistently remove residues to predetermined levels of acceptability, so as to prevent the contaminants from adversely affecting the safety and

quality of the next product manufactured. The cleaning validation study is the process that incorporates all the scientific means for achieving these goals.

2.4 Regulatory aspects and review of official documents

The requirement for clean equipment was one of the commonly implemented quality assurance actions mandated by GMP regulations as early as 1963. Part 133.4 of GMP states that “equipment shall be maintained in a clean an orderly manner” (CFR, 1963).

In 1978 the updated cGMP regulations have recognized that cleaning is a critical process that ensures product quality. The term prevention was added to the previous definition of cleaning. Part 211.67 of CFR (1978) states that “equipment and utensils shall be cleaned, maintained and sanitized at appropriate intervals to prevent malfunctions or contamination that would alter safety, identity, strength, quality or purity of the drug product”. Also, part 211.182 of the previously mentioned document states that “cleaning procedures must be documented appropriately, and that the use of a cleaning log book should be established”.

Although equipment cleaning has become over the time part of the GMP requirements, the term “cleaning validation” was not well established until the late 1980s.

In Europe the GMP directive of medicinal products for human use (CEEC, 1991) deemed cleaning as an integral part of the manufacturing process. Details were given on restrictions of the choice of equipment and their use: “washing and cleaning equipment should be selected and used so as to minimize the risk of contamination”. In Chapter 3 of the guide the purpose of cleaning was defined as ”to prevent contamination, cross-contamination, deposition of dust and stains and, as a general rule, any adulteration to the quality of the product”.

Vanhooydonck (2000) has reviewed the subject of cleaning for manufacturing equipment and cleaning validation tackled in various official documents. In this regard he mentions that the 1985 French cGMP guide talks about cleaning but does not make any references to cleaning validation. The paragraphs of the 1983 British cGMP dealing with cleaning issues are also discussed.

Although “cleaning validation” was not specifically stated in the cGMPs, the FDA has vigorously enforced the requirement since the early 1990s. In February 1993, a case court in US versus Barr Laboratories ruled that cleaning validation is implied in the cGMP 211.67 and that cleaning validation is a critical process that must be validated (CFR, 1978). This ruling was based on the interpretation that 211.67 require the firm to have cleaning procedures for equipment and utensils; therefore the firm must prove that the cleaning procedure is effective. The court also ruled that cleaning validation was not limited only to major equipment because 211.67 required cleaning procedures for utensils as well (United States of America, plaintiff vs. Barr Laboratories, 1993).

Such events made the FDA aware of the hazards associated with cross-contamination and urged FDA to carry out extensive research on topic. In July 1992, while the Barr case was being debated in court, the agency’s Mid-Atlantic Region staff developed an internal inspection guideline for cleaning validation (FDA, 1992). The FDA guide stated that “validation of cleaning procedures has generated considerable discussion since agency documents, including the Inspection Guide for Bulk Pharmaceutical Chemicals and the Biotechnology guide briefly addressed this issue”.

The FDA Mid-Atlantic Region Inspection Guide on Cleaning Validation, revised in 1993, has provided the pharmaceutical industry with the first directions in cleaning validation (FDA, 1993).

FDA (1993) “expects firms to prepare validation procedures which address the responsibility for performing and approving the validation study and the acceptance criteria. FDA expects a final validation report approved by management, which states whether or not the cleaning process is valid”. The data should support the conclusion that residue have been reduced to an “ acceptable level”.

The FDA guide (1993) states also that “a drug shall be deemed to be adulterated if it consists in part of any filthy, putrid or decomposed substance, if it has been prepared, packed or held under unsanitary conditions whereby it may have been contaminated by filth, or whereby it may have been rendered injurious to health or if its container is composed, in whole or in part, of any poisonous or deleterious substance which may render the contents injurious to health”.

Recognizing the importance of the relationship between cleaning and quality, regulatory agencies have started to demand greater evidence of cleaning effectiveness through validation and

verification. The PIC document (PIC/S, 2004) states that cleaning procedures should be validated. Also, the cGMP (CFR, 2005) clearly defines cleaning as a critical process requiring validation.

In South Africa, the 2004 GMP Guide requires “written standard operating procedures (SOPs) detailing the cleaning process for equipment and apparatus” and “written SOP detailing how cleaning processes will be validated” (PMA SA, 2004).

2.5 Field of applicability for cleaning validation

The importance of cleaning validation has been extended in time to a range of industries due to the need of controlling contamination hazards associated to the handling of drugs in various stages and forms. Irrespective of the scale or type of production, cleaning validation programs must be identified for chemical synthesis of active pharmaceutical ingredients (APIs), bulk pharmaceutical, biopharmaceutical, finished pharmaceutical liquid, dry, solid and semi-solid dosage forms in both sterile and non-sterile presentations, for both clinical and marketed product.

The cleaning principles and goals, such as method consistency and reproducibility or cleaning acceptable levels are the same irrespective of the type of production. However the techniques and methods used to achieve these principles may differ (Hwang, 2000).

2.5.1 Cleaning validation for galenical manufacturing facilities

Cleaning validation was firstly developed for the compounding processes specific to galenical manufacturing. This dissertation tackles mainly this subject and therefore it will be discussed in depth later in the chapter.

The development of highly active drug substances, commonly called potent compounds, such as cytotoxic, teratogenic and anti-thrombotic agents has placed more emphasis on cleaning validation due to their toxic or pharmacological potential. Handling such compounds has presented the manufacturing industry with special challenges including containment, cleaning, and decontamination (Krull and Swartz, 1999). Due to their low-dose therapeutic profile, these classes of products, besides the cross-contamination risk, can also present serious risks of exposure of humans working in such an environment (Valvis and Champion, 1999).

For such drugs, the rationale behind the selection of cleaning solvents, the design of a cleaning procedure and the choice of an analytical method must be developed carefully. In addition, acceptance criteria for asserting cleaning as successful must be agreed prior production and be well defined.

2.5.2 Cleaning validation for biotechnological manufacturing facilities

The cleaning validation strategy is not directly transposable from chemical pharmacy to biotechnology (Kitten and Zugmeyer, 2000). Because of the specificity of the active principle, production techniques and absence of historical record of these products, the determination of acceptance criteria requires adaptations and a particular approach. This approach is complicated with the transposition of cleaning validation to gene therapy where the active principle is no more a chemical or biochemical product but a living organism. The disinfection of all the equipment is imposed in this case and the living organisms must be inactivated through decontamination procedures (McArthur and Vasilevsky, 1995). Clean-in-place (CIP) and sterilize-in-place (SIP) technologies are frequently used in biopharmaceutical facilities (Marks, 1999).

A special case is the cleaning of aseptic fill areas, where pharmaceuticals that cannot be treated with terminal sterilization are handled and packed. Cleaning methods, their validation, sampling and monitoring of cleanrooms are discussed by Cooper (1997).

2.5.3 Cleaning validation for API manufacturing facilities

The cleaning validation in API facilities has its special challenges because a contaminated batch of API may be transferred into several batches of pharmaceutical dosage forms (Romanach, Garcia *et. al.*, 1999).

In API facilities cleaning involves reactors, product-transfer lines and pumps, filters, centrifuges and dryers that are different from those in the pharmaceutical production area. Many surfaces cannot be manually cleaned or visually inspected because big tanks, transfer lines and valves are inaccessible.

API manufacturing may also involve different chemical entities, which could be of concern if proved to be toxic (PhRMA Quality Committee, 1997). This may include product, non-reacted materials, by-products, degradation products, cleaning agents/solvents, and microorganisms or endotoxins. Therefore the nature of these materials needs to be considered when designing a cleaning procedure such as to ensure that all undesirable elements will be adequately removed by the solvents used for cleaning. Advantages and disadvantages of organic solvent cleaning versus aqueous cleaning for the case of APIs manufactured through chemical synthesis are discussed by Vergese (2003).

From an analytical point of view it would be also sensible to design a method capable of detecting a highly stable intermediate than a short-lived highly reactive intermediate (Romanach, Garcia *et. al.*, 1999).

An overview of the parameters to be considered for the cleaning validation of APIs is presented by Vincent (2000). Possible contaminants, the selection of cleaning materials, the characteristics of the equipment, means to calculate acceptance criteria and select sampling locations together with requirements for analytical methods are discussed with examples.

Current practices used in developing, establishing and monitoring validated cleaning procedures within the API manufacturing industry were discussed by the PhRMA's quality control bulk pharmaceuticals work group (1997) and by the APIC guideline (2000).

2.5.4 Cleaning validation for research facilities

In pharmaceutical research facilities cleaning validation has its own set of challenges. Compounds for new drug approval (NDA) are continually brought into the development cycle. Different manufacturing equipment is used from Phase I clinical trials, through scale up to Phase III trials, and demonstration runs. Forsyth and Haynes (1998) describe the establishment of a cleaning procedure that could be validated for a constantly changing product development line.

Cleaning validation versus cleaning verification aspects were discussed by Gerber, Perona, & Ray (2005). When the product mix is not well defined and the products processed do not have a

predictable lot size and formulation, cleaning verification may replace cleaning validation. For such cases the verification consists of routine monitoring of equipment cleanliness.

A strategy for cleaning validation in clinical trial packaging was developed by Ray, Pack *et al.*, (2006). Several model compounds consisting of solid dosage forms, were selected as worst cases for validation based on their chemical structure, pKa, functional groups and dose/ potency. A universal cleaning procedure that was aimed to be used for all trial products packed on the line was validated by means of swab sampling and visual observation.

The main difference to the production of drug dosage forms is in the safety factor one imposes when establishing limits (Forsyth and Haynes, 1998). For a compound of a relatively unknown toxicity this factor may need to be larger.

2.5.5 Cleaning validation in other industries

The issue of cleaning validation for the cosmetics and toiletries industry is also under debate. Cleaning validation was performed for a very potent ingredient, namely ethylhexyl (octyl) methoxycinnamate in conjunction with the equipment involved in the manufacture of sunscreen products (Mazonakis, Karathanassi *et al.*, 2002).

The importance of cleaning for the manufacture of diagnostic products in a multi-product facility was stressed by Hall (1997). In this case the products do not come into physical contact with the patient and they are rather use for the clinical testing of body fluids. However, the FDA concern was that product carryover may affect the performance of other diagnostics made in the same equipment. A false result in such a case may lead to an improper diagnosis from the physician's side (Hall, 1997).

2.6 General considerations for a cleaning validation program

There is no exact method for carrying out validation studies. The development of a universal approach to cleaning validation is also impractical due the variety of facilities, processes, products, equipment design, the number of cleaning methods and historical practices used by

individual companies (PDA, 1998). There is a wide variety of acceptable ways to accomplish the requisite proof that a cleaning method “does what it is intended to do” (Mendenhall, 1989).

Formal cleaning validation programs are relatively new to the industry. Developing a sound cleaning program is a rigorous and time consuming process which needs to be based on common sense, clear analysis and documentation. Any validation undertaken must prove in the end through scientific data that the system validated consistently does as expected and produces a result that consistently meets predetermined specifications (Laban, Cauwet *et. al.*, 1997). Therefore, the true purpose of cleaning validation must be kept in mind throughout the planning and execution stages (McCormick and Cullen, 1993).

The intention of setting detailed guidelines for cleaning validation is impossible in regulatory terms. General concepts have been elaborated by FDA (1993) to enable manufacturers to develop cleaning validation programs applicable in the context at a particular facility. According to the FDA guide (1993), when evaluating the cleaning process several questions should be addressed:

- i) at what point a piece of equipment or system becomes clean?
- ii) what does visually clean mean?
- iii) does the equipment need to be scrubbed by hand?
- iv) what is accomplished by hand scrubbing rather than just a solvent wash?
- v) how variable are manual cleaning processes from batch to batch and product to product?
- vi) what is the most appropriate solvent or detergent?
- vii) are different cleaning procedures required for different products in contact with a piece of equipment?
- viii) how many times need a cleaning process be applied to ensure adequate cleaning of each piece of equipment?

Companies must develop cleaning validation programs capable to offer answers to these questions by using scientific rationale. The decisions taken should become part of the design implemented in the cleaning validation study.

The design of the cleaning validation program indicates whether the study is meaningful and scientifically justifiable. An effective program design will ensure that effective cleaning

procedures pass and inappropriate procedures fail and that the cleaning capability will remain consistent in time (Hwang, 2000).

During inspections the following points would be envisaged by an auditor (Guerra, Graffard *et.al.*, 2000):

- i) Prerequisites for cleaning validation (including cleaning agents qualification, review of the cleaning procedures and training undertaken and review of equipment tested);
- ii) Examination of various steps of validation (including the definition of study objectives, criteria used in the selection of the contaminants, the acceptance criteria used and the rationale for them, the choice of sampling methods, the creation of a sampling plan highlighting critical points selected);
- iii) Examination of the cleaning validation plan and the report, supporting documentation and justifications of deviations;
- iv) Time frames for revalidation programs.

The development of a cleaning program identifies and correlates what must be achieved and what can be achieved given the time frame and resources available. Creation of such a program requires organizational ability, interdepartmental cooperation and determination. Generally, departments involved in cleaning validation programs are QC, QA, production and engineering. According to Voss and Masterson (2000) production executes the protocols and sometimes takes samples, which are analysed by the QC laboratory. Results are sent to the QA unit for reporting and documentation. Upon completion of the study, the results are analyzed and summarized, and the final report is written and approved.

Due to the interdisciplinary needs of such a project, fulfillment of requirements may compete with issues such as individual departmental workload, time needed to make batches of products, preparation of procedures and protocols, availability of test equipment and time needed for review and approval.

2.7 Prerequisite conditions for cleaning validation

Before launching a cleaning validation program, sufficient time and resources should be allocated (Laban, Cauwet *et. al.*, 1997) to fulfill several prerequisites:

- i) The production equipment should be qualified;
- ii) The qualification of cleaning equipment should be performed. Qualification of cleaning agents should be performed with regards to their suitability for removing traces of the manufactured products (PDA, 1998). The following information regarding the cleaning agent needs to be obtained from the supplier: composition (at least qualitative), certificate of analysis, safety data sheet, directions for use, assaying method and the possibility for trace analysis (Laban, Cauwet *et. al.*, 1997). Cleaning agents should be identified and controlled from the moment they are received similarly to raw materials. Cleaning agent vendors should be qualified in the same way as the raw material suppliers (Agalloco, 1992).
- iii) The cleaning procedures must be properly documented. The purpose of these procedures is to offer detailed and sequential information required to implement the cleaning process.
- iv) Equipment surface area in contact with the product should be calculated. Critical points and difficult to access areas should be identified. All aspects should be fully documented including drawings, location of equipment and the technologic flux.

According to McCormick and Cullen (1993), without such work the validation work could be fruitless.

2.8 Approach for cleaning validation

Various products are manufactured in campaigns by using dedicated or non-dedicated equipment. Multiple cleaning procedures are used, depending on the product manufactured. The same product could be manufactured in a variety of strengths and dosage forms. It does not make good economic sense to scrutinize every product, in every piece of equipment, using every cleaning procedure, and looking for every possible contaminant.

Therefore appears the necessity of making the cleaning validation programs manageable. This can be achieved through grouping or adopting a matrix design that reduces the number of cleaning validation processes to be undertaken. Worst cases are selected, based on sufficient data and scientifically justifiable assumptions (Hwang, Kowalski, & Truelove, 1997) and studied to cover all the in between cases (PDA, 1998; Lee, 2000).

It is acceptable to use a matrix design by selecting a representative range of similar products and equipment, and to group them into families. Such a validation program is justified because it addresses critical issues relating to the selected products and equipment (Rousseau, 1997). The acceptability of the grouping concept must be mentioned in the cleaning validation master plan or cleaning validation policy statement. The PIC/S validation document (2004) refers to the grouping concept as “bracketing”.

The FDA accepts also extrapolation, matrixing and bracketing on a limited basis, when supported with sound scientific evaluation. Any approach for bracketing, extrapolating or matrixing should start by identifying all the manufacturing equipment, all products in a facility, all cleaning agents and cleaning procedures used (Lee, 2000).

Bousquet and Dumant (2000) have described the steps involved in the determination of the worst case scenarios throughout a matrix analysis.

The matrix design may not be appropriate for certain high risk products such as biologicals or highly potent substances that are not detectable below their acceptance limits.

2.8.1 Product grouping

The purpose of product grouping is to simplify the cleaning validation design in cases where there is a large number of “similar” products made on the same equipment and cleaned by using the same procedure.

There are several potential bases for selecting a product as being representative of other products (Mendenhall, 1989; Agalloco, 1992):

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- i) Least soluble product contains the most poorly soluble active ingredients. The selection should be based on the solubility of the APIs at the pH and temperature used during cleaning.
 - ii) Most toxic product is a criterion based on the lowest numerical values for toxicity data.
 - iii) Most potent product is a criterion based on the therapeutic and pharmacological properties of a drug product.
 - iv) Most difficult to clean product is a subjective selection based upon operational experience with various formulations.
 - v) New products: The validation of cleaning procedures for all new products is required by FDA (1993) regardless of the criteria utilized for existing products.
 - vi) Formulation attributes: There are significant differences in cleaning between solid and liquid products. The distinction between these formulation types is related to how contamination might be left on the equipment and dispersed in subsequent products. Liquids have greater ability to penetrate equipment seals and joints, hindering their removal. Bulk solids may form aggregations of product. Liquids are considered to have superior dispersion of active ingredients uniformly across surfaces, while solid products are expected to have more point to point variability, based primarily upon equipment configuration. Dosage forms could generate small amounts of dry powder during filling due to the uncoated tablets friability or leaking of capsules (Garvey, Levy & McLoughlin, 1998).
 - vii) Sterile vs. non-sterile products: The route of a product administration may affect the level at which the product is found to be allergenic, toxic or potent. Injectable products, intra-ocular formulations and some inhalants which provide direct access to the fluid circulatory systems are of much greater concern in terms of cross-contamination. The endotoxin and particulate levels must be controlled to a degree not common to non-sterile products.

When using the grouping strategy, all the products in a group should pass through the same equipment or same equipment chain, and must be cleaned by the same cleaning procedure. This includes identical cleaning agent concentration, times, temperatures and other process parameters. Validation must be undertaken for the largest batch size and highest strength of the potentially contaminant product and considering the smallest batch size of the product susceptible of being contaminated (McCormick and Cullen, 1993).

2.8.2 Equipment grouping

A similar approach is useful in limiting the number and type of equipment requiring full testing for cleaning validation. For non-dedicated equipment the prevention of cross- contamination becomes the main objective in the cleaning validation effort (Mendenhall, 1989).

The selection of equipment takes into account parameters such as design, shape, and size, function, operating mode, critical points, accessibility and materials of construction used. Similarities found for these characteristics may allow extrapolation of data collected from worst cases selected to several pieces of equipment which utilize the same basic cleaning procedure (Laban, Cauwet *et. al.*, 1997). For similar equipment with different dimensions, the largest one should be validated.

FDA has indicated that it would be inappropriate to extrapolate the results of a laboratory study to equipment cleaning, e.g., applying the removal of a drug residue from a piece of stainless steel, from a laboratory experiment, to all equipment made of stainless steel in production. Companies must have data that relate to a given piece of equipment (CFR, 1978).

Equipment design characteristics are driven by equipment functionality and requirements of the process. The “cleanability” is a major criterion in the design of equipment. cGMP Part 211.65 stipulates that “ equipment shall be constructed so that surfaces which contact components, in-process materials, or drug products shall not be reactive, additive or adsorptive so as to alter the safety, identity, strength, quality or purity of the drug beyond official or other established requirements” (CFR, 2005).

Other worst case scenarios for cleaning validation include the choice of the cleaning method and agent used, the sampling locations and establishing maximum standing times for dirty/ clean equipment (Lee, 2000).

2.9 Points to consider for cleaning validation

2.9.1 Sources of contamination

The cleaning validation program should determine what material is being removed through cleaning. It is also important to identify the sources of contamination in order to develop a sound basis for which residues are selected for monitoring (Agalloco, 1992).

Residues have physical and chemical properties affecting the mechanism through which they are removed from surfaces. The degree of solubility, hydrophobicity and reactivity should be assessed in order to establish the characteristics of these residues during the cleaning process (Gavlick, Ohlemeier & Kaiser, 1995). Selection of the worst contaminants must be based on the nature and the level of risk these materials represent to the manufactured product and its environment.

Sources of product adulteration may represent the previously manufactured product which may contain:

- i) APIs whose potential synergistic interactions could be of concern. Some actives could be adversely affected by other trace contaminants therefore exhibiting changes in stability or bioavailability (McCormick and Cullen, 1993).
- ii) Degradation products of APIs, generated during product formulation or by contact with cleaning agents;
- iii) Synthesis precursors and reaction by-precursors especially in API manufacturing;
- iv) Excipients such as colors and flavors. Decomposition materials of excipients should be also considered. Generally, inert ingredients used in drug products are recognized as safe or have been shown to be safe for human consumption.

Other sources of product contamination are listed below:

- v) Cleaning agents of various chemical nature (organic solvents, surfactants, sequestering agents, acids and bases);
- vi) Sanitizing agents (e.g., disinfectants, alcohol) require also the confirmation of their removal when used;

- vii) Water of various qualities used during the equipment rinse cycles;
- viii) Buffers, media and filtering agents;
- ix) Environmental dust;
- x) Food grade lubricants used for tablet compression and for agitator shafts;
- xi) Coolants used for products involved in exothermic reactions. cGMP Part 211.65 stipulates that such products should not become in direct contact with the product (CFR, 2005).
- xii) Surface coatings and sealants which should be reviewed during the equipment design so that they present no danger in leaving behind residues that will adulterate products (PDA, 1998).
- xiii) Particulates (e.g., specks, flecks, fibers and products of equipment wear). Contamination with such items can lead to exceeding the acceptable levels of particulate matter in parenteral products (McCormick and Cullen, 1993).

Associated with the above-mentioned contaminants is the potential for microbiological contamination that may result from an inadequate cleaning process. Formulations such as parenterals, ophthalmics, semi-solids and topicals may require control of microorganisms prior to further processing. Microbiological contamination could be induced by:

- i) Endotoxins which are of concern only for sterile products;
- ii) Microbes and objectionable organisms such as *S. aureus* and *E. coli*;
- iii) Pyrogens

Maintenance and storage conditions may also introduce microorganisms with the opportunity to proliferate within the equipment. This could cause serious problems for sterile product manufacture because the generation of high levels of pyrogens decreases the assurance of sterility achieved by equipment sterilization. It can also be a serious problem for the manufacture of non-sterile dosage forms, particularly non-preserved products that can support microbial growth.

2.9.2 Non-uniformity of contamination

Agalloco (1992) has discussed the theories of contamination and dispersion. Usually, the contamination is uneven distributed on the equipment. It is invalid to make assumptions that a

residual contaminant would be worn off the equipment surface uniformly or that the contamination might occur only in the beginning of a batch.

In theory, when calculating acceptance limits, the distribution of contamination is considered even on the whole equipment. In order to avoid unacceptable high limits some safety factors are introduced in the formula for calculating the acceptance limits. These factors will be discussed later in this chapter.

Non-uniform contamination of the subsequent product is more likely to arise during continuous processes rather than batch processes (LeBlanc, 1998). For example, in a batch process involving liquid drug blending, the contaminating residue (provided there is adequate mixing) is likely to be evenly distributed throughout the subsequently manufactured product. In a continuous process, such as a flow through piping system and through a filling nozzle during a packaging operation, residuals cannot be assumed to be diluted by the entire batch but rather progressively through a relative small portion of the subsequently manufactured batch (Mendenhall, 1989). This depends on the solubility of the residue in the subsequently manufactured product and on product flow characteristics.

A special case of continuous process is a tablet press. Any residues on the surfaces of the press will not be distributed evenly over all tablets manufactured in subsequently compressed batch, but they will appear in the first portion of it (LeBlanc, 1998). This would dictate more stringent acceptance limits for such equipment. The maximum number of tablets that could theoretically be at the maximum LOC plus an additional reasonable number of subsequently produced tablets should be discarded.

The theory of dispersion relates to how residual contamination would be dispersed into the subsequent production lot (Agalloco, 1992). In the best case, the residual contamination is fully dispersed. This will lower the potential for cross-contamination among products. In the worst case, the residual contamination is not dispersed. This would mean that contamination in the next product contacting a dirty surface could be isolated into a single dose of that product. This assumption forces for extremely tight limits on residual contamination.

One issue frequently confused with the dilution assumptions is the potential for residual product pockets to be formed in poorly accessible areas of equipment (Mendenhall, 1989). This problem is further compounded since these pockets of material are usually not well exposed to the bulk material flow. The potential for such places must be addressed by a validated cleaning procedure including appropriate training and rigorous compliance to the cleaning procedure which may call for disassembly and intensive cleaning of equipment parts which have this potential.

2.9.3 Critical points

Critical points are those in which a contaminant is in danger of affecting a single dose or a small number of doses with a high level of contamination. For such points a lack of control can lead to unacceptable safety hazards towards the product, equipment, operator and environment (Laban, Cauwet *et. al.*, 1997).

A critical point can be:

- i) a highly contaminated area (i.e., bottom of the vessels, machine housing, stirrers);
- ii) a difficult to clean location (i.e., grills, gas bubbling column, flexible pipes, ball valves);
- iii) an area that can lead to unacceptable contamination (i.e., drain flap door, tablet punches and dies, filling heads or nozzles);
- iv) environmental areas which present a contamination risk for the product (e.g., ceiling in manufacturing areas for solid forms, floor traps, air recirculation ducts).

The design of equipment should be carefully examined such that critical areas should be identified and reduced to a minimum particularly in large systems that use semi-automatic or fully-automatic cleaning (PIC/S, 2004).

2.9.4 Cleaning mechanisms

Soil can be attached to the equipment surface by a combination of van der Waal's forces, electrostatic effects and mechanical adhesion. Therefore, the basic mechanisms used for product residue removal from manufacturing equipment surfaces should include mechanical action, dissolution, desorption, detergency and chemical reaction (LeBlanc, Danforth & Smith, 1996).

Mechanical action refers to a diversity of non-chemical processes including scraping, brushing, scrubbing and moving the stream of water to dislodge particulates.

Dissolution involves dissolving the residue with an organic solvent or with water. In some cases dissolution can be achieved by using additives such as chelants and dispersants. Chelants help dissolution of metal ions by forming metal-ion complexes. Dispersants include polymeric materials such as low molecular weight polyacrylates that aid in suspending the residue so that they can be readily flushed away and not re-deposited in other parts of the system (LeBlanc, Danforth & Smith, 1996).

The key to detergency is the use of a surfactant, usually in an aqueous system. Surfactants are surface active agents often consisting of long-chain molecules in which one end is more hydrophobic and one end is more hydrophilic. Because of their polar-nonpolar nature, surfactants align themselves at the interface of solvent and the residue. High-foaming surfactants should be avoided, especially for automated cleaning systems, to improve the rinsability of the cleaning agent and residual product (LeBlanc, Danforth & Smith, 1996).

The primary actions of detergency are wetting, emulsifying and dispersing. Wetting refers to the process by which the surface of a residue accepts water. Emulsifying refers to the process by which a residue is broken up and suspended by the surfactant to form emulsion droplets. If the residue is incorporated into or onto micelles, the process is called solubilization. Dispersing refers to the property of a detergent that keeps the residue broken up and suspended in an aqueous solution. Although the three actions are distinct, in real situations they are concomitantly present in a good cleaning agent system.

A fourth mechanism for cleaning involves chemical reactions in which the basic chemical nature of the residue is changed usually by breaking larger molecules into smaller ones that can be more readily removed by detergent action (LeBlanc, Danforth & Smith, 1996). Sequestering and saponifying agents are often used in the composition of detergents.

2.9.5 Criteria for the selection of cleaning agents and methods

The choice of a cleaning agent or cleaning method must be based on the objectives, resources, and limitations of the cleaning validation program within each manufacturing facility (LeBlanc, Danforth & Smith, 1996).

Water offers many advantages as a cleaning agent because it is nontoxic and leaves no residue. It may be used for product residues that are readily water-soluble. Such residues can be eliminated through repetitive equipment rinsing. One requirement with regards to the water quality is the monitoring of microbial counts in the water supply.

Detergents often aid the removal of water poorly soluble residues from surfaces. The detergent type must be chosen according to the chemical and physical properties of the residues to be removed (PDA, 1998) and according to the ability of the detergent to enhance the dissolution and extraction of residues from the crevices and microcavities of a surface (Valvis and Champion, 1999).

Formulating a universal detergent that will clean every pharmaceutical soil is impossible due to the variety of starting materials, manufacturing processes and equipment used in the pharmaceutical industry. The general composition of detergents used by the pharmaceutical industry was discussed by Roshner and Serve (1995).

Key elements for the effectiveness of a detergent in contact with the residue to be removed include parameters such as detergent concentration, contact time, pressure, temperature, pH and rinsing procedure (LeBlanc, Danforth & Smith, 1996). Often, the interaction of detergents and product residue is not enough to result in effective cleaning. Therefore, additional techniques such as agitation, direct impingement, and disruption by ultrasonic vibration can facilitate residue removal.

For a cleaning agent to be effective, it should not only remove the target substance but also rinse freely from the surface, leaving no residue behind or very low levels (Biwald and Gavlick, 1997). In the area of cleaning agent residue, the FDA has emphasized (FDA, 1993) that the validation for cleaning agent removal is mandatory. The FDA guide states that “detergents are not part of

the manufacturing process and are only added to facilitate the cleaning”. Thus, they should be easily removable.

The FDA has reiterated this prerequisite and has included additional guidance and requirements (FDA, 1998). The current expectation is for companies to evaluate the efficiency of the cleaning process and to prove removal of detergent residues. The validation of the removal process is performed by collecting and analyzing samples and comparing the results to pre-determined limits.

Detergent properties that need evaluation are lower surface tension, higher solubility and surfactant characteristics (PIC/S, 2004). Often the manufacturer needs the chemical composition of the detergent, knowingly that detergents have relatively complex compositions. However the disclosure of the quantitative composition of the cleaning agent is at the latitude of the supplier. The supplier should notify the manufacturer of any critical changes in the formulation of the detergent.

Various types of compounds such as dyes, surfactants, metal silicates, filming amines, sequestering agents may be found in cleaning products and they may exhibit selective adsorption properties either alone or in conjunction with each other (Gavlick, Ohlemeier & Kaiser, 1995). The company should determine the worst case ingredient in a cleaning agent, namely the one that will be lastly rinsed off during the cleaning process (Smith, 1992). The use of a single cleaning agent can reduce the work required to determine if residues of the agent remain after cleaning.

It is also important to perform an evaluation of the cleaning agent’s compatibility with the equipment surfaces. Glass, stainless steel, ceramic, plastic and various synthetics fibers may interact differently with cleaning materials.

The most common detergents are aqueous alkaline cleaners. Alkaline products are particularly effective on acid salts, tableting inserts, proteins and fermentation products. They also act as emulsifiers for hydrocarbons, such as fats, greases and oils. The ingredients of the alkaline cleaners and the mechanisms through which they achieve the cleaning effect are explained by Roshner and Serve (1995).

Regulatory inspectors may be interested in how the company has taken into account the stability and efficiency of diluted cleaning materials if stored for long periods. Detergent dilutions should be kept in previously cleaned containers and should only be stored for defined periods unless sterilized (EU Guide to GMP, 1996).

2.9.6 Requirements for cleaning procedures

Cleaning procedures are considered among the most difficult to validate processes. When establishing the cleaning validation program the types of cleaning used in the facility must be identified in order to be able to control factors such as cleaning reproducibility and effectiveness (PDA, 1998).

Some general “golden rules” for cleaning procedures are presented by Laban (2000). Irrespective of the type of cleaning applied, the cleaning process must:

- i) not transfer the contamination from a surface to another;
- ii) not introduce additional contamination;
- iii) not alter the surface to be cleaned;
- iv) be compatible with the surface to be cleaned;
- v) not become adverse with the areas concerned (air, procedures, materials and personnel);
- vi) be documented in a written procedure;
- vii) must be performed by trained personnel;
- viii) must be in accordance with the safety rules for a given area being cleaned.

21CFR211.113 (CFR, 2005) requires also that “appropriate written procedures, designed to prevent objectionable microorganisms in drug products not required to be sterile, shall be established and followed”. For products that are more prone to microbial growth the frequency of cleaning should be increased. It is also stipulated that “appropriate written procedures, designed to prevent objectionable microorganisms in drug products required to be sterile, shall be established and followed. Such procedures shall include validation of any sterilization process.”

2.9.6.1 Dedicated vs. non-dedicated equipment

FDA expects companies to have written SOPs detailing the extent of cleaning for different situations (FDA, 1993). Ideally, a piece of equipment should be used for processing a single product, in which case the equipment is dedicated. Visual cleanliness is a satisfactory acceptance criteria for dedicated equipment or equipment used for products manufactured in campaigns. Such cleaning is called “serial” and does not require validation. However the cleaning intervals and the number of the batches of the same product consecutively manufactured must be determined and validated (PIC/S, 2004). In such cases the problem is not the carry-over of a small amount of product from one batch to another but the hazard of contaminating the product with microorganisms and degradation products (Laban, Cauwet *et. al.*, 1997).

For non-dedicated equipment, which is used for the manufacture of a variety of different products, the prevention of cross-contamination is the main objective for cleaning validation. In this case residues of one product could remain after cleaning onto the surface of the equipment and therefore migrate into the following manufactured product, therefore resulting in one product being contaminated by traces of other products(s). Non-serial cleaning procedures are more rigorous and their validation must demonstrate their ability to clean the equipment below the acceptable LOC calculated.

2.9.6.2 Automated vs. non-automated cleaning

When manual cleaning is used it is difficult to ensure consistent cleaning results even when validated procedures are used (Hwang, 2000). Agalloco (1992) has also mentioned the virtual impossibility of validating manual cleaning procedures. Still, manual cleaning is universal practice within the pharmaceutical industry. There are many pieces and portions of equipment whose construction and configuration makes manual cleaning a necessity. For validation purposes one has to identify worst case cleaning situations (e.g. operator experience and cleaning methodology).

It is difficult to train an operator to perform certain tasks the same way each time. Hence the problems of within-operator variability and operator –to–operator variability are inherent.

Several questions from the FDA inspection guide mentioned earlier under point 2.6 were pointing to sources of variability associated with manual cleaning. The operator variability was however omitted from the FDA document.

Critical cleaning parameters introducing variability in the manual cleaning include: the volume of cleaning agent, volume of rinse water, temperature of wash and rinse solutions, duration of wash and rinse cycles, pressure of solutions and detergent concentration and mechanical force applied (PDA, 1998).

Therefore concern must be also given to the ruggedness of the cleaning method. This can be achieved with the help of detailed cleaning procedures. Common steps required for a manual cleaning procedure are described by McCormick and Cullen (1993):

- i) equipment disassembly must specify to what extent the equipment is dismantled;
- ii) the pre-wash step with potable water eliminates gross product residues and it serves to improve the reproducibility of subsequent steps by ensuring same state of equipment dirtiness;
- iii) during the washing step solutions of pre-approved detergents in a certain concentration and at specified temperature are used to dislodge and dissolve residual materials;
- iv) repeated rinses provide serial reductions of the residual contaminants. For equipment rinsing deionized water or even potable water can be used;
- v) the final rinse is used to reduce the quantities of residual contaminants to their final level without introducing any other contaminants. Purified water or water for injection (WFI) should be used, especially for the final rinse. Potable water should not be used for the final rinse as it contains trace levels of organic and inorganic residues (HPFBI, 2000).
- vi) drying of equipment is compulsory in order to avoid microbiological growth. Processed air should be used for this;
- vii) the re-assembly of the equipment must be performed as specified by the cleaning SOP.

The control of manual cleaning is accomplished by operator training, strict adherence to well defined cleaning procedures, routine visual examination of equipment after use and prior to the next use, well-defined change control programs.

Companies should rely on clearly defined cleaning procedures and intensive training programs addressing not only what is to be done but why it is to be done in order to reduce operator variability. Such programs should train operators on equipment-disassembly procedures, detergent preparation, step-by-step cleaning procedures and drying process. When performed adequately by qualified personnel manual cleaning procedures can be superior to the automated methods.

Semiautomated cleaning is carried out by portable clean-in-place (CIP) systems such as dishwasher type equipment. They require partial operator intervention. Operations such as loading are critical to ensure that all equipment surfaces are cleaned by the jets and the items drain readily (McCormick and Cullen, 1993).

Automated cleaning does not involve personnel intervention and provides reproducible results without major disassembly and assembly work.

The technology of CIP systems is of great importance for the pharmaceutical, biotechnology and cosmetic industry where processing takes place in a hygienic or aseptic environment. Fully automated cleaning systems are CIP systems designed to clean large, stationary pieces of equipment such as tanks, vessels and inaccessible pipe ducts. A CIP system is designed to circulate cleaning and rinsing solvents through the equipment at controlled flow rates and temperatures for a predetermined period of time. The rules for optimum design of the clean-in-place and sterilize-in-place systems have been discussed by several authors (Marks, 1999; Greene, 2003; Shnayder and Khanina, 2005).

A typical CIP cycle should include the following steps (Greene, 2003):

- i) the pre-rinse step that is meant to remove gross product debris with potable water. The water temperature should be decided based on the solubility characteristics of the product;
- ii) an alkaline wash step with an appropriate selected cleaning agent;
- iii) intermediate hot rinse to remove product and detergent;
- iv) an acidic wash step used to neutralize the residual alkali and solubilise mineral deposits;

-
- v) one or several purified water rinses to remove traces of cleaning agents. A fast measure of the cleaning effectiveness may be usually obtained by in-line monitoring of pH, conductivity or total organic carbon.

The validation of an automated system requires a rugged design for the cleaning cycle in order to generate reproducible results under various operating conditions. The validation should be extended to all critical variable parameters of a cleaning cycle such as flow rates and pressures, temperatures, wash and rinse times, cleaning agent concentration, coverage studies for the spray balls and also the validation of the programmable logic controller (PLC) associated to the CIP system (Greene, 2003).

The validation of a CIP system for a capsule filling machine was performed by Nicoli, Bonora *et.al.* (2003). The evaluation of the CIP procedure was performed by using a placebo trial batch containing as tracer fluorescein which is detectable at very low levels. Both swab and rinse samples were collected.

For complex equipment such as fluid bed driers and tablet presses it is very difficult to design CIP systems able to meet the desired levels of cleanliness. Such systems should be rather equipped with wash-in-place (WIP) systems. This means that the automated cleaning is supplemented in the end for some difficult to clean parts by manual cleaning. Although in the form of WIP, automated cleaning is the method of choice especially when handling high potent products that require, due to operator safety, containment and minimum dust generation.

The benefits of automated systems include reliable and repeatable cleaning process through reduced operator variability, increased operator safety and minimized down time between product runs. A properly designed CIP system will save money in terms of higher plant utilization and will offer significant savings of cleaning agent and water used, through the recirculation of cleaning solutions.

2.9.7 Cleaning procedure - development and validation

The development of a cleaning procedure depends on equipment design, surface characteristics, residue composition and its solubility in the presence of cleaning agents. Sufficient time for

elaboration and testing should be allowed for the development of a cleaning procedure and before its validation.

“How many times need a cleaning process be applied to ensure adequate cleaning of equipment?” is one of the questions addressed by the FDA guide. It has been established that it is not acceptable to “test until clean” (FDA, 1993; HPFBI, 2000) or to repeat the cleaning until an acceptable result is achieved (EU Guide to GMP, 2001). For equipment with a validated cleaning procedure the practice of resampling must not be utilized as it defeats the purpose of validation. Constant retesting and resampling shows that the cleaning process is not validated since these retests actually document the presence of unacceptable residue and contaminants from an ineffective cleaning process.

Key points that must be addressed and latter included within a written cleaning procedure are: the objective of cleaning, responsibilities for cleaning, the level of cleanliness (e.g., visual, chemical, microbiological and particulate) that needs to be attained, equipment disassembly and cleaning instructions, equipment used for cleaning, concentration of cleaning agents used together with any safety precautions that need to be taken. The storage conditions and maximum standing time of clean equipment need to be specified. For any non-compliances from the procedure corrective actions must be in place. All the above aspects should be documented in a cleaning SOP.

Cleaning procedures should be validated for product-contact equipment only, because these are the surfaces that could introduce contamination into the next product. This includes the interior surface of vessels, agitators, piping, hoses, pumps, valves, gaskets, seals and other items that directly contact the manufactured product. Still, consideration should be given also to non-contact product surfaces where product may migrate (e.g., seals, flanges, mixing shaft, fans of ovens, heating elements) (PIC/S, 2004).

This is valid especially for very potent products to prevent product and operator contamination. Environmental monitoring was performed for non-contact product surfaces for the 5-fluorouracil which is the most frequently used antineoplastic agent for parenteral administration (Sessink, Timmersmans *et. al.*, 1994).

During validation, checklists may be used to provide sufficient documentation that the cleaning steps were carried out in proper sequence and sources of variability are under control.

2.9.8 Equipment storage and holding times

The control of bioburden through adequate cleaning and storage of equipment is important to ensure that subsequent sterilization or sanitation procedures achieve the necessary assurance of cleaning (FDA, 1993).

2.9.8.1 Dirty equipment holding time

Product may degrade, dehydrate, strongly adhere to equipment or sustain microbial growth after long time periods. The maximum holding time for dirty equipment is the time period after its use and before cleaning in which no product degradation and microbial contamination occurs.

The validation of dirty equipment holding time should be assessed for worst-case situations (e.g., weekends, holidays and delays) encountered during actual production (Lee, 2000). If the holding time is established too short there will be continuous deviations to be solved. If the holding time is established too long significant over-killing must be used in the cleaning process. This has been a major area of contention between the FDA and industry, as evidenced by 483 FDA citations. The major reason for this non-compliance is the extensive disruption on the production process when the cleaning validation has to include the challenge of normal production waiting time and delays, i.e., equipment must remain idle after use, awaiting for cleaning and cleaning validation, to simulate the worst-case conditions.

2.9.8.2 Clean equipment storage conditions and holding time

Precautions should be taken when storing equipment to avoid problems associated with microbial contamination (Harder, 1984). These consist of preventive measures rather than removal of contamination once it has occurred. FDA requires evidences that routine cleaning and storage of equipment does not allow microbial proliferation (FDA, 1993).

After cleaning, the equipment must be covered or closed and placed in areas designed for equipment storage, when appropriate. The evidence of cleaning should be reflected by the cleaning record attached to the equipment.

The maximum holding time of clean equipment represents the period in which a piece of equipment can be stored without re-cleaning. If the storage conditions (i.e., humidity, temperature, crevices and rough surfaces present on equipment surface or proximity to manufacturing areas) encourage growth, microorganisms can proliferate even after the equipment has been cleaned and passed the acceptance criteria.

This time requires microbiological validation. The testing starts after cleaning and ends when the alert or action microbiological limits are exceeded. The testing is performed under normal storage conditions to determine whether microbial growth is supported or encouraged. The administration route of a product gives a good indication with regards to its susceptibility to microbial contamination or its ability to support microbial growth (PhRMA environmental monitoring work group, 1997a).

Under no circumstances stagnant water should be allowed to remain in equipment after cleaning. Valves, pumps, mills, filters and low spots in pipes can cause problems if not handled properly (FDA, 1993).

If the maximum holding time of clean equipment is exceeded the SOP must state how the equipment should be re-cleaned and sanitized prior to use.

The conditions of equipment storage and the maximum standing time of dirty and clean equipment should be part of the validation program. All these provide confidence that routine cleaning and storage of equipment does not allow cross-contamination and microbial proliferation.

2.10 Setting acceptance limits for residue cleaning

When the effectiveness of a cleaning procedure needs to be evaluated it is necessary to define what is meant by “clean” (Harder, 1984). The FDA guide (1993) asks “at what point does a piece of equipment or system become clean?” This question addresses the issue of setting acceptance

limits for a cleaning validation program. A piece of equipment is considered clean when all chemical and microbiological residues remaining after cleaning are below pre-determined acceptable limits. The selection of setting acceptance limits is the most difficult aspect of cleaning validation because one set of acceptance criteria cannot be applied to all products and equipment types (Agalloco, 1992).

FDA has not published specific guidelines for setting of acceptance limits and has stated that it is every company's responsibility to establish acceptance limits and be prepared to prove the rationale for these limits. When limits are reviewed during inspections, the rationale for establishing limits is also reviewed (Avallone, 1989).

It is recognized that below a certain limit product residues have no measurable biological effect although they are still analytically detectable. Such limits represent the maximum allowed LOC that has been judged not to adversely alter the safety, efficacy, quality, stability and integrity of a pharmaceutical product (CFR, 1978).

The common understanding for setting an acceptable LOC or else the required level of cleanliness is determined based on multiple parameters such as:

- i) the nature of residues, the physical and chemical interactions likely to occur between them and the mechanisms of residue removal (Harder, 1984);
- ii) the potency of the contaminating drug and its pharmacological, toxicological and allergic effects;
- iii) equipment parameters such as capacity, critical points, product-contact surfaces;
- iv) parameters related to the following batch such as batch size, maximum daily intake and the use of the product in which residue could be potentially found (Zeller, 1993);
- v) other parameters such as safety factors.

In all cases a worst case scenario should be selected. Sometimes the most difficult to clean product does not have the lowest acceptable residue limit in a product group. In this case the lowest acceptance criteria within the group must be chosen. Such calculations are complicated when the worst-case product does not contact every piece of the equipment in the equipment chain.

An acceptable LOC for a product can be calculated for an individual piece of equipment or for the entire equipment chain involved in the manufacturing operations. The first approach is to determine LOCs for each individual piece of equipment in a production chain. The rationale for this approach is the fact that there should be delimitation between the equipment pieces with high contamination potential and those in which the residue is relatively uniformly distributed i.e., blenders, granulators (LeBlanc, 1998).

More stringent acceptance criteria should be used for equipment in which the residue could be transferred to an individual dosage unit (e.g., tablet press, encapsulating machine, and tablet filler). The maximum tolerable residue level should be calculated assuming that total residue can be carried over into the smallest batch size/ maximum dose combination of the next product to be processed (LeBlanc, 1998). This approach should be used with caution since by the time a product reaches the final step in processing, it could pick up residue of a previous product from several pieces of equipment used in the manufacturing chain, each with its own calculated LOC (LeBlanc, 1998). This would result in an intolerable accumulation of residue towards the last equipment pieces of the chain therefore the acceptance limits being exceeded.

To avoid this scenario, the concept of using cumulative surface area can be used to calculate cleaning validation acceptance limits. This approach, relating the contamination to high surface areas usually results in lower residue limits.

The calculation of equipment cleanliness is usually based on the assumption that all equipment surfaces are uniformly contaminated (Krull and Swartz, 1999). In reality it is less probable that contamination will be uniformly distributed throughout a system or those contaminants would be worn off the equipment surface uniformly because the equipment surfaces are not even (Valvis and Champion, 1999).

Also, for unit processing equipment such as a tablet press residuals cannot be reasonably assumed to be diluted by the entire batch, but rather contaminating a certain number of dose units of the subsequently manufactured batch. In practical sense, a unique product LOC based on a homogenous dilution is a reasonable assumption.

A calculated LOC must be:

- reasonably safe (Harder, 1984);
- practically achievable by the designed cleaning procedure, reproducible, and based upon the most hazardous contaminants (Mirza, George *et. al.*, 1998).
- verifiable by the analytical methodology existing. A limit is only meaningful in the context of detection capabilities (Mendenhall, 1989).

Most cleaning validation publications have focused on the residue acceptance criteria for finished drug products. The common strategies for setting acceptance limits for drug products are not applicable for NDA products where equipment needs to be as “clean” as possible for any next product.

In the same line, there is no guidance in setting residue limits for API manufacture except the directive that the limit should be scientifically sound (PhRMA Quality Committee, 1997). When cleaning APIs the principal concern about setting residue limits is that the residue can be transferred to an API subsequently manufactured in the same equipment and ultimately into a finished drug product. Equations for calculating various aspects of residue limits for APIs are presented by LeBlanc (2000).

2.10.1 Limits based on the pharmacological potency of a drug product

Medical dosage level is the common basis for setting acceptance criteria. It allows a fraction of a daily dose of a product to be carried over into the lowest therapeutic dose (LTD) of a following product (PDA, 1998). An arbitrary safety factor is utilized in conjunction with the medical dosage level to set acceptance criteria defining for “How clean is Clean?” (Agalloco, 1992).

Fourman and Mullen (1993) have proposed that no more than 1/1000th of a LTD of a product should be detectable in the largest daily dose of the product being manufactured subsequently in the same equipment, or the so-called 3-log-reduction rule.

This arbitrary safety factor of 1/1000 was justified by Fourman and Mullen (1993) and by Forsyth and Haynes (1998) as follows:

- i) the first 10 fold residue reduction is explained by the fact that pharmaceuticals are often considered inactive at one-tenth of their normally prescribed dosage;
- ii) the second reduction adds a built-in 10 fold safety factor;
- iii) the third reduction is a 10-fold robustness factor added;
- iv) a further 10- log reduction factor can be added when the LOC is calculated for the entire equipment chain made of less than pieces of equipment.

According to Zeller (1993) higher safety factors should to be employed for high-potency drugs, with LTD's lower than 1 mg (See Figure 2.1).

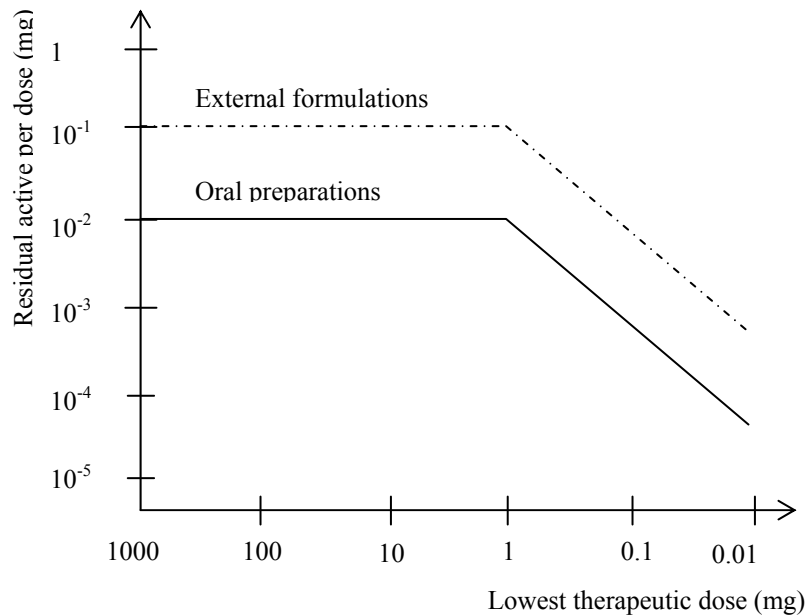


Figure 2.1 Allowable contamination limits that take into account the potency of the drug active (Zeller, 1993).

The Environmental Protection Agency (EPA) suggests that an acceptable level of a toxic material is correlated to the dosage form of pharmaceuticals and is no more than:

1/10th to 1/100th of LTD - for topical products;

1/100th to 1/1000th of LTD - for oral products;

1/1000th to 1/ 10 000th of LTD – for injections and ophthalmic products;

1/10 000 to 1/100 000th of LTD – for research and investigational products.

Such levels will not show any harmful biological effects in the most sensitive animal system known.

In Chapters 4.3.8 and 5.3.3 the 1/1000 safety factor or otherwise the 0.1% dose limit criterion will be used in determining acceptable LOC for products with defined therapeutic doses. This calculation method of LOC accounts for each product's LTD, minimum batch size and the maximum dose of the product likely to be contaminated. It also accounts for the equipment surface area in common between the products processed with the same equipment.

2.10.2 Percentage of toxic doses

In many cases the therapeutic dose of a compound is not known or the compound has no significant therapeutic efficacy (e.g., excipients, precursors and intermediates of APIs, cleaning agents, sanitizers, degradation materials, and other non-actives). The LOC in such situations can be calculated solely based on the potential toxicological effects of the compound and combined with an arbitrary safety factor. This approach was adopted by Layton, Mallon *et. al.*, (1987) and was used in establishing acceptable levels of chemicals in potable water and air.

Although the method known as “no observed effect level” (NOEL), makes use of toxicity data derived from animal studies it contains safety assumptions that allow its use for human and veterinary pharmaceutical products. For APIs in the NDA stages this could be the first known value and may be the only suitable limit for research and development (R&D) operations.

A similar approach was applied by Hall (1997) which has used toxicity data in the form of compound's LD₅₀ to pharmaceutical products. Layton, Mallon *et. al.* (1987) have proven that in the absence of chronic toxicity data (NOELs), the acute toxicity data expressed in the form of LD₅₀ used along with an animal-to-human uncertainty factor and the average adult weight could be used to establish an acceptable daily intake (ADI) level for a compound. It is important that the LD₅₀ is from the same route of administration as the product for which the limit is calculated (e.g., if the product is an oral product, then LD₅₀ should be from the oral route of administration). The computation of the uncertainty factor (ranging from 5×10^{-6} to 1×10^{-5}) was also explained by Layton, Mallon *et. al.* (1987).

ADI is defined as the amount of drug product (mg/day) contaminating another pharmaceutical product that a person can be exposed to without experiencing any adverse health effects, including pharmacological effects attributed to the contaminating drug (Forsyth and Haynes, 1998).

A maximum allowable residue (MAR) can be calculated as shown by Equations 5.5, 5.6 and 5.7. (see Chapter 5).

The use of MAR can also be employed for products with a defined therapeutic dose but will generally result in residue limits more stringent than the limits based on the LTD of a product. It should be noted that selecting more stringent criteria may result in unreasonable cleaning overkill and may also stretch the detection limits of available analytical methods.

For both acceptance criteria presented, if the next product is not known, then the smallest batch size of any product manufactured previously in the equipment should be used. When a new worst case is to be manufactured in the equipment, then this should be evaluated by the change control process and new limits could be imposed on the cleaning of the equipment.

2.10.3 No more than X ppm

For products without a defined therapeutic dose, no more than 5 or 10 ppm of product is allowed into the next product's maximum daily dose. This limit has roots in regulations applied for food industry. The limit can be applied to the product/equipment matrix approach. It is also usable for clinical trials, NDA, and for the manufacture of solid dosage forms (Garvey, Levy & McLoughlin, 1998). For packaging due to the difficulty in making meaningful assumptions regarding the number of doses be exposed to residues of a previous product this limit should be used carefully.

2.10.4 Limits based on analytical limitations

Part of the answer to the question “how clean is clean?” is connected with the performances of the analytical system used by the laboratory. The sensitivity of modern instruments has lowered the detection capabilities for trace levels of contaminants.

The cleaning limit should be based on the limit of analytical detectability. The analytical method must be able to quantify or at least detect the residue established by the cleaning limit. This means that “non-detected” or zero residue results cannot be reported. If the level of residue is below the detection capabilities of the analytical technique it does not mean that the residue was not present. The sensitivity of the method should be used in cases of “non-detected” to calculate the maximum amount of residue, which could be present. The sensitivity must be validated as part of the analytical method validation (Agalloco, 1992). The non-detectable limit is associated with a specific analytical procedure and it is reported in various ways to express the residue found in a sample: mg/L, mg/cm², mg/swab or mg/dose.

For certain allergenic ingredients such as penicillins, cephalosporins or potent steroids and cytotoxics, the limits should be below the limit of detection (HPFBI, 2000) by best available analytical methods. In practice this could mean that dedicated plants are used for such products (Garvey, Levy & McLoughlin, 1998; Avallone, 1989).

2.10.5 Visual acceptance limits

The minimum required from a cleaning method is the “visually clean” acceptance criteria. This means that no traces or particles should be visible to the naked eye when exposed to sufficient light (Laban, Cauwet *et. al.*, 1997). 21 CFR 211.67 demands that equipment should be visually inspected for cleanliness before use. Any procedure for establishing acceptance criteria for cleaning validation should take into account first the visual cleanliness of the equipment (McCormick and Cullen, 1993).

In some author’s experience (Fourman and Mullen, 1993), the limits calculated based on pharmacological and toxicological data for a number of products have yielded levels of residuals that can be visibly detected on the equipment. For drug products that have relatively safe toxicopharmacological profiles this matter does not raise concerns. The limit for visual verification can be experimentally demonstrated by spiking studies with active ingredients. For most products, according to Fourman and Mullen (1993), the active substances are visible from 4.0 µg/cm² if conditions of observation are satisfactorily. Jenkins and Vanderwielen (1996) have observed residues as low as 1.0 µg/cm² with a light source.

The ability to visually detect residue on a surface depends on factors such as: the composition formulation, the nature of the surface, background, solvent effects, and subjective factors such as light intensity, the angle of viewing, the viewing distance, viewing angle and the eye of the observer (Forsyth and van Nostrand, 2005).

For extremely potent or toxic products, calculated acceptance criteria based on pharmacological data are more stringent than the visually clean acceptance criteria. For potent drugs for which the acceptance criteria would be below $1\mu\text{g}/\text{cm}^2$ a determination of visual cleanliness would have no significance regarding the adequacy of cleaning (LeBlanc, 1998a).

Visual cleanliness, on its own, can be a qualitative acceptance criterion for dedicated equipment or equipment used in a campaign of several successive batches of the same product. For non-dedicated equipment a visual inspection is too subjective to be accepted as a decisive acceptance criteria (Fourman and Mullen, 1993). The role of using visual examination and the proper definition of “visual limit” were discussed by LeBlanc (2002).

The most stringent of the acceptance limits should be used in a cleaning validation study (Krull and Swartz, 1999; Fourman and Mullen, 1993).

2.10.6 Limits for microbiological contamination

A cleaning validation program involving microbial acceptance criteria should consist in the determination of possible contaminants, in both species and number (Voss and Masterson, 2000). The limits set must be realistic as well as justifiable for the products being manufactured.

One approach is to establish limits based on the route of administration and the product's microbiological contamination susceptibility (Docherty, 1999). A product with a very low or high pH or low water content is less susceptible to microbial contamination. Products manufactured with natural components or with high water content (i.e., liquids, or ointments) are highly susceptible to microbial contamination. Therefore, the manufacturing equipment should have lower limits than the equipment used to manufacture oral solids. A worst case product in each

classification (liquids, solids) should be chosen for its ability to support microbial growth, and limits based on that product should be established for all the products within the classification.

Another approach is to set acceptance limits based on historical data already generated. If the limit proves not to be realistic in time, the failures must be investigated.

Microbiological standards for both sterile and non-sterile manufacturing facilities should be established, validated and monitored to assure consistent conformance (Mendenhall, 1989). When discussing microbiological monitoring for non-sterile manufacturing environments, a general guideline that adequately sanitized production equipment in contact with a product should have a limit for bacteria of less than 2 CFU/cm² and for molds of less than 1 CFU/ cm² (Harder, 1984).

There are two types of microbiological limits that need to be established (PhRMA Environmental Monitoring Work Group, 1997a).

Alert limits are microbial levels that, when exceeded, may signify a change from normal operating conditions.

Action limits are microbial levels that, when exceeded, indicate a significant change from normal operating conditions and require corrective measures to prevent further increases in microbial activity. Such levels are designed to signal possible changes in the manufacturing environment so that action can be taken before the product quality is affected. Alert and action limits are established primarily through trend analysis.

In addition to numeric limits, consideration should be given to ensure the absence of objectionable such as *S aureus*, *E coli*, *Pseudomonas sp.* (McCormick and Cullen, 1993). Although many companies identify microorganisms, especially when the indicated microbial activity is above the action level, the decision to identify microorganisms depends on the origin of the sample and the type of activity in the area from which the sample was taken.

For parenteral products, similar controls are necessary to limit the amount of endotoxins on product contact surfaces and ensure the absence of pyrogens (Avallone, 1989).

2.11 Sampling methods

The type of sampling methods used will depend on the cleaning process, type of equipment used, acceptance limits chosen, the nature of the target contaminant and the percent sample recovery (PDA, 1998). A representative sampling plan is a key element of performing meaningful cleaning validation.

It is necessary to distinguish at this point between the sampling plans, based on statistical methods, which are frequently used in the pharmaceutical industry and the sampling strategy used for cleaning validation. For the second case there is a relatively reduced number of samples that can be collected from the manufacturing equipment due to surface area limitations. As with every validation approach, the worst case scenarios should be considered, for the case of cleaning validation the sampling points are determined by worst case surface areas in terms of cleaning capabilities and accessibility.

Equipment surfaces representing difficult-to-clean (drains, crevices, gaskets and seals, threaded piping and ball valves), difficult-to-reach (risers or dead-leg piping that protrudes from the equipment) and random locations should be considered (Laban, Cauwet *et. al.*, 1997). Since equipment is never homogeneously contaminated, several samples should be collected from each piece of equipment.

Generally, during the initial validation, sampling should include swabbing, rinsing and direct extraction to detect both insoluble and soluble residues (APIC, 2000). To shorten development times for cleaning validation assays, it may be preferable to utilize swab sampling and investigate other options if the swab test cannot be validated (Rossi and Ryall, 1996). The FDA expects the sampling site selection to include different surface types as well.

Some practical tips for sampling include:

- i) samples should not be collected if visual cleanliness is not achieved;
- ii) the area to be sampled should be in its final condition, as it would be when ready to use;
- iii) microbiological samples should be collected first. If not, the actions involved in other sampling (active and detergent residues) may lead to false positive results for micro;

- iv) with respect to the residual contamination levels, the sampling area should be large enough to enable the recovery of the contaminant in sufficient quantity so as to be detectable (higher than the analytical method LOQ or at least LOD) (Laban, Cauwet *et. al.*, 1997).

2.11.1 Swab sampling

FDA recommends direct surface sampling as the most desirable method (FDA, 1993). Direct sampling of inner equipment surfaces should be performed with an inert swab material (Hwang, 2000). The FDA Guide (1993) states that it is important to determine the type of sampling material used and its impact on the test data since the sampling material may interfere with the test. Most commonly used are cellulosic materials such as cotton and paper, spun quartz, non-woven cellulose/polyester blends, continuous filament double-knit polyester polypropylene/cellulose and hydroentangled polyester. Swabs should contact the surface in a manner as reproducible as possible with regards to the force used, the number and direction of strokes used over a defined surface area (Laban, Cauwet *et. al.*, 1997). Swabs should be changed regularly to avoid saturation or physical disintegration of the swab.

The use of swabs and wipers for cleaning validation studies was reviewed by Cooper (1996). The author describes requirements for swabs starting with the packaging methods that suppliers need to use to avoid contamination, continuing with the swabbing technique and requirements for solvents to be used, storage conditions for swabs including advantages and disadvantages of various analytical methods when using swabs.

The most important criteria for choosing the swab type were discussed by Miscioscio (1997). They include the following:

- i) minimum background: background is the amount of swab in a sample measured by the analytical technique when tested according to the analytical protocol. The sampling material must be tested in the presence of various solvents in order to choose the combination that will lead to the smallest background (the lowest release of swab components in extract reflected in the minimal blank contribution) (Zeller, 1993).
- ii) lack of interference with the analytes determined: the sampling material should be as inert as possible, should not interfere with the assay and should not cause degradation of

- compound analysed (Laban, Cauwet *et. al.*, 1997). The possible release from swab of substances, such as adhesives, interfering with the target contaminant is not acceptable (FDA, 1993).
- iii) high recovery rate: the swab material should allow the extraction of the compound to be analyzed. The recovery is the percent of contaminant measured by the analytical technique when the swab is spiked with a known quantity. A value of 60% recovery rate is acceptable, however, higher recovery rates are desirable.
 - iv) low particulate generation: it is critical that the swabbing material leaves the sampled surface free of particles that would further contaminate the surface.

The swabs are used dry or moistened with an appropriate solvent. In many cases as solvent is used purified water, mobile phase and non-toxic or volatile organic solvents. The solvent used to moisten the swab material is chosen based on criteria such as:

- i) the solubility and nature of targeted contaminant. The solvent used for swabbing should provide good solubility for the compound, should not encourage degradation and should not interfere with the analytical method being used (Chudzik, 1998).
- ii) type of surface sampled (silicone, PVC, stainless steel, etc);
- iii) the swab material used;
- iv) considerations with regards to the safety, ease of handling operations and its volatility at room temperature.

The amount of solvent used in extracting any contaminants from the swabbing material must be chosen to provide suitable extraction without diluting the contaminants to a point beyond the assay capabilities.

In some cases, it is reasonable to swab the entire product-contact surface. When this is not reasonable, a known surface area should be tested and the approximate overall surface area should be known. Test results are then extrapolated to the total surface area of the equipment (Hwang, 2000). This is necessary in order to convert analysis results from swabs into meaningful information about the total amount of contamination present in the equipment. The extrapolation of the test results on small, poorly accessible areas may transpose into exaggerate levels of

residue for the total equipment surface (Rossi and Ryall, 1996). The swab sampling method can be also applied to microbiological sampling.

Between sampling and testing the swabs should be protected from contamination and should be stored either dry or in solvent to prevent degradation of residuals. If the analysis is not carried out immediately after sampling, then it is imperative to perform stability studies to show that the contaminant is stable in the extracting and sampling solvents for at least the length of the time between sampling and analysis. This time period will be the limit for storage of cleaning validation samples. To assess the possible reduction in accuracy caused by degradation of the compound during handling and storage, the challenge swab/solvent can be held for various lengths of time (at various temperatures) prior to testing. Control samples should also be provided (unused swabs and solvent).

Smith (1992) has developed a swabbing technique for a CIP system which is based on filter-paper sampling. The technique has been used for the determination of detergent residues. The variables influencing the reproducibility of swab sampling were investigated by Yang, Burson *et. al.* (2005).

The swab sampling of coupons can be used when the equipment geometry does not allow the operator to perform in-place sampling. This sampling technique is quantitative but depends on whether coupons are equivalent to the surface of interest and requires removing the coupons from the system. The coupons are then swabbed and the swabs are treated as explained previously. Another alternative solution is to rinse the coupons.

Advantages and disadvantages of using swab samples are presented in Tables 2.1 and 2.2.

2.11.2 Sampling of rinse solution

Rinse sampling is performed by collecting a certain volume of the final rinse solvent. The sampling point is usually the one at the end of the cleaning circuit at which the rinse solvent exits the system. It should be observed that the notion of worst-case sampling points does not apply to rinse sampling because the entire product contact surface is sampled by the rinse solvent.

The solvent used should be selected based on the solubility of APIs, the type of surface sampled (silicon, PVC, stainless steel), the ease and safety of handling operations and costs involved.

For quantitative purposes, the volume of the final rinse must be accurately determined such that the amount found in the rinse sample aliquot can be related through calculations to the total amount of residuals left in the equipment.

This method is accepted by the FDA, although is not the method of choice. FDA (1993) considers that “When rinses are used a direct measurement of the residue or contaminant must be done in order to validate the cleaning process. For example, it is not acceptable to simply test the rinse water for water quality (whether it meets the compendial specifications) rather than test it for potential contaminants”. It is possible that the rinse water meets USP specifications and still be contaminated with residues. This is because the target residue is present either at a low level or as a chemical species that cannot be adequately detected by USP water tests.

One analogy is that of a dirty pot. When evaluating the cleanliness of the pot one must not look at the rinse but at the pot itself. Therefore, rinse sample procedures can provide upper-limit estimates of potential contamination in subsequently manufactured products (LeBlanc, 1998).

The non-uniformity of contamination should be also considered in evaluating residues. The fact that the residue may not be distributed homogeneously through the rinse should be accounted for (PIC/S, 2004). Steps should be taken to ensure the uniformity of residual material in the rinse prior to sampling. A mixing vessel should have the mixer activated for a period of time prior to discharge so any concentration gradients are eliminated. If the residual in question is insoluble and is therefore present as suspension, the rinse must be well mixed or samples must be taken from different levels of the vessel.

As with the swab samples the potential for degradation of the active in the solvent should be considered. The sample holding time prior to analysis must be validated or the assay method developed must be capable of detecting the degradation products.

Direct analysis of rinsates can be performed if the sample is enough concentrated. Otherwise the sample must be concentration or evaporation and then diluted with appropriate solvent (Laban, Cauwet *et. al.*, 1997).

The method is less suitable for fluid bed driers, granulation and milling equipment, powder filling equipment and it is unsuitable for tablet presses, most encapsulation equipment, packing equipment and other equipment that cannot be completely rinsed without risking damage to electrical components and instrumentation (Laban, Cauwet *et. al.*, 1997).

LeBlanc has dismissed one of the cleaning validation “myths” that rinse sampling is not acceptable by regulatory authorities (LeBlanc, 2005). He has explained that the improper use of rinse sampling in the past has lead to the situation when this method became less desirable. If used correctly, rinse sampling can offer valuable information regarding the overall equipment cleanliness, especially in the assessment of difficult-to-reach areas of equipment.

The combined testing of swabs and rinsates provides the highest level of assurance that the equipment is adequately cleaned and compensates for the disadvantages of each method (PIC/S, 2004; Zeller, 1993).

2.11.2.1 Rinse water sampling

It is suitable to sample and test water rinsates when the residues to be removed are known to be freely water soluble and therefore a high recovery rate of such residues from the manufacturing equipment can be achieved.

It is possible that the species found in rinse-water samples taken from process equipment might not be representative of chemical species found on product-contact surfaces (Smith, 1992) due to selective adsorption onto product-contact surfaces. Also, if the material of concern is insoluble in water, water rinsates cannot be taken and analyzed. In this case the analysis of water rinsates does not offer a realistic ground for the correlation of the residues found to the total residue left in the system. For such cases measuring residues in the rinse water is not preferred technique for validation purposes (LeBlanc, Danforth & Smith, 1996).

The surface rinse method can also be used to evaluate the microbiological cleanliness of the equipment after cleaning. This method gives a better overall assessment of cleaning when compared to the swab sampling for micro purposes.

Advantages of this method are its simplicity and the lack of analytical background. Direct analysis is possible when the sample is enough concentrated.

2.11.2.2 Solvent sampling

Solvent sampling uses a solvent not usually employed in the cleaning process to maximize recovery of expected residues. Solvent sampling is commonly used in bulk chemical facilities.

This method entails the application of a known volume of solvent to the surface examined. The solvent is then collected and an aliquot is analyzed. The solvent extraction is the only satisfactory way for determining product residuals for materials poorly water-soluble (Agalloco, 1992). When non-aqueous solvents are used, the potential for corrosive damage and the cost of solvent may be a limiting factor.

Boil-outs involve the refluxing of a solvent inside a closed vessel system in order to clean its interior surfaces and produce a representative sample. The cleaning effectiveness of a boil-out relies on the combined actions of dissolution, mixing and vapor extraction (Valvis and Champion, 1999).

The solvent sampling offers good analytical specificity and is applicable for actives, cleaning agents and excipients. It allows sampling of large surfaces and offers higher recovery rates.

However, solvent sampling may require operator protection and additional safety measures taken.

Advantages and disadvantages of using rinse samples are presented in Tables 2.1 and 2.2.

Table 2.1 Comparison of advantages of using swab and rinse samples

Swab sampling advantages	Rinse sampling advantages
i) adaptable to various surfaces types. It allows for direct sampling of a precisely defined area and the use of solvents which could not be used in bulk to rinse the equipment. This ensures the most complete extraction of contaminants from the areas sampled; ii) can be used for difficult-to-clean areas where rinsing is not practical; iii) applicable to the quantitative analysis of actives, cleaning agent residues and microbial counts; iv) allows specific determination of substances that are non-soluble or water poorly soluble; v) the FDA Guide (1993) explains that adherent material residues that are “dried out” or are insoluble can be dissolved and physically removed by swabbing; vi) economical and widely available. vii) it provides a better reflection of the equipment level of cleanliness since samples are collected from several equipment parts, which in turn may be differently contaminated.	i) allows sampling of large area surfaces and provides data for the entire product contact surface of the equipment (PDA, 1998); ii) it is useful for difficult-to reach areas of equipment that cannot be routinely disassembled (Laban, Cauwet <i>et. al.</i>, 1997); iii) it is adaptable to on-line monitoring, in CIP applications for obtaining fast results regarding the level of cleanliness achieved; iv) the treatment of samples is simpler than the treatment of samples collected by swabbing (Laban, Cauwet <i>et. al.</i>, 1997). v) easy to sample and therefore suitable for routine testing, usually after initial validation of the cleaning process; vi) less technique dependant than swabbing; vii) reduces variability in recovery; viii) the number of samples required is usually lower than for swabbing (Zeller, 1993).

Table 2.2 Comparison of disadvantages of using swab and rinse samples

Swab sampling disadvantages	Rinse sampling disadvantages
i) susceptible of interference from the swab material with the assayed compounds; ii) an invasive technique that, by swab shedding, may introduce fibers in the equipment; iii) difficult to standardize sampling technique. Results may be technique and operator variability iv) most solvents continually evaporate while the surface is being swabbed, and the extent of this evaporation can influence the results as redeposition of the contaminant is possible (Laban, Cauwet <i>et. al.</i>, 1997). v) not representative for complex equipment (APIC, 2000). The swabbing of flat surfaces can result in over-optimistic results (Zeller, 1993), whereas the swabbing of poorly accessible spots can produce high results that could lead to unreasonably high cleaning efforts. It is in the author's view (Mendenhall, 1989) that this method will also tend to overestimate the actual recoverable residue since the solvent utilized will be more efficient in recovering the residual material than the subsequently manufactured product would desorb during the process.	i) residue may be insufficiently soluble in the rinsing fluid, not homogenously distributed in rinsate or may be physically occluded in the equipment, thus giving erroneously low results (PDA, 1998); ii) only relatively harmless solvents (most commonly water) can be used for safety and environmental reasons; iii) rinse fluid may not come into contact with all the surface contacted by the product during manufacturing; iv) rinse volume is critical to ensure accurate interpretation of results; v) it provides limited information about the surface cleanliness. It is unable to detect the location of residue;

Table 2.2 Comparison of disadvantages of using swab and rinse samples - Continued

Swab sampling disadvantages	Rinse sampling disadvantages
vi) not practical for contact surfaces that are not easily accessible due to equipment design or process limitations (inner surface hoses, crevices, transfer pipes, tubing, large reactor tanks with small ports of handling toxic materials, cavities and small intricate equipment such as micronizers or microfluidizers); vii) subject to the arbitrary judgment of sampling points selection where the possibility of residue non-uniformity will misjudge the overall cleanliness of equipment (FDA, 1991). viii) requires the development and validation of an appropriate swabbing method;	vi) it may be difficult to accurately define and control the area sampled, therefore usually used for rinsing an entire piece of equipment; vii) may lower test sensitivity through dilution effects viii) requires recovery studies for cases when organic solvent is used.

2.11.3 Placebo sampling

Placebo sampling gives a satisfactory indication with regards to the type of residuals that might be carried over into the following product (Agalloco, 1992; Mendenhall, 1989). However this sampling method is not recommended by the FDA. The FDA guide (1993) states that one cannot assure that the contamination will be uniformly distributed into the next batch.

A true placebo with the same formulation as the finished product, but without the active substance may be used. Otherwise a specific placebo formulated from a restricted number of excipients may be used (Laban, Cauwet *et. al.*, 1997).

The placebo sampling is operationally costly and demanding from an analytical point of view. Any low level of contaminant must be determined in the presence of a complex matrix, which may require elaborate sample preparation and the use of selective and sensitive analytical techniques. Therefore the analytical results obtained may suffer significantly in terms of accuracy (Mendenhall, 1989).

Placebo sampling can only be applied under extraordinary circumstances and must be justified (Laban, Cauwet *et. al.*, 1997).

2.11.4 Product sampling

The product sampling is similar to the placebo sampling. A portion of a product batch is sampled and tested for traces of a previously manufactured product. Advantages include the fact that the next product contacts the same surfaces as the previous product. This method also facilitates the sampling of difficult to reach surfaces and requires no additional sampling steps. The limitations are connected to the fact that it is difficult to establish a recovery rate for contaminants as the residues may not be homogeneously distributed into the following batch. The sample matrix is also very complicated making therefore difficult to detect trace levels of a previously manufactured product. Furthermore, there is a significant risk for product rejection should any residual be found (Agalloco, 1992).

2.11.5 Contact sampling

A survey made by PhRMA association on 36 pharmaceutical companies for the microbiological monitoring of non-sterile pharmaceutical manufacturing has shown that product-contact surfaces are mainly monitored using contact plates (PhRMA Environmental Monitoring Work Group, 1997a).

A defined area of the equipment surface is contacted by a support imbibed with solvent or agar. The contact method is easier to standardize, since the only variables are the time of contact and the strength of application (Laban, Cauwet *et. al.*, 1997). This method has the convenience of the direct growth of microorganisms onto the media of the contact plate. However contact sampling cannot access irregular surfaces such as crevices and equipment corners that can be more contaminated than the flat surfaces.

2.11.6 Direct surface monitoring

Direct surface monitoring is used to evaluate the cleanliness without the equipment surface being contacted (PDA, 1998). Bioluminescence and other spectrophotometric measurements are rapid, non-invasive and economical. However, such techniques are not yet widely developed or available.

2.11.7 Visual examination

The visual examination of manufacturing equipment is a first step in the evaluation of cleaning which is required by cGMP. As a sampling method is economical, fast and can provide a semi-quantitative evaluation of the residue. It can be used when data from spiking studies have been previously performed and there is a history of satisfactory results. However it can be a subjective assessment (Fourman and Mullen, 1993).

2.12 Documenting a cleaning validation program

The success of cleaning validation relies on thorough documentation. To ensure compliance with FDA requirements (1993) the cleaning validation study should be supported by detailed

validation protocols, raw data and reports that are approved by the company's quality assurance unit (Lee, 2000).

2.12.1 The cleaning validation master plan

Scientific evidence is needed to establish which cleaning procedures need validation and why (Voss and Masterson, 2000). Sometimes called a Cleaning Validation Master Plan, this document should address objectives, responsibilities and the approach for validation. The document should also contain a list of all equipment types, product ranges and associated cleaning procedures.

The selection and rationale of the “worst cases” selected for validation together with priorities and deadlines for work should be also documented by the Master Plan. Analytical strategies, residue limit justifications and change control procedures need to be covered by such a document. Some companies tend to skip the master plan and to jump to writing the validation protocols. In the long run this may come to their disadvantage resulting in over work, because the initial risk analysis was not performed.

2.12.2 The operating procedure describing the cleaning

FDA (1993) expects companies to have written cleaning procedures specific for the cleaning of each piece of major equipment. The cleaning SOP must be enough descriptive to enable each operator to clean the equipment in a reproducible manner (McCormick and Cullen, 1993).

Cleaning SOPs should be developed in conjunction with the cleaning development phase. The SOP should make distinction between a serial cleaning procedure intended for use between batches of the same product manufactured consecutively and a non-serial cleaning procedure used at the end of a campaign or between different products.

Major equipment should be subject of individual SOPs. Cleaning of minor equipment such as utensils, small parts and smaller equipment may be described in one SOP being often cleaned using broadly defined procedures.

According to Agalloco (1992), the following aspects of the cleaning procedure should be evaluated, validated, documented and included in the SOP for equipment cleaning:

- i) assignment of responsibilities for cleaning;
- ii) list of products for which the procedure is intended to;
- iii) equipment and materials used for cleaning and the extent of equipment disassembly together with methods for disassembling and reassembling equipment (CFR, 2005 Part 211.67; FDA, 1991);
- iv) visual examination for equipment wear, excessive product residuals and foreign material;
- v) list of critical sites or difficult to clean areas that may require special cleaning;
- vi) type and concentration of cleaning agents;
- vii) the volume of cleaning solution;
- viii) type and quality of wash and rinse solvent used and agitation, where applicable;
- ix) the volumes and time allocated for wash and rinse and their number and sequence in the process;
- x) temperatures, flow rates and pressure, gas purges, evacuations and drain times;
- xi) coverage of the system: in complex systems, it may be necessary to establish that all portions of the vessel interior are contacted by the wash and rinse solution; the ability to adequately clean locations which may be behind baffles, inside nozzles, under agitators must be confirmed;
- xii) procedures for proper handling and disposal of rinse solutions;
- xiii) storage conditions for clean equipment in order to protect it from being contaminated;
- xiv) inspection of equipment for cleanliness immediately before use.

Check lists should be available to document each step as the cleaning procedure is carried out, so steps are executed in the proper sequence.

The cleaning validation should test the cleaning SOP not just the effectiveness of a cleaning cycle (Voss and Masterson, 2000). This includes the preparation of equipment for cleaning, operation of the cleaning system and/ or process system during cleaning and hold times. These steps generate the data used to assess the effectiveness and reproducibility of the cleaning procedure being validated. If procedures are not recorded in sufficient detail, changes will inevitably take place (Harder, 1984) and the validated state of a cleaning procedure becomes void.

2.12.3 Cleaning records

Parts 211.180 and 182 of CFR (2005) state that records should be kept for the cleaning, sanitization inspection and maintenance of major equipment. Major manufacturing equipment should bear an identification number and a cleaning record documenting:

- i) the date, time (if appropriate), product and batch number of each batch processed;
- ii) area of the piece of equipment cleaned;
- iii) person who carried out cleaning;
- iv) the SOP defining the cleaning process;
- v) the product previously processed with the equipment being cleaned;
- vi) signatures of the operator performing the cleaning and the person responsible for inspecting the equipment (PIC/S, 2004)

2.12.4 The cleaning validation plan/ protocol

A validation protocol should be written and approved prior to validation. This is the document that defines “how to” perform the validation for a particular cleaning procedure associated to a product or a group of products.

The protocol should include:

- i) clear objectives of the validation study (PIC/S, 2004);
- ii) responsibilities for performing, documenting and approving the validation study;
- iii) detailed description of the equipment type, products and cleaning type;
- iv) other products, processes and equipment for which the planned validation is valid according to a “bracketing” concept;
- v) methods, materials, extent of cleaning, parameters to be monitored and controlled;
- vi) training records reflecting the competence of the personnel carrying out the cleaning (Harder, 1984);
- vii) demonstrate the compatibility between the cleaning agent and the drug product;
- viii) solubility, reactivity and potency of the product;

- ix) specify the acceptable LOC including rationale for setting it. The calculation used for determining the LOC should be clearly shown;
- x) analytical test methods needed to test samples for residual drug content and microorganism levels. The validation must not be executed until all the associated analytical methods have been validated (PIC/S, 2004);
- xi) data on recovery rate studies;
- xii) visual inspection of all identified critical points. If the visual inspection is successful, the next step is implemented; if not, cleaning operations are repeated, and followed by a subsequent visual inspection;
- xiii) methods used for sampling including the rationale for why a certain sampling method is used. There must be clearly defined sampling locations. An accurate sampling plan (drawing, picture) must be available indicating the sites to be sampled;
- xiv) sampling methodology: type and reference of sampling materials, labeling and transport of samples, time interval between sampling and analysis, nature of container, sample preparation;
- xv) description of tests carried out;
- xvi) intervals between the end of production and the beginning of cleaning process;
- xvii) Period intervals for revalidation;
- xviii) the criteria by which a cleaning procedure will be judged with respect to its reliability and effectiveness and the mechanism by which the procedure will be documented as having been validated (Harder, 1984).

A good protocol is one that is clearly written and has been reviewed and approved by scientifically and technically competent individuals. Such a protocol is also a good guarantee that a properly validated cleaning procedure will be in place when the validation studies have been completed.

2.12.5 The cleaning validation report

Once the validation study is completed, a validation report is generated. The purpose of this document is to take the decision whether a cleaning procedure can be declared as validated. The report summarizes results, documents deviations encountered throughout the study and presents conclusions and recommendations (Laban, Cauwet *et. al.*, 1997).

Results obtained are discussed in connection to the previously set acceptance criteria, summarized and interpreted. Conclusions of the report should be clear and objective. These conclusions should lead to proposals and recommendations on improvement, changes or approval of the cleaning procedures. The final conclusions of the cleaning validation report should be approved by management (PIC/S, 2004).

2.13 Change control

All aspects of cleaning should fall under the auspices of a change control policy and must be reviewed periodically. Once the initial validation exercise is completed, the different departments involved (QC, QA, engineering, production) should agree on the rules to adopt for controlling changes (major, minor or critical) that could lead to a partial or complete revalidation (Laban, Cauwet *et. al.*, 1997).

Changes of cleaning SOPs, assay methods, equipment, detergents and product formulations will require formal documentation and approval. If a change deemed to be fundamental to the grouping strategy on which the validation program was founded the change may require revalidation.

2.14 Revalidation of cleaning procedures

Revalidation, in contrast to review, may require that portions of the initial cleaning validation program be repeated. Revalidation may differ from review by the amount or type of sampling that is performed. Typically the sampling and testing that are performed during revalidation are more stringent than those performed during routine or occasional monitoring.

The need for revalidation of cleaning procedures may arise for several reasons. An initial, simple approach to revalidation might be to require routine revalidation based on the length of the time since the original or last validation studies were performed. This approach represents a very conservative philosophy and requires an extraordinary commitment of resources to validation.

A more practical approach is to revalidate for a cause. This links revalidation to change control in an important way. Responsible individuals approve the implementation of changes in materials,

equipment, and procedures and assess the potential impact of these changes on the original validation work. With such a system in place, the risk of problems arising from compromising previous validation work can be minimized. It can also be argued that such a system allocates resources in the most efficient way possible assuming proper and sound validation studies were conducted initially, it is reasonable to limit revalidation studies to situations in which something about the materials, equipment, or procedures originally validated has changed in such a way as to put the validity of the original work in significant jeopardy.

There shall be a quality assurance system in place, which requires revalidation whenever there are:

- i) formula or product changes;
- ii) acceptance criteria changes;
- iii) new products manufactured in non-dedicated equipment;
- iv) significant changes in equipment configuration or equipment chain: a major change in the equipment used to make a product, such as the use of a fluid bed dryer rather than tray drying, would require revalidation. Minor changes, such as batch size change, in which the capacity of some of the equipment used has been changed, might require only limited work to verify that the cleaning procedure is still effective;
- v) cleaning agent changes: when cleaning agent formulation is changed, revalidation may be or may not be warranted. If only minor changes are made, it may be sufficient to compare the effectiveness of the new and old formulations under simulated conditions, using selected products that are known to be difficult to clean. Some evaluation of the ease of removal of residual cleaning agent should be performed. A major change in the cleaning agent such as use of an entirely new product, normally would require full revalidation;
- vi) cleaning method changes: minor changes, such as use of distilled water rather than municipal water or using an additional rinse step, or the use of different cleaning tools are not likely to affect the overall procedure adversely and should not require revalidation work. Modifications in the procedure to make it more effective require validation. Significant changes would require at least some revalidation to verify the equivalence or improvement in results. Major changes such as cleaning a piece of equipment intact rather than disassembling it, should be fully evaluated by conducting comprehensive validation studies on that piece of equipment.

The careful planning and execution of the revalidation program will allow for compliance in the ongoing operations of the facility.

2.15 Maintaining the cleaning procedures in a validated state

Completing the cleaning validation study is a major task. But what does such study really demonstrate? When a minimum of three cleaning validation trials are completed and the results meet the acceptance criteria, the cleaning procedures have demonstrated to sufficiently and consistently remove contaminants and cross-contaminants from equipment surfaces (Hwang, 2000).

However, in time certain factors can decrease the efficiency and consistency of the cleaning program. Such factors are operator variability, equipment aging and repair and changes to product, equipment, process or batch size. Therefore it is not only important to design a cleaning validation program but also to maintain and sustain in time the validity of the program. Another challenge of cleaning validation is how to design an effective maintenance program to sustain the validity of the cleaning validation program. An effective maintenance program overcomes the limitations of the cleaning validation process and provides a high degree of assurance for consistent cleaning results

Validation follow-up can reveal eventual deviations from proper methodology (Laban, Cauwet *et al.*, 1997). Any deviation should result in an investigation and suitable corrective action. If the corrective action follows due to an operator error (e.g., deviation from the manual cleaning techniques) the process does not need to be altered and the validation is still valid. If corrective actions lead to modifications in the cleaning process or change in validated parameters, a new validation exercise is required (e.g., change in equipment or cleaning agent, change in controlled parameters). When deviations or abnormalities are observed preventive actions need to be taken.

A systematic and scientific approach can be used to design an effective maintenance program that overcomes these limitations.

2.15.1 Operator variability and an effective and continuous training program

Highly variable cleaning results indicate that the process is more likely to fail in the future. Cleaning procedures that provide results with unacceptable high variability require major changes. Procedures with moderate variability can be improved by an effective and continuous training program.

Training should be conducted periodically for existing operators, and new operators should be properly trained before they are allowed to perform cleaning procedures. An effective and continuous training program minimizes the between-operator and within-operator variability and therefore enhances the consistency of cleaning results (Hwang, 2000).

2.15.2 Equipment aging, repair and maintenance program

Through the normal use, the smoothness and structural integrity of the equipment surfaces can change over time. Changes in equipment or in the configuration of the equipment chain can render an effective validated cleaning procedure ineffective (Harder, 1984).

As the surface becomes rougher, it is more difficult to clean because it has a greater contact area that can adsorb and trap more chemical residues. Repairing a piece of equipment or installing new parts could create new stress centers, which could result in difficult-to clean surfaces. The potential effects of equipment aging and repair on cleaning can be managed by the equipment maintenance program (Hwang, 2000).

The preventative maintenance program minimizes unnecessary damage to equipment caused by improper installation and operation. The routine functionality check and mechanical maintenance are designed to detect equipment aging and malfunction before cleanability is affected. Such programs provide a mechanism to prevent cleaning failure resulting from equipment aging and repair.

2.15.3 Potential non-representative results and monitoring program

Statistically, cleaning validation results from only three trials do not provide a high degree of confidence, especially for manual cleaning procedures, unless the results are extremely consistent and well below the acceptance limits. Operators may introduce a bias in their cleaning during the cleaning validation process. If acceptable results from cleaning validation are the result of extra efforts on the part of the operators, it is questionable whether future cleaning would produce the same satisfactory results. To confirm the validity of extrapolating validation results to future operations, the implementation of a validation review in the form of a monitoring plan must ensure consistent cleaning capability after the cleaning validation has been completed.

The validation review is not part of the validation (Laban, Cauwet *et. al.*, 1997). This-post-validation step is necessary to check if procedures and parameters are consistently observed. A monitoring program provides a mechanism to verify the capability of the cleaning procedures, the efficiency of the training program, and the effectiveness of the equipment maintenance program. It also provides a mechanism for trend analysis and early warning of problems associated with the cleaning system. The review concerns the following two items: the control of results and the control of means.

When good cleaning results are obtained consistently through periodic testing being conducted for a sufficient period of time (e.g., a number of years) the program can be reduced to a random monitoring program.

The quality of cleaning is based on the good implementation of the validated cleaning processes. For this purpose, the review should also include periodic audits. These audits enable to check that appropriate human and material means are continuously allocated to cleaning (follow-up of equipment, qualification, staff training). Manual cleaning should be specifically scrutinized through periodic verification due to its high variability (PDA, 1998). Verification should confirm the ongoing appropriateness of the training program as well as the operator's ability to perform the cleaning process.

Audits should be carried out at scheduled frequencies to check that cleaning operations are consistently and properly implemented. The frequency with which a cleaning procedure should be

verified after validation depends upon several factors (Harder, 1984). For liquid and semi-solid products, especially those known to be prone to microbial growth, verification should be carried out at least annually or even more often until a reliable history has been established. For solid products, particularly those that are dry-processed, if the initial validation and subsequent revalidation establish a wide margin of safety, verification can be performed less frequent.

2.15.4 Changes to the product, equipment, process, or batch size: the expansion program

After the study has been completed, establishing an expansion program can help manage the addition of new products, equipment and changes in manufacturing process and batch sizes. When new products or pieces of equipment are added to the matrix, the acceptance limits may change. The manufacturing process can affect the degree of difficulty in cleaning and, consequently, the cleaning results. A well designed cleaning validation expansion program provides scientific and systematic means for managing new products, new equipment, changes in the manufacturing processes or batch size, after the completion of the cleaning validation study. Such an expansion program maximizes the sustainability of a cleaning validation program (Hwang, 2000).

2.16 Conclusion

Cleaning and decontamination operations and their validation are not isolated, but integrated part of the manufacturing process. Validation of cleaning procedures like the validation of any other process, does not have standard answers. The success of the cleaning validation process resides in a logical and realistic approach. Every company should develop its own cleaning validation policies based on its own type of activity and justify its choices by a scientific rationale. This logic is based on the legislation, which defines the context, as well as on a precise knowledge of the conditions of validation, the methodology of implementation and documentation of methods.

Good common sense is a vital factor in the establishment of validated cleaning procedures for pharmaceutical manufacturing equipment. By knowing the products, the manufacturing processes and the equipment characteristics a greater probability of success for validation is expected.

The effectiveness and reliability of a validated cleaning procedure depends first upon the relevance of initial documentation, and secondly upon the adherence to plans and degree to which the operators understand and follow cleaning procedures (Harder, 1984).

Chemical and microbiological contamination of pharmaceutical products still can occur even after validation studies have been carried out unless the purpose and implications of validation are understood clearly. Because most cleaning procedures are manually operated, obtaining and maintaining a reasonably high degree of confidence on the capability and consistency of the procedures is challenging.

Implementing a maintenance program overcomes the highly variable nature of cleaning and provides a high degree of confidence to ensure efficient and consistent cleaning.

The company must be aware of the current regulatory requirements. The company must also have knowledge of the validation practices that are unacceptable to regulators. These include the substitution of validation with testing after each cleaning, the use of placebo batches, and the reliance on rinse fluid results without measuring residues still adhering to the equipment surface, and the lack of data to support the selected cleaning limit.

There are no absolute answers (Mendenhall, 1989) for cleaning validation. Different solutions to the encountered problems can be offered as long as they are all consistent with the end objective of establishing a meaningful and fully documented cleaning validation program. As with most other interfaces of quality and manufacturing, the desire for high quality products to meet the public's health needs must be weighted against the costs of achieving them. Sound technical judgments provide a realistic tempering medium to achieve both objectives.

Chapter 3: Analytical methods used for cleaning validation - review of literature

3.1 General considerations

Cleaning validation studies may be facilitated by the choice of an appropriate analytical method. Many methods are complementary to each other. The suitability of using various analytical techniques for cleaning validation together with their advantages and disadvantages will be examined in this chapter.

Commonly, existing analytical methods used for product release purposes, or stability studies may be altered and validated at trace levels to become assays for drug residuals (Harder, 1984). Because the amount of drug present in the sample being assayed is very low, and due to sample matrix differences from the one drug product to another, changes in analytical methods may be required to overcome the dilution effect and interferences from other compounds present in a cleaning validation sample.

It is important to establish which analytes need to be measured to assess the equipment cleanliness. The method developed should be able to detect at least one of the drug APIs and sometimes toxic degradation products at a sufficiently low level to provide the desired information about the equipment LOC achieved through cleaning.

If a cleaning agent is effective in removing the drug-product residue but leaves behind its own residue, then the cross-contamination has been eliminated but the equipment has been contaminated with detergent and consequently the equipment has not been cleaned effectively (Gavlick, Ohlemeier & Kaiser, 1995). Therefore, the analytical strategy needs to include as well an assay for the determination of the cleaning agent residues, together with the scientific rationale for selecting a particular assay.

Before choosing a suitable method of analysis it is mandatory to establish the limits for the residue allowed to remain after cleaning (Kaiser and Minowitz, 2001).

Rossi and Ryall (1996) have discussed the selection of a suitable analytical method for cleaning validation in conjunction with the choice of sampling methods and acceptance criteria determination.

3.2 Requirements for operational parameters of analytical methods

Once a method of analysis has been selected, the following step is to validate it for the concentration range of interest for cleaning validation. According to Erni, Steuer & Bosshardt, (1987) “validation is the procedure used to prove that a test method consistently yields what it is expected and required to do with adequate accuracy and precision”. The validation approach used to assay residues of potential contaminants follows the same principles used for method validation of dosage forms.

The degree of analytical validation should reflect the purpose of the analysis (Krull and Swartz, 1999). The USP 28 (2005) divides the data required for assay validation into three categories based on the requirements of the method’s application. Kirsch (1998) has chosen to validate cleaning methods as a Category II- quantitative assays for impurities in bulk drug substances or degradation products, because for validation purposes residue analyses can be resembled to the quantification of impurities in drug products.

According to Kirsch (1998) the following analytical performance parameters need to be evaluated for cleaning assessment methods: accuracy, specificity, range, linearity of response, limit of detection and limit of quantification, precision, reproducibility and robustness.

3.2.1 Method accuracy

The accuracy of an analytical method is the closeness of the test results obtained by that method to the true value of the sample (Green, 1996; USP 28, 2005). Accuracy may be assessed through the assay of a known quantity of analyte spiked into a sample matrix within the proper concentration range spanning the anticipated result. The percentage recovery of the known quantity of analyte added in the sample is then calculated (USP 28, 2005).

3.2.2 Method specificity and selectivity

Once a method has been developed and demonstrated to be suitable for its intended use, its specificity and selectivity should be determined. The ability of an analytical method to accurately separate and quantify the analyte studied and its related compounds is essential in the assessment of equipment cleanliness (Kirsch, 1998).

It should be stressed that the analytical method must be able to detect the analyte after exposure to the cleaning environment. The International Conference on Harmonization (ICH) Guideline defines specificity as “the ability to assess unequivocally the analyte in the presence of components which may be expected to be present” in the sample matrix (ICH, 1996).

There are many sources of interferences that could bias the cleaning validation analytical results. In the case of samples collected after equipment cleaning, this would include: excipients, cleaning agent compounds, swab extracts, extracts from process equipment surfaces, compounds introduced by the sampling procedure (acids, bases, solvents) and maintenance materials (Kaiser and Minowitz, 2001).

Ideally, the analyte assayed should have no interference from extraneous compounds and should be well resolved from them (Brittain, 1998). It is desirable to identify all sources of interferences and minimize them as much as possible. For example, for chromatographic separations no peaks should coelute along with the analyte peak and baseline separation between the peak of interest and all other components should be obtained (Debasis, Boehlert *et. al.*, 1982).

3.2.3 Precision and reproducibility

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of a homogenous sample (USP 28, 2005). This quantity is expressed in terms of relative standard deviation obtained during a multiple series of measurements.

The precision expressed as repeatability is the precision under the same operating conditions, by using the same instrument, over a short interval of time when experiments are carried out by the same analyst. Repeatability is also known as intra-assay precision.

The intermediate precision (IP) expresses within-laboratory variations such as different days, different analysts working on different instruments and in different laboratories (Bruce, Minkinen & Riekkola, 1998).

For the purpose of cleaning methods, the repeatability and intermediate precision need to be evaluated as part of the method validation (Kirsch, 1998).

3.2.4 Linearity of response and range

The linearity of an analytical method is its ability to prove that the test results are directly proportional to the concentration of analytes in sample within given ranges (USP 28, 2005; Brittain, 1998).

Cleaning sample concentrations can vary widely from undetectable to a higher level that cannot be predicted. Therefore a wide analytical range should be validated, ideally from 50% of the cleaning limit to ten times this concentration (Kirsch, 1998). At levels far above the LOC, accurate quantification is not required because the cleaning process is certainly not sufficient.

According to Kirsch (1998) acceptable linearity criteria for cleaning validation are:

- i) Correlation coefficient (r^2) ≥ 0.9900 (i.e., 99% or more of the data are accounted for by the regression line);
- ii) Y-intercept $\leq 5\%$ relative to response at 100% working concentration;
- iii) Residuals are randomly dispersed and exhibit no obvious trend.

A linear regression equation applied to the results should have an intercept that is not zero (Huber, 1998). A non-zero intercept demonstrates that there is no effect on the accuracy of the method.

The range of the method is the interval between the upper and lower levels of analyte concentration that have been demonstrated to be determined with a suitable level of precision, accuracy and linearity using the specified method (Brittain, 1998, USP 28, 2005).

3.2.5 Limits of sensitivity

According to Jenke (1998) sensitivity is the ability of a method to reliably respond in a consistently recognizable manner to decreasingly smaller amounts of analyte. Frequently utilized measures of sensitivity are the limit of detection (LOD) and limit of quantification (LOQ).

For cleaning validation, the analytical method selected must offer a sensitivity level at least equal to that of the acceptable residual level (Hwang, Kovalski & Truelove, 1997). A limit is only meaningful in the context of detection capabilities (Mendenhall, 1989).

The LOD is defined as the lowest amount of analyte in a sample that can be detected but not quantified (USP 28, 2005). For chromatographic procedures LOD is defined as approximately three times the standard deviation of the baseline noise level around the analyte peak (Kirsch, 1998).

The LOQ is defined as the lowest concentration of an analyte that can be determined with acceptable precision and accuracy under the stated operational conditions of the method (USP 28 2005). For chromatographic techniques the LOQ can be calculated as the concentration that is 9-10 times the standard deviation of the baseline noise level around the analyte peak (Kirsch, 1998).

To obtain accurate and reliable results for cleaning validation it is recommendable that the method LOQ be at least two times more sensitive than the concentration corresponding to the LOC expected (Chudzik, 1998). If this requirement cannot be met, the method should be re-evaluated to optimize detection sensitivity.

If levels of contamination of residual are not detected, it does not mean that there is no residual contaminant present after cleaning. It only means that levels of contaminant greater than the detection limit of the analytical method are not present in the sample (FDA, 1993).

Usually test results greater than LOQ are considered reliable. The term non-detectable (ND) is used to measure cleanliness relative to the LOQ (Valvis and Champion, 1999). A test result below the method LOQ is considered unreliable because of the increased noise of the analytical method at these levels. It is not recommendable to report ND results but rather calculate the LOC found on equipment by using the method's LOQ.

If the calculated LOC produces a concentration which is below the method's LOD and the concentration of the contaminant is found below the LOQ but still above the LOD, it is acceptable to use the method LOD to demonstrate the presence or absence of the contaminant on the equipment (Kirsh, 1998).

3.2.6 Robustness and ruggedness

The robustness or ruggedness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations of method parameters and provides an indication of its reliability during normal usage (ICH, 1994).

Typical variations in liquid chromatography can be the pH and composition of mobile phase, different temperature, flow rate or analysis columns (ICH, 1996).

For cleaning validation, a validated method must be rugged and robust enough to measure the residue limit established.

3.3 Analytical requirements for percent sample recovery and sample stability

FDA states that "the firm should challenge the analytical method in combination with the sampling method(s) used to show that contaminants can be recovered from the equipment surface and at what level (i.e., 50% recovery, 90% etc). This is necessary before any conclusions can be made on the sample results" (FDA, 1993). Therefore, the assessment of the percent sample recovery and its validation is a compulsory step (Laban, Cauwet *et. al.*, 1997). Failure to carry out recovery studies is commonly cited in FDA's observations.

The purpose of validation of recovery is to determine the amount of a chemical compound that can be recovered from a surface (Kaiser and Minowitz, 2001). The validation of sample recovery is translated into a sampling procedure, which defines the area to be sampled, the sampling technique and the medium for sample elution. The yield is assessed in the laboratory by imitating normal sampling conditions.

A sampling procedure is effective if it provides reasonably high and consistent recovery (Hwang, 2000). Each company is responsible for setting a minimum percent sample recovery, below which the sampling method needs to be improved or altered (Laban, Cauwet *et. al.*, 1997). Then, the percent recovery will be included in the calculation of residual contamination level.

A recovery greater than 80% is good. If the recovery is greater than or equal to 50% with a 10% maximum coefficient of variation is considered acceptable (Kaiser and Minowitz, 2001). However, if the recovery is less than 50 % the source of poor recovery should be investigated. When the recovery of a chemical residue is low, the accuracy of the test tends to be affected significantly even when corrections are made to adjust for the low recovery. If the recovery is inconsistent, a worst-case approach may be used by correcting the actual sample results with the lowest recovery value obtained.

According to Chudzik (1998) several solutions may be tested for recovery: drug substance alone, drug substance with known degradation products, degradation products alone, cleaning agent alone, cleaning agent with drug substance, cleaning agent with drug substance and degradation products, and blank solvent are usually tested.

It should be emphasized that the validation of analytical methods and the validation of recovery rate are two completely different validations.

3.3.1 Recovery rate from swab material

The overall loss of analyte due to swabbing a surface is a result of loss due to swab retention as well as retention on the surface of the equipment (Kirsch, 1998). It is essential to verify that the contaminant is thoroughly extracted from the sampled surface. It is equally important to ensure that the contaminant is fully recovered from the sampling medium (swab, solution, or placebo)

prior to the analysis. Therefore, swab and surface recovery must be determined and evaluated independently. If the evaluating procedures are not effective in recovering the total amount of residual drug remaining in the equipment the validation studies will be invalid (Harder, 1984).

The analyte recovery rate from swab materials is determined by spiking clean dry swabs with amounts of analyte in rinse solution that are equivalent to the amount of analyte that should theoretically be removed from a certain surface area. The spiking is performed for a concentration range of 50 to 200% of the LOC. The spiked analyte is allowed to dry on the swab and is then extracted. The recovery is calculated by using an external standard solution at the used concentration. The method precision and accuracy tests should be performed. The method is acceptable when $\geq 90\%$ of the analyte is recovered from the swab at the limit concentration (McCormick and Cullen, 1993). A possible cause of a poor recovery may be that the residue is being too tightly held by the swab. If the analyte is held too tightly by the swab, another type of swab material should be investigated.

3.3.2 Recovery rate from coupon materials

Surface recovery is performed to determine the extent of analyte recovery from the process surfaces (Kirsch, 1998) and it is performed similarly to swab recovery. The main difference consists of the analyte being spiked on coupons made of the same material as the production equipment (e.g., stainless steel, teflon, silicone, plexiglas).

A general guide to recovery studies using swab sampling is presented by Chudzik (1998). The combined sampling and test method should be challenged for accuracy by testing the recovery of contaminants – through spiking studies over a range of potential contamination levels, i.e., at the acceptance limit, as well as above and below the acceptance limit. This ensures that the limit can be measured in the analysis.

Amounts of analyte ranging from 50 to 200% of the LOC may be used. The dried analyte residue on the coupon is removed with a pre-moistened swab by moving the swab across the coupon in a series of vertical swipes so that the entire surface is covered using a single side of the swab. Among the issues to be considered are: the amount of solvent on the swab, the exact motions of

the swab over the sampled area, the number of strokes over the sample site, the composition, size and shape of the swab and the amount of the time spent swabbing the site.

Swabbing or wiping tests are not easy to standardize for the following reasons: rubbing is not consistent as the movement and speed of execution are difficult to reproduce, and re-deposition of the contaminant is possible (Laban, Cauwet *et. al.*, 1997). To enhance recovery, one could use several swabs.

The idea of achieving linearity in recovery studies, when the analyte is spiked at several concentration levels has been disputed by LeBlanc (2005a). He bases his arguments on the variability of recovery associated with manual sampling and on the observation that the higher spiked amount the lower the percentage recovery. In order not to overcomplicate cleaning validation studies he suggests that recovery should be performed at two contamination levels, namely at the LOC and 20% of the LOC.

The criterion for recovery from coupon surface will vary widely depending on factors such as analyte solubility, analyte-metal binding strength, reactivity of surface with analyte, and the swabbing technique of the analyst. When the recovery is less than 70% the method should be modified to optimize recovery from surface. Also the variability of the analyte recovery from the surface should show an imprecision of less than 5-10% RSD at the LOC. The calculations included in the final analytical procedure should be adjusted by the mean recovery value obtained for all concentrations in a study. All results obtained should be multiplied by a factor compensating for surface and swab analyte loss (Chudzik, 1998).

Lombardo, Inampudi *et al.*, (1995) have presented a case study consisting of the development and validation of surface swabbing procedures as a part of the cleaning validation program for the recombinant human neurotropic factor (rHCNTF) manufacturing process.

Laban, Cauwet *et. al.*, (1997) have presented the practical steps of performing recovery studies:

- i) spreading, over a predefined area the targeted chemical substance solution at the concentration equivalent to the LOC;
- ii) sampling of the total contaminated area with sampling material and solvent of choice, in a reproducible manner with regards to direction, speed and strength;

- iii) extraction of the contaminant from the sampling material;
- iv) sample preparation (evaporation, dilution with another solvent, filtration etc.);
- v) repeating the above operations for several times (at least three times) to determine the reproducibility of the methodology.
- vi) results should be compared with those from check samples.

3.3.3 Recovery rate from rinsates

When rinse samples are used for the evaluation of equipment cleanliness it is useful to perform rinse-recovery studies to verify that a sampling rinse procedure can adequately recover residues from surfaces. The best way to approximate a sampling rinse-recovery is to conduct laboratory studies involving coupons (Le Blanc, 1998).

Rinse recovery is simulated by spiking various amounts of analyte dissolved in the rinse solution on the surface of a flat bottom stainless steel pan or beaker. Once the analyte is distributed across the surface, the solvent should fully evaporate and then the spiked surface should be rinsed with solution. The analyte amount placed on the surface should correspond to the cleaning limit (Kirsch, 1998). The analyte concentration recovered is compared with the theoretical one and a percent recovery is calculated. The procedure is repeated by spiking amounts of analyte corresponding to 50% to 200% of the limit application level to determine whether rinsate recovery is concentration-dependent. The amount of analyte recovered should not be less than 80%.

A well designed recovery study is the keystone of a good cleaning validation. It must show that the analytical method is accurate and reliable as well as demonstrate that the sampling procedure is capable of recovering drug substance, cleaning agent, or any other related contaminants that might be present following the completion of the cleaning procedure. The recovery protocol must be written, reviewed and approved prior sampling.

3.3.4 Sample stability

If the residue being detected is a drug active, and the method used for its detection is the same method that is used for product release purposes, it should be established that the active has not changed its chemical nature during the cleaning process.

This may be performed by assessing the stability of the API contaminant in swab samples which are stored in a dry or moisten state for the time interval required between sampling and analysis. The sample storage conditions should be clearly defined.

3.4 Criteria for the selection of analytical methods

There are many different analytical techniques available that can be used to detect trace residues, ranging from simple titrations to more complex LC-MS methods. The selection of analytical methods should reflect an optimization between practicality and reliability (Mendenhall, 1989) and is determined by factors such as the type of sampling method used and the concentration of a component within the product, its potency, toxicity and solubility characteristics and the existence of an analytical assay method for it (Zeller, 1993).

3.5 Specific vs. nonspecific methods

One question raised often is whether to use specific or nonspecific methods of analysis. One may choose specific analysis methods targeting a defined chemical compound in the presence of potential contaminants and this is usually the case for the analysis of API traces. Otherwise a non-specific property of the entire residual present in a sample may be measured to gather information about the level of equipment cleanliness.

Special questions are raised especially when selecting analytical methods suitable for assessing the level of detergent residue after cleaning. The rationale for cleaning agent residue analysis is somehow different from the rationale used when analysing product traces. The differences are dictated by the fact that cleaning agents are usually formulations of several active components, each with its own role in the overall cleaning ability (Gavlick, Ohlemeier & Kaiser, 1995).

When using a single assay for detergent analysis, the focus is on determining whether any residue from the cleaning agent is present without trying to identify which particular component is present. A targeted component may be alternatively identified but it may not be straightforward to decide which component to analyze because of the multitude of active ingredients in a cleaning agent. Possible approaches that can be used are listed by Gavlick, Ohlemeier & Kaiser (1995):

- i) determine which component of the cleaning agent is present in the greatest amount;
- ii) look for the component that is the easiest to assay (yields greatest response for a given analytical method);
- iii) look for the most toxic component based upon acute oral toxicity LD₅₀ or other toxicity data;
- iv) alternatively, look for a selective adsorber i.e., the particular component that adsorbs on the equipment surface and is the last to rinse off during the cleaning process. The rationale is that if it can be demonstrated that the selective adsorber has been removed, then it can be assumed that all other cleaning agent components have been removed as well (Smith, 1993).

The multitude of analytical methods suitable for cleaning agent determination during the validation of cleaning procedures has been reviewed by Gavlick, Ohlemeier and Kaiser (1995).

FDA site inspectors generally give strong preference to product specific cleaning validation assays, especially for multiple product facilities. Annex 15 (EU Guide to GMP, 2001) stipulates also that analytical methods should be specific/selective for the substance to be assayed and provide a sensitivity that reflects the level of cleanliness determined to be acceptable by the company.

When non-specific methods are used it is important to be able to compare results obtained via specific methods to the results from other nonspecific methods. A comparative study for the effectiveness of a cleaning agent recovery from surfaces has evaluated the use of several specific and non-specific analytical techniques: inductively coupled plasma, atomic emission spectroscopy, ion chromatography, total organic carbon, pH, titration and conductivity (Kaiser, Tiery & LeBlanc, 1999).

However it is not mandatory to correlate such results obtained from nonspecific methods to the results obtained through specific methods such as HPLC (LeBlanc, 2005a). During validation the availability of a specific method can offer precise indication of the residue level present after cleaning. Conductivity, pH, and TOC may be especially used in routine monitoring of cleaning effectiveness (PDA, 1998), after the validation phase has been finalised.

Sometimes two methods can be used to complement each other. For the case of a drug in the presence of excipients, the excipients are water-soluble, while the drug actives have extremely low solubility in water. The excipients will contribute to the TOC values while the drug active, not detected by TOC, may be assessed through HPLC.

Although there should not be used alone, the USP purified water assays may be performed for chloride, heavy metals, oxidizable substances, sulfates, ammonia, carbon dioxide, calcium and total solids.

All analytical techniques present advantages and disadvantages. A review of the specific and nonspecific analysis methods used for cleaning validation is presented further in this chapter.

3.5.1 Specific analysis methods

A specific method is a method that detects a unique compound in the presence of potential contaminants. Some examples of specific methods are high performance liquid chromatography (HPLC), gas chromatography (GC), ion chromatography (IC), thin layer chromatography (TLC), atomic absorption spectrophotometry (AAS), inductively coupled plasma (ICP) and capillary electrophoresis (CE).

If a drug active is highly toxic, a specific method should be used for its detection. For specific methods, there should be no interference with the analyte of interest if the method is properly designed.

3.5.1.1 High performance liquid chromatography (HPLC)

HPLC is one of the most popular methods used for cleaning validation because it satisfies all the requirements for cleaning validation (Kirsch, 1998). HPLC is sensitive for most pharmaceutically

active and inactive species and if validated properly it provides the necessary specificity. It has also the advantage of detecting degradation products.

There are various detectors used for HPLC: ultraviolet – visible (UV-VIS), fluorescence, electrochemical, refractive index, conductivity, evaporative light scattering detector (ELSD).

The UV-VIS is the most commonly used detector being generally inexpensive and readily available. Many compounds have chromophores and therefore they can be easily detected by UV. Many instruments are equipped with diode array detectors (DAD). This allows for easy detection of impurities or potential contaminants within peaks. The UV detection usually requires no additional reagents, post-column or pre-column reactions. Also, molar absorptivities are generally not affected by the temperature and there is no need for heating or cooling the detector.

However, UV detectors cannot detect all types of compounds and therefore are not considered universal. Also, not all compounds have chromophores. This is particularly true of surfactants that are used in pharmaceutical industry. Dirty cells, air bubbles and the use of gradients can affect baseline drift and detection capability. The limits of detection can be higher than other detector types due to background interferences (Kaiser and Minowitz, 2001).

An HPLC-UV method was developed for the determination of cefadroxil and of semisynthetic cephalosporin antibiotics cefsulodin, cefmenoxime in swab samples. The cephalosporins can be quantified as low as 1 µg/ 100 cm² (Gorski, Plaszczyńska *et.al.*, 1991).

For the determination of meclizine hydrochloride in swabs collected from cleaned equipment surfaces, an RP-HPLC method was developed and validated. Visual and pharmacologically based acceptance limits were established and recovery rates from swab material and stainless steel were determined (Mirza, Lunn *et. al.*, 1999).

HPLC methods have been developed for controlling the cleaning procedures used for acetylsalicylic acid (Nozal, Bernal *et. al.*, 2000), analgesic narcotics (Lambropoulos, Spanos & Lazaridis, 2000), amlodipine residues (Klinkenberg, Steel & Ceccato, 2003) and cisplatin (Raghavan, Burchett *et.al.*, 2000). Other HPLC-UV methods for the assays at trace levels of the

highly potent compounds losoxantrone (Shea, Shamrock *et. al.*, 1996) and bisnafide (Segretario, Cook *et.al.*, 1998) have been developed;

For the determination of detergent traces of 4-tert-amylphenol and 2-phenylphenol a HPLC assay was developed and validated by Zayas, Colon *et. al.* (2006)

HPLC coupled with electrochemical detection was used to determine isoproterenol sulfate in cotton swabs. The assay was used as a rapid quantification means in order to minimize down times of shared manufacturing equipment (Elrod, Schmit & Morley, 1996).

An RP-HPLC method with ELS detection was developed for the determination of LY354740 which is a weak UV absorbing structural analog of glutamate. The method was used to determine the compound in cleaning validation swab samples (Risley, Hostettler & Peterson, 1998). The ELSD may be the suitable detector for cleaning agents. ELS is claimed to be a universal detector because it can detect any type of compound. ELSDs are simple, versatile and rugged in use. Drawbacks of ELSD detection are the very limited choice of buffer salts that can be used and the fact that any buffers that will not evaporate will also produce signal and cause interferences. (Risley, Hostettler & Peterson, 1998).

3.5.1.2 Ion chromatography (IC)

IC was used for the determination of ionic surfactants (Hoeft and Zollars, 1994). In addition to its use for surfactants, ion chromatography can be used for the analysis of inorganic and some organic compounds present in cleaners (Weston, 1998). Very low levels of detergent can be detected by using IC.

3.5.1.3 Thin layer chromatography (TLC)

TLC analysis is used in many applications for semi-quantitative purposes. TLC is specific, but is not sensitive in all cases. Henrich (1992) has described the TLC analysis for over 150 surfactants in six different TLC systems. Novakovic (2000) has used high performance TLC for trace analysis of two drugs affecting gastrointestinal function, namely ranitidine hydrochloride and ursodeoxycholic acid. The method was used for the determination of the two drugs in swabs, in the final rinse water, and in the placebo batch manufactured after the cleanup process.

A sensitive overpressured layer chromatographic procedure was developed to control the cleaning of five steroid hormones processed in the same manufacturing equipment by Katona, Vincze *et. al.*, 1999.

The Canadian cleaning validation guidelines (HPFBI, 2000) recommends the use of TLC for cleaning validation as a screening technique along with other specific methods.

3.5.1.4 Gas chromatography (GC)

GC has characteristics comparable to HPLC but is less commonly used for cleaning validation. It is used especially for organic volatile compounds at raised temperatures. Mirza, George *et.al.*, (1998) have developed and validated a GC method for the determination of methenamine hippurate in swab samples.

The analysis of product traces for a dermal solution containing camphor and menthol was performed by GC. The method was used for the evaluation of a rinsing- based cleaning method for pipes (Prosek, Krizman & Kovac, 2005).

3.5.1.5 Capillary electrophoresis

Capillary electrophoresis methods have been developed mainly for surfactant determinations (Heining and Vogt, 1999). The method is less commonly used for trace drug residues analysis. However the potential of using CE as a routine tool in the pharmaceutical analysis and specifically for cleaning validation has been explored by Altria, Chen & Chlos (2001). Two of the most popular modes of operation of CE, namely capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC) can be used alongside HPLC. The two separation techniques allow the separation of a wide variety of analytes differing in size, charge, hydrophobicity and stereochemistry.

The CZE was applied to routinely monitor the equipment cleanliness after the manufacture of a wide range of basic drugs. The method is able to quantify residue levels as low as 25 ng/ml (Altria and Hadgett, 1995). Detergent residues were routinely monitored following product manufacture by Altria, Gill *et. al.* (1995).

The advantages of CZE over HPLC consist of relatively simple instrumentation, less use of solvents and little waste generation, versatility and detectability of a high number of compounds that can be separated based on charge to mass properties. However, detection limits typically can be higher than those obtained with HPLC due to on-column detection. This can be overcome by pre-concentrating the samples on the capillary itself, generating the so-called stacking effect. Trace residue determinations through CZE could be used especially in the biotechnology industry for trace determinations of aminoacids, peptides, proteins, and cleaning agents.

Capillary ion analysis (CIA) is a technique derived from CE. It has been used for the detection of ionic contaminants in cleanroom knitted polyester wipers (Attebury and Bhattacharjee, 2000).

3.5.1.6 Atomic absorption spectrophotometry and inductively coupled plasma

Atomic absorption spectrophotometry (AAS) and inductively coupled plasma (ICP) are specific techniques used often for inorganic trace analysis. These techniques can detect inorganic contaminants down to extremely low levels. The cisplatin was determined at ppb levels in rinse water samples (Raghavan and Mulligan, 2000).

3.5.1.7 Ultraviolet – Visible spectrometry

UV-VIS scans provide an evaluation of various types of residues in a single test. It is a relatively fast way to establish the presence of various organic materials. Some degree of quantification and correlation to assay residuals is possible (Laban, Cauwet *et. al.*, 1997). UV-VIS spectrometry requires that the analyte should contain a strong chromophore to allow detection at trace concentrations. The technique is nonselective if more than a single UV-active species is present in the product formulation.

An on-line analyzer that uses UV spectroscopy was developed to detect within a few seconds most drug substances with chromophores (<http://www.zeiss.de>, 1995). The system comprises a UV diode array spectrometer coupled to an external flow cell by the use of fibre optics. The cell is mounted in a by-pass loop that can be moved from reactor to reactor in a manufacturing facility.

3.5.1.8 Fourrier transform infra-red (FTIR) spectroscopy

FTIR spectroscopy is not used as a stand-alone method for analyzing residues on equipment. This is because of the semi-quantitative nature of the reflectance techniques used for this type of analysis.

However, it is very useful in performing screening studies and evaluating potential residues of cleaning agents. This may be done by soiling coupons with the cleaning agent, allowing to dry and perform static rinsing studies. Such studies can indicate whether or not the cleaning agent is readily removed from surfaces. The height or area of a particular peak is measured versus the concentration of the standard spiked on the coupon. It is relatively easy to implement for the characterization of contaminants present in molecular layers (Biwald and Gavlick, 1997).

The technique can be insufficient sensitive and is not always adapted to the analysis of opaque or rough surfaces (Laban, Cauwet *et. al.*, 1997).

FTIR analysis can be used to complement TOC in the analysis of cleaning agent residues. Grazing-angle IR spectroscopy was used for detection and semi-quantitation of residues on surfaces (Biwald and Gavlick, 1997). The in-line grazing-angle-incidence attachment for IR spectroscopy is excellent for recording spectra of thin films on metal surfaces, such as stainless steel, allowing for direct surface analysis. The angle of incidence is fixed at 75° resulting in excellent spectral contrast. Large panel samples can be analyzed, because the sampling surface is above all optical components. Detection is specific because a particular band due to a functional group in the material of interest (the cleaning agent) is monitored as the substrate undergoes various cleaning processes.

Shortcomings of the classical methods used for cleaning validations include the need to sample the surface, incomplete analyte recovery from surfaces and from the swabs after sampling. An ideal validation method for a cleaning procedure would be the rapid, automated, in –situ, multi-component analysis of the entire surface. A remote sensing FT- IR reflectance device was used to assess the equipment cleanliness proving fast and quantitative results (Ciurczak, 1998). A fiber-optic, mid-IR spectroscopy probe combined with a grazing- angle reflectance sampling head was also used as a solvent-free, in situ, method for validating the equipment cleanliness (Mehta, Pollo *et.al.*, 2002).

Further improvement was brought by Urbas and Lodder (2003) with the development of a multifunctional active-excitation spectral analyser (MAESA) capable of illuminating a line on a surface. The returned light from multiple spectroscopic phenomena such as absorbance, scattering, polarisation and luminescence is analysed and small concentrations of a contaminant on surfaces can be accurately assessed.

Research was also directed to develop techniques for the direct evaluation of surfaces using diffuse reflectance spectroscopy (DRS) and near infrared (NIR) (Krull and Swartz, 1999). The advantage of such techniques is that there is no necessity to allow for incomplete recovery as in the classical sampling methodology.

To find out which component of the detergent is the least rinsable, the industry has developed a technique using FTIR as an analytical tool to determine the last component to rinse from a multicomponent cleanser on a given substrate. If this last component can be determined qualitatively, then it becomes possible to quantitatively analyze for it and thus, to obtain a more accurate upper limit estimate of the cleaning product present (LeBlanc, Danforth & Smith, 1996).

3.5.1.9 Time of flight – secondary ion mass spectrometry (TOF-SIMS)

TOF-SIMS is an extremely sensitive method of surface analysis, enabling the determination of the organic or inorganic nature of contaminants present in molecular layers. Analyses can be performed on any type of surface (rough, opaque or insulating). Upon bombarding a surface by primary ions the surface excitation leads to the emission of secondary ions characteristic of the chemical composition in the uppermost surface monolayer. The interpretation of spectra enables the accurate determination of the molecular nature of residues as almost organic contaminants give typical fingerprint peaks in the mass spectrum (EAG, 2005).

3.5.1.10 Ion mobility spectrometry (IMS)

Ion mobility spectrometry (IMS) has been recently used in the pharmaceutical industry for cleaning verification (DeBono, Stefanou *et. al.*, 2002; Munden, Everitt *et. al.*, 2002). IMS is similar to TOF-MS. Pharmaceutical compounds are thermally desorbed from the surface. The vaporized sample is introduced into the IMS via a carrier gas stream before being selectively ionized by an atmospheric chemical ionization source. The resulting ions are separated according

to mass and charge and accelerated towards a detector. On impact a specific current is produced, amplified and displayed as an ion mobility spectrum or plasmagram. IMS exceeds all validation requirements for specificity, precision, linearity, LOD/LOQ, accuracy and stability. It offers an ultrafast alternative to HPLC for the validation of cleaning, with measurements performed in ca. 30 seconds, thus ensuring fast turn-around of production (Munden, 2004; Payne, Fawber *et. al.*, 2005).

3.5.1.11 Dynamic contact angle analysis

Dynamic contact angle (DCA) analysis is a surface analytical sensitive technique that was used to study the kinetics of protein removal from steel, glass, polytetrafluoroethylene, polypropylene, ethylenepropylene rubber and silicone surfaces (Davies, Nunnerley *et. al.*, 1996).

3.5.1.12 Ion selective electrodes (ISE)

Specific ion-meters may be useful for confirming the level of residual from acidic or basic detergents in the final water rinse (Laban, Cauwet *et. al.*, 1997).

3.5.1.13 Bioluminescence methods

Bioluminescence or TPA-metry is useful in biopharmaceutical facilities. It is based on the reaction of adenosin triphosphate (ATP) with luciferin/luciferase followed by the emission of luminescence (Davidson, Griffith *et.al.*, 1999). It has extremely high sensitivity and very high reproducibility. The instruments can be used at the equipment site. This very rapid technique, with immediate results, is not used for identification but rather as a semi-quantitative method. It has low sensitivity, therefore being applicable to highly contaminated samples. The results obtained are expressed in units of bioluminescence (Laban, Cauwet *et. al.*, 1997).

3.5.1.14 Immunoassays and LAL test

Immunoassays can be used for biological drugs to monitor the presence of biological carry-over. A negative test may be the result of denaturation of the protein epitope. Product-specific assays can be used in addition to TOC for the detection of the protein residues (HPFBI, 2000).

For biopharmaceutical manufacturing facilities, because TOC analysis is a nonspecific assay, most cleaning validation programs employ TOC analysis in conjunction with more specific assays such as Bradford/Lowry protein assay, enzyme-linked immunosorbent assay (ELISA), DNA hybridization assays and Limulus Amoebocyte Lysate (LAL) -based endotoxin assays (Lombardo, Inampudi *et. al.*, 1995).

The Limulus Amoebocyte Lysate (LAL) test is a rapid and sensitive assay for the detection and quantification of the levels of bacterial endotoxin present. The LAL test is suitable for sterile products and it is complementary to the total counts testing method. A positive result can reveal previously existing microbiological contamination. The LAL test is described by the European Pharmacopoeia (EP, 2005).

There are also organic compounds for which enzymatic assay kits of excellent specificity and limit of detection are available (Laban, Cauwet *et. al.*, 1997).

3.5.2 Nonspecific analysis methods

Nonspecific methods are those methods that detect any compound producing a certain response. Some common examples of nonspecific methods are total organic carbon, pH, titrations and conductivity. Such methods reach sufficient sensitivity for cleaning validation purposes and do not involve major sample preparation.

For nonspecific methods any compound with the analysed property present in the sample will interfere. For example, if the method used is TOC, atmospheric carbon entering the sample could cause interference. With all nonspecific methods, there is a need to identify potential sources of interference. These interferences can be either positive or negative. After measuring a non-specific property the residue is calculated as if all of the measured property is due to that residue. For example, if the cleaning agent was the analyte and TOC was the method used, all of the TOC would be assumed to have come from the cleaning agent and calculated as such. This would then provide a worst-case upper-limit value (Kaiser and Minowitz, 2001) and sometimes this approach can lead to an overestimation of the residue present on equipment. If the calculated LOC is exceeded, it would not be known where the failure is originating from. The failure could be due to the active, excipients, detergent system or even an unknown contamination source. Therefore

non-specific methods are used more suitably for routine purposes after the validation phase has been successfully finalized.

3.5.2.1 Total organic carbon analysis

The total organic carbon is determined by the oxidation of the organic compounds into carbon dioxide. This oxidation can occur through a number of mechanisms depending on the instrument being used. Some typical methods through which oxidation is induced are:

- i) photocatalytic oxidation;
- ii) oxidation by combined action of persulfate chemical oxidation in the presence of UV radiation;
- iii) direct combustion assisted by high temperatures (700-900°C).

The carbon dioxide that is produced from these oxidations is either measured using conductivity or infrared techniques. Instruments generally measure the total and inorganic carbon content of a sample. The inorganic carbon consists of carbon dioxide, bicarbonate and carbonate. Then the TOC is computed by subtracting the inorganic carbon concentration from the total carbon concentration of the sample.

Initially, TOC was developed to monitor contamination of drinkable (Thomas, 1991) and waste waters. Later the TOC technique was adopted by pharmacopoeias and it is currently used to monitor the quality of pharmaceutical waters such as water for injection and purified water. TOC is also applied for analysis of high-purity waters in areas such as nuclear power plants and semiconductor production. Nowadays TOC is widely used in the pharmaceutical and biopharmaceutical industry to test for residues of previously manufactured products (Holmes and Vanderwielen, 1997, Wallace, Stevens & Purcell, 2004), cleaning agents (Jin and Woodward, 2001; Westman and Karlsson, 2000; Clark, 2000), and proteins (Lombardo, Inampudi *et. al.*, 1995).

Although TOC is nonspecific, it is a sensitive (LOD < 0.5 ppm) and less time-consuming method (Agalloco, 1992). This makes it a better choice for cleaning validation than the liquid chromatographic selective methods. This is especially true when validating cleaning procedures for biopharmaceutical manufacturing facilities, where the residue is constituted by proteins, lipids,

nucleic acids, recombinant DNA, endotoxin, carbohydrates and materials with complex chemical matrices such as detergents (Baffi, Dolch *et. al.*, 1991; Jenkins, Vanderwielen *et. al.*, 1996; Guazzaroni, Yiin & Yu, 1998). For such facilities swab sampling combined with TOC analysis seems to be the ideal method (Lombardo, Inampudi *et. al.*, 1995) because it can determine the whole range of potential contaminants that are not able to generate a response through other assay methods.

The TOC is optimally used for monitoring of cleaning processes once the validation is completed. It does not identify the specific residue material and therefore is useful for equipment cleanliness evaluation before use for the next production lot.

Applying TOC assumes that all residues detected are due to the most potent or toxic potential contaminant's carbon content. All carbon is calculated back to the most toxic material. A disadvantage of using this nonspecific method for the entire cleaning validation analysis is that, if there is a failure in the future, it would not be known where the failure originally occurred.

To make the TOC analysis as sensitive as possible, it is necessary to minimize sources of carbon from the sampling system (such as swab material, water and vial). When used for swab sampling a swab material with low TOC content must be selected. Jenkins, Vanderwielen *et. al* (1996) have reviewed the suitability of various commercially available swab materials for TOC analysis.

TOC is a versatile method suitable for the analysis of swabs and rinse water, detecting virtually every water-soluble compound and cleaning agent residue (Gavlick, Ohlemeier & Kaiser, 1995). Other advantages of TOC include little number of variables in the actual analysis, quick method development and validation, a broad range (from 0.2 to 50 000 ppm), good linearity, excellent precision and accuracy.

Disadvantages include the fact that it is only applicable to organic product or substances water-soluble. Organic solvents cannot be used in collecting the swab sample. As it does not identify the specific residue material, if results exceed the determined limit, specific analytical methods may be required.

Strege, Stinger *et. al.* (1996) have used TOC for the analysis of swab samples collected from the fermentation equipment used in the production of biopharmaceuticals. The TOC method proved to offer better results than a colorimetric method developed for the determination of total proteins in swab samples.

Westman and Karlsson, (2000) have compared the sensitivity of TOC with three potential analytical method candidates: visual detection of foam, pH and conductivity measurement in the detection of cleaning agents residues.

Guazzaroni, Yiin & Yu (1998) have examined the use of TOC for the analysis of detergents, endotoxins, biological media and polyethylene glycol.

3.5.2.2 pH measurements

pH measurements are readily adapted to the production area in order to determine the presence of alkaline or acidic cleaning materials in the final water rinse. It is applicable for determining cleaning agent traces and it may be carried out in the plant or laboratory providing immediate results.

3.5.2.3 Conductivity measurements

Conductivity is a rapid way for determining the ionic concentration of a water sample. It is applicable for cleaning agent traces monitoring with conductivity probes placed in-line for the end-point assessment of cleaning.

Conductivity of rinse samples is commonly used to detect any ionic or inorganic material residue at low levels. This technique is useful for contaminants such as cleaning agents or detergents when the composition may be unknown and the sample contains one or more ionic species. However, the technique is not sensitive to neutral or even highly polar compounds and is not specific for a particular analyte (Kirsch, 1998).

Indirect tests such as conductivity testing, may be of value for routine monitoring once a cleaning process has been validated (FDA, 1993). This would be particularly true for drug bulk substance

manufactured where reactors, centrifuges and piping can only be sampled by using rinse water samples.

3.5.2.4 Titration

If acidic or alkaline materials are being measured, titration is a very useful technique. In some cases, titration can be more sensitive than performing TOC analyses. It is applicable to detect cleaning agent traces in the final rinse water and provides accurate quantification.

The tandem use of TOC along with conductivity, pH, and titrimetry has proven to be effective in ensuring the absence of both acidic and alkaline detergent components used in pharmaceutical cleaning processes.

3.5.2.5 Gravimetric analysis

Non-volatile materials may be quantified as total solids for both organic and inorganic materials in the liquid extracts of either rinses or swab samples. It is a time-consuming technique providing nonspecific results.

3.5.2.6 Particulate count

The principle of this technique is the use of an IR laser source particle counter (Laban, Cauwet *et. al.*, 1997). The sampling is achieved via a pump and a probe capable of removing particles from the surface. The surface must be flat, dry and smooth. It has the capability of counting particles of up to 10µm. Main applications are in the control test for the validation of cleaning procedures for clean rooms. The results obtained are difficult to be used in absolute terms. Particulate count provides subjective acceptance criteria.

Table 3.1 presents the applicability and characteristics of various analytical techniques for cleaning validation (Laban, Cauwet *et. al.*, 1997) and their associated advantages and limitations (PDA, 1998).

Table 3.1 Characteristics of physiochemical analytical methods used for cleaning validation (Laban, Cauwet *et. al.*, 1997; PDA, 1998).

Analytical Method	Characteristics					Applications			Observations	
	Sensitivity	Specificity	Result availability	Ease of implementation	Cost	Chemical residue	Excipient	Cleaning agent	Advantages	Disadvantages
Conductivity	••	•	•••	•••	••	-	-	✓	- rapid - inexpensive - on-line capability	- nonspecific - limited sensitivity
pH	••	•	•••	•••	••	-	-	✓	- rapid - inexpensive - on-line capability	- nonspecific - only aqueous soluble samples
Acido-basic assay	••	•	••	••	••	-	-	✓		
UV-VIS	•••	••	••	•••	••	✓	✓	-	- moderate to high specificity - high sensitivity - use as screening method	- not quantitative
TLC	••	•••	••	••	••	✓	✓	-	- highly specific - moderate to high sensitivity - fairly inexpensive	- visual endpoint - not quantitative - long sample preparation
HPLC	•••	•••	••	•	•••	✓	✓	✓	- highly specific; quantitative - moderate to high sensitivity	- Long analysis time - expensive
GC	•••	•••	••	•	•••	✓	✓	✓		
TOC	•••	-	••	••	•••	✓	✓	✓	- broad spectrum - low level detection - minimal sample preparation - on-line capability - rapid sample turn-around	- nonspecific - only aqueous soluble samples
Gravimetry	•	••	••	••	•	✓	-	-		- labor- intensive - time consuming

•: low, •• : average; •••: high; ✓: yes; -: no

3.6 Data analysis for estimating contamination

The limited number of runs performed in a cleaning validation program makes it difficult to obtain accurate and precise measurements of the total possible contamination. After completing a cleaning validation study, based on several cleaning assessments, each sampling site for each piece of equipment has associated with it a set of residual results. Usually, the mean of the results from a particular sampling site is used as a measure of the amount of residue found at that site (Hwang, 2000).

Still, this mean value does not address operator variability, as some individual runs can result in much higher residues than the mean. The reliability of the estimated amount of residue increases as the number of runs increases.

One approach to estimate future levels of contamination would be in the opinion of Hwang, Kovalsky & Truelove (1997) to use the worst possible results. This means that the mean of three runs plus three standard deviations is proposed to estimate the possible contamination at each site. This approach offers a high degree of confidence that the operator variability is addressed and provides reasonable validity to the cleaning validation results.

Alternatively, N standard deviations may be used, depending on the desired level of confidence for determining possible contamination.

3.7 Conclusion

Selecting the most suitable analytical method for cleaning validation is equivalent to choosing the right tool for the job. Irrespective of the analytical technique selected, the method developed needs to be sensitive enough to detect low trace of potential equipment contaminants. Principally, tests performed during the active validation phase should be based on a selective analytical method capable of determining unequivocally the analyte of interest. After the initial validation, non-selective analytical methods may be used for the fast monitoring of the level of equipment cleanliness.

The other important analytical aspect is the validation of the analytical method. Proper validation means that the method range suitably covers the the level of concentrations encountered during

cleaning validation including the concentration level corresponding to the acceptance criteria set. The method should also be robust enough such as not to be influenced by the small variations of the sampling matrix or other minor changes connected to the experimental set-up. Also, fast sample throughput is required for cleaning validation assays as the production down times are highly influenced by the rapidity with which the cleaning validation results are generated.

Finally, when interpreting the raw data obtained after cleaning validation trials it is important to look at the overall results obtained and not only at the target analyte. The results should be always interpreted within a given context. Sometimes it is difficult to assess the level of detergent residue through one technique only. By corroborating results obtained from various techniques one can assess the level of detergent residues left behind. If the TOC results obtained from swab or rinse samples are above the LOC it is not conclusive that there is detergent residue on the equipment. Such results can be easily biased by the colorants and other excipients such as maize starch and sugars present in the product and should be compared to results obtained through other analytical techniques, such as pH measurements or titrations, if dealing with acidic/ alkaline cleaners. Another case of judging the results within the context is when the peak of the analyte targeted in a sample is not found, but the chromatogram has got unknown peaks. It is obvious that there may be some other equipment contaminant(s) present in the sample, such as the previously manufactured products, and one should always try to find out what is the source of contamination in order to be able to eliminate it.

Sample preparation steps such as extractions, filtrations and concentrations are sometimes necessary but they should be kept to minimum such as to avoid further sample contamination. A problematic issue related to cleaning validation is also the limited sample quantity that has to go through the sample preparation processing. Also, non-destructive sample analysis is preferred in the case the sample needs to be re-analysed. An alternative way of assessing the equipment cleanliness would be the more frequent use of in-situ assay methods that would reduce many of the obstacles related to the validation of classical analysis methods.

In conclusion, good analytical science constitute a key factor for the success of a cleaning validation program.

Chapter 4: A validated HPLC method for determining residues of a dual active ingredient antimalarial drug on manufacturing equipment surfaces

4.1 Introduction

In the pharmaceutical industry it is of critical importance to establish documented evidence that cleaning procedures applied to the manufacturing equipment are able to remove residues to predetermined levels of safety. The validation of cleaning ensures that subsequent product batches are not contaminated by previously manufactured products or by the cleaning process itself. Much literature has been published on cleaning validation in the past 10 years (PDA, 1998; Hwang, 2000; LeBlanc 1998, Rossi and Ryall, 1996; Laban *et.al.*, 1997) and official organizations (e.g., FDA and PIC) have regulated the subject through guidelines (FDA, 1993; PIC/S, 2004). Still, there is limited practical guidance available.

Challenges for cleaning validation are encountered especially when developing sensitive analytical methods capable of detecting traces of APIs, which are likely to remain on the surface of the pharmaceutical equipment after cleaning (Nozal, Bernal *et.al.*, 2002; Lambropoulos, Spanos & Lazaridis, 2000). The fact that some residuals are not detected by a method could mean that either the method is not sensitive enough for the residual in question or the sampling procedure is inadequate.

HPLC coupled with UV detection is widely used to monitor the efficiency of the cleaning methods due to its high sensitivity, selectivity and automation characteristics. All the compounds presenting chromophores (e.g., APIs, impurities and degradation products, placebo components, cleaning agents) whether they are hydrophilic or hydrophobic can be detected through HPLC-UV.

In non-dedicated manufacturing facilities, where there is a potential risk for cross-contamination, cleaning validation forms an integral part of the manufacturing process. One of the products that

could cause the cross-contamination of other products manufactured with the same equipment is the anti-malarial drug Fansidar®. Fansidar® tablets (Hoffman-La Roche, Basle, Switzerland) contain as active ingredients pyrimethamine (PYR) and sulfadoxine (SUL) in a weight ratio of 1:20. Both compounds present low water solubilities (PYR < 1 mg/ml and SUL < 0.1 mg/ml at 20°C). PYR is more toxic than SUL (acute toxicity values: LD₅₀ = 128 mg/Kg oral, rat for PYR; LD₅₀ = 5 200 mg/Kg oral, mouse for SUL). Due to its low water solubility and high toxicity, Fansidar® was chosen as the worst case among a range of products sharing several equipment pieces (a granulator, a wet mill, a fluid bed drier, a vacuum transfer system, a blender, a compression and a packaging machine).

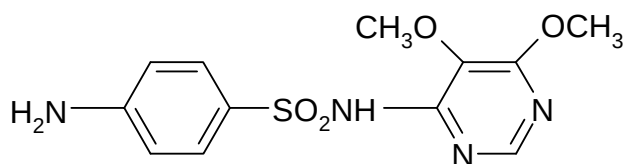
Pyrimethamine is a dihydrofolate reductase inhibitor, which is indicated in the treatment and prevention of parasitic diseases like malaria caused by *Plasmodium falciparum*, congenital toxoplasmosis caused by *Toxoplasma gondii*, meningeal leukaemia and against coccidiosis (Midskov, 1984a). In malaria chemotherapy, pyrimethamine is combined in synergistic effect with sulfadoxine, a long-acting sulfonamide used for the treatment of bacterial infections. Fansidar acts by reciprocal potentiation of its two actives, producing a sequential blockade of two enzymes, namely dihydrofolate reductase and dihydropteroate synthetase, involved in the biosynthesis of folic acid within the parasites (Fairlamb, 1989).

The independent determination of SUL through GC (Midskov, 1984) and supercritical fluid chromatography (Bhoir, Bhoir *et.al.*, 2001) has been reported. Determination of PYR was achieved by fluorimetry (Parimo, 1998), GC (Harris, Baker and Munday, 1977) and HPLC (Bangchang Tan-ariya *et.al.*, 1997; Coleman, Edwards and Mihaly, 1984; Horii, Nishi *et.al.*, 2001) in biological fluids, animal tissue and animal feeds. A variety of analytical methods for the simultaneous determination of both drugs in pharmaceutical preparations through spectrophotometry (Onah and Odeiani, 2002) in biological fluids through HPLC (Midskov, 1984a; Astier, Renard *et.al.*, 1997; Green, Mount and Nettey, 2002; Bergqvist, Eckerbom *et. al.*, 1991; Edstein, Lika *et.al.*, 1991; Dua, Sarin and Sharma, 1994; Eljaschewitsch, Padberg *et.al.*, 1996) and through micellar electrokinetic chromatography in veterinary preparations (Berzas, Penalvo & Guzman, 1999) have been reported.

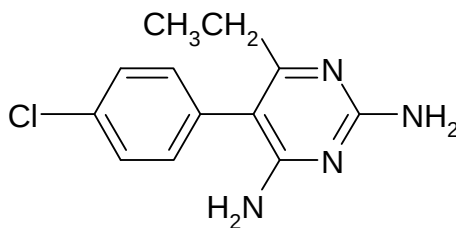
The simultaneous determination of PYR and SUL is impeded by the differences in their chemical properties. PYR is a weak base (pK_a = 7.3) and SUL is an ampholyte (pK₁ = 1.8; pK₂ = 6.1)

(Astier, Renard *et.al.*, 1997). The analysis is even more complicated by the presence in the sample of the detergent components. The detergent contains sodium xylene sulfonate (SXS) with acidic properties (acute toxicity value: $LD_{50} = 1\ 000\ \text{mg/Kg}$ oral, rat).

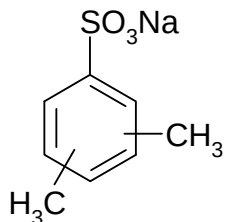
A method for analyzing all three components has not yet been reported. The chemical structures of these compounds are shown in Figure 4.1.



Sulfadoxine



Pyrimethamine



Sodium Xylene Sulfonate

Figure 4.1 Chemical structures of sulfadoxine, pyrimethamine and sodium xylene sulfonate

4.2 Materials and methods

4.2.1 Equipment

The HPLC system was an Alliance Waters 2690 (Mildford, MA, US) equipped with Waters 996 photodiode array detector and a detection cell of 8 μ l. Waters Millenium software (version 3.20) was used for data acquisition and processing.

4.2.2 Chemicals and reagents

All chemicals were of analytical grade. HPLC grade methanol was purchased from Lab-Scan (Dublin, Ireland). *ortho*-Phosphoric acid 85% (v/v) was purchased from Merck (Darmstadt, Germany). Sodium hydroxide (1M) Fixanal[®] (Riedel-de-Häen), and SXS (Aldrich) were purchased from Sigma–Aldrich (Johannesburg, South Africa). Sulfadoxine (N'-(5,6-dimethoxy-4-pyrimidinyl) sulfanilamide) and pyrimethamine (5-(4-Chlorophenyl)-6-ethyl-2,4-pyrimidine-diamine) certified standards were supplied by Roche Diagnostics (Mannheim, Germany). Detergent was purchased from Health & Hygiene (Johannesburg, South Africa). Solutions were prepared with deionized water, produced by a MilliQ water purification system from Milipore (Bedford, MA, US). The sampling was performed with Bemcott M₃ wipers purchased from Asahi Chemical Industry (Japan). Samples were filtered through PVDF Millex HV-25 syringe filters purchased from Microsep (Johannesburg, South Africa).

4.2.3 Chromatographic conditions

Chromatographic separations were performed on a Waters 150 mm $\times$ 4.6 mm Symmetry 5 μ m, C18 analytical column, 100 Å pore diameter.

The chromatographic experiments were performed under isocratic elution. The mobile phase consisted of methanol and 0.06 M *ortho*-phosphoric acid (35:65 v/v). The mixture was allowed to cool down to 20°C in a water bath and it was adjusted to pH* 3.0 with 1 M sodium hydroxide. The mobile phase was degassed by sonication under low vacuum prior to use. The flow-rate was set to 1.0 ml/min. The autosampler temperature was 15°C and the column temperature was set at 26°C. The injection volume was 50 μ l. The detector was programmed to record the absorbance of

the samples between 190 and 300 nm with a sampling frequency of 1 spectrum/second. The raw data acquired with the photodiode array detector were processed at 270 nm.

4.2.4 Preparation of calibration solutions

A stock solution containing approximately 250 µg/ml PYR and 250 µg/ml SUL was prepared in methanol. Intermediate stock solutions with concentrations ranging between 5 µg/ml to 200 µg/ml were prepared in methanol at seven concentration levels. The final dilutions were prepared as follows: 1 ml of 0.1M urea solution was spiked with 0.2 ml methanolic intermediate stock solution and the solution was made up to 10 ml with mobile phase. The concentrations of the calibration solutions are: 0.10, 0.20, 0.50, 1.01, 2.02, 3.03, and 4.03 µg/ml for PYR and 0.10, 0.20, 0.51, 1.02, 2.03, 3.05 and 4.07 µg/ml for SUL.

4.2.5 Sample preparation

Bemcott M₃, a non-woven, entangled fabric made of continuous filaments of 100% cellulose, fabricated without any adhesive binders was selected for its highly absorbent properties. Three swabs cut in pieces of 2 cm × 2 cm were placed into 15 ml test tubes. The swabs were spiked with various quantities of active compounds resulting in final solutions with the same theoretical concentrations as for the standard calibration solutions when diluted with 10 ml of solvent. The swabs were left to dry. Then, 1 ml of 0.1 M urea solution and 9 ml of mobile phase was added. The test tubes were vortexed for ca. 30 seconds and then the solution was filtered through PVDF (0.45µm) syringe filters, discarding the first 2 milliliters of the solution. Four replicates per level were prepared as described above.

Three replicate blank samples for the swab material were prepared as above.

A detergent sample was prepared by diluting 200 mg of detergent concentrate into 100 ml MilliQ water and further diluting 5 ml of this solution with mobile phase in a 20 ml volumetric flask. The final detergent solution has a concentration of 12.5 µg/ml SXS. An independent standard solution for SXS was prepared in order to identify the SXS peaks obtained for the detergent solution.

4.3 Results and discussion

4.3.1 Method optimization

A proprietary method, which was developed, validated and approved for Roche's affiliates, was re-evaluated. This initial method, which is used to assay PYR and SUL in pharmaceutical formulations at relatively high concentrations, was modified in order to accommodate the requirements of trace analysis specific for a cleaning validation study.

A Symmetry® column was used to improve the peak symmetry and obtain the best overall chromatographic separation of the cleaning validation samples.

The initial method detects PYR and SUL at 227 nm. For tablet content assay, an optimum simultaneous detection of both components is ensured at this wavelength where the much lower absorption of SUL is compensated by the high content of SUL per tablet. The UV spectra of PYR and SUL in the mobile phase (see Figure 4.2) show stronger absorption maxima for both compounds around 270 – 273 nm.

For the cleaning validation assay, the detection was performed at 270 nm in order to improve the method sensitivity towards SUL which is less soluble than PYR and therefore likely to remain in higher quantities on the pharmaceutical equipment after cleaning. At this wavelength possible interferences from the detergent and other sample compounds are minimized.

The injection volume was increased from 20 µl, in the initial method, to 50 µl in order to enhance the method sensitivity.

4.3.2 Selectivity

The solutions for detergent and for the active components were chromatographed on the Symmetry column but maintaining the mobile phase from the initial method. It was observed that the detergent components are interfering with the active components of the drug. A peak was also observed when solutions prepared for the swab blank were injected.

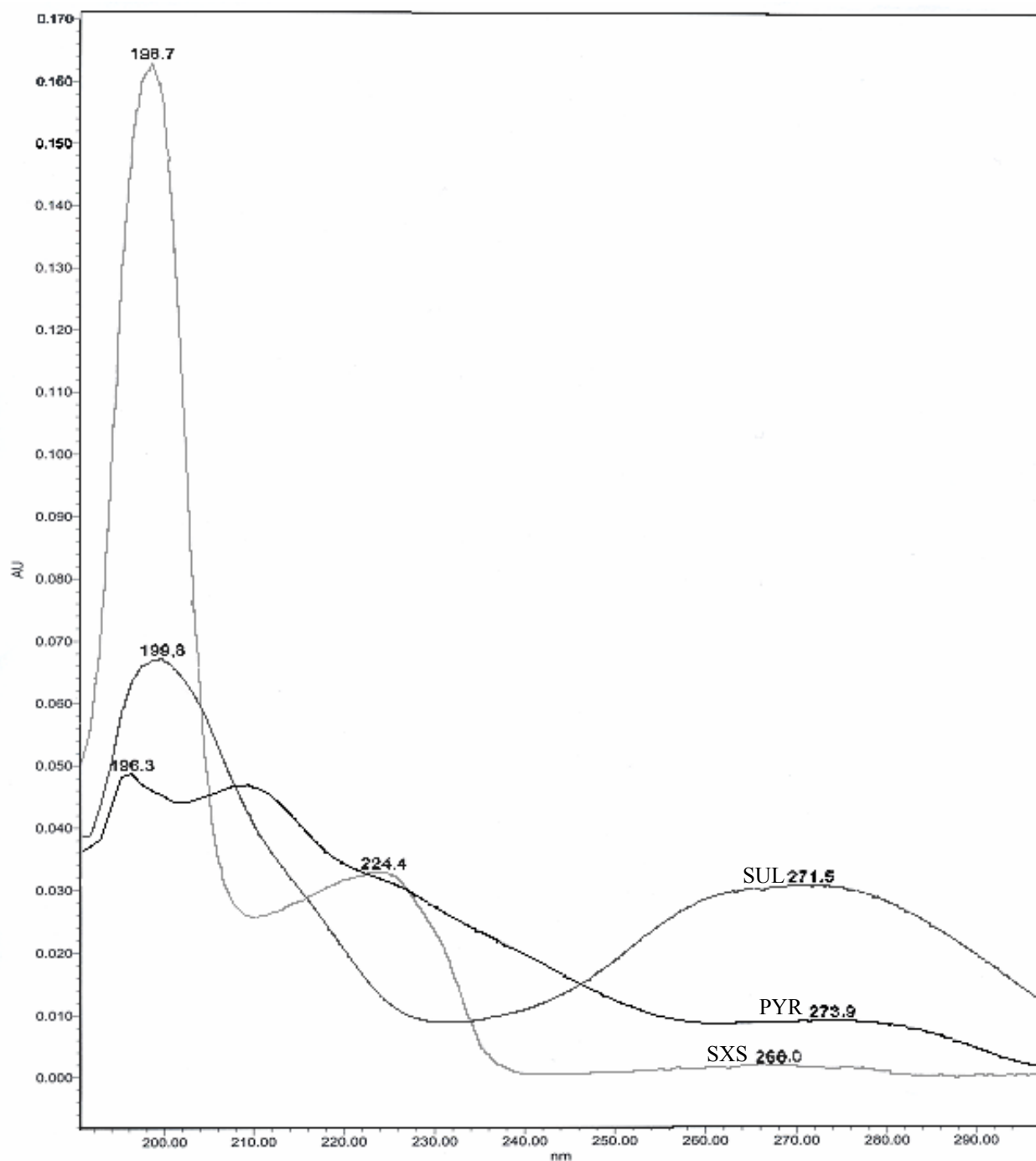


Figure 4.2 Absorption spectra of PYR (2 $\mu\text{g/ml}$), SUL (2 $\mu\text{g/ml}$) and SXS (12.5 $\mu\text{g/ml}$) in mobile phase

Several parameters of the mobile phase were simultaneously optimized in such a way until baseline resolution was obtained for all the peaks. The final optimum separation conditions were achieved when the mobile phase composition was methanol and 0.06 M *ortho*-phosphoric acid (35:65 v/v) adjusted to pH* 3.0 with 1 M sodium hydroxide.

With the optimized separation conditions, HPLC runs with detergent solution and swab blanks indicated that no swab or detergent component is detected at the retention time of SUL and PYR. Figure 4.3 shows separation profiles of the samples injected to assess the selectivity of the method.

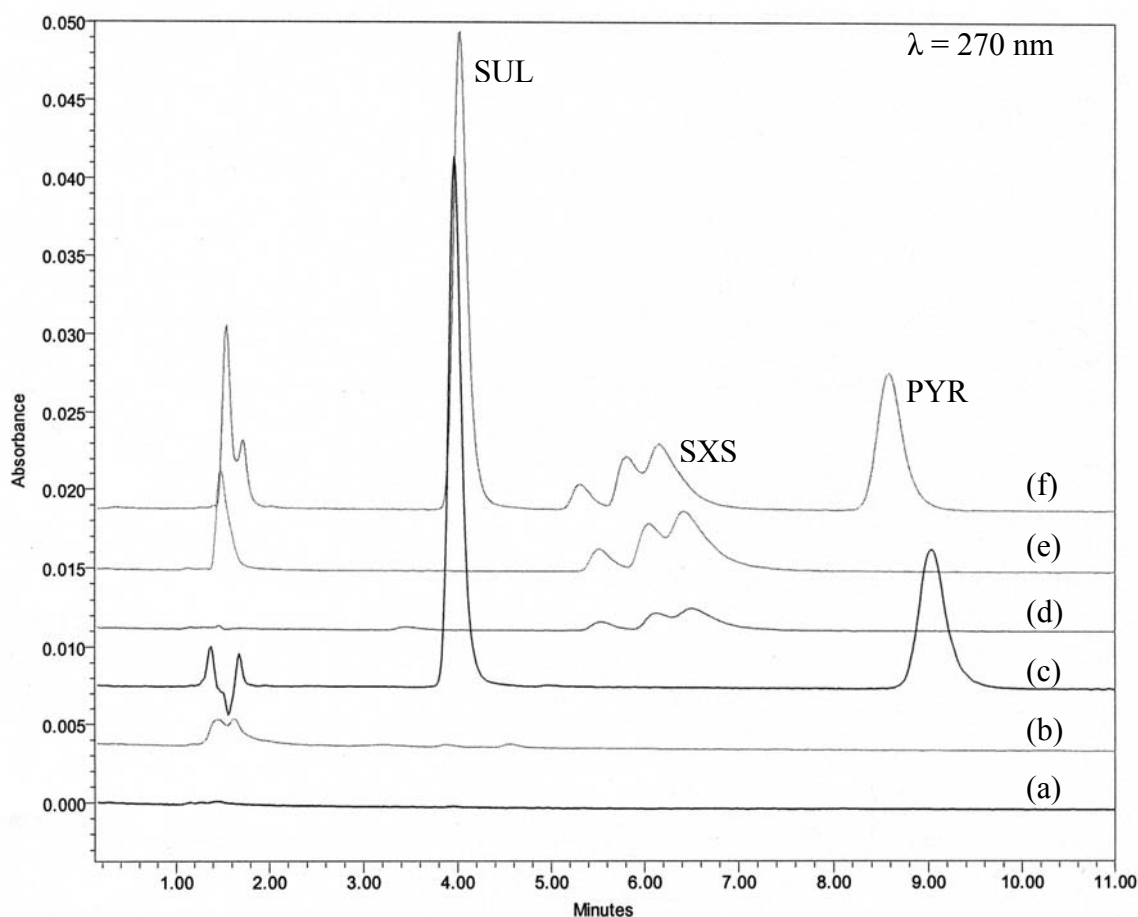


Figure 4.3 Typical chromatograms for: mobile phase (a), an extracted swab blank (b), the chromatogram obtained for the 100% level (c), an SXS solution (d), a detergent solution (e) and a combined sample containing detergent, swab and the drug actives (f)

SXS is a mixture of five constitutional isomers. With the current separation conditions it elutes as a group of three peaks in the time interval from 5 to 7 minutes. SUL and PYR are eluting at 4.2 and 8.6 minutes respectively. The retention time of PYR in Figure 4.3f was slightly earlier than in 4.3c probably due to matrix differences. The identity of PYR in figure 4.3c was confirmed by the UV spectrum. The complete sample separation was achieved within 10 minutes.

4.3.3 Method range

The new method was developed for the concentration range of interest for cleaning validation, unlike the case of the methods, which are validated for APIs assay on a relatively small concentration range from 80% to 120% of the expected concentration. For cleaning validation, a wider concentration range with at least five concentration levels should be validated. This is due to the fact that it is difficult to predict the actual level of contaminants found on the production equipment and to avoid re-diluting and re-injecting too concentrated samples.

4.3.4 Linearity

Linearity was studied for swab samples (7 concentration levels; four replicates per level). Linearity data were obtained by plotting the peak area of PYR and SUL expressed in area units against the concentration of PYR and SUL expressed as $\mu\text{g/ml}$. A linear regression least square analysis was performed in order to determine the slope, intercept and correlation coefficient of the standard curve.

Standard curves were linear from 0.129 to 4.02 $\mu\text{g/ml}$ PYR and from 0.120 to 4.06 $\mu\text{g/ml}$ SUL. The coefficients of correlation (r) are higher than 0.999 for both PYR and SUL indicating a good relationship between the peak area and the concentration of PYR and SUL, respectively in these concentration ranges.

The intercepts of the regression lines are situated for both PYR and SUL within the 95% confidence band of $\pm 10.0\%$ of $\langle Y \rangle_{\text{Ref}}$ indicating that there are no constant systematic errors.

The limits of detection and quantification were calculated with the following formulas:

$$\text{LOD} = 3.3s/S$$

$$\text{LOQ} = 10s/S$$

(Eq. 4.1 and 4.2)

Where S is the slope of the calibration curve and s the standard deviation. For the estimation of the standard deviation s , the standard deviation of the y-intercept was used. The linearity data are presented in Table 4.1.

Table 4.1 Parameters of linearity for pyrimethamine and sulfadoxine

Analyte	Calibration range ($\mu\text{g/ml}$)	y-intercept	Slope	r^2	LOD ($\mu\text{g/ml}$)	LOQ	
						($\mu\text{g/ml}$)	($\mu\text{g/swab}$)
PYR	0.042 – 4.020	– 2.78E+03	1.030E+05	0.9987	0.042	0.129	1.29
SUL	0.040 – 4.060	– 3.391E+03	2.198E+05	0.9989	0.040	0.120	1.20

4.3.5 Precision (by repeatability)

The intra-assay precision of the chromatographic method, reported as relative standard deviation, was assessed by measuring the repeatability of the results obtained for four replicate swab samples, at seven concentration levels. The preparation of the swab samples was performed as described earlier in the article. The % RSD values obtained per level are presented in Table 4.2.

The overall precision expressed as %RSD is 2.1% for PYR and 2.0% for SUL.

Table 4.2 Intra- assay precision (1 day) for PYR and SUL

Concentration level (%)	%RSD ($n=4$)	
	PYR	SUL
5	2.1	1.5
10	1.3	1.9
25	2.4	2.2
50	1.6	1.4
100	2.4	2.7
150	1.8	1.0
200	3.3	3.2
Average	2.1	2.0

4.3.6 Precision (by reproducibility)

The inter-assay precision was assessed on six replicate swab samples for three different concentration levels (50%, 100% and 150%) (see Table 4.3). The assays were carried out over three days and involved the same instrument and operator ($n = 18$).

Table 4.3 Inter-assay precision (three days, one instrument, one operator) for PYR and SUL

Concentration level (%)	%RSD ($n=18$); 3 days	
	PYR	SUL
50	3.2	4.8
100	4.3	1.7
150	2.9	2.3
Average	3.5	2.9

These values demonstrate that the precision of the method is adequate over the range of concentrations expected in cleaning validation samples.

4.3.7 Accuracy (by recovery)

The method accuracy was determined on spiked and dried swabs that were then extracted in mobile phase. The accuracy of the procedure was assessed by comparing the analyte amount recovered from swabs versus the known amount in the standard injection solutions at seven concentration levels with 4 replicates ($n=4$) for each concentration level investigated.

Recoveries from swabs higher than 100% were obtained especially for SUL (see Table 4.4). This was seen as a positive systematic error caused by the low SUL solubility. Various methods were used in order to increase the solubility of SUL. It was observed that a solution of 0.1M urea, when added to the samples, increases the SUL solubility. It is assumed that urea neutralizes the hydrogen bonds between the SUL molecules, therefore preventing their association and precipitation phenomena.

In the case of spiking swabs with SUL it is believed that the cotton acts as a “buffer” medium between the methanolic standard solution containing SUL and the aqueous mobile phase,

therefore preventing SUL precipitation. However, precipitation is more readily to occur for the samples prepared without cotton swabs.

Table 4.4 Accuracy (by recovery) for PYR and SUL

Concentration level (%)	Recovery (%) (n=4)	
	PYR	SUL
5	96.6	127.6
10	103.2	136.9
25	108.2	124.3
50	115.1	126.4
100	102.7	106.9
150	99.9	103.6
200	100.2	102.7

4.3.8 Establishing limits for the cross-contamination level permitted on clean equipment

It is to be remarked that contamination refers to any chemical, microbiological or particulate contaminant likely to remain on the equipment surface after its cleaning, whereas cross-contamination refers to the contamination of a product by a previously manufactured product.

The maximum permitted quantities (R) of PYR and SUL as potential cross-contaminants were calculated through several methods (Fourman and Mullen, 1993) (see Table 4.5). The total surface area of the equipment chain in direct contact with the product was accounted for in the calculations. The lowest obtained values were selected as LOC for this study.

Table 4.5 Maximum permitted levels of cross-contamination with PYR and SUL obtained through two different calculation methods

Criterion	Permitted cross-contamination ($\mu\text{g}/\text{cm}^2$)	
	PYR	SUL
0.1% dose limit	4.99 (LOC)	39.88
10 ppm	19.14	19.14 (LOC)

A lowest calculated value of 4.99 µg PYR / cm² was obtained when the 0.1% dose limit criterion was used for the total equipment chain. The 0.1% dose limit criterion is justified by the principle that an API at a concentration of 1/1000 of its lowest therapeutic dose (LTD) will not produce any adverse effects to one's health. This criterion as explained by Fourman and Mullen (1993) accounts also for the maximum daily intake of a following product and for the batch size of the product that will be manufactured next with the same equipment (see Equation 4.2).

$$R = \frac{D \times S}{I \times F} \times \frac{10^9}{A} (\mu g / cm^2) \quad (\text{Eq. 4.2})$$

For SUL, the lowest calculated value was obtained when the 10 ppm acceptance criterion was applied. When no more than 10 ppm of SUL was allowed into the next manufactured product, a limit of 19.14 µg SUL/ cm² was determined as LOC (see Equation 4.3).

$$R = 10 \times 10^9 \times S \times \frac{1}{A} (\mu g / cm^2) \quad (\text{Eq. 4.3})$$

Where R is the maximum residue of API permitted after cleaning, allowed into the next product; it is assumed that the total amount of residue is distributed homogenously into the following product; D is the daily LTD of the contaminant; S is the lowest batch size of the product to follow; I is the maximum daily intake of the product to follow; F is a safety factor (can vary from 10 to 100 000 depending on the product nature e.g., topical, oral or injectable preparations); A is the total surface area of equipment in direct contact with the products, calculated based on the assumption that all the products come into contact with all the equipment pieces of the chain.

4.3.9 Determination of recovery rate of contaminants from stainless steel

Recovery studies were performed in order to determine to what extent the residue could be retrieved from the production equipment with the sampling procedure chosen. The recovery experiment was performed at the LOC level of PYR and SUL on the equipment.

PYR and SUL were separately spiked on 316 stainless steel coupons (10 cm × 10 cm). The spiked coupons were allowed to dry (~ 2 hours) at room temperature. Since the swabbing is an operator

dependant technique, each analyte was recovered six times from coupons in order to obtain a representative mean of the recovery rate. The swab sampling simulation was performed by one operator.

A pair of tweezers was used to perform the swabbing. Various proportions of water - methanol were considered as swabbing solvents. A mixture of methanol - MilliQ water 70:30 (v/v) swabbing solvent provided the highest recovery rate. Better recovery results were obtained when the spiked analytes were recovered by using three swabs per sample rather than two. The first swab was wetted with 0.2 ml of swabbing solvent. The coupon was swabbed horizontally with one side of the swab and vertically with the other side. The procedure was repeated two more times with two more wetted swabs. All three swabs were collected into the same test tube. The swab samples were prepared as described above. Appropriate dilutions were performed in order to bring the concentration of the samples within the validated range of the analytical method.

Table 4.6 Recovery of PYR and SUL from stainless steel coupons

Quantity of contaminant spiked	Recovery rate (%)	Mean recovery (%) $\pm$ IC (%)	% RSD (n=6)
Pyrimethamine, 498.5 μ g	96.4	90.5 $\pm$ 6.5	7.4
	81.2		
	84.8		
	93.9		
	97.9		
	88.8		
Sulfadoxine, 1880.7 μ g	84.8	83.9 $\pm$ 4.2	5.1
	84.4		
	83.2		
	80.7		
	91.3		
	79.0		

Table 4.6 presents the recovery results. The recovery experiment provided a good indication regarding the reproducibility of the swabbing procedure. RSD values of 7.4% for PYR and 5.1% for SUL were obtained (n=6).

4.3.10 Sample stability

The stability of the APIs in the swab matrix was tested. Several series of samples were prepared by spiking swabs with a quantity of analyte that would result in the nominal concentration level when diluted with 10 ml solvent. The first lot ($n=4$) of samples were stored in a moistened state. The second lot ($n=4$) was kept in a dry state. All samples were stored in amber test tubes, in the refrigerator (4°C) for seven days. It is known that a methanolic stock solution containing 5.0 mg SUL /ml and 0.5 mg PYR /ml which is stored in amber glassware is stable at 4°C for over six months (Edstein, 1984). Before analysis the samples were allowed to reach room temperature. Urea solution 0.1M (1 ml) and 9 ml of mobile phase was added to the dry stored samples. A fresh sample was prepared on the day of the analysis. All the samples were then vortexed, filtered through PVDF ($0.45\mu\text{m}$) syringe filters and analyzed.

No changes in the chromatography of the stored samples were found and no additional peaks appeared when compared with chromatograms of freshly prepared samples. It was observed that SUL is slightly more stable than PYR in swab samples. With each compound, similar recoveries were obtained for both dry and moistened stored samples and it was decided that samples should be stored in a dry state in order to avoid the double preparation of the mobile phase (see Table 4.7).

Table 4.7 Sample stability for PYR and SUL (samples kept at 4°C in the dark for seven days)

Contaminant	Concentration ($\mu\text{g/ml}$)	Sample storage conditions	Recovery (%)	% RSD ($n=4$)
PYR	2.01	Dry	95.8	1.3
		Soaked	96.7	1.7
SUL	2.02	Dry	97.9	1.4
		Soaked	98.0	1.8

4.3.11 Assay of swab samples collected from the equipment train

Swab samples collected from different locations of the manufacturing equipment train were analyzed with the new method. The results found are presented in Table 4.8.

Table 4.8 Results obtained for the determination of PYR and SUL in actual swab samples collected from the manufacturing equipment chain

Processing step	Contaminant ($\mu\text{g}/\text{cm}^2$)									
	Pyrimethamine (LOC= $4.99 \mu\text{g}/\text{cm}^2$)					Sulfadoxine (LOC = $19.14 \mu\text{g}/\text{cm}^2$)				
	Run 1	Run 2	Run 3	Run 4	Mean+2s	Run 1	Run 2	Run 3	Run 4	Mean+2s
Granulation	0.197	2.038	0.302	0.164	2.496	0.583	4.724	0.286	0.179	5.831
Milling	0.020	0.057	0.063	0.059	0.090	0.355	0.173	0.634	0.136	0.779
Drying	0.476	0.250	0.218	0.162	0.552	11.164	1.264	4.543	0.882	13.983
Blending	0.004	0.055	0.056	0.055	0.094	0.131	0.085	0.166	0.084	0.196
Vacuum transfer	0.007	0.041	0.059	0.040	0.080	0.098	0.252	0.166	0.157	0.295
Compression	0.010	0.050	0.081	0.050	0.106	0.167	0.174	1.287	0.128	1.570
Packing	0.077	0.327	0.087	0.072	0.389	3.275	0.837	0.481	0.113	4.036

Since the hand cleaning is generally less reproducible than the automated cleaning, the validation results obtained for manual cleaning procedures must be consistent and well below the LOC. This is to ensure that residues will be removed efficiently and below the acceptance levels in the conditions of routine cleaning of the manufacturing equipment.

For the current study it was observed that all data obtained lie within 2s of the sample mean and well below the LOC. This gives the confidence that the manual cleaning procedures tested do provide sufficient removal of the residues from the equipment chain.

4.3.12 The place of the analytical method within a cleaning validation strategy

A successfully validated analytical method, sensitive enough for the level of contamination determined by calculating acceptance criteria, and supported by an appropriate sampling method for which validated recovery studies are established constitute the answers to the question often encountered in the cleaning validation literature of “How clean is clean” (Hwang, Kowalski and, Truelove, 1997; Krull and Swartz, 1999). The analytical method measures the level of cleanliness of the equipment at the time of validation and monitors any trends and deviations from the validated status of a cleaning process susceptible to develop in time.

4.4 Conclusion

An accurate, reproducible, sensitive and selective HPLC method for the simultaneous determination of PYR and SUL residuals on pharmaceutical manufacturing production surfaces has been validated to control the efficiency of the equipment cleaning. The chromatographic separation is achieved within 10 minutes facilitating a high sample throughput. The level of contamination found after equipment cleaning was monitored during several consecutive runs. The results obtained confirm that the cleaning procedures used are able to remove residues from equipment surfaces well below the calculated limit of contamination.

Chapter 5: Assessment of MEKC suitability for residue drug monitoring on pharmaceutical manufacturing equipment

5.1 Introduction

This chapter presents the development and validation of a micellar electrokinetic chromatographic (MEKC) method for the determination at trace levels of co-trimoxazole drug, some of its decomposition products and detergent residues likely to remain on pharmaceutical manufacturing equipment after cleaning.

Co-trimoxazole is a bacteriostatic antibiotic combination of sulfamethoxazole (SMZ) and trimethoprim (TMP) in a 5:1 (w/w) ratio, formulated as injections, oral suspensions and tablets under different brand names (Bactrim®, Septra®, Sulfatrim®). SMZ is a sulfonamide, having a structure analogue of *p*-aminobenzoic acid, and acts as a competitive inhibitor of dihydropteroate synthetase. TMP acts by interfering with the action of bacterial dihydrofolate reductase, inhibiting the synthesis of tetrahydrofolic acid. When TMP is used in combination with SMZ, co-trimoxazole exhibits a synergistic antibacterial effect by inhibiting successive steps in the folic acid metabolism of bacteria (see Figure 5.2) (Conn and Stumpf, 1976; White, Handler and Smith, 1973).

Co-trimoxazole is used in the treatment of sinusitis, pneumonia, chronic bronchitis, renal and urinary tract infections, gastrointestinal tract infections, skin and wound infections, septicaemias and other infections caused by sensitive organisms (Delgado and Remers, 1991).

Typically, for cleaning validation, assay methods are developed for API's or for the most toxic, potent or difficult to remove compounds of a drug. Sometimes it is recommendable to also test for the presence of potential toxic decomposition products. In practice this could result in extensive sample preparation followed by analysis via several different techniques.

SMZ and TMP were simultaneously determined in biological samples (Gochin, Kanfer & Haigh, 1981; Kebriaeezadeh, Zarghi *et. al.*, 2000), veterinary formulations (Nevado, Penalvo & Bernardo, 2001) and commercial samples (Mulcahey and Taylor, 1992; Liu, Hu & Ding, 1991).

Simultaneous RP-HPLC determinations of SMZ and TMP have been described in a number of publications (Avgerinos, Athanasiou & Malamataris, 1991; Bergh, Breytenbach & Dupreez, 1990; Ricci and Cross, 1996).

A pharmacopoeial method for their simultaneous determination by HPLC is also available (USP28, 2005a). For the determination of TMP, SMZ and its byproducts sulfanilamide (SAM) and sulfanilic acid (SAA) a TLC method was developed and validated (Agbaba, Radovic *et. al.*, 1996). However, TLC is a semi-quantitative method and therefore less suitable for cleaning validation. Through RP-HPLC the elution of SAM and SAA takes place at or near the void volume, causing problems with their identification and quantification (Nickerson, Scypinski *et. al.*, 1995).

Water, potable and purified, is most commonly used to remove product residues from the pharmaceutical manufacturing equipment. To increase the effectiveness of cleaning, aqueous solutions of cleaning agents in pre-defined conditions of concentration and temperature are used. The detergents could constitute themselves a source of product contamination. A complete cleaning validation study requires the availability of a validated method capable to detect traces of detergent after the manufacturing equipment is cleaned and thoroughly rinsed.

Due to the very low water solubilities of SMZ (0.5 mg/ml) and TMP (0.4 mg/ml) at 25°C (data taken from Roche Material Safety Data Sheets database) an alkaline detergent solution in a dilution of 1% (v/v), and a temperature of ca. 60°C was used to clean the equipment, following the manufacture of co-trimoxazole syrup. Nonoxynol-9 (NP9), a complex of non-ionic surfactants which are miscible with water, is used in the detergent composition as a wetting agent. NP9 consists of mixtures of oligomers of polyethoxylated nonylphenol. US Pharmacopoeia describes an assay method for NP9. However, according to (Shifflet, Shapiro *et. al.*, 2001) the USP method does not provide enough sensitivity for determining NP9 in the lower concentration range required for cleaning validation. An HPLC method was validated by Shifflet, Shapiro *et. al.* (2001) for the determination of residual NP9 in cleaning validation samples collected from stainless steel, polytetrafluoroethylene, and acrylic substrates.

At present there is no analytical method available capable to determine in a single run the API's of co-trimoxazole, its decomposition products and traces of detergent potentially present in the cleaning validation sample.

Capillary electrophoresis has the advantage of separating compounds differing in their charge-to-radius ratio. It is also sensitive enough to allow the quantification at trace levels of drug- related impurities and residues found in cleaning validation samples. A single assay would offer savings in time, effort and convenience (Altria and Hadgett, 1995).

CZE separations have been possible for SMZ and TMP due to their nature. SMZ is a weak acid ($pK_a=5.6$) and TMP is a weak base ($pK_a=6.6$). SMZ and TMP were simultaneously determined through CZE with UV detection (Li, Zhang *et. al.*, 2000; Luo, Chen & Zhang, 2000; Nevado, Penalvo & Bernardo, 2001a) in fused silica capillaries. A polyamine coated capillary was used to separate TMP and some of its decomposition products including 3,4,5 – trimethoxybenzoic acid (TBA) (Assi, Altria & Clark, 1997). CZE with amperometric detection (Wang, Gong *et. al.*, 1999) was used to determine TMP and several sulfonamides. MEKC was used in the determination of several sulfonamides and TMP (Dang, Sun & Ling, 1992; Hows, Perrett & Kay, 1997) and also in the determination of SMZ, TMP and other drugs in human plasma without sample pretreatment (Kunkel, Gunter & Watzig, 1997). The migration behaviour of thirteen sulfonamides was studied by both CZE and MEKC (Lin, Lin *et. al.*, 1997).

Here is reported the development and validation of a MEKC method for the simultaneous determination at trace levels of compounds likely to remain on pharmaceutical manufacturing equipment after cleaning. The cleaning validation sample could potentially contain APIs of co-trimoxazole syrup (SMZ, TMP), some decomposition products (SAM, SAA, TBA) and detergent components such as NP9

The chemical structures of the compounds of interest are shown in Figure 5.1.

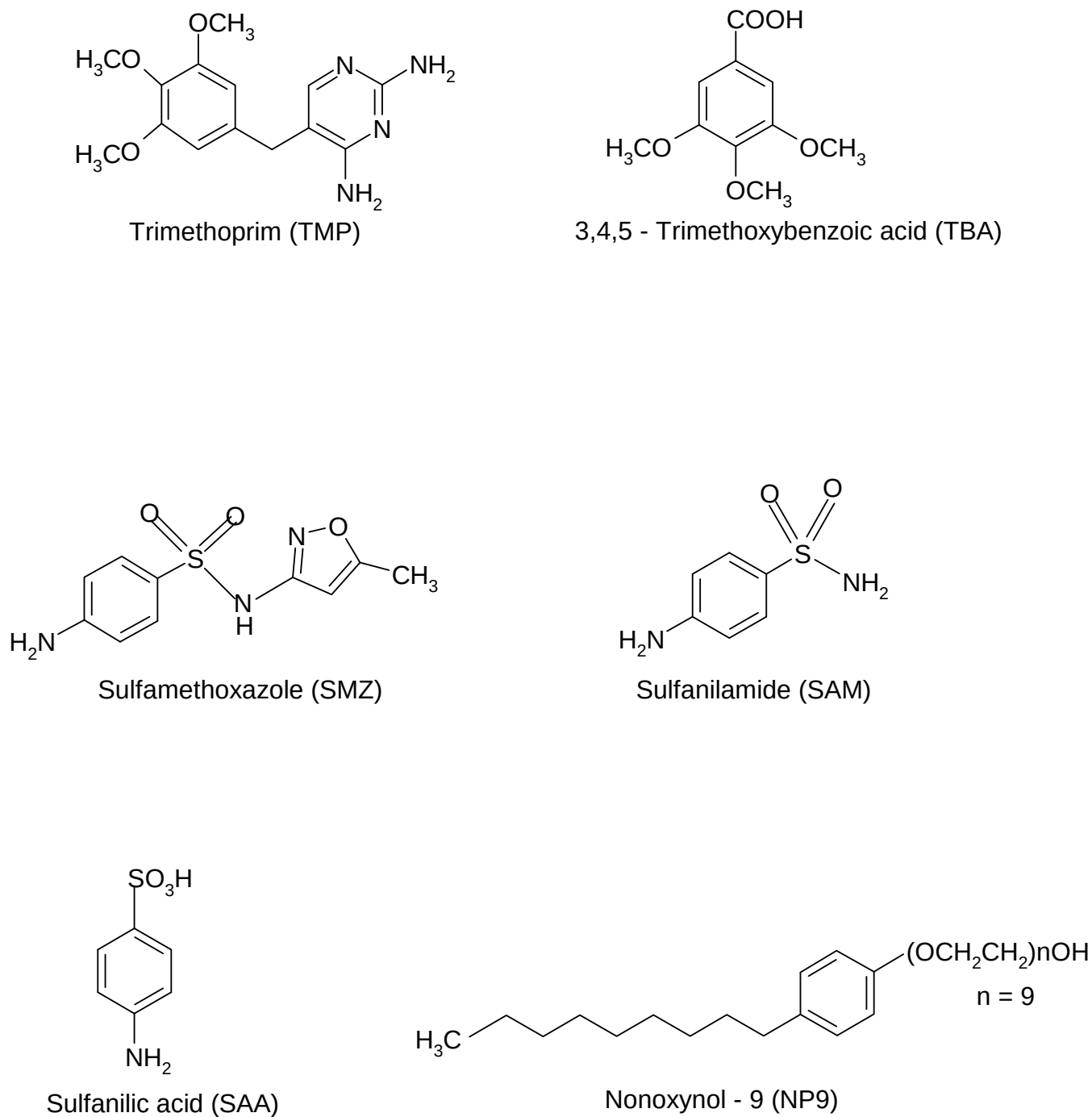


Figure 5.1 Chemical structures of trimethoprim, 3,4,5 – trimethoxybenzoic acid, sulfamethoxazole, sulfanilamide, sulfanilic acid and nonoxynol – 9.

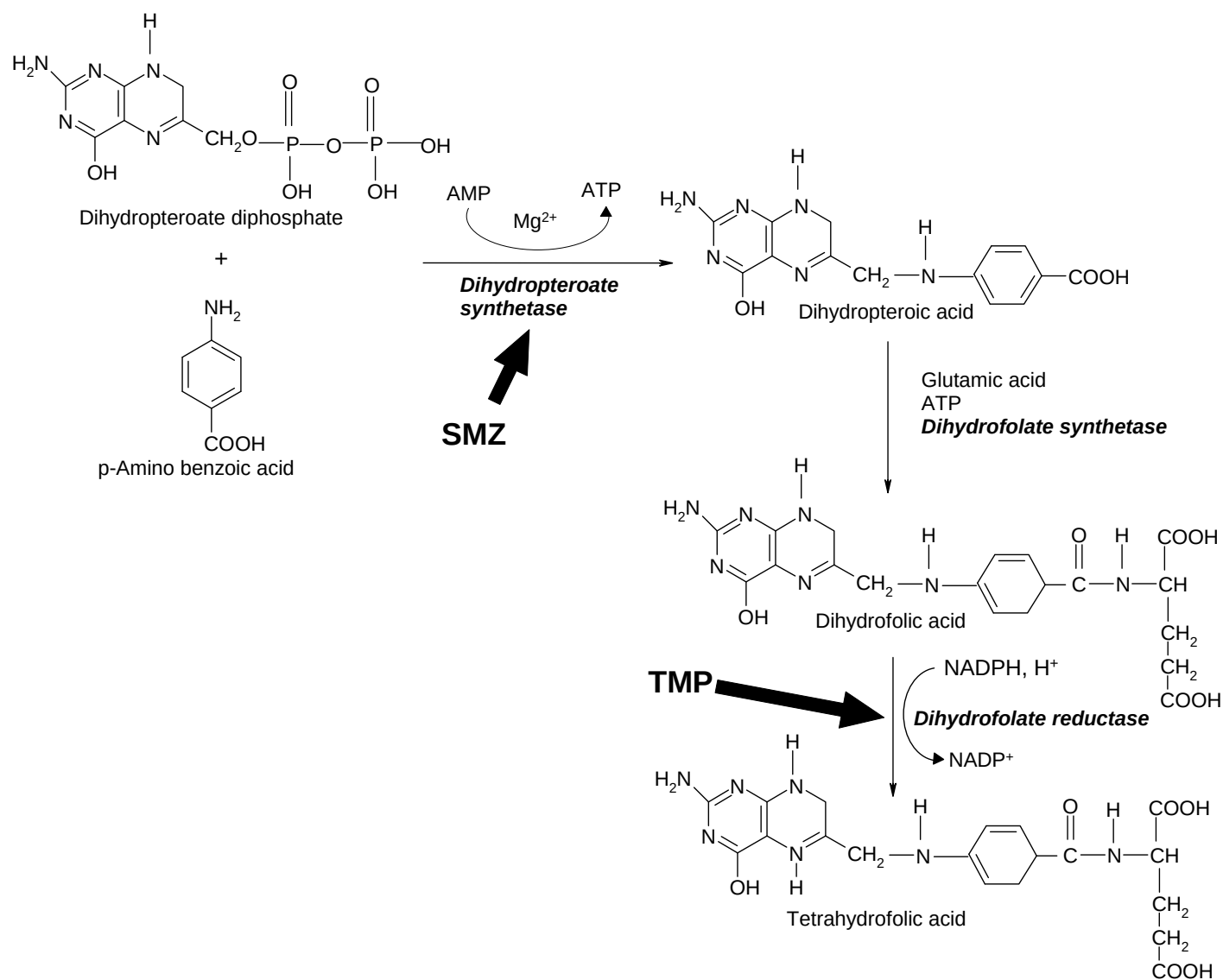


Figure 5.2 Metabolic pathways of tetrahydrofolic acid and inhibitive actions of SMZ and TMP over the dihydropteroate synthetase and dihydrofolate reductase (adapted from Conn and Stumpf, 1976; White, Handler and Smith, 1973).

5.2 Materials and methods

5.2.1 Equipment

CE separations were performed on a Hewlett Packard 3D CE instrument with HP ChemStation software. Uncoated silica capillaries were purchased from Separations (Johannesburg, South Africa). The typical capillary dimensions were 50 μm i.d., 375 μm o.d., 58 cm total length (L) and 50 cm effective length (L_{det}) from the inlet to the detector window. On-line UV detection was performed at 210 nm with a diode array detector. The capillary was maintained at 25°C with forced air circulating in the capillary oven. The high voltage power supply was set to 22 kV (normal polarity equivalent to a field strength of 482.8 V/cm) resulting in a typical current of 50 μA . The detection window was at the cathodic side. Injections were made at the anodic side of the capillary in the hydrodynamic mode (0.75 psi $\approx$ 50 mbar) for 4 s of sample followed by 2 s for background electrolyte (BGE).

Before the first use, the capillary was conditioned with freshly prepared 0.1 M NaOH for 6 h at 25°C in order to regenerate the silanol groups on the capillary surface. At the beginning of a working day the capillary was regenerated by rinsing with 0.1M NaOH (15 min), water (5 min) and BGE (15 min). Between-run washings were performed with water (1 min), 0.1 M NaOH (1 min.), water (1 min) and BGE (2 min.). At the end of every day the capillary was rinsed with water (5 min.), 0.1 M NaOH (15 min.), water (5 min.) and methanol (5 min.). Air was flushed for 15 minutes prior to capillary storage.

For pH measurements a Mettler Toledo MPC 227 pH-meter with a precision of 0.01 pH units was used. Daily calibrations of the pH sensor were performed with buffers of the following pH values: 4.01, 7.00 and 9.21.

5.2.2 Chemicals and reagents

All chemicals were of analytical grade. Sodium hydroxide, di-sodium hydrogen phosphate, sodium di-hydrogen phosphate and sodium dodecyl sulphate were purchased from Merck (Wadeville, South Africa). HPLC grade methanol was purchased from Lab-Scan (Dublin,

Ireland). Nonoxynol-9 (Sigma), Sulfanilic acid (Fluka) and sulfanilamine (Fluka) were purchased from Sigma– Aldrich (Kempton Park, South Africa).

Sulfamethoxazole (4- amino-N-(5-methyl-3-isoxazolyl) benzenesulfonamide), trimethoprim (5-[(3,4,5 - trimethoxyphenyl) methyl]-2,4-pyrimidinediamine) and 3,4,5-trimethoxybenzoic acid certified standards were supplied by Roche Diagnostics (Mannheim, Germany).

Solutions were prepared with deionized water, produced by a MilliQ water purification system from Milipore (Bedford, MA, US). Swab samples were prepared with Bemcott M3 wipers purchased from Asahi Chemical Industry (Tokyo, Japan).

5.2.3 Background electrolyte, standard solutions and sample preparation

The phosphate solutions were prepared by mixing solutions of di-sodium hydrogen phosphate and sodium di-hydrogen phosphate of equal molarities and adjusting the resulting solution to the desired pH value. Various quantities of SDS were dissolved in the buffers already adjusted to the final pH in order to obtain the desired SDS concentrations.

Independent stock solutions were prepared in 25 ml volumetric flasks by dissolving SMZ (248.9 mg), TMP (76.3 mg) and TBA (126.1 mg) in 15 ml methanol. All three volumetric flasks were then brought to volume with MilliQ water. Other independent stock solutions were prepared in 25 ml volumetric flasks for NP9 (661.5 mg), SAA (176.9 mg) and SAM (301.6 mg) by dissolving in MilliQ water.

Intermediate independent stock solutions were prepared at eight concentration levels (between 5% up to 200% of the nominal concentration level) by further diluting in 25 ml volumetric flasks 5 ml of the initially prepared stock solutions with a mixture of methanol – MilliQ water 50:50 (v/v).

Working standard solutions were prepared at eight concentration levels by mixing 0.2 ml of each of intermediate stock solutions into a 10 ml volumetric flasks. Each of the eight volumetric flasks prepared for each concentration level was brought to volume with 3 mM NaOH. The final injection solution corresponding to the 100% level contains 39.82 µg/ml SMZ, 12.21 µg/ml TMP, 20.18 µg/ml TBA, 28.30 µg/ml SAA, 48.26 µg/ml SAM and 105.84 µg/ml NP9.

Swab samples were prepared for each concentration level studied in order to assess the method accuracy by recovery. Three pairs of swabs were placed into 15 ml test tubes. The swabs were spiked with 0.2 ml of the intermediate stock solution corresponding to each analyte. The swabs were allowed to dry and then were extracted in 3 mM NaOH.

5.3 Results and Discussion

5.3.1 MEKC method development and optimisation

To ensure the experiments result is reproducible all runs were carried out at least in triplicate.

In MEKC the BGE pH, the surfactant concentration and the voltage are the most important experimental parameters influencing the electrophoretic mobility of solutes. To optimise the separation of the discussed sample mixture the effects of the BGE type, the pH, ionic strength, concentration of surfactant and organic modifier, applied voltage and temperature on the overall separation were studied. Each parameter was optimised individually.

5.3.1.1 pH influence

Initial separation conditions chosen were 25 mM phosphate, 20 mM SDS, 20 kV and 25°C. The pH influence was studied in the range from 6.6 up to 8.2 in increments of 0.2 pH units.

The pH of the BGE determines the degree of ionization of individual solutes and their net charge in solution. SAM has a pKa of 10.43 and therefore at any pH below 9.43 is neutral and elutes close to the EOF. The migration times of SMZ and TBA remained fairly constant with increasing the pH. SMZ (pKa's not found in literature) is a large anion with possibly several ionization configurations due to the three nitrogen groups and one sulphony group. TBA is a monocarboxylic acid, negatively ionized in the pH range studied. For SAA a slight decrease in the migration times was observed with increasing of pH. SAA is a small anion and the pH variation could influence more readily its charge/mass ratio. It is believed that in the pH range studied SAA and SMZ are dissociated and negatively charged. They migrate slowly towards the cathode as a result of their electrophoretic behaviour.

By increasing the pH of the BGE the overall separation time has increased especially due to the migration behaviour of TMP and NP9. Changes were more significant for TMP which can dissolve in both acidic and basic media. According to the Henderson – Hasselbach equation for weak bases, when pH shifts from acidic to basic TMP suffers a variation from cationic to neutral (Dang, Sun & Ling, 1992).

Two problems were identified in the separation while studying the pH influence. In the lower range of the pH studied (6.6 up to 7.2) the TMP and NP9 peaks were not separated. The complete baseline resolution was obtained only above pH 7.8. But as the pH increases the selectivity factor (α) between SMZ and TBA decreases. Above 7.2 pH units α remains with a constant value of 1.02 between these peaks. However, notwithstanding the low α , baseline resolution of the two peaks was constantly achieved for the whole pH range.

The electrophoretic mobility of test solutes was calculated with the Equation 5.1.

$$\mu_{ep} = \mu - \mu_{eo} = \frac{L_{det}L}{V} \left(\frac{1}{t_m} - \frac{1}{t_{eo}} \right) \quad (\text{Eq. 5.1})$$

in which μ_{ep} is the electrophoretic mobility of the solute tested, μ is the apparent mobility and μ_{eo} is the electroosmotic mobility. L (cm) is the total length of the capillary, L_{det} (cm) is the length of the capillary between injection and detection and V (Volts) is the applied voltage. The migration time of the solute, t_m , and the migration time of the neutral marker, t_{eo} , were measured, in seconds, from the electropherogram. Methanol was used as neutral marker in this study.

Figure 5.3 shows the effect of BGE pH on the electrophoretic mobilities of the solutes studied.

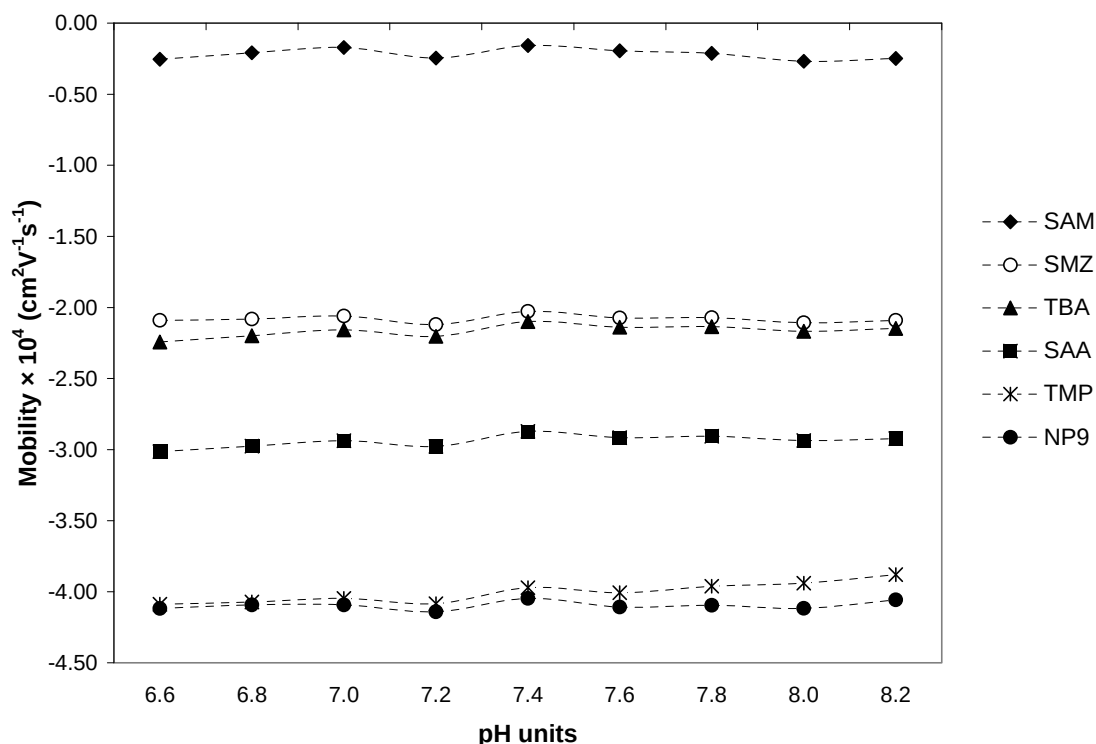


Figure 5.3 Electrophoretic mobilities of solutes obtained as a function of pH in the range 6.6 – 8.2. BGE: 25 mM phosphate containing 20 mM SDS. Operating conditions: 20 kV; 25°C; injection: 4 s sample followed by 2s BGE.

A phosphate BGE with a pH of 7.8 was chosen as optimum for further method optimization because it offers good buffering capacity. Optimum values in terms of resolution, selectivity, number of plates were also obtained at this pH for all the compounds studied.

5.3.1.2 Voltage influence

Voltage influence was studied in the range between 18 kV up to 28 kV in increments of 2 kV by using the previous found optimum pH value of 7.8 for the phosphate BGE. All the other parameters were kept constant. The variation of electrophoretic mobility values are represented in Figure 5.4 as a function of voltage for all solutes studied. In accordance with the principle that the electroosmotic velocity is proportional to the the voltage it was observed that overall separation time has decreased to nearly half as the voltage was increased. Currents ranging from 37 μ A to 80 μ A were recorded.

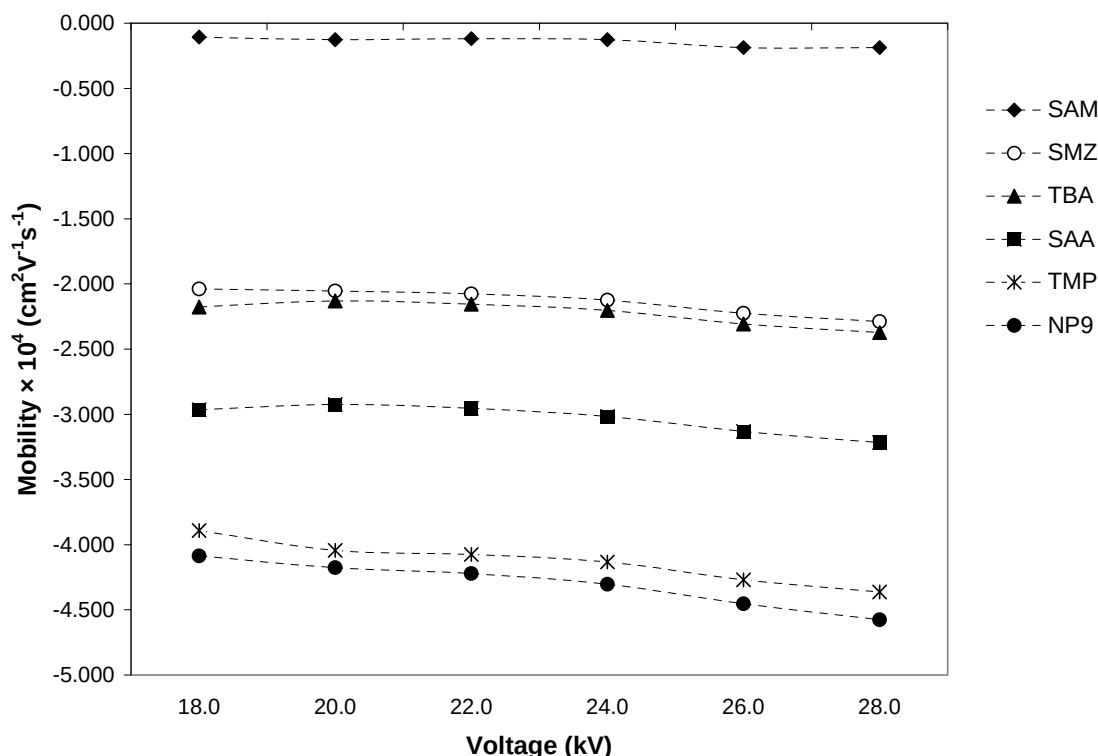


Figure 5.4 Electrophoretic mobilities of solutes obtained as a function of voltage in the range 18 kV – 28 kV. BGE: 25 mM phosphate pH 7.8 containing 20 mM SDS. Operating conditions: 25°C; injection: 4 s sample followed by 2s BGE.

A general decrease in resolution, selectivity and number of plates was observed for all the solutes as the voltage was increased. The total separation time was reduced from 27.3 minutes at 18 kV to 11.4 minutes at 28 kV.

At low voltage values the selectivity between SMZ and TBA is slightly better. Above 20 kV the selectivity factor remains constant to 1.02. It was observed that the selectivity between TMP and NP9 which are more retained by the SDS micelles when compared to all the other compounds is relatively constant. However, as the voltage decreases the resolution between these last two compounds and all the others is slightly increased.

A voltage value of 22 kV was chosen as optimum for further method optimization as the heating caused by the Joules effect does not influence the separation negatively. Fast sample analysis and moderate current values of ca. 58 μ A and a power consumption of ca. 1.62 W were obtained at 22 kV.

5.3.1.3 Influence of surfactant concentration

Sodium dodecyl sulphate (SDS) was used as surfactant. The SDS influence was studied for the concentration interval ranging from 10 to 50 mM SDS in increments of 5 mM units. All the other parameters were kept constant while varying the SDS concentration.

Although by increasing the SDS concentration the total separation time and therefore the elution window is decreased, the selectivity between TMP and NP9 is also decreasing. As illustrated by Figure 5.5 the selectivity between SMZ and TBA was not improved by varying the SDS concentration. At higher SDS concentrations problems related to capillary blockage were encountered. A concentration of 10 mM SDS was chosen for this separation.

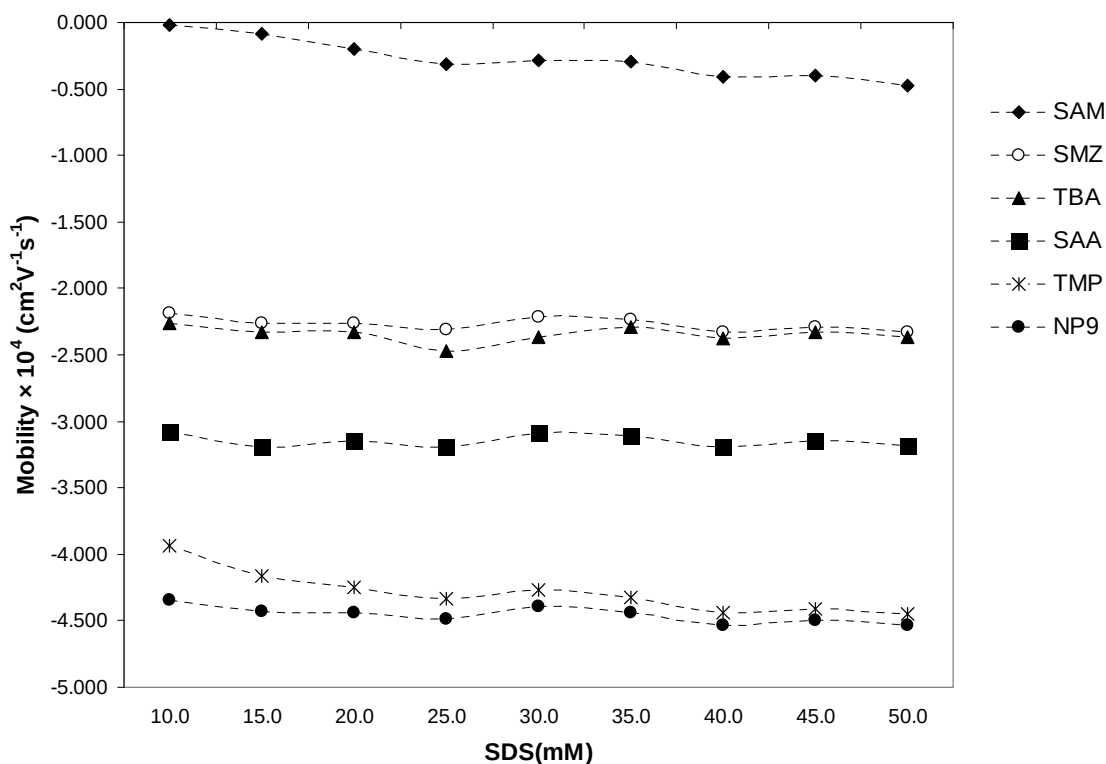


Figure 5.5 Electrophoretic mobilities of solutes obtained at varied SDS concentrations ranging from 10 mM up to 50 mM. BGE: 25 mM phosphate pH 7.8; 20 kV; 25°C; Injection: 4 s sample followed by 2s BGE.

5.3.1.4 Ionic strength influence

The influence of ionic strength was studied for the phosphate BGE over the range from 10 mM to 50 mM phosphate and by maintaining the SDS concentration to a constant 10 mM. The experiments were performed with the already optimized parameters. The variation of electrophoretic mobility values are represented in Figure 5.6.

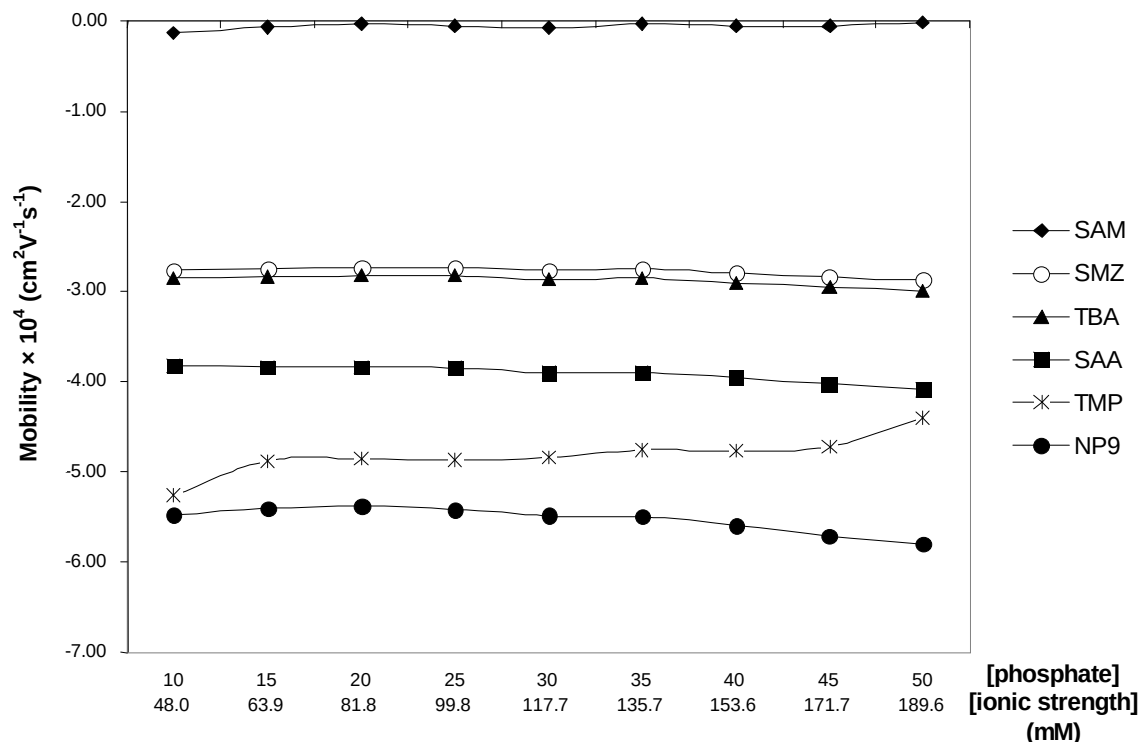


Figure 5.6 Electrophoretic mobilities of solutes obtained at various BGE concentrations ranging from 10 mM to 50 mM. BGE pH 7.8 contains 10 mM SDS. The calculated ionic strength of BGE solutions is also given on the X axis. Operating conditions: 25°C; 22 kV; injection: 4 s sample followed by 2s BGE.

Currents ranging from 24 μ A up to 102 μ A were obtained. By increasing the phosphate electrolyte concentration the buffering capacity increases with benefits for separation. Above 35 mM phosphate the Joule effect becomes noticeable through heating and consequently peak broadening, especially towards the end of the separation. Also, the total separation time increases from 7 minutes up to 15 minutes. However, at low phosphate concentration values of ca. 10 mM, the TMP and NP9 peaks are overlapping. A concentration of 30 mM phosphate was chosen to ensure an optimum ionic strength of the BGE. In terms of ionic strength this corresponds to a

value of ca. 107.7 mM that was determined with the help of Equation 5.2. The proportions of weak acid and weak base present in solution were calculated based on the Henderson – Hasselbalch equation. It is known that the BGE is made of di-sodium hydrogen phosphate, sodium dihydrogen phosphate and sodium dodecyl sulphate.

$$I = \frac{1}{2} \sum_i c_i z_i^2 \quad (\text{Eq. 5.2})$$

where,

I = ionic strength

c_i = molar concentration of ion i in solution

z_i = net charge of ion i in solution

5.3.1.5 Temperature influence

It was observed that the temperature which was varied between 19°C and 29°C does not have a major overall effect on the present separation. However, a temperature of 23°C was chosen as optimum because it is easy for the instrument to maintain it without overloading the cooling system.

Injection times between 3 to 5 seconds for sample were evaluated. An injection time of 4 seconds of sample followed by 2 seconds of BGE was selected.

Optimum final conditions were found as 30 mM phosphate BGE, pH 7.8, containing 10 mM SDS and an applied voltage of 22 kV. With the above optimised separation conditions the six components were resolved within 14 minutes. A typical electropherogram of the six compounds separated is shown in Figure 5.7. Each compound is completely resolved.

The method optimization and validation were performed on a dedicated capillary over a period of ca. seven months. During this time the capillary had to be changed once due to blockage. Also, the pH was moved across the range for a number of times during method development. These changes had limited effect with regards to the capillary behaviour and similar results were obtained in all situations. This fact is very important for the highly regulated pharmaceutical

environment that requires robust analysis methods and reproducible results over extended time periods.

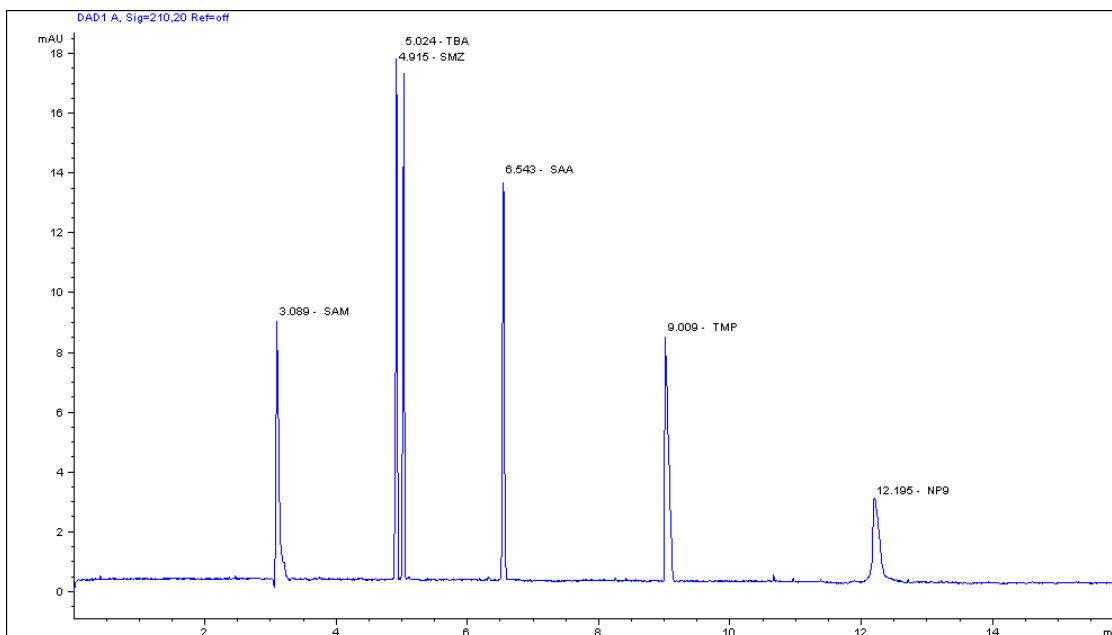


Figure 5.7 Electropherogram obtained with the optimized migration conditions (30 mM phosphate BGE pH 7.8 containing 10 mM SDS, applied voltage of 22 kV and 23°C). Injection: 4 s sample followed by 2s BGE.

5.3.2 MEKC method validation

5.3.2.1 Linearity

Linearity was studied by injecting standard solutions prepared at eight concentration levels with four replicates per level. Linearity data were obtained by plotting the peak area of the analytes expressed in area units against their concentration expressed as $\mu\text{g/ml}$. A linear regression least square analysis was performed in order to determine the slope, intercept and correlation coefficient of the standard curves.

Table 5.1 Parameters of linearity for the Co-trimoxazole mixture compounds

Analyte	Concentration range ($\mu\text{g/ml}$)	y-Intercept	Slope	r ²	LOD	LOQ
					($\mu\text{g/ml}$)	($\mu\text{g/ml}$)
SAM	2.41 – 96.51	-2.043E-01	5.367E-01	0.998	1.423	4.312
SMZ	1.99 - 79.65	-4.567E-01	6.841E-01	0.999	0.753	2.283
TBA	1.01 – 40.35	-9.314E-02	1.201E+00	0.999	0.431	1.306
SAA	1.42 – 56.60	-7.177E-02	8.348E-01	0.998	0.825	2.501
TMP	0.61 – 24.42	1.135E-01	2.745E+00	0.997	0.394	1.193
NP9	5.29 – 211.68	1.559E+00	3.184E-01	0.993	5.358	16.237

The coefficients of correlation (r) are higher than 0.996 for all the compounds studied indicating a good relationship between the peak area and the concentration of these compounds in the concentration range studied.

One disadvantage of using MEKC for cleaning validation is the on-capillary detection capability of capillary electrophoresis, which limits the sensitivity of the method due to the small optical path length. To increase method sensitivity larger injection times were used cautiously so that capillary overloading and peak distortion/tailing phenomena were avoided.

The limits of detection and quantification were calculated with Equations 4.1 and 4.2. The linearity data are presented in Table 5.1.

5.3.2.2 Precision (by repeatability)

The intra-assay precision of the method, reported as relative standard deviation, was assessed by measuring the repeatability of the peak areas and of the mobility times obtained for four replicate samples at eight concentration levels. The % RSD values obtained per level are presented in Table 5.2.

Table 5.2 Intra- assay precision (one day) for Co-trimoxazole mixture compounds

Level	Peak Area (% RSD)						Migration Time (% RSD)					
	SAM	SMZ	TBA	SAA	TMP	NP9	SAM	SMZ	TBA	SAA	TMP	NP9
5 %	3.2	4.9	5.5	3.0	6.8	-	0.2	0.6	0.4	0.5	2.3	-
10 %	5.6	3.8	3.7	2.9	6.6	8.4	0.6	0.9	0.9	1.2	3.3	1.7
25 %	3.1	3.1	3.0	1.6	3.9	6.8	0.4	0.6	0.5	0.7	1.5	1.3
50 %	2.5	1.1	1.4	1.5	3.9	3.3	0.3	0.4	0.3	0.3	1.9	0.8
75 %	3.0	0.8	0.8	1.8	3.9	1.4	0.1	0.2	0.2	0.3	2.8	0.7
100 %	2.5	1.2	1.6	1.4	2.5	1.3	0.4	0.6	0.6	0.8	1.6	1.1
150%	1.7	0.6	0.8	0.9	1.3	1.6	0.1	0.2	0.2	0.2	1.4	0.5
200 %	2.7	0.5	0.6	0.8	1.2	4.1	0.1	0.1	0.1	0.1	1.9	0.3
Overall %RSD	3.2	2.5	2.7	1.9	4.2	4.6	0.3	0.5	0.5	0.6	2.2	1.0

5.3.2.3 Precision (by reproducibility)

The inter-assay precision was assessed based on peak area and migration times obtained for three concentration levels (50%, 100%, and 150%) with six replicate swab samples per level (see Table 5.3). The assays were carried out over three days on the same instrument by one operator (n=18).

Table 5.3 Inter-assay precision (three days, one instrument, one operator) for Co-trimoxazole mixture compounds

Level	Peak Area (% RSD)						Migration Time (% RSD)					
	SAM	SMZ	TBA	SAA	TMP	NP9	SAM	SMZ	TBA	SAA	TMP	NP9
50 %	4.2	2.6	3.0	3.1	4.8	5.7	0.7	1.0	1.0	1.4	1.4	3.1
100 %	4.3	4.3	4.4	4.7	3.7	5.6	0.7	1.0	1.0	1.4	3.1	2.4
150 %	3.9	3.3	3.3	3.5	4.5	5.8	0.6	0.6	0.6	0.7	4.8	0.8
Overall % RSD (n = 54)	4.1	3.5	3.6	3.8	4.3	5.7	0.8	1.0	1.0	1.4	3.7	2.6

These values demonstrate that the precision of the method is adequate over the range of concentrations expected in cleaning validation samples.

5.3.2.4 Accuracy (by recovery)

The method accuracy was determined on spiked and dried swabs that were then extracted in 3 mM NaOH. The accuracy of the procedure was assessed by comparing the analyte amount recovered from swabs versus the known amount in the standard injection solutions at seven concentration levels with 3 replicates (n=3) for each concentration level investigated. Lower recoveries were obtained for NP9 (see Table 5.4).

Table 5.4 Accuracy by recovery for Co-trimoxazole mixture compounds

Concentration Level	Recovery (%) (n=3)					
	SAM	SMZ	TBA	SAA	TMP	NP9
10 %	138.0	113.2	106.7	104.2	105.5	83.6
25 %	96.3	89.9	92.3	89.5	104.8	49.4
50 %	107.4	101.9	103.0	102.0	96.0	58.0
75 %	98.6	98.4	100.7	95.7	92.9	60.3
100 %	93.8	99.1	103.3	103.7	95.9	82.6
150 %	96.6	98.7	99.9	97.4	92.2	82.0
200 %	95.4	97.0	98.6	95.9	90.7	87.0
Average	103.7	99.7	100.64	98.3	96.9	71.8

5.3.3 Establishing limits for the contamination and cross-contamination level permitted on cleaned equipment

For products with a defined therapeutic dose, the maximum permitted quantity (R) of residue allowed on the equipment surface can be calculated by using the 0.1% dose limit criterion. The 0.1% dose limit criterion is justified by the principle that, generally, an API at a concentration of 1/1000 of its lowest therapeutic dose will not produce any adverse effects to one's health. This criterion as explained by (Fourman and Mullen, 1993) accounts for the maximum daily intake of a following product and for the batch size of the product that will be manufactured next with the same equipment (see Equation 5.3) and for the total surface area of the equipment chain in direct contact with the product.

$$R = \frac{D \times S}{I \times F} \times \frac{10^9}{A} (\mu g/cm^2) \quad (\text{Eq. 5.3})$$

The values obtained by using Eq. 5.3 for the calculation of R were 4.28 $\mu g/cm^2$ for SMZ and 0.86 $\mu g/cm^2$ for TMP.

The 10 ppm criterion is another way of establishing limits of contamination for SMZ and TMP (see Equation 5.4). However, when this criterion was applied, a limit of 85.70 $\mu g/cm^2$ was obtained for both SMZ and TMP, which is much higher than the limits calculated with the 0.1% dose limit criterion.

The lowest obtained values were selected as limit of contamination (LOC) for this study.

$$R = 10 \times 10^9 \times S \times \frac{1}{A} (\mu g/cm^2) \quad (\text{Eq. 5.4})$$

Where,

R = maximum residue of API, in $\mu g/cm^2$, permitted after cleaning, allowed into the next product; it is assumed that the total amount of residue is distributed homogenously into the following product.

D = lowest daily therapeutic dose of the contaminant, in mg.

S = smallest batch size for any subsequent product, in Kg.

I = maximum daily intake of any product manufactured with the same equipment, in mg.

F = safety factor (can vary from 10 to 10 000 depending on the route of product administration e.g., topical, oral or injectable preparations). More stringent figures down to 1/100 000th of LTD are used for research, investigational products and products such as hormones and antibiotics that can still cause an allergenic effect even in very small doses.

A = total surface area of equipment in direct contact with the products, in cm^2 , calculated based on the assumption that all the products come into contact with all the parts of the manufacturing equipment train.

10^9 is a transformation factor from Kg to μg .

So far, the problem of cross- contamination was assessed by establishing limits for SMZ and TMP. Cross-contamination refers to the contamination of a product by a previously manufactured product. Whereas contamination refers to any chemical, microbiological or particulate contaminant likely to remain on the equipment surface after its cleaning. Therefore NP9 is referred to as an equipment and product contaminant.

For the detergent an acceptable daily intake (ADI) can be defined based on toxicological limits (Layton, Mallon *et. al.*, 1987). The maximum allowable residue (MAR) for NP9 per process step or equipment can be calculated with the Equations 5.5 and 5.6 and 5.7.

$$ADI = NOAEL \times AAW / F \quad (\text{Eq. 5.5})$$

$$NOAEL = LD_{50} \times EF \quad (\text{Eq. 5.6})$$

$$MAR = ADI \times \frac{S}{I} \times \frac{10^6}{A} (\mu g/cm^2) \quad (\text{Eq. 5.7})$$

where,

ADI = Acceptable daily intake, in mg.

NOAEL = No Observed Adverse Effect Level, in mg/Kg/day

AAW = average adult weight, in Kg (= 70 Kg)

EF = empirical factor derived from animal model developed by Layton, Mallon *et. al.*, (1987) (=0.0005)

LD₅₀ = lethal dose 50 percent kill (LD₅₀ for NP9 = 1310 mg/Kg oral, rat)

MAR = maximum allowable residue, in $\mu g/cm^2$ will be calculated taking into account all equipment surface area.

For NP9 a MAR value of 19.65 $\mu g/cm^2$ was calculated as follows:

$$\begin{aligned} MAR_{NP9} &= LD_{50} \times EF \times \frac{AAW}{F} \times \frac{S}{I} \times \frac{10^6}{A} = \\ &= 1310 \text{ mg/Kg} \times 0.0005 \times \frac{70 \text{ Kg}}{1000} \times \frac{900 \text{ L}}{20 \text{ ml}} \times \frac{10^6}{105019 \text{ cm}^2} = 19.65 \mu g/cm^2 \end{aligned}$$

As Co-trimoxazole is manufactured as a liquid preparation, the smallest batch size ($S = 900 \text{ L}$) and maximum daily intake ($I = 20 \text{ ml}$) were expressed in volume units.

5.3.4 Determination of recovery rate of contaminants from stainless steel and plexiglas surfaces

Recovery studies were performed in order to determine to what extent the residue could be retrieved from the production equipment with the sampling procedure chosen. The recovery experiment was performed at the LOC level of SMZ and TMP and NP9 on the equipment.

SMZ, TMP and NP9 were independently spiked on 316 stainless steel and plexiglas coupons (10 cm $\times$ 10 cm). The spiked coupons were allowed to dry (ca. 2 hours) at room temperature. Since

the swabbing is an operator dependant technique, each analyte was recovered five times from coupons in order to obtain a representative mean of the recovery rate. The swab sampling simulation was performed by one operator.

A pair of tweezers was used to perform the swabbing. Various solvents were considered as swabbing solvents. A mixture of methanol - MilliQ water 70:30 (v/v) swabbing solvent provided the highest recovery rate for SMZ and TMP. Ethanol 96% (v/v) was used to obtain the best recovery rates for NP9. Better recovery results were obtained when the spiked analytes were recovered by using three swabs per sample rather than two. The first swab was wetted with 0.2 ml of swabbing solvent. The coupon was swabbed horizontally with one side of the swab and vertically with the other side. The procedure was repeated two more times with two more wetted swabs. All three swabs were collected into the same test tube. The swab samples were prepared as described under sample preparation. Appropriate dilutions were performed in order to bring the concentration of the samples within the validated range of the analytical method. Table 5.5 presents the recovery results.

Comparative recovery rate values were obtained for both stainless steel and plexiglas surfaces. The recovery experiment provided a good indication regarding the reproducibility of the swabbing procedure.

Table 5.5 Recovery of SMZ, TMP and NP9 from stainless steel and plexiglas coupons

	Analyte type spiked on stainless steel coupons			Analyte type spiked on plexiglas coupons		
	SMZ	TMP	NP9	SMZ	TMP	NP9
Quantity of analyte spiked on coupons ($\mu\text{g}/100\text{ cm}^2$)	428.1	85.5	1971.3	428.1	85.5	1971.3
Quantity of analyte recovered from coupons ($\mu\text{g}/100\text{ cm}^2$)	413.1	81.6	1944.6	413.9	77.5	1832.0
	402.0	76.8	1840.4	405.5	80.4	1778.0
	414.2	82.9	1809.8	409.2	82.7	1869.2
	413.7	79.9	1835.1	406.9	79.3	1989.8
	401.8	77.9	1918.6	412.2	86.6	1889.2
Mean recovery	96.3	94.7	94.9	96.0	94.4	95.4
(%) $\pm$ IC (%)	± 1.4	± 3.1	± 3.0	± 1.3	± 2.8	± 4.1
% RSD (n=5)	1.5	3.4	3.2	1.4	3.1	4.4

5.4 Conclusion

Due to the composition of the current cleaning validation sample a CZE method could not be developed for this study. Since NP9 is a non-ionic surfactant it was possible to obtain its separation through MEKC.

The MEKC method was developed and optimised for the separation of the six potential contaminants likely to be found on the pharmaceutical manufacturing equipment, after the manufacture of Bactrim® syrup. The electrophoretic separation was achieved within 14 minutes.

The advantages of the present method are derived from the complex separation mechanisms governing MEKC. Compounds that cannot be separated on the same RP-HPLC chromatographic column due to their diverse chemical nature can be separated through MEKC based on differences in their ionization state in the presence of a suitable BGE. MEKC also offers the advantage of a small consumption of solvents. The higher number of theoretical plates, the broad range of possibilities for tuning the selectivity and less solvent waste makes MEKC as good, or better than HPLC for cleaning validation applications.

MEKC requires less setup time than the HPLC for the analytical equipment and therefore is suitable for the monitoring of equipment cleanliness in a routine-type working environment. The results obtained while validating the analytical method indicate that MEKC may be a useful technique for cleaning validation in the pharmaceutical analysis.

Chapter 6: Design of the cleaning validation program for the pharmaceutical manufacturing plant of Roche SA - working model

The purpose of this chapter is to discuss the cleaning validation program that was implemented for Roche, South Africa. This program was implemented based on the literature reviews and research discussed in the previous chapters.

Roche, South Africa manufactures within the same manufacturing facility, by using non-dedicated equipment, the following range of products: Fansidar, Triprim, Bayer Aspirin, Dyna Jets, Cal-C-Vita, Berocca and Supradyn. Therefore, the equipment must be cleaned after product manufacture and the cleaning procedures need to be validated in order to ensure that no contamination or cross- contamination of products will occur.

The purpose of developing the Roche, SA cleaning validation program was to ensure that the cleaning validation activities taking place within the manufacturing facility are planned, carried out and documented in a structured, prioritized, controlled and consistent way, in compliance with the applicable regulations. Therefore, the proper design and implementation of such a cleaning validation program is a key success factor in obtaining meaningful cleaning validation results.

In planning the current cleaning validation program for Roche SA, one of the first validation approaches designed by Harder (1984) was followed. He has summarised the minimum requirements for a cleaning validation program:

- an SOP for cleaning;
- a procedure for determining cleanliness (e.g., rinse or swab then analyze);
- an assay for testing for residual drug levels;
- pre-set chemical and microbial limits to which the equipment must be cleaned;
- a protocol for cleaning validation.

Therefore, the following sections describe the implementation of the Roche SA cleaning validation program.

6.1 Placing the cleaning validation program for Roche SA within the broader validation frames

The various elements or steps of a cleaning validation program have been discussed in detail in Chapter 2. Their place within the broader process validation framework needs to be localized in order to ensure that the cleaning validation program is carried out in synchronization with other validation activities. Figure 6.1 and the following paragraphs show the various elements of the cleaning validation program on a general process validation time scale, and present the approach used in the development of the cleaning validation program for the pharmaceutical manufacturing plant of Roche, South Africa.

For both new and existent manufacturing processes and equipment the preparations for cleaning validation need to start early within the process validation. In the current manufacturing facility at Roche SA, the majority of equipment was already existent or refurbished. Therefore, the initial work for cleaning validation was to assess the existing equipment. This was done by measuring and calculating the internal equipment surface areas in contact with the product. Also, the sampling points were selected for areas that were anticipated as difficult-to-clean locations. This was done by taking the equipment geometry into account or from previous cleaning experience.

After the initial evaluation process at Roche SA was completed, it was decided to use a new cleaning agent, product X. This new cleaning agent was tested, validated and approved for use on equipment parts in direct contact with the manufactured products. All the regulatory requirements for the selection and qualification of such cleaning agent, as described in Chapter 2, were fulfilled. Briefly, this include:

- assessing the ability of the detergent in removing residues of Roche selected products, through cleaning simulation experiments;
- develop and validate an analytical method suitable to assess the level of detergent residues after cleaning;
- develop a procedure for receiving, releasing, handling, storing and disposing of the detergent.

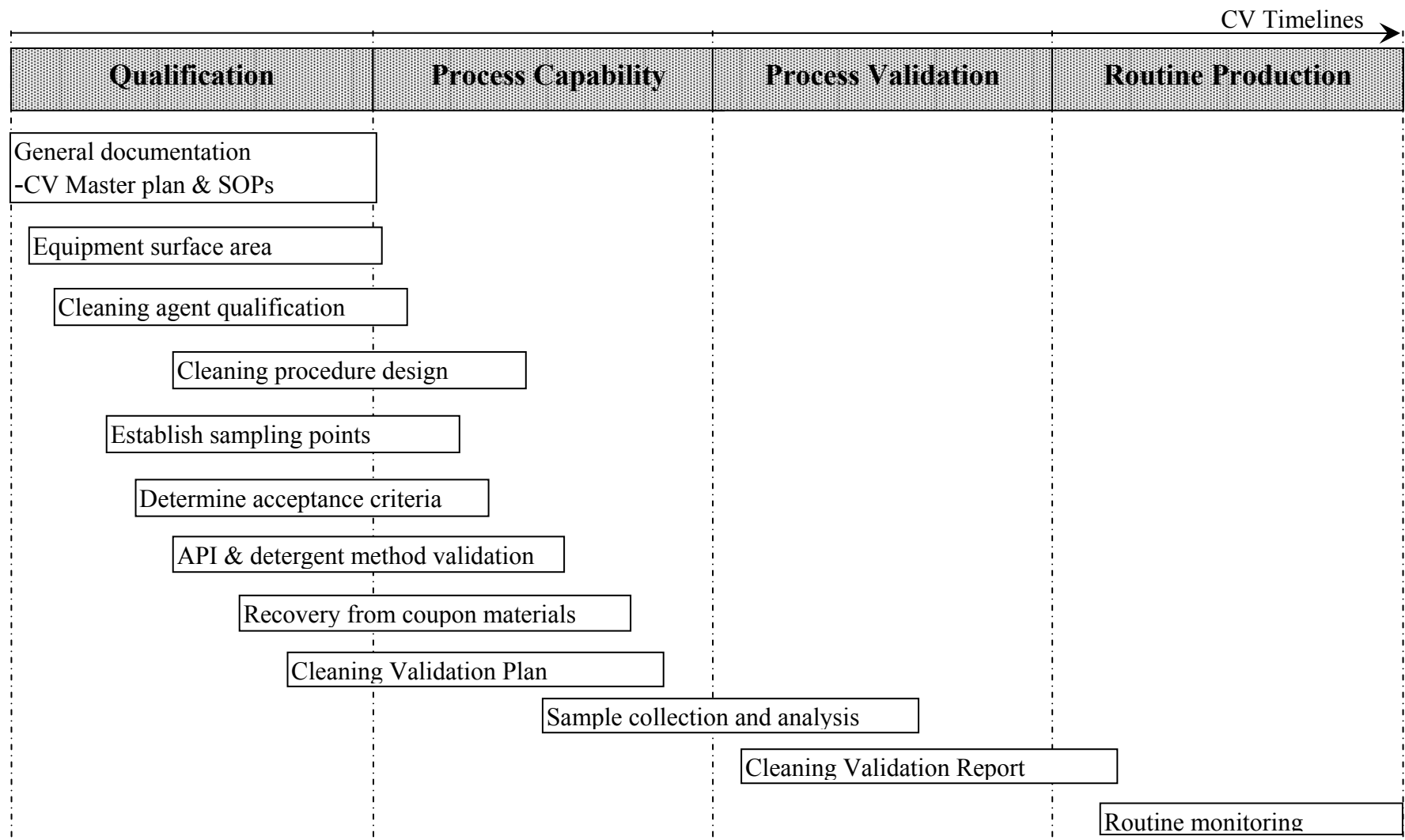


Figure 6.1 Place of cleaning validation steps within the broader validation program

As no former cleaning experience was associated with this new cleaning agent (product X), a long period of time of approximately six months was dedicated to the design, documentation, cleaning trials, analytical method validation, personnel training and implementation of the new cleaning procedures.

The documentation supporting the cleaning validation program was implemented in parallel with the above-mentioned activities and far prior to the active phase of validation. The structure of the documentation prepared is presented in Figure 6.2.

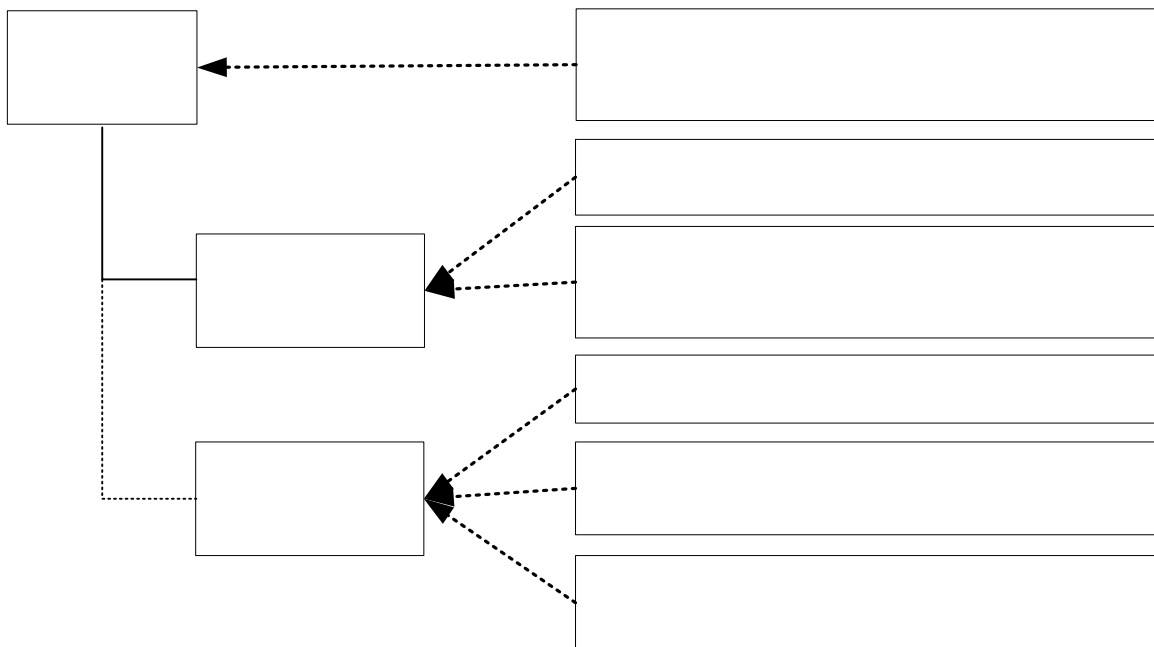


Figure 6.2 Structure of documentation used for cleaning validation

While the first trial batches for newly introduced products were manufactured, the cleaning validation trials also took place. This is known as the process capability phase. Cleaning procedures which did not produce successful results, according to previously calculated cleaning limits, were further refined and, when necessary, the level of equipment dismantling was supplementary detailed.

For every project undertaken all activities required in preparation for cleaning validation and all necessary documentation (e.g., cleaning validation plan, sampling plan, acceptance criteria and

analytical method validation documentation) was generated prior to production and timeously approved. Therefore, for new products, cleaning validation took place at the same time when the process validation batches were being manufactured.

After each manufacturing campaign, cleaning took place according to the newly documented procedures and samples were collected and analysed in accordance to validated sampling methods and analytical methods.

After the active validation phase and upon gathering sufficient successful cleaning results, the cleaning procedures were declared validated and released for routine use. The results acquired during validation, together with their data interpretation, recommendations handling of deviations, where applicable, and conclusions were gathered for every product specific project in a cleaning validation report.

6.2 Detailing the cleaning validation program

The core document describing the overall approach for cleaning validation is the *cleaning validation master plan*, and this was therefore the first document that was created. The objectives of the cleaning validation program, listed in the master plan, are as follows:

- identify the documentation required to support the cleaning validation activities;
- outline the validation approach for cleaning procedures;
- describe the functional responsibilities for performing the cleaning validation tasks;
- identify all the production equipment, cleaning procedures and products in the manufacturing facility;
- select, based on a risk analysis, the cleaning procedures requiring validation;
- outline the key testing principles and acceptance criteria for cleaning validation;
- define procedure through which changes, deviations and investigations are handled throughout the entire validation program;
- establish timelines for the execution and monitoring stages of the validation program.

The objectives listed above will be discussed in the following paragraphs.

6.2.1 The documentation structure elaborated for cleaning validation

The document structure and the correlations between all supporting cleaning validation documents were presented in Figure 6.2.

In addition to the core document, the following supporting operating procedures were also created to respond to questions related to **how** specific aspects and activities related to cleaning validation have to be carried out:

- General procedure for cleaning validation;
- Sampling procedures for cleaning validation;
- Procedure for analytical method validation, recovery from coupon materials and assessment of sample stability;
- Analysis of cleaning validation samples and data interpretation.

In order to facilitate the validation activities, working templates were created for several document types. The reason was to try to standardize the cleaning validation documentation as much as possible, while still leaving the necessary room for adapting it for the specific needs of every project. Such templates were made available for the cleaning validation plan (see Appendix D), the sampling plan as well as for the cleaning validation report (see Appendix E).

Also, for the documentation of routine cleaning validation activities, working forms were created for sampling records, results and data interpretation (see Appendices F to I). These forms were designated to be completed by the user at the time of carrying out specific activities.

The procedures for handling and filing the various working templates and forms were documented in the above mentioned “General procedure for cleaning validation”.

6.2.2 Validation strategy

The overall validation strategy was based on equipment and product grouping (or bracketing) due to the variety of manufacturing equipment and products handled in the facility. The assumption that was made in adopting the grouping approach is the following: if a cleaning procedure successfully removes residues of the worst case product below pre-determined levels of safety, it

will also successfully remove residues of all the other products handled with the same production equipment.

Also, the validation approaches intended to be used were then identified as follows:

- for new equipment, processes and products, cleaning validation is to be carried out before routine production is approved (prospective validation);
- concurrent validation is performed when cleaning validation activities take place at the same time with process validation;
- retrospective validation takes place for existing cleaning procedures;

6.2.3 Assigning responsibilities for cleaning validation

For assessment, planning and execution of the validation program, detailed responsibilities were assigned in the master plan for every department involved. An example is presented in Table 6.1.

Throughout the entire process, the person responsible for cleaning validation assumed the leading role. However, the optimum correlation of all activities was dependant on interdepartmental cooperation, communication and good timing of all activities.

As the majority of cleaning procedures validated in the facility rely on manual cleaning, the role of the operator was carefully considered. It seems important that a cleaning procedure is only as good as how well the operator adheres to the prescribed cleaning validation strategy. In turn, this will depend on the accuracy of operator training. It is therefore important that operators in training should “shadow” existing operators and their techniques should constantly be spot-checked and tested (quality control) as the standardization of the techniques will depend largely on the accuracy of the implementers of the procedures. Operators have been educated not only in how to clean but why the cleaning step is of such a great importance in relation to the safety of the final product.

Table 6.1 Responsibilities for cleaning validation

Department/ Function	Responsibilities
Quality Control/ Staff member responsible for cleaning validation	• Is responsible for the periodic review of the CVMP and its associated attachments. • Updates the relevant SOP system associated to cleaning validation. • Is responsible for organizing the cleaning validation activities. • Coordinates the selection of cleaning agents and the activities performed for assessing the cleaning process capabilities. • Creates and reviews product specific validation plans and reports • Ensures that the equipment surface area calculation is available. • Selects the sampling methods and sampling locations based on a scientific rationale (e.g., difficult-to clean areas). Creates the sampling plans. • Decides on the chemical compounds tested for cleaning validation. • Calculates acceptance criteria for the level of contamination and cross-contamination permitted after cleaning. • Carries out sampling simulation for assessing the recovery rate from coupon materials. • Generates the documentation for the validation of analytical methods, recovery rate from coupons and stability studies of sample performed to support the cleaning validation program. • Reviews cleaning procedures • Coordinates activities during validation by making the necessary arrangements with the Production, QA and QC departments. • Verifies that the training of cleaning procedures being validated has been performed and documented. • Collects chemical and microbiological samples for cleaning validation in accordance to the relevant SOP and validated sampling methods. • Carries out analysis of samples and data interpretation with every cleaning trial. • Organizes periodic re-testing activities after the initial validation.

Table 6.1 Responsibilities for cleaning validation - continued

Department/ Function	Responsibilities
Quality Assurance/ Manager	• Approves the CVMP and cleaning validation-related SOPs. • Approves CVPs, CVRs, sampling plans and acceptance criteria – project specific. • Approves the documents created to support the cleaning validation program: method validation plans and reports (referring to analytical method validation, recovery studies from coupons, stability studies) and methods of analysis.
Production/ Manager	• Approves the CVMP and cleaning validation SOPs related to the Production department. • Approves the product specific cleaning validation plans, reports and sampling plans. • Ensures that the outcome of the cleaning validation exercise is implemented.
Production/ Pharmacist in charge of the area where validation activities take place	• Reviews the CVMP and cleaning validation SOPs related to the Production department. • Reviews CVPs, CVRs, sampling plans and acceptance criteria documents. • Elaborates or delegates the creation and update of cleaning procedures for production equipment. • Ensures that training of operators for the currently used cleaning procedures is implemented and documented. • Ensures that any changes for equipment, product and cleaning procedures are covered by the change control procedure. • Provides relevant information related to the manufacturing equipment on request from the QA staff. • Participates in the process of establishing and justifying the sampling points and reviews sampling plans. • Is responsible for the execution of the validation activities. • Acknowledges the results of the cleaning validation trials. • Cooperates with QC responsible in the selection of cleaning agents and the activities performed for assessing the cleaning process capabilities.
Production operator	• Cleans the manufacturing equipment by adhering to the instructions set in the cleaning procedures approved by the Quality unit.

Table 6.1 Responsibilities for cleaning validation - continued

Department/ Function	Responsibilities
Quality Control/ Manager	• Approves the CVMP and cleaning validation related SOPs. • Approves the CVPs, CVRs and sampling plans. • Approves the documents created to support the cleaning validation program: method validation plans and reports (referring to analytical method validation, recovery studies from coupons, stability studies) and methods of analysis. • Delegates to the relevant Lab staff the execution lab activities for the analytical method validation, recovery rate and stability study of sample and sample analysis. • Reviews the analytical data generated for method validation and analysis of cleaning validation samples. • Ensures that the laboratory instrumentation required for cleaning validation is qualified and in a calibrated state.
Engineering/ Manager	• Ensures equipment drawings are available upon request from the QC responsible and delegates the calculation of equipment surface areas.
Technical Director	• Approves the CVMP.

6.2.4 Identification of production equipment, cleaning procedures and products existent in the manufacturing facility

As an important part of the program, the cleaning procedures requiring validation were identified. For every work centre in the facility an inventory of all equipment together with their cleaning procedures was compiled. The production equipment was divided into several classes according to its designation:

Class 0

- Non-dedicated equipment, shared by several products, but not in direct contact with the product (e.g., drums lined with plastic bags, scales). No potential risk for product contamination.

Class I

- Non-dedicated and dedicated equipment in direct contact with excipients (e.g., sugar or binder) that needs visual verification to assess its cleanliness. No chemical testing required. The necessity of micro testing has to be assessed on a case by case situation.
- Dedicated equipment in contact with product containing APIs that needs only visual verification to assess its cleanliness. The necessity of micro testing has to be assessed on a case by case situation.

Class II

- Non-dedicated equipment shared by several products, but having dedicated tools. Cleaning validation required only for the non-dedicated parts of the equipment. Visual verification is required to assess the cleanliness of the dedicated equipment parts.
- Non-dedicated equipment shared by several products that necessitates cleaning validation.

A matrix of equipment/ cleaning procedures and products was then compiled. The information contained by this matrix is presented in a simplified format in Table 6.2.

Thereafter, information about product physical and pharmacological properties was gathered. The product properties reviewed were related to the dosage form, strength and potency, toxicity and scheduling data, the water solubility of the active ingredients, the overall water solubility of the product known from previous cleaning experience. A simplified example is given in Table 6.3.

All product data together with the previous data accumulated in Table 6.2 have been used to perform a risk assessment analysis for the selection of cleaning procedures for validation.

6.2.5 Performing the risk assessment analysis

The final conclusions as to which cleaning procedures to validate in conjunction to which product are presented in Table 6.4. The stars in the table (*) indicate the range of products are in contact with every piece of equipment. The symbol (©) in the table indicates for which product cleaning validation will be performed.

Wherever possible, products were grouped and the bracketing strategy was adopted for cleaning validation. For example, all effervescent tablets have been grouped as a family product due to the fact that they all contain multivitamins and minerals. In this product group the product Cal-C-Vita was selected as the worst case for cleaning validation. The manufacture of Cal-C-Vita has been newly introduced in the facility; also the product contains vitamins at a concentration equal to the maximum recommended daily allowance.

As it can be seen from Table 6.4 a large group of equipment pieces are shared by the products, Dyna Jets, Aspirin, Fansidar and Triprim. Among these products Fansidar was selected as the worst case for cleaning validation due to the poor solubility of its actives and high toxicity and potency characteristics. Fansidar was as well a newly introduced product in the manufacturing facility and therefore according to regulatory requirements it necessitates cleaning validation.

Information similar to the one presented in tables 6.2, 6.3 and 6.4 was formal part of the appendices of the master plan and was updated at appropriate time intervals.

6.2.6 Outlining the key testing principles for cleaning validation

The *Roche SA cleaning validation master plan* has defined a minimum of three consecutively successful cleaning trials (e.g., cleaning after the manufacture of three batches or three campaigns) after which a cleaning procedure can be declared as validated. This approach applies to any cleaning validation project undertaken in the manufacturing facility. Although no statistical data interpretation is allowed through this approach, for the pharmaceutical industry

this is a reasonable tactic for validation that balances process feasibility and operating costs on one side and the product quality and safety on the other side. Of course, as with every other validated processes the routine monitoring of cleaning after the initial validation phase will allow timeous detection of potential deleterious trends and deviations from the validated state.

Table 6.2 Matrix of equipment, cleaning procedures and products (dispensing, solids manufacture and packaging operations)

Work centre/ manufacturing operations	System Name	Cleaning procedure ID.	Products handled	Equipment Class	In scope for cleaning validation (Yes/No)
Dispensing	Electronic floor scales	DP1	All raw materials	0	No (no product contact)
	Sugar mill	DP2	Sugar	I	Yes (not contacted by API, assess cleanliness from microbiological point of view)
	Scoops, spatulas	DP3	All raw materials and bulk tablets	II	Yes
Granulation and drying	Gral granulator	SP1	Fansidar Triprim	II	Yes
	Quadro Comil	SP2	Dyna Jets Fansidar Triprim	II	Yes
	Glatt fluid bed drier	SP3	Fansidar Triprim	II	Yes
	Moritz granulator	SP4	Cal-C-Vita Supradyn Berocca	II	Yes
	Buhler mill	SP5	Cal-C-Vita Supradyn Berocca	II	Yes
	Condux mill	SP6	Supradyn	I	Yes (not contacted by API, assess micro only)
	Vibrating sieve	SP7	Cal-C-Vita Supradyn Berocca Aspirin Dyna Jets Fansidar Triprim	II	Yes
	Turbostirrer	SP8	Fansidar	I	Yes (not contacted by API, assess micro only)

Blending	Bertrams mobile blending container	SP9	Cal-C-Vita Supradyn Berocca Aspirin Dyna Jets Fansidar Triprim	II	Yes
	PIAB vacuum transfer system	SP10	Fansidar Triprim	II	Yes
	AZO vacuum transfer system	SP11	Cal-C-Vita Supradyn Berocca Aspirin Dyna Jets	II	Yes
	100L and 200 L drums	SP12	All products	II	Yes
Compression	Manesty Betapress	SP13	Aspirin Dyna Jets Fansidar Triprim	II	Yes
	Manesty Novapress	SP14	Cal-C-Vita Supradyn Berocca	II	Yes
Packaging*	Alu-alu stripping machine	PP1	Fansidar	I	Yes (assess micro only) – dedicated equipment
	Effervescent brassele packaging	PP2	Cal-C-Vita Supradyn Berocca	II	Yes
	Uhlmann blister machine	PP3	Aspirin Dyna Jets Triprim	II	Yes

* Cleaning validation performed for primary packaging operations only where there is direct product – equipment contact.

Table 6.3 Physical characteristics of the products manufactured

Product identification	Product physical form	Active ingredients	Concentration of active ingredients	API water solubility (mg/ 100 ml at 20°C)	Toxicological data expressed as LD ₅₀ (mg/Kg)	Product schedule	Comments
Berocca	Effervescent tablet	Biotin Vitamin C Nicotinamide Calcium carbonate	150 µg/4800 mg 300 mg/4800 mg 25 mg/48000 mg 100 mg/4800 mg	80 33 000 69.1 PI*	> 20 000 mg/Kg oral, rat 11900 mg/Kg oral, rat 2 500 mg/Kg oral, mouse 6450 mg/Kg oral, rat	None	Same family product as Cal-C-Vita
Cal-C-Vita	Effervescent tablet	Vitamin C Vitamin D ₃ Vitamin B ₆	300 mg/4800 mg 1000 µg /4600 mg 250 mg/4600 mg	33 000 PI 22 000	11900 mg/Kg oral, rat 42 mg/Kg oral, rat 4000 mg/Kg oral, rat	S ₀	API concentration at recommended daily allowance level; new product
Supradyn Recharge (Orange/ Lemon)	Effervescent tablet	Vitamin A palmitate Vitamin E acetate Vitamin D ₃ Vitamin C	4000 i.u./4700mg 16.39 mg/4700mg 240 i.u./4700 mg 198 mg/4700 mg	PI PI PI 33 000	>4000 mg/Kg oral, mouse > 4000 mg/Kg oral, rat 42 mg/Kg oral, rat 8 000 mg/Kg oral, mouse	None	Same family product as Cal-C-Vita
Dyna Jets	Tablets	Vitamin A Vitamin D ₃ Vitamin E acetate	720 µg/560 mg 7.5 µg/560 mg 1.0 µg/560 mg	PI PI PI	>4000 mg/Kg oral, mouse 42 mg/Kg oral, rat > 4000 mg/Kg oral, rat	None	Multivitamins and minerals formulation; same equipment used for Fansidar
Aspirin 300mg	Tablets	Acetylsalicylic Acid	300 mg/600 mg	330	1500 mg/Kg oral, rat	S ₀	Easy to clean due to high water solubility
Fansidar 25/200 mg	Tablets	Pyrimethamine Sulfadoxine	25 mg/700 mg 200 mg/700 mg	10 100	128 mg/Kg oral, rat 5200 mg/Kg oral, mouse	S ₄	New potent product
Triprim 200mg	Tablets	Trimethoprim	200mg/500mg	30	2000 mg/Kg oral, rat	S ₄	Same equipment used for Fansidar

*PI = practically insoluble; for PI solutes, according to the USP definition, 10 000 parts of solvent are required to solubilize one part of solute

Table 6.4 Final selection of cleaning procedures/ equipment/ products for cleaning validation

Equipment identification	Cleaning procedure ID	Products							Reasons for cleaning validation
		Berocca	Cal-C-Vita	Supradyn Recharge	Dyna Jets	Aspirin	Fansidar	Triprim	
Sugar mill	DP2	*	*©	*					As sugar is a raw material that can stand microbial growth, CV is required for micro CV performed for pyrimethamine raw material as being highly toxic and poor water
Scoops, spatulas, sampling tools	DP3	*	*	*	*	*	*©	*	
Moritz granulator	SP4	*	*©	*					
Buhler mill	SP5	*	*©	*					
Azo vacuum transfer system	SP11	*	*©	*					
Manesty Novapress	S14	*	*©	*					
Effervescent packaging	PP2	*	*©	*					
Condux mill	SP6			*©					Used for excipients only; CV required for micro
Bertrams container	SP9	*	*	*	*	*	*©	*	CV performed for the biggest size as equipment is similar in geometry and function
Vibrating sieve	SP7	*	*	*	*	*	*©	*	
Gral granulator	SP1						*©	*	
Quadro Comil	SP2				*		*©	*	
Glatt fluid bed drier	SP3						*©	*	
PIAB vacuum transfer system	SP10				*	*	*©	*	
Manesty Betapress	SP13				*	*	*©	*	
Turbostirrer	SP8						*©		Used for excipients only; CV required for micro
100 L drums	SP12	*	*	*	*	*	*	*	CV required for Triprim as being the most potent/ least soluble product
200 L drums	SP12	*	*	*	*	*	*©	*	
Uhlmann blister machine	PP3				*	*		*©	Dedicated equipment; CV required for micro only
Alu- alu striping machine	PP1						*©		

6.2.7 Tackling a cleaning validation project – Fansidar tablets

Every project identified was appropriately prioritized in the master plan and deadlines were set. The cleaning validation for Fansidar tablets was the most comprehensive project initiated in the manufacturing facility, covering a large spectrum of cleaning procedures and manufacturing operations. Several manufacturing related aspects of this project will be discussed under this heading as the analytical aspects of the project have already been covered in Chapter 4. Briefly, the already discussed aspects include:

- analytical method optimization and validation at trace levels for the Fansidar APIs;
- establishing limits for the level of Fansidar residues allowed on equipment after cleaning;
- selection of a suitable swab material and recovery solvent for sampling;
- performing recovery rate studies on stainless steel coupons;
- assessing the analytes stability in swab samples;
- assaying Fansidar residue level in samples collected after equipment cleaning.

6.2.7.1 Manufacturing flow for Fansidar tablets

The manufacturing workflow for Fansidar tablets is presented schematically in Figure 6.3. After dispensing of excipients and active components, the first manufacturing step was producing a granulation paste containing excipients only. Upon homogenization, the paste is added together with the APIs into the Gral granulator, where the wet granulation process takes place. This is followed by the granule drying process that takes place into the Glatt fluid bed drier. In order to bring the granules to the desired size, the granulate is then passed through the Quadro Comil. Thereafter, the active granulate is sieved and blended into the Bertrams container together with an inactive compression mix. The final mixture is compressed on the Betapress compression machine. Then the tablets are sealed in aluminium foil with the help of the striping machine.

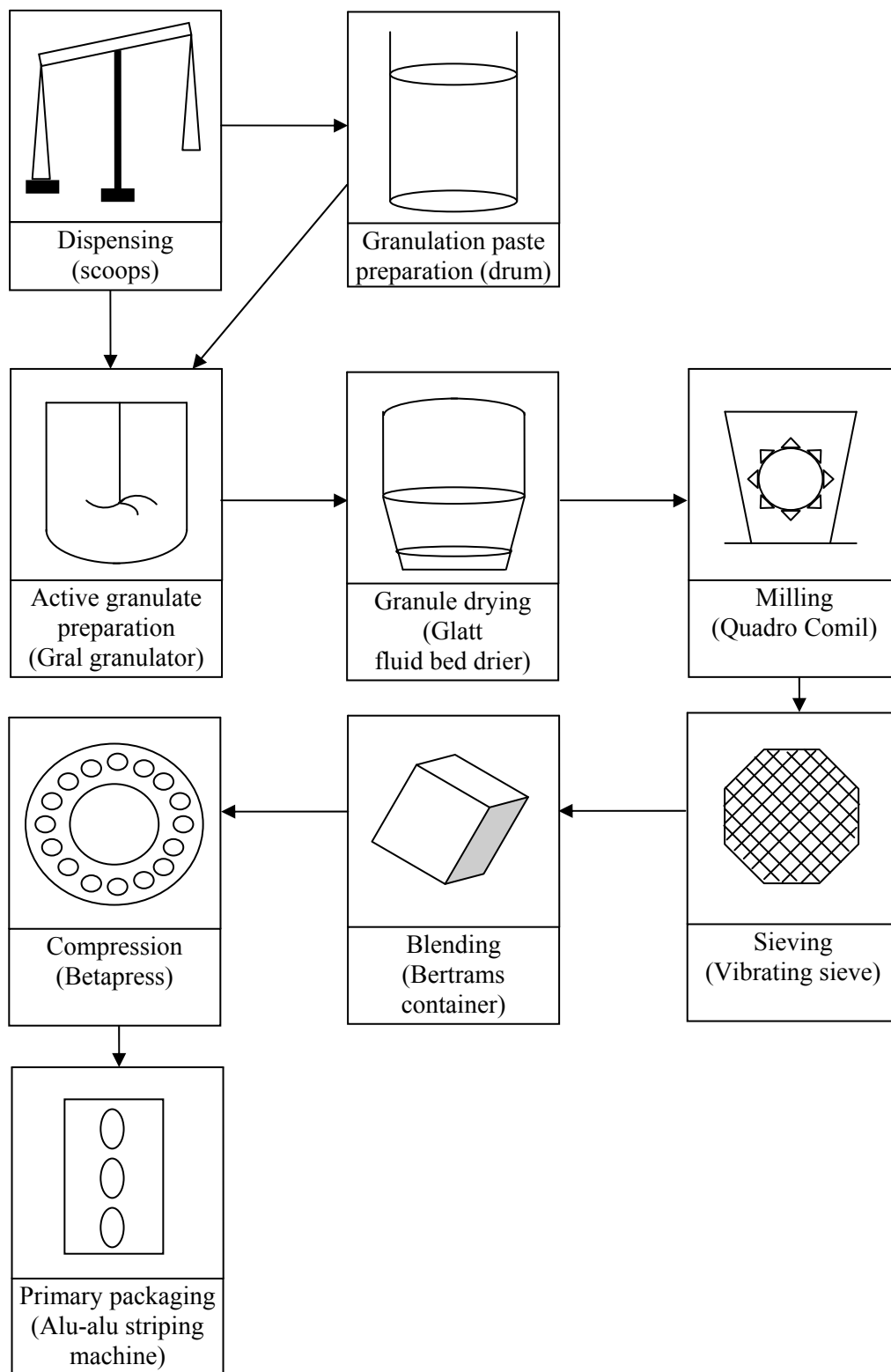


Figure 6.3 Workflow of manufacturing process for Fansidar tablets

6.2.7.2 Compiling the cleaning validation plan

The cleaning validation plan for Fansidar was compiled according to the requirements mentioned under heading 2.12.4 (see also the Cleaning Validation Plan template presented in Appendix D).

These include:

- listing of reasons for selecting this product for validation;
- enumerate the equipment and cleaning procedures used in conjunction with Fansidar;
- calculating limits for the level of Fansidar and detergent residues allowed on equipment after cleaning;
- description of sampling methodology and tests carried out;

For validation there were selected procedures used to clean dispensing tools, solids manufacturing equipment as well as primary packaging equipment (see Table 6.4).

6.2.7.3 Setting acceptance criteria for the chemical and microbiological level of cleanliness

Special issues were encountered when setting acceptance criteria for the level of residue allowed for dispensing tools which are used to dispense the entire range of raw materials processed in the facility. The main difficulty was determining acceptance criteria for the level of cleanliness. As the material dispensed is pure raw material and not a mixture of API and excipients the formula of setting acceptance criteria based on percentage dosages could not be applied.

Also, it is practically impossible to anticipate what would be the next product dispensed with the same scoop or the quantity dispensed. Therefore the 10 ppm criterion could not be applied either.

Through spiking studies it was demonstrated that the traces of PYR and SUL spiked on stainless steel surfaces can be visualized down to very low concentration levels:

- between 5 $\mu\text{g}/\text{cm}^2$ and 2.5 $\mu\text{g}/\text{cm}^2$ there can be seen visible traces (white stains) on the stainless steel coupon;
- at levels between 2.5 $\mu\text{g}/\text{cm}^2$ and 1.0 $\mu\text{g}/\text{cm}^2$ the shape of the drop on the stainless steel coupon can be distinguished easily. However, the drop does not form white stains.

Therefore, for dispensing operations a visual acceptance criterion of $1.0 \mu\text{g}/\text{cm}^2$ was selected.

For manufacturing operations the calculation of acceptance criteria was outlined under heading 4.3.8. Formulas based on percentage of dose limits and based on 10 ppm criterion were used in calculating the acceptance criteria. A lowest calculated value of $4.99 \mu\text{g PYR}/\text{cm}^2$ was obtained when the 0.1% dose limit criterion was used for the total equipment chain. For SUL, the lowest calculated value of $19.14 \mu\text{g SUL}/\text{cm}^2$ was obtained when the 10 ppm acceptance criterion was applied.

For the microbiological monitoring of internal surfaces of equipment in direct contact with the product the following acceptance criteria were established initially as guidance:

- Alert Limit: 20 CFU/plate
- Action Limit: 100 CFU/plate

These limits were based on the data available from the Roche Internal Guideline for the microbiological monitoring of the manufacturing areas. The guideline specifies for clean zones, in which open product is handled, an alert limit of 50 CFU/plate and an action limit of 250 CFU/plate. Since there were no historical data available for the level of microbiological contamination permitted inside manufacturing equipment the limits given by the guideline and an additional safety factor of 2.5 was used as a starting point. It was decided that after acquiring several results the limits will be revised and adjusted accordingly.

6.2.7.4 Selecting sampling methods and compiling the sampling plan

For chemical testing the sampling method chosen was swabbing of equipment surfaces. The sampling procedure as well as the validation of recovery rate from coupon materials was presented under the heading 4.3.9. This includes:

- selection of a suitable swab material and recovery solvent for sampling;
- performing recovery rate studies on coupon materials;

The sampling for the microbiological assessment of the manufacturing equipment was performed by using Rodac plates filled with Trypticase Soy Agar (TSA). This way the level of total microbiological counts after equipment cleaning was assessed. After establishing the acceptance criteria, the sampling plan was elaborated. Table 6.5 presents the sampling plan.

Table 6.5 Sampling plan for cleaning validation of Fansidar tablets

Equipment/ Manufacturing operations	Sampling Point	Sample No.*	Sampled area (cm ²)	Testing for**
Dispensing				
Scoop	Inner part of the scoop	1.1 1.4	100 --	PYR VC
Solids operations				
Gral	Chopper	2.1	129.3	PYR, SUL
		2.2	129.3	Detergent (TOC)
	Stirrer	3.1	270	PYR, SUL
		3.2	270	Detergent (TOC)
	Lid	4.1	100	PYR, SUL
		4.2	100	Detergent (TOC)
		4.3	--	TC
	Wall	5.1	100	PYR, SUL
		5.2	100	Detergent (TOC)
		5.3	--	TC
	Seal	6.1	52	PYR, SUL
		6.2	52	Detergent (TOC)
Discharge port	7.1	--	TC	
Glatt	Bowl	8.1	100	PYR, SUL
		8.2	100	Detergent (TOC)
		8.3	--	TC
	Plenum	9.1	100	PYR, SUL
		9.2	100	Detergent (TOC)
		9.3	--	TC
	Screen	10.4	--	VC
	Seal	11.1	60	PYR, SUL
Quadro Comil	Milling chamber	12.1	100	PYR, SUL
		12.2	100	Detergent (TOC)
		12.3	--	TC
	Stirrer paddle	13.1	68	PYR, SUL
		13.2	68	Detergent (TOC)
	Screen	14.1	182	PYR, SUL

Equipment/ Manufacturing operations	Sampling Point	Sample No.*	Sampled area (cm2)	Testing for**
Mobile blending container	Blending lid	15.1	100	PYR, SUL
		15.2	100	Detergent (TOC)
		15.3	--	TC
	Wall	16.1	100	PYR, SUL
		16.2	100	Detergent (TOC)
		16.3	--	TC
	Pipe of transfer lid	17.1	78.5	PYR, SUL
Ball valve	18.1	156	PYR, SUL	
Butterfly valve	19.4	--	VC	
PIAB	Wall	20.1	100	PYR, SUL
		20.2	100	Detergent (TOC)
		20.3	--	TC
	Lid	21.1	153.9	PYR, SUL
Inlet pipe	22.1	212	PYR, SUL	
Turbostirrer	Impeller	23.2	85.8	Detergent (TOC)
Vibrating sieve	Screen	24.4	--	VC
200 L drum	Wall	25.1	100	PYR, SUL
		25.2	100	Detergent (TOC)
		25.3	--	TC
Betapress	Reject chute	26.2	49	Detergent (TOC)
	Off take chute	27.1	176	PYR, SUL
	Hopper	28.1	100	PYR, SUL
		28.2	100	Detergent (TOC)
		28.3	--	TC
	Granule feeder	29.1	91.9	PYR, SUL
Metal tray (feeder)	30.2	100	Detergent (TOC)	
Vacuum transfer pipe	Metal connections at inlet and outlet	31.1	78.5	PYR, SUL
		31.2	78.5	Detergent (TOC)
	Inner of pipe	32.2	--	Detergent (TOC)
		32.4	--	VC
Filter house	Chamber	33.4	--	VC
Packaging operations				
Alu-Alu Stripping Machine	Hopper	34.3	--	TC
	Feeding tray	35.1	100	PYR, SUL
		35.2	100	Detergent (TOC)
	Springs	36.4	--	VC
	Guiding unit	37.1	48	PYR, SUL
		37.2	48	Detergent (TOC)
	Vibrating tray	38.1	100	PYR, SUL
		38.2	100	Detergent (TOC)

*The sample number is described as: XX.Y, where XX is the serial number of the sampling point, Y means the notation for the test.

1= testing of active

2 = testing for detergent

3 = testing for total counts performed with agar plates

4 = visual check

**Notations used under “Test for” are abbreviations for testing of the following:

PYR = Pyrimethamine

SUL = Sulfadoxine

Detergent (TOC) = Detergent, analyzed for total organic carbon

TC = total counts

VC = visual check

6.2.7.5 Compiling the cleaning validation report

The average results for the four sampling campaigns were summarized in Table 4.8. All results obtained during validation for both chemical and microbiological samples were found below the set limits. This gives the confidence that the manual cleaning procedures are able, in routine working conditions, to consistently remove residues from equipment surfaces below the calculated limit. Based on these findings the cleaning procedures applied to the production equipment used for Fansidar manufacture were declared validated.

6.3 Conclusion

From the experience gathered during this project it can be concluded that in order to make a cleaning validation program manageable, especially for large manufacturing facilities, cleaning validation activities should not be undertaken in isolation, but rather as a part of the broader validation program set for the manufacturing facility. Another major learning was the fact that the success of the cleaning validation program depends on how thorough certain pre-requisite activities for cleaning validation are carried out. In this line the proper selection of cleaning agents and design/ revision of cleaning procedures are of great importance.

Documentation is another key factor of the cleaning validation program. The better the initial documentation structure is planned, the easier is to tackle individual projects later in the program. Having a comprehensive master plan is also very important as all the elements of the cleaning validation program are blended into and reflected by this document. The validation risk assessment performed in the initial phase of the project and periodic updates for it will offer the

piece of mind one needs that the cleaning procedures, when routinely applied not only for the cleaning procedures selected for validation but for any of the products manufactured, will provide the expected results and therefore ensuring product safety and quality characteristics.

The volume of analytical work involved by any cleaning validation project is usually very high. This makes the validation work time consuming and costly. Therefore elaborating meaningful sampling plans covering critical areas of equipment, rather than performing extensive sampling is of great importance. Also, the selection of the most suitable analytical methods would provide reliable results.

Calculating acceptance criteria is one of the most difficult tasks in cleaning validation. This is because various calculation formulas would provide various limits. One needs to put such limits in the practical context and select based on scientific rationale those limits providing meaningful and achievable cleaning results.

The existence of a documented change control system that tackles all the changes and deviations during validation is of utmost importance. Such a system allows for the assessment of the impact changes would have during validation and also in the future. This system points also at changes that would invalidate cleaning procedures and decides when re-validation should take place.

For a general view on how the elements of the cleaning validation program interrelates to each other a workflow diagram was elaborated for the manufacturing facility of Roche and is presented in Figure 6.4. This diagram is a working model that can be adopted by any manufacturing facility where cleaning validation is required.

The current cleaning validation program has sustained scrutiny from local regulatory authorities, the Medicines Control Council of South Africa, from third parties such as Bayer, South Africa as well as from Roche, Switzerland headquarters. After being thoroughly implemented in South Africa this cleaning validation model was further recommended for implementation to other Roche manufacturing sites and is currently being implemented by Roche Pharmaceuticals Shanghai, P.R. China in a facility which is dedicated to the manufacture of highly potent products, in fully contained systems.

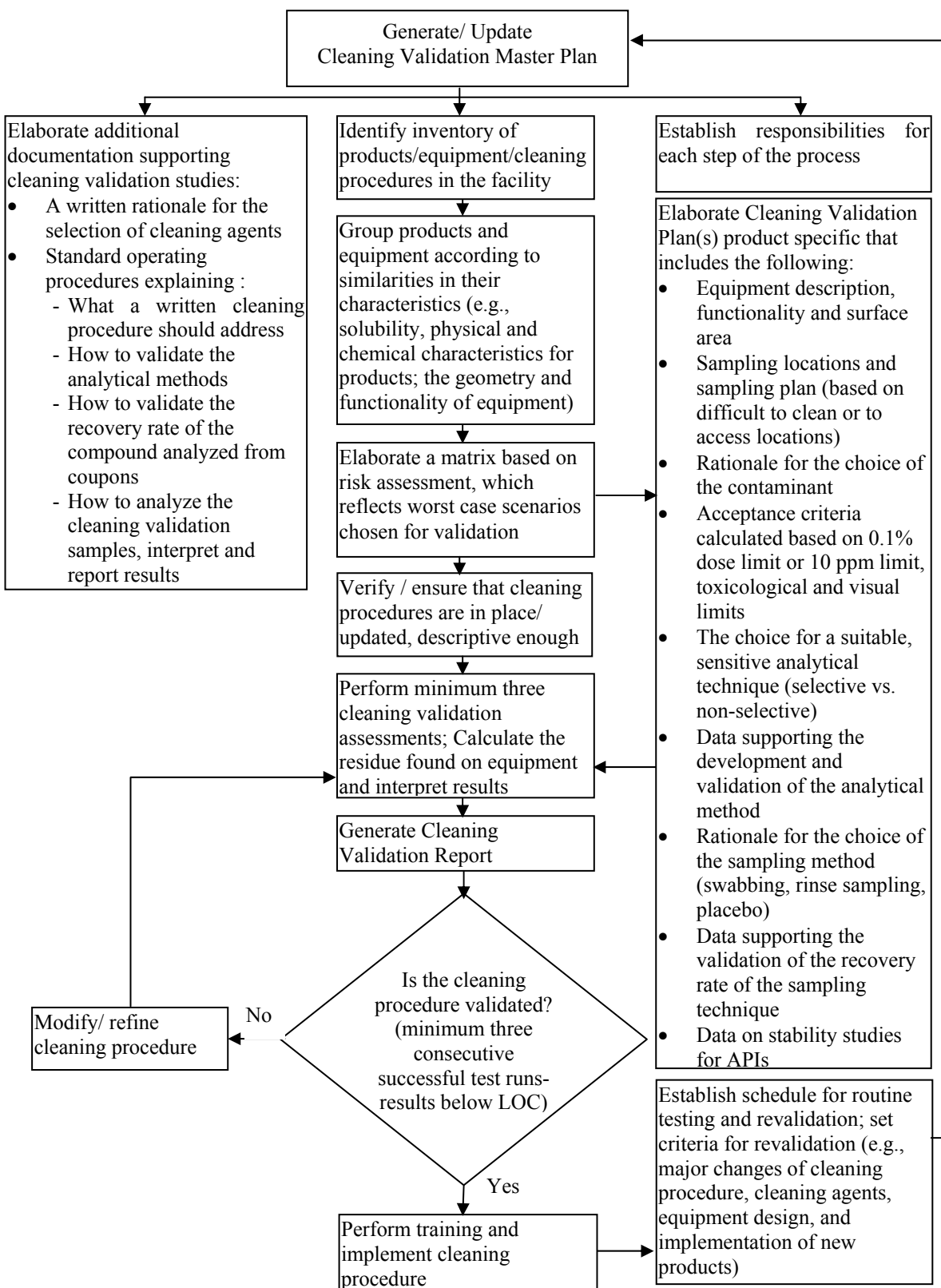


Figure 6.4 Flowchart expressing the relationships between the elements of the cleaning validation program.

Chapter 7: An overview on the validation approach for moist heat sterilization

7.1 Introduction

This chapter offers a working model for the qualification of steam sterilization equipment and the validation of its associated steam sterilization process. It is also intended to bring together the practical information one needs when validating an autoclave from procurement through routine use.

7.2 The sterility concept and sterilization principles

Sterility is defined as the absence of any micro-organism capable of reproduction (Bigelow, 1921). Sterility can be achieved through the destruction of all viable life forms, by applying a lethal agent to the item that must be sterilized. Several sterilization principles exist according to the lethal agent used (Soper and Davies, 1990). Each treatment destroys micro-organisms in a unique manner and with a different degree of effectiveness. A few examples of these treatments are:

- i) Heat sterilization with dry or moist heat;
- ii) Radiation sterilization with gamma- and X-rays, which produce positively charged ions in their passage through the matter, thereby further generating free radicals with lethal effects in the cells;
- iii) Chemical sterilization with glutaraldehyde, chlorine, iodine, hydrogen peroxide, quaternary ammonium salts, ozone, peracetic acid and phenolic compounds;
- iv) Gaseous sterilization with chemical agents such as ethylene oxide, propylene oxide and formaldehyde, all of which have a lethal effect that consists of the alkylation of proteins, DNA and RNA;
- v) Filtration sterilization which physically removes of the micro-organisms from the product by means of retentive filters.

New technologies have also been applied, such as the sterilization with free radicals generated by the combination of UV radiation with hydrogen peroxide, or sterilization with chlorine dioxide.

The sterility criterion is a mandatory requirement for parenteral products, ophtalmic preparations and products applied to injured skin or used to irrigate the body cavities. All products that must be manufactured as sterile products must pass the sterility test described by the pharmacopoeias.

When exposing homogenous populations of micro-organisms to a sterilizing agent, the microbial inactivation follows an exponential decrease curve (Equation 7.1). Mathematically, the inactivation of micro-organisms can be expressed as a first-order kinetic process. A finite probability of surviving organisms, independently from the magnitude of the delivered sterilizing agent, can be expressed as:

$$N_t = N_0 \times e^{-kt} \quad \text{Eq. 7.1}$$

in which N_t is the number of surviving organisms after time t , N_0 is the number of micro-organisms at time zero, (i.e., the bioburden), t is the exposure time and k is a microbial inactivation rate constant.

For a given process, the probability of survival is determined by the number, type and resistance of the micro-organisms present and by the environment in which the organisms exist during the treatment (e.g., moisture content, thermal energy and time for steam sterilization) (EP, 1997).

For pharmaceutical products, the term sterile is applied to products which have been treated in such a manner that, on completion of the process, individual items have a probability of being non-sterile or a sterility assurance level (SAL) equal to 1×10^{-6} or more (BP, 2001). Such a level of sterility assurance is required for terminally sterilized injectable products. This definition of sterility as a probability function does not assume that one in a million products is allowed to be non-sterile but “admits a finite statistical probability that a micro-organism may survive the sterilizing process” (EP, 1997).

7.3 Sterilization by saturated steam in the autoclave

When heat is used as sterilizing agent, the vibratory motion of every molecule of a micro-organism is increased to levels which induce the cleavage of intra-molecular hydrogen bonds

between proteins. Death is therefore due to an accumulation of irreversible damage to all metabolic functions of the organism (De Santis and Rudo, 1986).

Heat can either be used alone or mixed with steam. In the autoclave chamber, terminal sterilization is based on highly efficient heat transfer from the saturated steam to the autoclave load (Newsom, 2000). Heat transfer occurs by the release of the latent heat from saturated steam under pressure as it condenses.

Heat transfer has maximum efficiency if the steam is kept along the phase separation line of water-steam phase diagram. Heat transfer from saturated steam to the chamber environment is much more effective and timeless for the coagulation and denaturation of nucleic acids and proteins than from dry heat or superheated heat (Agalloco, 2000). Moist heat in the form of saturated steam under pressure is therefore the most reliable sterilizing agent and is the method of choice whenever it can be used, especially for aqueous preparations (Soper and Davies, 1990; Nagpal and Shriniwas, 1978).

7.4 Types of cycles used in moist heat sterilization

For saturated steam sterilizers, the physical parameters governing the efficiency of the sterilization process are exposure time, temperature and pressure. The last two parameters vary in a direct proportional relationship to each other.

Generally, a cycle comprises heating, sterilizing and cooling phases (ANSI 11134, 1993). The choice of a cycle for a particular load depends on the heat sensitivity of the load material and on the knowledge of heat penetration into the articles. Figures 7.1 and 7.2 illustrate the cycles typically used in the moist heat sterilization process.

For a saturated steam-vented cycle (Figure 7.1) the steam is injected at the top of the chamber and displaces the cold air that will exit through a trap. This cycle is recommended for containers filled with aqueous preparations (i.e., parenterals, opthalmics, media and buffers). The pre-vacuum cycle (Figure 7.2) ensures a more effective penetration of the load by saturated steam and is generally used for porous loads (e.g., surgical dressings and wrapped materials).

For moist heat sterilization, the accepted range of sterilizing temperatures is 118-134°C. The US Pharmacopeia (USP) explains in a footnote that “an autoclave cycle, where specified in the compendia for media or reagents, is a period of 15 minutes at 121°C, unless otherwise indicated” (USP24, 2000). The European Pharmacopeia (EP) and the British Pharmacopeia (BP) recommend a heating process at a minimum of 121°C for 15 minutes as a reference condition for aqueous preparations (EP, 1997; BP, 2001). The text of both books states that other conditions of time and temperature may be used provided that the process chosen has been satisfactorily demonstrated to deliver an adequate and reproducible level of lethality when operating routinely within the established tolerances (EP, 1997; BP, 2001).

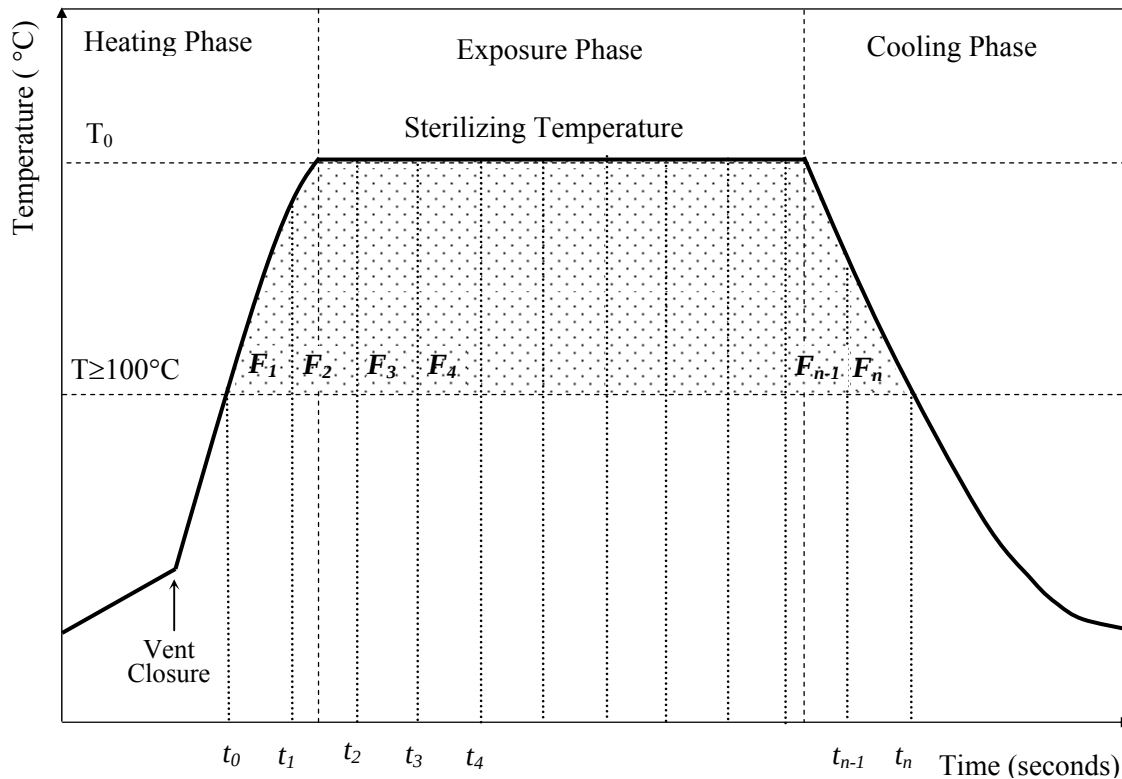


Figure 7.1 The temperature profile for a saturated steam-vented cycle. t_0 to t_n is the time interval accounted in the computation of F -value. F_1 to F_n areas represent the lethal rates in the product calculated for each discrete time interval. The shaded area under the curve obtained through graphical summation of F_1 to F_n -values represents the total calculated F -value per cycle. In the heating phase, saturated

steam is admitted into the chamber displacing the cold air until the exposure temperature and corresponding saturated steam pressure are attained. In the exposure phase, the sterilizing temperature is maintained in the chamber by saturated steam for the prescribed exposure time. The cooling phase can be achieved by slow exhaust (for containers filled with liquids) or fast exhaust (for goods required to be dry after sterilization). This phase is completed when the pressure in the chamber reaches atmospheric pressure.

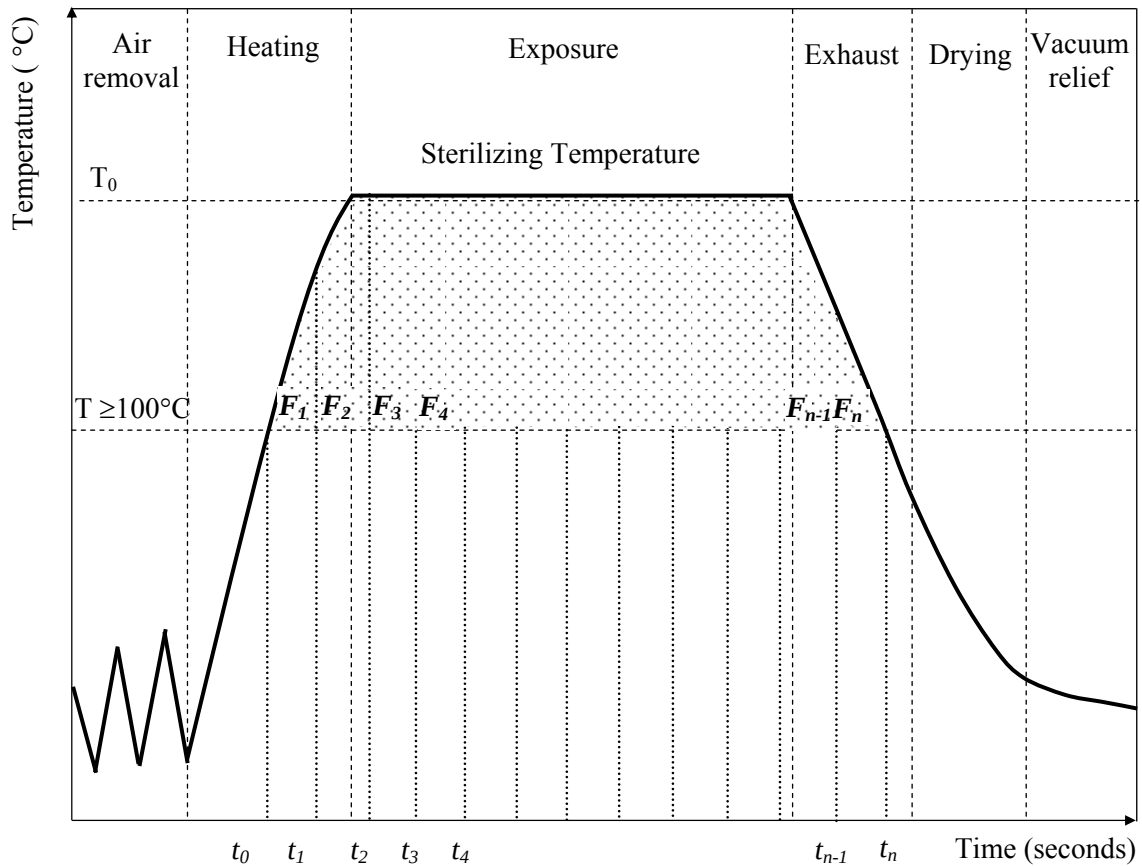


Figure 7.2 The temperature profile for a saturated-steam with forced air removal cycle. t_0 to t_n is the time interval accounted in the computation of F -value. F_1 to F_n areas represent the lethal rates in the product calculated for each discrete time intervals. The shaded area under the curve obtained through graphical summation of F_1 to F_n -values represents the total calculated F -value per cycle. In the air removal

phase, air is removed from the chamber and load by a number of vacuum pulses. In the heating phase, the cold air is displaced by saturated steam, which enters the chamber until the sterilization temperature and pressure are attained. In the exposure phase the sterilizing temperature and pressure are maintained in the chamber by saturated steam for the specified exposure time. In the exhaust phase steam is exhausted from the chamber until the atmospheric pressure is reached. In the drying phase, the temperature in the jacket and the vacuum in the chamber are maintained for a predetermined time period for products required to be dry. In the vacuum relief phase, air is admitted to the chamber through a microbiologically-retentive filter until atmospheric pressure is reached.

7.5 Development of sterilization cycles

Theoretically, the timing of the exposure phase begins when the temperature sensor placed in chamber drain (i.e., the coldest spot, theoretically) reaches the set sterilizing temperature. Experiments using thermocouples have shown that the temperature profile of the chamber under loaded conditions is different from the one obtained for the empty chamber. The rate of heating and cooling of a product in a container is a function of container type and size, viscosity for liquids, and the size and arrangement of the load (Soper and Davies, 1990). Therefore, to ensure sterilization efficiency, the timing for sterilization must begin after a certain equilibration time when the temperature of material being sterilized has reached the sterilizing temperature. This means that a lag time must be added to the cycle exposure time representing the time necessary for the coldest spot inside the load to reach the sterilizing temperature. The lag time must be determined experimentally and validated with each loading configuration that will be used in the autoclave for any further routine sterilization. The larger the containers and volumes to be sterilized are, the longer the lag time is.

The modern design of a sterilization cycle can be based on either the initial population of micro-organisms in the product examined during a suitable time period or on the measurement of the physical parameters attained in the load during the sterilization process. BP indicates that "sterilization methods must be validated with respect to both the assurance of sterility and integrity of the product and to ensure that the final product complies with requirements of the monograph" (BP, 2001). USP states that "the design or choice of a cycle for given products or

components depends on a number of factors described under the validation program” (USP 24, 2000) Three approaches are used in designing sterilization cycles: the bioburden approach, the overkill approach and a combination of the two approaches.

The bioburden approach is based on the determination of the number, type and resistance characteristics of the organisms comprising the bioburden (Pflug and Evans, 2000). For heat-sensitive products, the heat exposure has to be minimized in such a way to reach an optimum balance between an acceptable degree of sterilization and an acceptable stability of the product after its sterilization (PDA, 1978). In the bioburden approach, the process will provide less than 1×10^{-6} probability of micro-organisms survival. The sterilizing conditions used for the bioburden approach are less extreme than the conditions used for an overkill approach.

The design of a sterilization cycle can also be based on the heating characteristics of the containers located in the slowest heating zone of the load. The overkill approach stems from the concept that the sterilization process will inactivate a high microbiological challenge with an additional safety factor. The microbiological challenge will consist of a biological indicator with a specific number of micro-organisms ($10^3 - 10^6$), and a worst case assumption is made that the heat-resistance of bioburden is equivalent to that of the biological indicator. Therefore, the cycle conditions established are more severe than those required to inactivate the real product bioburden, and a theoretical spore reduction of 10-12 is expected to prove the overkill assurance. Considerably exceed the sterilization time is recommended for heat -resistant products (PDA, 1978). Therefore F0-values (see definition in the “Mathematical approach” section) in the range of 30-60 are to be expected.

The bioburden approach emphasizes the stability of the final product while the optimum sterility is achieved by knowing the characteristics of the bioburden; however, the overkill approach places the emphasis on sterility. A combination of the two approaches is sometimes useful to reach a balance between the maximum acceptable SAL and the maximum allowable concomitant adverse effect upon the material to be sterilized.

7.6 Measuring the efficiency of the sterilization process

Assessment of sterility is based on the demonstration of the absence of growth following the sterility test. Performance of the sterility test is not always a guarantee of sterility for a product (Hoet, 1997). Therefore, two methods that incorporate microbial inactivation data have been developed to measure, control and demonstrate the efficiency of a sterilization process.

7.6.1 Biological approach

In the biological approach known also as the *Latin approach* one determines the lowest probability to detect a non-sterile unit in a sterile load, and uses biological indicators (BIs) (Galtier, 1982). BIs are standardized preparations of micro-organisms specific to the type of sterilization process being studied. These BIs will be used to show a reproducible logarithmic inactivation. Many types of BIs consist of a given population of bacterial spores considered to be the most resistant forms against a particular sterilization principle.

In sterilization microbiology the *D*-value is frequently used instead of *k* as a measure of the rate of microbial death. Equation 7.1 can be formulated in a logarithmic manner as follows (see Eq. 7.2):

$$\log \frac{N_0}{N_t} = \frac{kt}{2.303} = \frac{t}{D} \quad \text{Eq. 7.2}$$

The *D*-value represents the temperature coefficient for the lethal process and is the exposure time in minutes required to cause a 1-logarithm or 90% reduction in the population of a particular micro-organism (ANSI 11134,1993; PDA, 1978). The resistance of an organism changes with alterations in temperature. The smaller the *D*-value, the more sensitive the organism is to the lethal agent.

The *z*-value is defined as the number of degrees of temperature required for a 1-logarithm change in the *D*-value. The *z*- and *D*-values are significant only under precisely defined experimental conditions and are generally assumed for aqueous solutions. For saturated steam sterilization, a BI containing spores of *Bacillus stearothermophilus* strain ATTC 7953 with a *D*-value higher than

1.5 minutes, a z -value of 10°C and a population higher than 5×10^5 is recommended (Pfeiffer, 2000). *Bacillus stearothermophilus* is considered the reference organism for saturated steam sterilization because it is known for its great resistance to the lethal agent when compared to the organisms contained the bioburden (BP, 2001). For a standard cycle, as recommended by BP, no growth should be recorded for the BI after an exposure time of 15 minutes at $121 \pm 1^{\circ}\text{C}$ and a post study incubation period.

USP states that for *Bacillus stearothermophilus* spores with a D -value of 1.5 minutes under total process parameters (i.e., 121°C), if they are treated for 12 minutes, the lethality input is $8D$ (USP 24, 2000). However, it is not specified that, after 15 minutes, no growth of the reference organism should exist.

For meaningful results, at least 20 BIs per cycle should be used to ensure that statistical distribution is differentiated from true deviations and to demonstrate homogenous conditions within the chamber autoclave. Although the biological approach presents the difficulties specific to statistical methods it allows the determination of lethality effect within the loads in which it is difficult or impossible to place temperature probes (e.g., inside ampoules).

7.6.2 Mathematical approach

The F -value is a unit of lethality and is a measure of the microbial inactivation capability of a heat sterilization process. It is defined as “the equivalent in minutes at 250°F of all heat considered with respect to its capacity to destroy spores or vegetative cells of a particular organism” (Soper and Davies, 1990) and allows the comparison of lethal effects at various temperatures. Sterilization cycle parameters can be found by using other temperatures than 121°C with the help of the F_0 concept. F_0 , as defined by USP 24 (2000) at a particular temperature other than 121°C , is the time, in minutes, required to provide the lethality equivalent to that which is provided at 121°C for a stated time. The temperature of 250°F or 121.1°C represents the temperature of saturated water vapor at $\sim 100 \text{ kPa}$. The F -value allows the comparison of lethal effects at various temperatures.

The total F -value of a process can be practically calculated by the integration of lethal rates with respect to time at discrete temperature intervals (Akers and Anderson, 1993.). Integrating the

lethality constant $10^{(T-T_0)/z}$ between two time points, t_0 and t_n , will yield the shaded area under the curves indicated in Figures 7.1 and 7.2.

The mathematical approach, also known as the *Anglo-Saxon approach* uses the F_0 -value as the reference unit of lethality. The F_0 -value can be calculated using Equation 7.3. Product - temperature data accumulated during the entire process are converted to the equivalent lethality at 121.1°C. For practical purposes, temperatures higher than 100°C are considered in calculations. It should be emphasized that the F_0 -value must be calculated at 121.1°C, not at 121°C, because the difference of 0.1°C introduces an error of 2.4% in the computation of the F_0 -value.

$$F_0 = \int_{t_0}^{t_n} 10^{(T-121.1)/z} dt \quad \text{Eq. 7.3}$$

In a non-mandatory section of the EP, General Text 5.1.5 offers guidance concerning the validation of the steam sterilization process by means of the F_0 concept, whose value can be determined from Equation 7.4 (EP, 1997). D_{121} is the exposure time at the indicated temperature required to cause a 90% reduction in the spore population. The text of BP states that the application of F_0 concept requires microbiological validation.

$$F_0 = D_{121} (\log N_0 - \log N_t) \quad \text{Eq. 7.4}$$

The lethality of the sterilization process is influenced by the effectiveness of heat penetration into actual articles and the time of sterilization. Pfeiffer, quoting BP 1983, indicates that the steam sterilization process must be sufficient to produce an F_0 -value of at least 8 minute equivalents (Pfeiffer, 1998). This means that the coolest location in the autoclave loading configuration must be exposed to an equivalent time of at least 8 minutes of exposure to a temperature of 121.1°C. This represents the most conservative estimate of the lethality and therefore the safest conditions for determining cycle times.

An F_0 of 8 minute equivalents is considered a realistic minimum value because most mesophilic spore-forming micro-organisms have D -values between 30 seconds and 1 minute at 121.1°C. When designing a cycle, one should introduce an additional safety factor that takes into account

the extra time that may be required for steam to penetrate certain containers in the middle or cool locations of the chamber. For practical purposes one should design sterilization cycles with an F_0 -value of 12 minute equivalents.

Depending on the nature of the load, one may use non-standard conditions and try to achieve an equivalent level of kill from new conditions (Pfeiffer, 2001). A high activation energy needed to kill spores can lead to chemical changes, especially in aqueous preparations (Soper and Davies, 1990). To avoid product degradation, a longer cycle at lower temperatures is recommended. For other products such as vitamin media that may suffer decomposition processes from a prolonged treatment at a lower temperature, it would be more advantageous to apply a short-time exposure at a higher-temperature (Dewhurst and Hoxey, 1990). To evaluate the effect of the killing process at a temperature different than 121.1°C the following equation can be used when calculating the lethality factor, F_T .

$$F_T^Z = \frac{F_0}{10^{(T-121.1)/z}} \text{ (min)} \quad \text{Eq. 7.5}$$

in which D - and z -values are known for a given micro-organism, and F is the effectiveness of killing at the specified process temperature, T (°C).

Table 7.1 presents equivalent combinations of temperature and time, providing the F_0 -value of 8- and 12- minute equivalents. The use of 110°C as sterilizing temperature requires very long exposure times to reach the target F_0 -values. Such exposure times would be unrealistic.

The biological and mathematical approaches are complementary to each other. Lethality using physical process data should be determined in conjunction with appropriate microbiological studies.

Table 7.1 Combinations of temperature and time calculated to provide F_0 -values of 8- and 12- minute equivalents based on reference spores of *Bacillus stearothermophilus* with a D_{121} of 1.5 minutes and a z -value of 10°C

Temperature ($^\circ\text{C}$)	Sterilization Exposure Time (minutes) for $F_0=8$ minute equivalents	Sterilization Exposure Time (minutes) for $F_0=12$ minute equivalents
110	103.05	154.58
115	32.59	48.88
118	16.34	24.50
121	8.18	12.27
124	4.10	6.15
126	2.58	3.88
130	1.03	1.54

7.7 Qualifying an autoclave and validating the sterilization process

Absolute sterility cannot be practically demonstrated without the complete destruction of the load. For practical purposes, the industry and the regulatory agencies approach the concept of sterility on a probabilistic basis. Validation must prove that the sterilization process delivers a treatment that ensures a certain statistical probability of sterility for an initially non-sterile unit. Therefore, any sterilization process must be validated before use and must be routinely monitored, as required by many official texts (EP, 1997; BP, 2001; PDA, 1978).

It should be emphasized that the terms qualification and validation are not identical. Equipment is qualified, and processes are validated. Qualification provides assurance that the autoclave can consistently kill a microbial load of 1×10^6 colony-forming unit (CFU) per container during a defined cycle, by using a specific loading configuration. The actual process validation of the cycles is performed by recording and interpreting the physical parameters (i.e., time, temperature, and pressure for steam sterilization) required to prove that the process will consistently yield a product that complies with previously established specifications.

During routine monitoring, the assurance of sterility is gained by demonstrating that validated conditions, known to produce the required level of microbial inactivation, have been attained.

Therefore, the careful design and validation of the sterilization process enables sterility to be addressed with an increasing probability of success (Pflug and Evans, 2000). Such a system is called “parametric release” and has been defined as a “sterility release procedure based upon effective control, monitoring, and documentation of a validated sterilization process in lieu of release based upon end-product sterility testing” (CFR, 1989).

7.8 The qualification - validation procedure

As recommended by American National Standard Institute (ANSI 11134, 1993), the qualification - validation of a moist heat sterilization process involves:

- i) qualification of the autoclave by checking its performance against the design specifications;
- ii) validation of the process by establishing the actual effectiveness and reproducibility of the cycle in relation to the product and the loading configurations;
- iii) assessment of possible changes in the product that could have occurred during sterilization.

A typical qualification program is outlined in the USP 24 (2000). However, choosing a particular qualification approach depends on the type of autoclave, its actual condition (whether it is new or refurbished), the critical parameters controlling the process, the geometry of the chamber, the type of goods that are to be sterilized and the SAL required. Therefore, before the qualification process is started, the user must become acquainted with the type of the autoclave and its characteristics. It is important to assemble a validation team consisting of at least a user representative and a member of the quality assurance division.

After the decision has been made to qualify an autoclave, the process of collecting information must be performed in a logical manner. Because extensive literature about this topic is available, one should be selective when consulting information sources and retrieve only the type of information applicable to the appropriate sterilizing principle and the operating parameters of the sterilizer being validated.

The first step in collecting information is to consult national standards. If these are not available, then one should consult international standards. Because validation cannot be performed in

general, the type of materials that will be routinely sterilized should be considered at all times when setting objectives for validation (British Standard 2646, 1993). In accordance with the purpose of the validation, scientifically sound acceptance criteria that can be accurately evaluated by monitoring the physical parameters of the process should be established.

The qualification -validation procedures should be performed using approved protocols developed before the procedures are initiated (CDER, 1994). A description of the autoclave process, cycle types, parameters and performance specifications must be completed. The protocols should clearly state the objectives of the validation and define the scope of the activities for each stage of the process. Time frames and general acceptance criteria should also be part of the protocols. Each stage of validation must be documented and approved.

Qualification -validation consists of a series of tests, each of which includes an objective, a method, acceptance criteria, and test results. The responsibility for testing must be assigned to either the manufacturer or user, agreed on, and documented.

To perform a proper validation, several items are required such as a temperature recorder (data logger) that can record and accumulate temperature data collected by thermocouples, a calibrated thermometer, a computer that can analyze raw data and compute F0-values (Trotter, 1996). A laminar-flow hood, biological indicators (BIs), access to an incubator (55-60°C), a temperature bath and ice bath for accuracy verification of the thermocouples also are compulsory elements.

7.8.1 Commissioning or pre-installation qualification (IQ) phase

As a pre-requisite to qualification of both new and refurbished autoclaves, a user requirement specification document containing key equipment requirements should be developed and approved. Referring to this document helps prevent purchases of unsuitable and inappropriate equipment that can be difficult to qualify for the intended purpose. After its approval and authorization, the document must be passed to relevant vendors before receipt of quotes or offers for work.

Commissioning is the first step of the qualification (Ransdell, 1996) and consists of obtaining the evidence that equipment has been provided and installed in accordance with its specifications and

that it functions within predetermined limits when operated as directed by its instructions. The company installing the unit must deliver and commission the autoclave and must offer technical support to the owner until the autoclave is in proper condition for use. Performing a proper commissioning saves time and money and provides the necessary confidence in the performance of the equipment.

7.8.2 Installation qualification (IQ) – operational qualification (OQ) phase

The IQ and OQ phases can be instituted separately or simultaneously. During the IQ and OQ phases, various features of the autoclave are examined and tested for proper functioning. This procedure includes tests of the chamber design, pressure vessel, and door safety interlock system and inspections of the chamber jacket, steam traps, electrical circuits, pressure and temperature indicators, vacuum pumps, vent filters, and steam supply to the chamber.

Usually the IQ document indicates the location of the documentation for equipment (which must include a checklist containing specifications and identification of equipment or components by description, model, and serial number), the instruction manual, the troubleshooting and preventive maintenance schedule, the electrical and piping drawings, and the diagram of the programmable logic controller (PLC).

7.8.2.1 Calibration of command and control instruments

During the IQ phase, the calibration of all critical instruments – process controllers, temperature sensors, pressure gauges, timers, measuring and recording instruments - should be performed. It is also important to know the degree of accuracy of the instruments used to calibrate the gauges. The accuracy level of the calibrating instruments must exceed the accuracy level of the autoclave's control, measuring and recording systems (ANSI 11134, 1993; British Standard 2646, 1993a). Likewise, the control, measuring and recording instruments must have an accuracy level that fits the purpose of the autoclave. When the desired level of accuracy is not known, standards must be consulted.

The performance of calibration test instruments should be traceable to national reference standards. All calibration procedures must be fully documented. Calibrations must be completed before any other validation tests are started. The calibration can be performed either before

installation of the unit or in situ after the unit is placed in its operating site. The advantages of calibrating in situ include the ability of a sensor or gauge to provide accurate information about the environment that the unit is monitoring when compared with a known standard (Ransdell, 1996). This type of calibration is more rugged and provides a more accurate image of the process being monitored.

7.8.2.2 Requirements for thermocouples

To monitor temperatures attained at different locations throughout the chamber, temperature-measuring devices, also called thermocouples are used. The thermocouples are connected to computerized multi-channel recording systems that can record and print temperature data. For validation purposes, type T thermocouples are recommended, because they are stable throughout a wide temperature range. Depending on the chamber size, 10 - 20 thermocouples must be used per cycle. Thermocouples should not be placed into the chamber through the door gasket because they can be easily damaged when loading or unloading the chamber, which can also create a leak that will affect the normal working conditions of the autoclave. One should place the thermocouples in the autoclave chamber by means of a feed-through assembly connected to a suitable port. After purchasing a new autoclave, this port must be documented during the IQ phase.

One must also verify the accuracy of the thermocouples to traceable secondary standards at two significant temperatures (e.g., the freezing and boiling point of water) at the beginning and at the end of each phase of the qualification. This procedure can be performed by immersing the thermocouples and a reference calibrated thermometer in water maintained at the previously mentioned temperatures and monitoring the temperature values of both thermocouples and the reference thermometer for a certain period of time. Accuracy of the thermocouples should be at least $\pm 0.5^{\circ}\text{C}$. Temperature accuracy is especially important in steam sterilization validation because an error of just 0.1°C in temperature measured by a faulty thermocouple will produce an error 2.4% in the calculated F0-value (Akers and Anderson, 1993). All inaccurate thermocouples should be discarded.

One thermocouple must always be located next to the autoclave temperature probe, which is usually placed in the chamber drain. The reason for this is that, theoretically, the “cold spot” of a

chamber is in the drain. Depending on various chambers' geometry, sometimes the location of the cold spot in the chamber drain is not confirmed in practice.

The OQ phase consists of a verification of the equipment functionality. A series of checks and tests should be performed after the unit is installed. The proper operation of alarms and safety devices also must be tested. For a prevacuum cycle involving an air-removal phase, the attainability, effectiveness, and consistency in time of vacuum conditions must be tested.

Chamber mapping consists of heat distribution studies throughout the chamber for each sterilizing temperature used. Heat distribution studies determine during processing whether the temperature is uniform and reproducible throughout the empty chamber and localize the cold spots within the chamber. These studies also confirm that the system works within the specified limits throughout the entire range of operational parameters.

The sterilizing temperatures are programmed into the autoclave controls. For each sterilizing temperature being validated, the empty chamber should be monitored three times during a period of one hour, and the reproducibility of the results obtained must be compared. The comparative readings of the reference temperature sensor, the thermocouple near the temperature sensor and the temperature displayed by the autoclave recorder should be $\pm 1^{\circ}\text{C}$. During the exposure phase, a difference not greater than $\pm 2.5^{\circ}\text{C}$ between the temperature of the chamber's coolest spot and the mean chamber temperature is acceptable (Akers and Anderson, 1993).

7.8.3 Performance qualification (PQ) phase

PQ represents the confirmatory phase of the validation program and consists of tests performed with the autoclave chamber under loaded conditions. During the PQ phase, which is sometimes referred as process validation, the following objectives must be attained (ANSI 11134, 1993):

- i) demonstration of the uniformity and effectiveness of the process in inactivating or removing micro-organisms to the required safety level ;
- ii) demonstration of the reproducibility of the process - through the use of sufficient cycles;
- iii) Demonstration of the compatibility of the process with the items to be sterilized – through the assessment of the influence of the sterilization process on the products.

The PQ phase consist of studies that use thermocouples inserted into the articles being sterilized and studies that uses BIs in operationally fully loaded autoclave conditions (PDA, 1978).

Heat penetration studies are considered the most critical component of the entire validation program. These studies are intended to find areas of the loads that are difficult to penetrate or heat. When selecting the monitoring sites, one must take into account the cold spots previously found during the monitoring of the empty chamber.

Heat penetration studies must demonstrate the reproducibility of a cycle in relation to the loads and the effectiveness of the killing effect throughout the chamber and load. Selecting loading configurations must be performed very carefully and should be based on worst case situations. The maximum number of items in the loads for the most critical or used products should be decided. Ideally three consecutive validation runs should be performed per loading configuration. During testing, if some configurations cannot be successfully sterilized because they are too big the worst case in regard to the size of the loading configuration should be redesigned. If the results are still unsatisfactory, then the cycle should be redesigned, which usually can be achieved by modifying the exposure time.

Heat penetration results must show the slowest heating points in the load and must ensure that the minimum time and temperature requirements are met. The success of a qualified cycle depends on the determination of F0-value measured inside the item located at the coldest spot. It should be confirmed that a minimum prescribed F0-value is delivered consistently through the autoclave chamber.

Microbiological validation, which consists of tests involving BIs, should be performed concomitant with the penetration studies, to verify independent of temperature data results that the minimum F0 is met at the coldest spot of the load. For terminal moist heat sterilization, heat penetration studies must demonstrate a SAL of 1×10^{-6} or higher. The BIs should be placed in operational, maximum loads at the locations presumed to be the least accessible to the sterilizing agent. One should place the BIs next to thermocouples and inside the item being tested. They must not be placed directly on surfaces or outside the loads because the heating process is rapid in those areas and the BIs may get killed faster. The number of BIs used per load depends on the size and the complexity of the load. For statistical significance, tests with BIs that are performed

during the PQ phase should be conducted at least three times per loading configuration. After sterilization, the BIs should be incubated for seven days and checked daily for growth. As a part of the documentation, information provided by the manufacturer concerning the spore lot number (i.e., D- and z-values for the current lot) must be available.

Demonstrating the integrity of the product after sterilization represents another aspect of process validation. The product must be tested for possible physical and chemical changes that may have occurred during sterilization. In this regard, the pH of solutions and the physical condition and appearance of goods before and after sterilization must be verified. For culture media, tests for growth promotion that are recommended by USP should prove whether the culture media shows growth following the sterilization process.

7.9 Qualification - validation report

The final stage of the validation program requires the documentation of all acquired data. The qualification -validation report summarizes the overall results of validation. It includes the calibration certificates for calibrating instrumentation, calibration records, and methods for calibrating the measuring instruments, gauges, and recorders as well as the accuracy verification data of thermocouples (CDER, 1994). Test data such as high -low temperature ranges, average temperatures during exposure time, minimum and maximum F0-values achieved for every load configuration, run date and time, and autoclave records must be included.

The qualification -validation report is not complete unless it contains evidence checks for the availability of an instrument logbook, the SOPs used with the autoclave, procedures for preventive and unscheduled maintenance and re-calibration programs. The validation report also should include the user's training records. Training should begin only after the validation has been accomplished to ensure that users will implement already-validated loading configurations.

The qualification - validation report must include the drawings of all loads tested. The location of the thermocouples and BIs must be specified in each drawing. Any specification deviations that were encountered during validation activities and the procedures that followed their discovery should be reviewed. After the validation activities are completed, all data and documents that were accumulated must be revised, approved and certified by both the owner of the autoclave and

the contractor in cases when the validation was performed in collaboration with third parties or by the manufacturer.

Once the autoclave is qualified and the loading configurations are validated, the following documents must be present in the laboratory at all times:

- i) diagrams of various loading configurations stamped with their effective dates;
- ii) a list of the determined lag times determined for each loading configuration;
- iii) temperature - pressure recorder printouts;
- iv) user, calibration, and maintenance SOPs.

7.10 When requalification - revalidation activities are required

Once a unit has been installed and qualified, it does not need requalified unless the unit is reinstalled in another location or has undergone major modifications. Only revalidation is performed periodically. Generally, the extent of requalification -revalidation depends on the nature of the changes that occur and how they affect the already-validated sterilization cycles. The responsibility for deciding which validation activities should be repeated must be assigned. Validation frequency must be stipulated in the validation documents. The need for possible revalidation should be assessed every 12 – 24 months, and revalidation should occur whenever major repairs have been performed.

Any modifications to control systems should be evaluated to confirm that the process conditions delivered to the load are comparable to those originally validated. However, the validation should be repeated whenever new sterilization cycles, loading configurations, or major changes in the sterilization procedure are introduced.

7.11 Change control

A system for change control should be implemented that establishes when the qualification - validation should be repeated (ANSI 11134, 1993). The nature of the change must be assessed to determine the potential effects on the cycles and load. Changes that could affect the sterilization conditions are not allowed be introduced without documentation. The change control procedure must follow a change control SOP.

Changes in any of the following items can invalidate an autoclave validation: pressure gauge, temperature gauge, control panel programming, temperature control valve, steam trap and steam source. Changes introduced by preventive or unplanned maintenance procedures must be evaluated and documented in regard to a decision for revalidation.

During routine use, personnel should ensure that the process specifications established during qualification testing are followed and remain valid. The critical parameters governing the process must be monitored routinely to confirm that previously determined conditions are achieved.

7.12 Estimation of probability of non-sterility of a load during the validation of steam sterilization process

7.12.1 Introduction

Qualification - validation activities were performed for a steam sterilizer, model 2020, from Amsco AHSC – South Africa (Pty) Ltd. The following example demonstrates the validation of a saturated steam– vented cycle used with a load containing 50 of 250 ml bottles filled with 200 ml trypticase soy broth (TSB). TSB is a heat–sensitive medium that is prone to alteration during the sterilization process. Therefore, the parameters for sterilization should be carefully chosen. The microbiology laboratory referred to here uses the sterilized broth for microbiological testing of nonsterile products and stab cultures.

7.12.2 Materials and Methods

As recommended by pharmacopeias (EP, 1997; BP, 2001; USP24, 2000) the reference sterilization conditions for liquid media (121°C for an exposure of 15 minutes) were tested. A preliminary experiment was performed to determine the lag time characteristic for this loading configuration. A simulation load comprising 50 bottles filled with 200 ml tap water was used first.

To monitor the temperatures attained in the chamber under loaded conditions, 9 thermocouples were placed in various locations within the chamber, and another 10 were placed in the middle of the liquid contained in the bottles. The placement of the thermocouples in the chamber and the

loading configuration are presented in Figure 7.3, which shows the overhead view of the autoclave chamber.

Timing the lag time began when the thermocouple placed in the drain next to the sterilizer temperature probe reached the sterilizing temperature. Timing stopped when the last thermocouple placed in the load reached the sterilizing temperature.

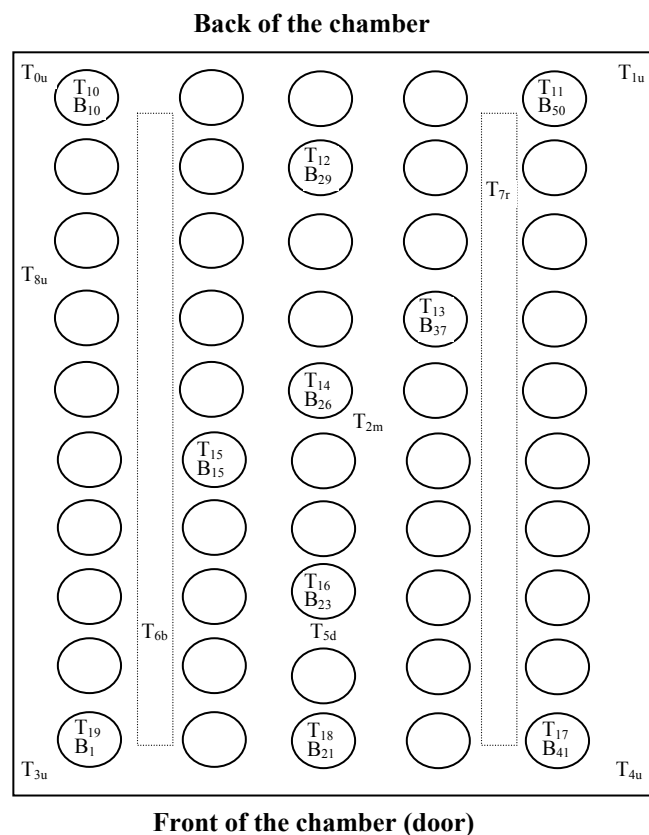


Figure 7.3 Representation of the autoclave chamber (overhead view). B is the number of the BI ampul, and T is the thermocouple number. Heat-distribution thermocouples are marked with a number and letter indicating their position in the chamber: u = upper position, m = middle position, b = bottom position, r = under the rail, d = in the chamber drain. T₈ was placed in the piping heading to the pressure gauge. The dotted rectangles represent the two rails of the autoclave chamber. The circles represent the 50 bottles, some of them containing thermocouples and BI ampuls.

The heat penetration studies were conducted concomitant with the BIs studies. An ampul containing a population of $\sim 10^6$ spores of *Bacillus stearothermophilus* was placed next to each thermocouple inserted into the load. In the loading diagram (See Figure 7.3) the positioning of the thermocouples and BIs is indicated by the letters T and B. For the ampuls, the number marked on each of the 50 bottles was assigned. The sterilized ampuls were incubated afterwards together with positive controls (nonsterilized ampuls) for seven days at 55-60°C, and were checked daily for growth. As a means to verify the sterilization efficiency, 5 of the 50 bottles containing TSB broth were incubated after sterilization for five days at 32°C. The broth was checked daily for growth.

To check the effectiveness of the culture medium after its sterilization, the TSB was tested in accordance to the USP Growth Promotion Test (USP 24, 2000). The broth was tested for growth in the presence of *Bacillus subtilis*, ATCC 6633 spores and incubated at 37°C for 48 hour.

Another means for verifying the integrity of the physical properties of the broths and buffers is to measure their pH values before and after the sterilization. For TSB a pH of 7.3 ± 0.2 should be obtained after its preparation.

7.12.3 Results

For the simulation load, a lag time of ~ 13 minutes was measured. The TSB has a slightly higher viscosity than tap water. A lag time of 15 minutes was considered sufficient to be added to the required exposure time of 15 minutes.

Temperature profiles recorded by using thermocouples inserted into the simulation load have indicated that during the exposure phase the temperature of the load is slightly below the set temperature of 121°C. In order to achieve the prescribed sterilization temperature of 121°C inside the load, the set temperature of the PLC was raised to 122°C. Therefore the experiments for the validation loads were performed with the new set parameters of 122°C and 30 minutes.

As an example, Figure 7.4 shows the middle portion of the temperature profiles achieved within the autoclave chamber, in the chamber drain, and inside the load during the second run.

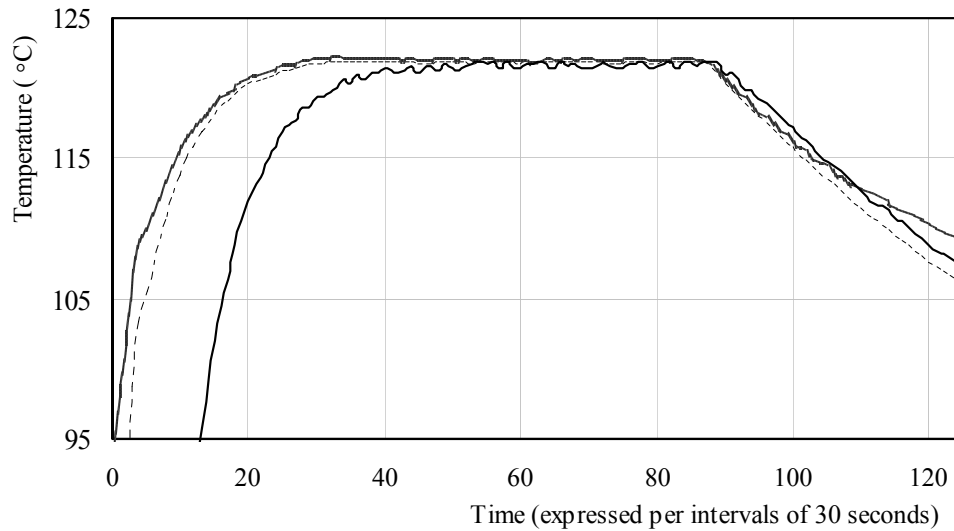


Figure 7.4 Representation of the middle portion of the temperature profiles in the chamber recorded during the second run.

- Profile of average temperatures recorded by the heat distribution thermocouples;
- Temperature profile recorded by thermocouple placed in the chamber drain;
- Profile of average temperatures recorded by the heat penetration thermocouples placed in the bottles filled with broth.

The data acquired by the heat distribution thermocouples during four runs are summarized in Table 7.2. The data acquired by heat penetration thermocouples introduced into the bottles filled with broth during four runs are summarized in Table 7.3.

When observing the mean temperature values per run calculated for heat distribution (see Table 7.2), one will note that position 4 is the hottest spot in the chamber, and positions 6 and 7, situated under the rail, can be considered as the coldest spots within the chamber. Analysis of the data with the t-test indicated no statistically significant differences in mean temperature values at a 95% level of confidence between the drain location and any of the arbitrarily chosen points in the chamber, with the exception of thermocouple T3, which is situated on the top corner of the chamber farther from the steam inlet. This fact is not critical because loads will not be placed in the top corner.

Table 7.2 Temperature data interpretation for a heat distribution study during exposure phase

Function	Run Number	Values within the Run									Values across the Run
		T ₅ (Drain)	T ₀	T ₁	T ₂	T ₃	T ₄	T ₆	T ₇	T ₈	
Minimum temperature	1	120.43	121.87	121.85	121.37	121.79	121.71	121.53	121.62	121.63	120.43
	2	121.71	121.81	121.82	121.77	121.92	121.81	121.39	121.58	121.78	121.39
	3	121.35	121.91	121.92	121.84	121.75	121.71	121.53	121.62	121.70	121.35
	4	121.85	122.09	122.07	122.01	122.02	122.10	121.66	121.29	121.83	121.28
Maximum temperature	1	123.27	122.02	122.02	121.96	122.16	122.14	121.92	122.05	122.03	123.27
	2	121.91	122.13	122.02	121.98	122.15	122.20	122.02	122.07	122.25	122.24
	3	122.37	122.22	122.14	122.08	122.30	123.45	122.00	122.12	122.16	123.44
	4	122.16	122.44	122.42	122.34	122.35	122.46	122.16	122.83	122.39	122.82
Mean temperature	1	121.76	121.95	121.93	121.87	121.99	121.98	121.73	121.82	121.81	121.89
	2	121.84	121.97	121.94	121.89	122.06	122.02	121.72	121.85	122.03	121.92
	3	121.88	122.05	122.03	121.96	122.15	122.18	121.77	121.85	121.93	121.98
	4	122.00	122.21	122.19	122.11	122.12	122.22	121.89	121.94	122.01	122.08
Mean of the four runs		121.87	122.05	122.02	121.96	122.09	122.10	121.84	121.84	121.95	--
Standard deviation for the means	1										0.09
	2	0.10	0.12	0.12	0.11	0.06	0.11	0.12	0.08	0.09	0.10
	3										0.13
	4										0.12
t-value	1										--
	2	--	0.07	0.10	0.28	0.01	0.02	0.71	0.69	0.30	0.59
	3										0.15
	4										0.002

The heat penetration temperature data (see Table 7.3) indicate positions 11 and 17 as the hottest spots and positions 14 and 19 as the coldest spots in the chamber. The temperature data recorded at various points in the load proved not to be statistically different from each other at 95% level of confidence.

Temperature readings taken every 30 seconds were used for the computation of F_0 -value (Akers and Anderson, 1993). The value was calculated with the Equation 7.6.

$$F_0 = \Delta t \sum_{1}^n F_n = \Delta t \sum_{1}^n (10^{(T_n - 121.1)/z}) \quad \text{Eq. 7.6}$$

in which $\Delta t = (t_n - t_0)$.

The sterilizing effects for temperatures higher than 100 ° C attained during the total set time of the cycle, including the entire exposure phase and portions of the heating up and cooling phases, were considered in the calculations. The time at which the autoclave temperature probe reached the sterilizing temperature was considered as t_0 and therefore the timing of the cycle started. The lethality values (see Table 7.4) were analyzed to determine the 95% confidence interval for the mean F_0 -value per individual run.

Table 7.3 Temperature data interpretation for a heat penetration study during exposure phase

Function	Run Number	Values Within the Run										Values across the Run
		Thermocouple Numbers for Heat Penetration										
		T ₁₀	T ₁₁	T ₁₂	T ₁₃	T ₁₄	T ₁₅	T ₁₆	T ₁₇	T ₁₈	T ₁₉	
Minimum temperature	1	120.26	120.91	121.05	120.76	120.69	120.86	120.57	120.67	120.91	121.15	120.26
	2	120.84	121.35	121.04	120.80	121.08	120.73	120.80	120.87	121.07	121.14	120.73
	3	120.62	120.95	121.18	120.96	120.86	120.92	120.45	121.15	120.22	120.87	120.21
	4	120.81	121.48	121.31	121.33	121.19	121.38	121.27	121.20	121.14	121.23	120.81
Maximum temperature	1	123.10	122.33	122.20	122.31	122.66	122.34	122.49	122.52	122.28	121.92	123.10
	2	122.35	121.92	122.11	122.31	121.99	122.40	122.10	122.17	122.04	121.98	122.40
	3	122.83	122.27	122.01	122.04	121.91	122.09	121.85	121.71	122.40	121.84	122.83
	4	122.91	122.14	122.26	122.19	122.32	122.20	122.37	122.27	122.30	122.25	122.90
Mean temperature	1	121.67	121.68	121.69	121.61	121.64	121.60	121.53	121.55	121.54	121.56	121.61
	2	121.53	121.63	121.55	121.53	121.56	121.57	121.56	121.63	121.64	121.63	121.58
	3	121.63	121.62	121.54	121.53	121.45	121.54	121.33	121.52	121.33	121.39	121.49
	4	121.58	121.77	121.81	121.81	121.84	121.83	121.82	121.84	121.81	121.82	121.79
Mean of the four runs		121.60	121.67	121.64	121.62	121.62	121.63	121.56	121.63	121.58	121.60	--
Standard deviation for the means	1											1.04
	2											0.04
	3	0.06	0.06	0.12	0.13	1.66	0.13	0.2	0.14	0.19	0.17	0.10
	4											0.07
t-value	1											--
	2	--	0.17	0.57	0.83	0.4	0.69	0.71	0.69	0.83	0.98	0.35
	3											0.007
	4											0.00002

Table 7.4 Accumulated F_0 -value interpretation for heat penetration study

Function	Run No.	Thermocouple Number										Mean F_0 per Run	St. Dev. per Run	Std Error of the Mean	95% Confidence interval		t-value across the Runs
		T_{10}	T_{11}	T_{12}	T_{13}	T_{14}	T_{15}	T_{16}	T_{17}	T_{18}	T_{19}						
F_0	1	25.82	25.04	24.92	23.86	24.50	24.35	23.76	24.42	24.42	24.51	24.56	0.59	0.18	24.14*	24.98*	--
	2	23.95	24.81	24.01	23.50	24.20	23.91	24.25	24.80	24.90	25.12	24.35	0.53	0.16	23.97*	24.73*	0.4
	3	23.85	24.55	24.26	23.96	23.03	24.42	22.02	24.08	22.03	22.69	23.49	0.96	0.31	22.80*	24.17*	0.009
	4	24.68	26.05	26.13	26.14	26.05	26.43	25.65	26.32	25.15	26.06	25.87	0.55	0.17	25.47*	26.26*	0.00008
Mean of the four runs		24.58	25.11	24.83	24.36	24.45	24.78	23.92	24.91	24.13	24.60	24.57	1.08		24.23**	24.90**	
Standard deviation for the means		0.90	0.66	0.95	1.20	1.24	1.12	1.50	0.99	1.43	1.42						
t-value		--	0.38	0.71	0.78	0.87	0.79	0.49	0.64	0.62	0.98						

* The confidence interval per run was calculated considering t for 0.05 (95%) confidence level = 2.262 (N-1=9 degrees of freedom).

** The confidence interval across runs was calculated considering the critical value for 95% level of confidence =1.96

The SAL achieved was determined for each of the four runs. The calculated lower limit of the 95% confidence interval was introduced in Equation 7.4 indicated by EP (1997) for the calculation of F_0 -value. In rearranging equation 7.4, one can define a probabilistic term P as the absolute value of N_t expressed as:

$$P = N_t = \text{antilog} \left(\log N_0 - \frac{F_0}{D_{121}} \right) \quad \text{Eq. 7.7}$$

To calculate the probabilities of non-sterility of the product, the equations obtained were solved for N_t .

As indicated in the certificate of conformity provided by the manufacturer for the *Bacillus stearothermophilus* spores, D_{121} was considered 1.5 minutes and N_0 as 10^6 spores per vial. The results of the N_t calculated at the lower limit of the 95% confidence interval are shown in Table 7.5.

Table 7.5 Sterility assurance levels (SALs) attained for each run

Run No.	F_0 values representing the calculated lower limit of the 95% confidence interval	SAL
1	24.14	$10^{-10.09}$
2	23.97	$10^{-9.98}$
3	22.80	$10^{-9.2}$
4	25.47	$10^{-10.98}$

It can be concluded that, when reproducible sterilization conditions are applied to the studied load, a 95% level of confidence exists that the probability of a single organism's survival will be $10^{-9.2}$ or better.

7.12.4 Discussion

The previously described approach is based on a worst case assumption that a population of 10^6 spores per item exists and must be sterilized and that the micro-organisms in the bioburden have the same heat resistance as the spores of *Bacillus stearothermophilus*. However, achieving a SAL

of 10^{-12} is not necessary for this situation because the raw materials used for the preparation of the broth are pure and possess a low bioburden.

An adequate SAL also was indicated by the results of the experiment using BIs. After sterilization, which was followed by seven days of incubation at 55-60°C, all the spores contained in the ampuls were killed. The positive controls have shown growth after the incubation period.

The visual examination of the broth after five days of incubation indicated no growth. A bottle containing unsterilized TSB was incubated as positive control, and the medium turned turbid after the incubation period. This confirmed the conclusion that the sterilization process was efficient.

The effectiveness of culture medium after its sterilization was also confirmed by the growth promotion test. After its sterilization, the broth, spiked with *Bacillus subtilus* and incubated for 48 hours at 37°C, showed a positive spore growth. The fact that the medium turned turbid after the prescribed incubation period confirmed that its stability and chemical composition were not altered through the sterilization process and that the medium still supports growth when spiked with a suitable micro-organism.

Four pH measurements for the sterilized broth were performed. All pH values ranged between 7.15 – 7.17, which confirmed the preservation of the initial physical and chemical properties of the broth after its sterilization. Also, a visual test performed by three independent observers confirmed no color changes or precipitation phenomena in the broth after its sterilization.

It can be concluded that, for the studied load, the study's objectives for the PQ phase have been fulfilled. Monitoring the physical parameters attained during the sterilization phase revealed an even distribution of temperatures in the chamber during the run, even penetration at all points within the load, and reproducible conditions across three of the four runs. The second run was significantly different from the first, but a SAL of $10^{-9.98}$ was attained. This ensured compliance with the BP which states that a SAL equal to 10^{-6} or higher must be achieved (BP, 2001).

The conditions used to sterilize the load are halfway between an overkill approach and the bioburden approach. They provide an optimum balance between an acceptable degree of sterilization and an acceptable stability of the broth after sterilization. The conclusion for the

discussed step of qualification is that the autoclave works properly within its functional parameters in four consecutive runs, providing a SAL of $10^{-9.2}$ or higher.

7.13 Conclusion

Although the validation approach follows several standard steps, exact methods for validation cannot be offered because various autoclave types exist and each unit has specific operating parameters. Practical examples and requirements for validation were offered in the article for readers who may be inexperienced at qualifying steam sterilizers. Some of the principles presented can be applied to other sterilization procedures.

As long as the strategy for validation is based on a logical, systematic approach, and meaningful tests are performed to prove the suitability of the autoclave for its intended use, the validation can only be successful. A key factor for a comprehensive validation is the meticulous documentation of all activities performed during the process. Documentation provides a comprehensive review of validation results that is essential for acceptance by the quality control unit and also serves to satisfy the scrutiny of regulatory bodies.

Chapter 8: Final conclusions

The need to qualify manufacturing or analytical equipment as well as to validate their associated processes is encountered in the pharmaceutical manufacturing industry on a daily basis due to regulatory requirements. However, as stated in the introductory part of the thesis, what constitutes qualification or validation is subject to broad interpretations, and sometimes missinterpretations, as there are no universally accepted industry practices, due to the different nature of the processes within the industry.

Validation was initially enforced by regulatory authorities as mandatory and without the manufacturers having too much input into the process. Therefore, for quite some time validation was seen as a burden rather than benefit.

In order to prove the benefits offered by validation and to offer a better understanding as to what validation is and how it should be performed, this thesis was intended to provide the industry, and in particular Roche South Africa, with the knowledge of enabling the successful implementation of various validation programs.

Several chapters of the thesis were dedicated to issues dealing with cleaning validation aspects. It was stated in the introductory part of the thesis that there is a general preconception that manual cleaning procedures cannot be successfully validated. It was demonstrated that such an opinion is based on a superficial approach towards equipment cleaning and that with adequate documentation and using the right level of detail a manual cleaning procedure can be as effective and reproducible as the automated cleaning methods.

It was shown that the large volume of work involved in a cleaning validation program can be successfully handled through logical planning and prioritization of tasks as well as through systematic documentation. In order to ensure that every cleaning validation project is treated with the same thoroughness, standard operating procedures as well as working templates for documentation were generated. In this way it was possible to approach the cleaning validation project not only for Fansidar tablets but for any subsequent cleaning validation project undertaken in the Roche, South Africa manufacturing facility. This approach has also ensured that all

cleaning procedures in the manufacturing facility are under control and that the procedures are written in such a way that the process runs effectively and can be supervised by any person with adequate understanding of the cleaning validation processes.

Discussing the analytical methods suitable for cleaning validation studies was another focus point of this work. The first method developed and validated for the cleaning validation of Fansidar tablets, was based on liquid chromatography. This part of the study was aimed at setting minimum requirements for analytical method validation, selecting justified acceptance criteria for the level of equipment cleanliness required and developing suitable sampling methods for the recovery of residuals left on equipment after cleaning.

The possibility of using an alternative method such as capillary electrophoresis as opposed to the classical HPLC method was further investigated successfully. It was shown that complex cleaning validation samples can be optimally resolved by the capillary electrophoretic techniques. This was seen as a simplification of the approach used by the industry when different analytical techniques are generally used to determine traces of product residues on one side and traces of detergent on the other. The direct benefits of using such an approach resulted in cost reduction of the cleaning validation study and reducing production down-times.

Special attention was granted to the validation of steam sterilization processes, knowingly that the conventional approach is that all products deemed to be sterile had to pass the sterility test as described by the pharmacopoeias. This is a costly approach that results in the destruction of a large number of product units. Therefore, the parametric release approach relying on the careful design and validation of the sterilization process was seen as a salutary alternative to the classical approach. It was demonstrated that effective control, monitoring, and documentation of a validated sterilization process offers the guarantee of sterility and integrity for any batch of product undergoing sterilization.

Setting the example on how to tackle validations the author wishes to stress once more the need for soundness of any validation programs elaborated. For both validation approaches discussed it was highlighted that meaningful validation results can be obtained when the validation activities are preceded by a risk analysis of processes undergoing validation.

For the case of cleaning, the risk analysis resulted in the selection for validation of only several representative products from the broad range that is processed in the manufacturing facility.

For the steam sterilizer, parameters such as temperature, pressure and time were identified as critical and therefore were subjected to validation. In addition, it is obvious that for terminally sterilized products the parameters defining the steam quality need to be validated. Still, at which point validation has to stop is decided on a case by case situation and justified logically. Whereas it makes scientific sense to qualify the steam generator and the steam lines connecting the generator to the sterilizer it would be impractical to attempt to qualify the plant generating the energy source for the steam generator.

In all validation examples offered, the outcome results have demonstrated that, by controlling all critical process parameters, reliable results are obtained every time when a process is applied. Meticulous documentation also is necessary for any protocol. Therefore the theoretical models that have been developed will require continual evaluation over time, periodical review and modifications such as to comply with the latest standards set by the industry's regulatory bodies and at the same time to reflect the actual specific conditions of a dynamic environment of a pharmaceutical manufacturing plant.

Through the work involved in this study it was possible to demonstrate that with the right understanding of the validation concept and its implications, the industry may reap the benefits of performing the validation. While the long term benefits of validation cannot be contested, the short term ones are less obvious. For those manufacturers who embark on systematic validation activities the initial efforts start to pay out the moment the product reliability, safety and consistency are demonstrated. Other immediate or middle term benefits confirming the usefulness of the validation efforts are summarized below:

- a decrease in the number of product rejects and reworks and associated with this is the reduction of manhours dedicated for a batch of product;
- a decrease in the number of laboratory analyses required to release a product;
- a thorough review of the practices of the company with emphasis in reconsidering and logically justifying all aspects and steps of manufacturing. In a sense, this means looking with new eyes at existing processes and improving their efficiency and sometimes even

correcting past deficiencies. This in turn would result in a better understanding of the parameters influencing the manufacturing processes and discovering ways of controlling them;

- avoiding sanctions upon inspections from local and global regulatory authorities;
- improved communication and level of trust between departments that strengthens the teamwork spirit and encourages initiative and proactiveness in solving problems associated with a manufacturing type environment;
- timeous discovering or even avoiding deleterious trends and deviations that could be caused by unreliable and non-reproducible processes which in turn can lead to recalls.

The need for demonstrating the quality of the products manufactured within a competitive marketing environment is currently becoming the driving force of validation. Safe and effective products and cost effectiveness are the ultimate targets sought by validation. Therefore, validation is becoming the core of a total quality management system that integrates the high requirements for quality products with the limitations of time, money and human resources inherent within any manufacturing facility.

Having a new perspective over the meaning and implications of validation it can be stated that process validation consists of defined programs which in combination with routine production checks and quality control methods will provide the necessary assurance that a system performs as intended and the associated product conforms to its pre-determined specifications.

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Summary

The main aim of this research work was to assist the pharmaceutical manufacturing industry with elaborating strategies and practical ways of validating their various processes. At present, regulatory bodies set general guidelines but do not offer clear, detailed guidance with regards to validation and qualification. Although there is consensus reached with regards to WHY validation is vital for the industry, there are not enough practical examples with regards to HOW to perform validations. In this study validation approaches were proposed for processes such as cleaning and sterilization which are often encountered in the pharmaceutical manufacturing industry.

A validation program was developed for the cleaning procedures used on the production equipment existent in the galenical manufacturing facility of Roche South Africa. The program was elaborated after consulting the available literature on the cleaning validation topic and selecting the most suitable particulars for the current manufacturing facility.

An inventory of the products, equipment and cleaning procedures was performed as part of the cleaning validation master plan initiated. As the pharmaceutical equipment in the facility is non-dedicated and therefore used for the processing of a broad range of products a risk assessment evaluation was performed targeting the most difficult to clean products. A grouping or bracketing strategy was applied based on certain criteria and among each group of products the worst case situation was identified and selected for validation. The selection criteria studied when performing the risk analysis were the product potencies, toxicity, solubility data, schedule rating and hystorical knowledge of products cleanability. Suporting documentation such as operating procedures working templates and forms were also elaborated in order to standardize as much as possible the activities required to be performed with each validation project.

Fansidar® tablets were one of the products selected as worst case for the manufacturing of solid dosage forms. The cleaning procedures used for the equipment involved in the manufacturing process for Fansidar® were validated with regards to their ability to consistently remove residues to predetermined levels. Acceptance criteria for the LOC desired were established by using several calculation approaches and the lowest values obtained were selected (4.99 µg/cm² for PYR and 19.14 µg/cm²). An RP-HPLC method was developed and validated down to 0.129 ppm for PYR and 0.120 ppm for SUL for the assay of these contaminants in the cleaning validation

samples. Other validation parameters studied were the method selectivity, linearity, precision by repeatability and reproducibility) and the method accuracy. The validated analytical method was challenged in combination with a swab sampling method to prove that the residues of potential contaminants can be reproducibly recovered from stainless steel material coupons at a satisfactory level. The recovery experiment provided a good indication regarding the reproducibility of the swabbing procedure. Mean recoveries of 90.5% for PYR and 83.9% for SUL were obtained with the swabbing method developed. The stability of the analytes in the swab samples during storage was also assessed. A sampling plan was elaborated such as to cover the most difficult to clean and reach locations. The level of residues present after equipment cleaning was monitored after four consecutive cleaning trials. The swab samples were analysed with the validated analytical method and the levels of residues obtained were found well below the calculated LOC. It was proven that the manual cleaning procedures can be validated and therefore used confidently during routine production.

Bactrim® syrup was selected as the worst case for the manufacturing of liquid products. The purpose of this study was to develop an analytical method capable of analyzing, in a single run, residues of co-trimoxazole drug together with some decomposition products, potentially present in the cleaning validation samples. The sample analysed contained: sulfamethoxazole, trimethoprim, sulfanilic acid, sulfanilamide, trimethoxybenzoic acid and nonoxynol-9, a non-ionic surfactant present in the detergent used for cleaning. The suitability of using a MEKC method for the analysis of cleaning validation samples was assessed. A suitable separation method was firstly developed and optimized with regards to the following parameters: pH, instrument voltage, SDS surfactant concentration, electrolyte ionic strength and instrument temperature. The method was further validated in order to be of suitable use for cleaning validation. Acceptable levels of sensitivity, recovery, precision and linearity were obtained with the optimised separation conditions of 30 mM phosphate buffer pH 7.8, containing 10 mM SDS and an applied voltage of 22 kV. Good recovery results (above 90% for SMZ, TMP and NP9) were obtained from both stainless steel and plexiglas coupons proving that the swabbing method developed is reliable and reproducible. The analytical method proved to be enough sensitive, fast and therefore could be used as part of a cleaning validation study in a routine-type work environment.

Another validation approach was proposed for moist heat sterilization and for the qualification of a steam sterilizer. The sterilizer was retrospectively qualified. The media used were categorized based on their physio-chemical characteristics and a media was chosen as worst case in each group. For each worst case a sterilization cycle was validated. Heat distribution studies were performed to establish the coldest spots into the sterilizer. Heat penetration studies were performed in connection with microbiological validation for each media being validated in order to establish whether the minimum time and temperature are reached for the attainment of a certain SAL for each cycle designed. The effectiveness of the media after its sterilization was verified. For the TSB broth, a saturated –steam vented cycle was designed, having a lag time of ca. 15 minute, an exposure phase of 30 min and 122°C. A SAL of $10^{-9.2}$ was achieved with the current sterilization conditions. It was proven that a combination of the mathematical and bioburden approach can be used in order to obtain the guarantee of sterility the product undergoing the sterilization process.

Although each validation strategy follows standard principles, exact methods for validation cannot be defined. Each validation approach is unique due to the multitude of variables involved. As a general rule for validation, a risk assessment needs to be performed in order to identify worst case situations or critical parameters that are likely to change the output result of a process. Variations of the critical process parameters need to be monitored during actual consecutive validation trials. If the monitoring stage produces results which are consistent, reproducible and rugged, there is a high probability that the process will generate the expected results during routine. A process where the critical variables are controlled such that they cannot introduce unintended changes can be declared validated. Particularly, for the pharmaceutical industry, validations need to finally demonstrate that the quality, efficacy and safety of manufactured products are guaranteed for the customers.

Appendices

Appendix A

Boca B.M., Pretorius E., Gochin R., Chappoullie R., Apostolides Z. 2002. An overview of the validation approach for moist heat sterilization, Part I, *Pharmaceutical Technology*, 26(9): 62-70.

Appendix B

Boca B.M., Pretorius E., Gochin R., Chappoullie R., Apostolides Z. 2002. An overview of the validation approach for moist heat sterilization, Part II, *Pharmaceutical Technology*, 26(10): 96-106.

Appendix C

Boca M.B., Apostolides Z., Pretorius E. 2005. A validated HPLC method for determining residues of a dual active ingredient anti – malarial drug on manufacturing equipment surfaces, *Journal of Pharmaceutical and Biomedical Analysis*, 37: 461 – 468.

Appendix D

Cleaning Validation Plan – working template

Appendix E

Cleaning Validation Report – working template

Appendix F

Sampling Record– working form

Results of microbiological testing– working form

Results of chemical testing– working form

Data interpretation for chemical testing– working form

An Overview of the Validation Approach for Moist Heat Sterilization, Part I

B.M. Boca, E. Pretorius*, R. Gochin, R. Chapoullie, and Z. Apostolides

This article illustrates a **qualification-validation strategy for moist heat sterilization** and briefly discusses the sterility concept and common sterilization principles. In Part I, the authors present examples for cycle types, parameter requirements for a standard cycle as defined by pharmacopeias, methods used to design sterilization cycles, and various approaches used to measure the efficiency of the sterilization process.

This article provides an update of the validation of moist heat sterilization. It brings together practical information one needs when validating an autoclave, from procurement through routine use. In Part I of this article, the sterility concept, sterilization principles, development of sterilization cycles, and the measurement of sterilization efficiency are discussed. Part II will be published in *Pharmaceutical Technology*'s October issue and will review the qualification-validation procedure and the probability of nonsterility of a load during the validation of the steam sterilization process.

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The sterility concept and sterilization principles

Sterility is defined as the absence of any microorganism capable of reproduction (1). Sterility can be achieved through the destruction of all viable life forms by applying a lethal agent to the item that must be sterilized. Several sterilization principles exist according to the lethal agent used (2). Each treatment destroys microorganisms in a unique manner and with a different degree of effectiveness. A few examples of these treatments are

- heat sterilization with dry or moist heat
- radiation sterilization with gamma and X-rays, which produce positively charged ions in their passage through the matter, thereby further generating free radicals with lethal effects in the cells
- chemical sterilization with glutaraldehyde, chlorine, iodine, hydrogen peroxide, quaternary ammonium salts, ozone, peracetic acid, and phenolic compounds
- gaseous sterilization with chemical agents such as ethylene oxide, propylene oxide, and formaldehyde, all of which have a lethal effect that consists of the alkylation of proteins, DNA, and RNA
- filtration sterilization, which physically removes microorganisms from the product by means of retentive filters.

New technologies also have been developed, including sterilization with free radicals generated by the combination of UV irradiation with hydrogen peroxide and sterilization with chlorine dioxide.

The sterility criterion is a mandatory requirement for parenteral products, ophthalmic preparations, and products applied to injured skin or used to irrigate the body cavities. All products that must be manufactured as sterile should pass the sterility test described by the pharmacopeias.

When exposing homogenous populations of microorganisms to a sterilizing agent, the microbial inactivation follows an exponential decrease curve (see Equation 1). Mathematically, the inactivation of microorganisms can be expressed as a first-order kinetic process. A finite probability of surviving organisms, independent from the magnitude of the delivered sterilizing agent, can be expressed as:

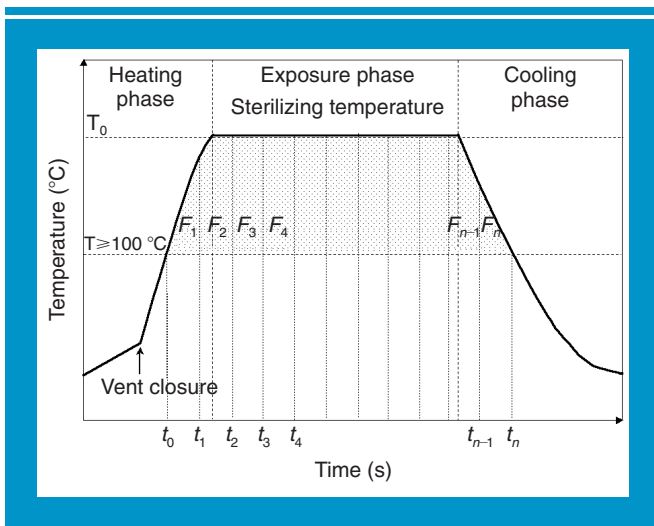


Figure 1: The temperature profile for a saturated steam-vented cycle. t_0 to t_n is the time interval in the computation of the F value. F_1 to F_n areas represent the lethal rates in the product calculated for each discrete time interval. The shaded area under the curve obtained through graphical summation of F_1 to F_n values represents the total calculated F value per cycle. In the heating phase, saturated steam is admitted into the chamber, displacing the cold air until the exposure temperature and corresponding saturated steam pressure are attained. In the exposure phase, the sterilizing temperature is maintained in the chamber by saturated steam for the prescribed exposure time. The cooling phase can be achieved by slow exhaust (for containers filled with liquids) or fast exhaust (for goods required to be dry after sterilization). This phase is completed when the pressure in the chamber reaches atmospheric pressure.

$$N_t = N_0 e^{-kt} \quad [1]$$

in which N_t is the number of surviving organisms after time t , N_0 is the number of microorganisms at time zero (i.e., the bioburden), t is the exposure time, and k is a microbial inactivation rate constant.

For a given process, the probability of survival is determined by the number, type, and resistance of the microorganisms present and by the environment in which the organisms exist during the treatment (e.g., moisture content, thermal energy, and time for steam sterilization) (3). For pharmaceutical products, the term *sterile* is applied to products that have been treated in such a manner that, on completion of the process, individual items have a probability of being nonsterile or have a sterility assurance level (SAL) equal to 1×10^{-6} or more (4). Such a level of sterility assurance is required for terminally sterilized injectable products. This definition of sterility as a probability function does not assume that one in a million products is allowed to be nonsterile but admits a finite statistical probability that a microorganism may survive the sterilizing process (3).

Sterilization by saturated steam in the autoclave

When heat is used as a sterilizing agent, the vibratory motion of every molecule of a microorganism is increased to levels that induce the cleavage of intramolecular hydrogen bonds between proteins. Death is therefore caused by an accumulation of irreversible damage to all metabolic functions of the organism (5).

Heat can be used either alone or mixed with steam. In the autoclave chamber, terminal sterilization is based on highly efficient heat transfer from the saturated steam to the autoclave load (6). Heat transfer occurs by the release of the latent heat from saturated steam under pressure as it condenses.

Heat transfer has maximum efficiency if the steam is kept along the phase separation line of the water-steam phase diagram. Heat transfer from saturated steam to the chamber environment is much more effective and timely for the coagulation and denaturation of nucleic acids and proteins than from dry heat or superheated heat (7). Moist heat in the form of saturated steam under pressure is therefore the most reliable sterilizing agent and is the method of choice whenever it can be used, especially for aqueous preparations (2,8).

Types of cycles used in moist heat sterilization

For saturated steam sterilizers, the physical parameters governing the efficiency of the sterilization process are exposure time, temperature, and pressure. The last two parameters vary in a direct proportional relationship to each other.

Generally, a cycle comprises heating, sterilizing, and cooling phases (9). The choice of a cycle for a particular load depends on the heat sensitivity of the load material and on the knowledge of the heat penetration in the articles. Figures 1 and 2 illustrate the cycles typically used in the moist heat sterilization process.

For a saturated steam-vented cycle (see Figure 1) the steam is injected at the top of the chamber and displaces the cold air, which exits through a trap. This cycle is recommended for containers filled with aqueous preparations (i.e., parenterals, ophthalmics, media, and buffers). The prevacuum cycle (see Figure 2) ensures a more effective penetration of the load by saturated steam and is generally used for porous loads (e.g., surgical dressings and wrapped materials).

For moist heat sterilization, the accepted range of sterilizing temperatures is 118–134 °C. The *US Pharmacopeia* (USP) explains in a footnote that “an autoclave cycle, where specified in the compendia for media or reagents, is a period of 15 min at 121 °C, unless otherwise indicated” (10). The *European Pharmacopoeia* (EP) and the *British Pharmacopoeia* (BP) recommend a heating process at a minimum of 121 °C for 15 min as a reference condition for aqueous preparations (3–4). The text of both books states that other conditions of time and temperature may be used provided that the process chosen has been satisfactorily demonstrated to deliver an adequate and reproducible level of lethality when operating routinely within the established tolerances (3–4).

Development of sterilization cycles

Theoretically, the timing of the exposure phase begins when the temperature sensor placed in the chamber drain (i.e., the coldest spot, theoretically) reaches the set sterilizing temperature. Experiments using thermocouples have shown that the temperature profile of the chamber under loaded conditions is different from the one obtained for the empty chamber. The rate of heating and cooling a product in a container is a function of the container type and size, the viscosity of liquids, and the size and arrangement of the load (2). Therefore, to ensure sterili-

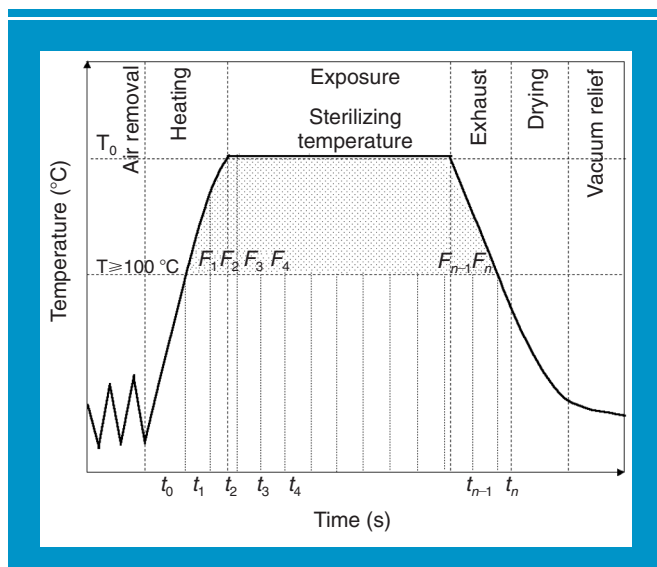


Figure 2: The temperature profile for a saturated-steam with forced air-removal cycle. t_0 to t_n is the time interval in the computation of the F value. F_1 to F_n areas represent the lethal rates in the product calculated for each discrete time interval. The shaded area under the curve obtained through graphical summation of F_1 to F_n values represents the total calculated F value per cycle. In the air-removal phase, air is removed from the chamber and load by several vacuum pulses. In the exhaust phase, steam is exhausted from the chamber until the atmospheric pressure is reached. In the drying phase, the temperature in the jacket and the vacuum in the chamber are maintained for a predetermined time period for products required to be dry. In the vacuum-relief phase, air is admitted to the chamber through a microbiologically retentive filter until atmospheric pressure is reached.

zation efficiency, sterilization must begin after a certain equilibration time when the temperature of the material being sterilized has reached the set sterilizing temperature. This means that a lag time must be added to the cycle exposure time that represents the time necessary for the coldest spot inside the load to reach the sterilizing temperature. The lag time must be determined experimentally and validated with each loading configuration that will be used in the autoclave for any further routine sterilization. The larger the containers and the volumes to be sterilized are, the longer the lag time is.

The modern design of a sterilization cycle can be based on either the initial population of microorganisms in the product that is examined during a suitable time period or on the measurement of the physical parameters attained in the load during the sterilization process. *BP* indicates that “sterilization methods must be validated with respect to both the assurance of sterility and integrity of the product and to ensure that the final product complies with the requirements of the monograph” (4). *USP* states that “the design or choice of a cycle for given products or components depends on a number of factors, including the heat lability of the material, knowledge of heat penetration into the articles, and other factors described under the validation program” (10). Three approaches are used in designing sterilization cycles: the bioburden approach, the overkill approach, and a combination of the two approaches.

The bioburden approach is based on determination of the

number, type, and resistance characteristics of the organisms contained in the bioburden (11). For heat-sensitive products, the heat exposure must be minimized in such a way to reach an optimum balance between an acceptable degree of sterilization and an acceptable stability of the product after its sterilization (12). In the bioburden approach, the process will provide less than 1×10^{-6} probability of microorganism survival. The sterilizing conditions used for the bioburden approach are less extreme than the conditions used for an overkill approach.

The design of a sterilization cycle also can be based on the heating characteristics of the containers located in the slowest heating zone of the load. The overkill approach stems from the concept that the sterilization process will inactivate a high microbiological challenge with an additional safety factor. The microbiological challenge will consist of a biological indicator with a specific number of microorganisms (10^3 – 10^6), and a worst-case assumption is made that the heat resistance of the bioburden is equivalent to that of the biological indicator. Therefore, the cycle conditions established are more severe than those required to inactivate the real product bioburden, and a theoretical spore reduction of 10^{-12} is expected to prove the overkill assurance. Considerably exceeding the sterilization time is recommended for heat-resistant products (10). Therefore, F_0 values (see definition in the “Mathematical approach” section) in the range of 30–60 are expected.

The bioburden approach emphasizes the stability of the final product while the optimum sterility is achieved by knowing the characteristics of the bioburden; however, the overkill approach places the emphasis on sterility. A combination of the two approaches is sometimes useful to reach a balance between the maximum acceptable SAL and the maximum allowable concomitant adverse effect upon the material to be sterilized.

Measuring the efficiency of the sterilization process

Assessment of sterility is based on the demonstration of the absence of growth following the sterility test. Performance of the sterility test is not always a guarantee of sterility for a product (13). Therefore, two methods that incorporate microbial inactivation data have been developed to measure, control, and demonstrate the efficiency of a sterilization process.

Biological approach. In the biological approach, known also as the *Latin approach*, one determines the lowest probability to detect a nonsterile unit in a sterile load and uses biological indicators (BIs) (14). BIs are standardized preparations of microorganisms specific to the type of sterilization process being studied. These BIs are used to show a reproducible logarithmic inactivation. Many types of BIs consist of a given population of bacterial spores considered to be the most resistant forms against a particular sterilization principle.

In sterilization microbiology the D value is frequently used instead of k as a measure of the rate of microbial death. Equation 1 can be formulated in a logarithmic manner as follows:

$$\log \frac{N_0}{N_t} = \frac{kt}{2.303} = \frac{t}{D} \quad [2]$$

The D value represents the temperature coefficient for the lethal process and is the exposure time in minutes required to

cause a 1-logarithm or 90% reduction in the population of a particular microorganism (9,10). The resistance of an organism changes with alterations in temperature. The smaller the D value, the more sensitive the organism is to the lethal agent.

The Z value is defined as the number of degrees of temperature required for a 1-logarithm change in the D value. The Z and D values are significant only under precisely defined experimental conditions and are generally assumed for aqueous solutions. For saturated-steam sterilization, a BI containing spores of *Bacillus stearothermophilus* strain ATTC 7953 with a D value greater than 1.5 min, a Z value of 10 °C, and a population greater than 5×10^5 is recommended (15). *B. stearothermophilus* is considered the reference organism for saturated-steam sterilization because it is known for its great resistance to the lethal agent when compared with the organisms contained in the bioburden (4). For a standard cycle, as recommended by BP, no growth should be recorded for the BI after an exposure time of 15 min at 121 ± 1 °C and a poststudy incubation period. USP states that for *B. stearothermophilus* spores with a D value of 1.5 min under total process parameters (i.e., 121 °C), if they are treated for 12 minutes, the lethality input is $8D$ (10). However, it is not specified that, after 15 min, no growth of the reference microorganism should exist.

For meaningful results, at least 20 BIs per cycle should be used to ensure that statistical distribution is differentiated from true deviations to demonstrate homogenous conditions within the chamber autoclave. Although the biological approach presents difficulties specific to statistical methods, it allows the determination of the lethality effect within the loads in which it is difficult or impossible to place temperature probes (e.g., inside ampuls).

Mathematical approach. The F value is a unit of lethality and is a measure of the microbial inactivation capability of a heat sterilization process. It is defined as “the equivalent in minutes at 250 °F of all heat considered with respect to its capacity to destroy spores or vegetative cells of a particular organism” (2) and allows the comparison of lethal effects at various temperatures. Sterilization cycle parameters can be found by using other temperatures than 121 °C with the help of the F_0 concept. F_0 , as defined by USP (10) at a particular temperature other than 121 °C, is the time (in minutes) required to provide the lethality equivalent to that which is provided at 121 °C for a stated time. The temperature of 250 °F or 121.1 °C represents the temperature of saturated water vapor at ~100 kPa. The F value allows the comparison of lethal effects at various temperatures.

The total F value of a process can be calculated by the integration of lethal rates with respect to time at discrete temperature intervals (16). Integrating the lethality constant $10^{(T - T_0)/Z}$ between two time points, t_0 and t_n , will yield the shaded area under the curves indicated in Figures 1 and 2.

The mathematical approach, also known as the *Anglo-Saxon approach*, uses the F_0 value as the reference unit of lethality. The F_0 value can be calculated using Equation 3. Product-

Table I: Combinations of temperature and time calculated to provide F_0 values of 8- and 12-min equivalents based on reference spores of *Bacillus stearothermophilus* with a D_{121} of 1.5 min and a Z value of 10 °C.

Temperature (°C)	Sterilization Exposure Time (min) for $F_0 = 8\text{-min Equivalents}$	Sterilization Exposure Time (min) for $F_0 = 12\text{-min Equivalents}$
110	103.05	154.58
115	32.59	48.88
118	16.34	24.50
121	8.18	12.27
124	4.10	6.15
126	2.58	3.88
130	1.03	1.54

temperature data accumulated during the entire process are converted to the equivalent lethality at 121.1 °C. For practical purposes, temperatures higher than 100 °C are considered in calculations. It should be emphasized that the F_0 value must be calculated at 121.1 °C, not at 121 °C, because the difference of 0.1 °C introduces an error of 2.4% in the computation of the F_0 value.

$$F_0 = \int_{t_0}^{t_n} 10^{\left(\frac{T - 121.1}{Z}\right)} dt \quad [3]$$

In a nonmandatory section of the EP, General Text 5.1.5 offers guidance concerning the validation of the steam sterilization process by means of the F_0 concept, whose value can be determined from Equation 4 (3). D_{121} is the exposure time at the indicated temperature required to cause a 90% reduction in the spore population. The text of BP states that the application of the F_0 concept requires microbiological validation.

$$F_0 = D_{121} (\log N_0 - \log N_t) \quad [4]$$

The lethality of the sterilization process is influenced by the effectiveness of heat penetration into actual articles and the time of sterilization. Pfeiffer, quoting BP 1983, indicates that the steam sterilization process must be sufficient to produce an F_0 value of at least 8-min equivalents (17). This means that the coolest location in the autoclave loading configuration must be exposed to an equivalent time of at least 8 min of exposure to a temperature of 121.1 °C. This represents the most conservative estimate of the lethality and therefore the safest conditions for determining cycle times.

An F_0 of 8-min equivalents is considered a realistic minimum value because most mesophilic spore-forming microorganisms have D values between 30 s and 1 min at 121.1 °C. When designing a cycle, one should introduce an additional safety factor that takes into account the extra time that may be required for steam to penetrate certain containers in the middle or cool locations of the chamber. For practical purposes one should design sterilization cycles with an F_0 value of 12-min equivalents.

Depending on the nature of the load, one may use nonstandard conditions and try to achieve an equivalent level of kill from new conditions (18). A high activation energy needed to

kill spores can lead to chemical changes, especially in aqueous preparations (2). To avoid product degradation, a longer cycle at lower temperatures is recommended. For other products such as vitamins media that may suffer decomposition processes from a prolonged treatment at a lower temperature, it would be more advantageous to apply a short-time exposure at a higher temperature (19). To evaluate the effect of the killing process at a temperature different from 121.1 °C, the following equation can be used when calculating the lethality factor, F_T :

$$F_T^z = \frac{F_0}{10^{(T-121.1)/z}} \text{ (min)} \quad [5]$$

in which D and Z values are known for a given microorganism, and F is the effectiveness of killing at the specified process temperature, T (°C).

Table I presents equivalent combinations of temperature and time, providing the F_0 value of 8- and 12-min equivalents. The use of 110 °C as a sterilizing temperature requires very long exposure times to reach the target F_0 values. Such exposure times would be unrealistic.

The biological and mathematical approaches are complementary to each other. Lethality using physical process data should be determined in conjunction with appropriate microbiological studies.

Qualifying an autoclave and validating the sterilization process

Absolute sterility cannot be practically demonstrated without the complete destruction of the load. For practical purposes, the industry and regulatory agencies approach the concept of sterility on a probabilistic basis. Validation must prove that the sterilization process delivers a treatment that ensures a certain statistical probability of sterility for an initially nonsterile unit. Therefore, any sterilization process must be validated before use and must be routinely monitored as required by many official texts (3,4,10).

It should be emphasized that the terms *qualification* and *validation* are not identical. Equipment is qualified, and processes are validated. Qualification provides assurance that the autoclave can consistently kill a microbial load of 1×10^6 colony-forming units per container during a defined cycle by using a specific loading configuration. The actual process validation of the cycles is performed by recording and interpreting the physical parameters (i.e., time, temperature, and pressure for steam sterilization) required to prove that the process will consistently yield a product that complies with previously established specifications.

During routine monitoring, the assurance of sterility is gained by demonstrating that validated conditions, known to produce the required level of microbial inactivation, have been attained. Therefore, the careful design and validation of the sterilization process enables sterility to be addressed with an increasing probability of success (12). Such a system is called *parametric release* and has been defined as a "sterility release procedure based upon effective control, monitoring, and documentation of a validated sterilization process in lieu of release based upon end-product sterility testing" (20).

Part II of this article will be published in *Pharmaceutical Technology's* October issue and will review the qualification-validation procedure and the probability of nonsterility of a load during the validation of the steam sterilization process.

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An Overview of the Validation Approach for Moist Heat Sterilization, Part II

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This article illustrates a qualification-validation strategy for moist heat sterilization and briefly discusses the sterility concept and common sterilization principles. In Part II, the authors discuss the qualification-validation procedure, including the installation, operational, and performance qualification phases, when requalification-revalidation is required, and a system for change control.

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Part I of this article was published in the September issue of *Pharmaceutical Technology* and provided an update of the validation of moist heat sterilization, reviewing the sterility concept, sterilization principles, development of sterilization cycles, and the measurement of sterilization efficiency. Part II discusses the qualification-validation procedure and the probability of nonsterility of a load during the validation of the steam sterilization process.

The qualification-validation procedure

As recommended by the American National Standards Institute (1), the qualification-validation of a moist heat sterilization process involves

- qualification of the autoclave by checking its performance against the design specifications
- validation of the process by establishing the actual effectiveness and reproducibility of the cycle in relation to the product and the loading configurations
- assessment of possible changes in the product that could have occurred during sterilization.

A typical qualification program is outlined in the *United States Pharmacopeia (USP)* (2). However, choosing a particular qualification approach depends on the type of autoclave, its actual condition (whether it is new or refurbished), the critical parameters controlling the process, the geometry of the chamber, the type of goods that are going to be sterilized, and the sterility assurance level (SAL) required. Therefore, before the qualification process is started, the user must become acquainted with the autoclave type and its characteristics. It is important to assemble a validation team consisting of at least a user representative and a member of the quality assurance division.

After the decision has been made to qualify an autoclave, the process of collecting information must be performed in a logical manner. Because extensive literature about this topic is available, one should be selective when consulting information sources and retrieve only the type of information applicable to the appropriate sterilizing principle and the operating parameters of the sterilizer being validated.

The first step in collecting information is to consult national standards. If these are not available, then one should consult international standards. Because validation cannot be per-

formed in general, the type of materials that will be routinely sterilized should be considered at all times when setting objectives for validation (3). In accordance with the purpose of the validation, scientifically sound acceptance criteria that can be accurately evaluated by monitoring the physical parameters of the process should be established.

The qualification-validation procedures should be performed using approved protocols developed before the procedures are initiated (4). A description of the autoclave process, cycle types, parameters, and performance specifications must be completed. The protocols should clearly state the objectives of the validation and define the scope of the activities for each stage of the process. Time frames and general acceptance criteria should also be part of the protocols. Each stage of validation must be documented and approved.

Qualification-validation consists of a series of tests, each of which includes an objective, a method, acceptance criteria, and test results. The responsibility for testing must be assigned to either the manufacturer or user, agreed on, and documented.

To perform a proper validation, several items are required such as a temperature recorder (data logger) that can record and accumulate temperature data collected by thermocouples, a calibrated thermometer, and a computer that can analyze raw data and compute F_0 values (5). A laminar-flow hood, biological indicators (BIs), access to an incubator (55–60 °C), a temperature bath, and an ice bath for accuracy verification of the thermocouples also are compulsory elements.

Commissioning or pre-installation qualification (IQ)–operational qualification (OQ) phase. As a prerequisite to qualification of both new and refurbished autoclaves, a user requirement specification document containing key equipment requirements should be developed and approved. Referring to this document helps prevent purchases of unsuitable and inappropriate equipment that can be difficult to qualify for the intended purpose. After its approval and authorization, the document must be passed to relevant vendors before receipt of quotes or offers for work.

Commissioning is the first step of qualification (6) and consists of obtaining evidence that equipment has been provided and installed in accordance with its specifications and that it functions within predetermined limits when operated as directed by its instructions. The company installing the unit must deliver and commission the autoclave and must offer technical support to the owner until the autoclave is in the proper condition for use. Performing a proper commissioning saves time and money and provides the necessary confidence in the performance of the equipment.

IQ–OQ phases. The IQ and OQ phases can be instituted separately or simultaneously. During the IQ and OQ phases, various features of the autoclave are examined and tested for proper functioning. This procedure includes tests of the chamber design, pressure vessel, and door safety interlock system and inspections of the chamber jacket, steam traps, electrical circuits, pressure and temperature indicators, vacuum pumps, vent filters, and steam supply to the chamber.

Usually the IQ document indicates the location of the documentation for the equipment (which must include a checklist containing specifications and identification of equipment or

components by description, model, and serial number), the instruction manual, the troubleshooting and preventive maintenance schedule, the electrical and piping drawings, and the diagram of the programmable logic controller (PLC).

Calibration of command and control instruments. During the IQ phase, the calibration of all critical instruments—process controllers, temperature sensors, pressure gauges, timers, and measuring and recording instruments—should be performed. It is also important to know the degree of accuracy of the instruments used to calibrate the gauges. The accuracy level of the calibrating instruments must exceed the accuracy level of the autoclave's control, measuring, and recording systems (1,7). Likewise, the control, measuring, and recording instruments must have an accuracy level that fits the purpose of the autoclave. When the desired level of accuracy is not known, standards must be consulted.

The performance of calibration test instruments should be traceable to national reference standards. All calibration procedures must be fully documented. Calibrations must be completed before any other validation tests are started. The calibration can be performed either before installation of the unit or in situ after the unit is placed in its operating site. The advantages of calibrating in situ include the ability of a sensor or gauge to provide accurate information about the environment that the unit is monitoring when compared with a known standard (6). This type of calibration is more rugged and provides a more accurate image of the process being monitored.

Requirements for thermocouples. To monitor temperatures attained at various locations throughout the chamber, temperature-measuring devices called *thermocouples* are used. The thermocouples are connected to computerized multichannel recording systems that can record and print temperature data. For validation purposes, type T thermocouples are recommended because they are stable throughout a wide temperature range. Depending on the chamber size, 10–20 thermocouples must be used per cycle. Thermocouples should not be placed in the chamber through the door gasket because they can be easily damaged when loading or unloading the chamber, which can also create a leak that will affect the normal working conditions of the autoclave. One should place the thermocouples in the autoclave chamber by means of a feed-through assembly connected to a suitable port. After purchasing a new autoclave, this port must be documented during the IQ phase.

One must also verify the accuracy of the thermocouples to traceable secondary standards at two significant temperatures (e.g., the freezing and boiling point of water) at the beginning and end of each phase of the qualification. This procedure can be performed by immersing the thermocouples and a reference calibrated thermometer in water maintained at the previously mentioned temperatures and monitoring the temperature values of both the thermocouples and the reference thermometer for a certain period of time. Accuracy of the thermocouples should be at least ± 0.5 °C. Temperature accuracy is especially important in steam-sterilization validation because an error of just 0.1 °C measured by a faulty thermocouple will produce an error of 2.4% in the calculated F_0 value (8). All inaccurate thermocouples should be discarded.

Table 1: Temperature data interpretation for a heat distribution study during exposure phase.

Function	Run Number	Values within the Run									Values across the Run
		Thermocouple Numbers for Heat Distribution									
		T ₅	T ₀	T ₁	T ₂	T ₃	T ₄	T ₆	T ₇	T ₈	
Minimum temperature	1	120.43	121.87	121.85	121.37	121.79	121.71	121.53	121.62	121.63	120.43
	2	121.71	121.81	121.82	121.77	121.92	121.81	121.39	121.58	121.78	121.39
	3	121.35	121.91	121.92	121.84	121.75	121.71	121.53	121.62	121.70	121.35
	4	121.85	122.09	122.07	122.01	122.02	122.10	121.66	121.29	121.83	121.28
Maximum temperature	1	123.27	122.02	122.02	121.96	122.16	122.14	121.92	122.05	122.03	123.27
	2	121.91	122.13	122.02	121.98	122.15	122.20	122.02	122.07	122.25	122.24
	3	122.37	122.22	122.14	122.08	122.30	123.45	122.00	122.12	122.16	123.44
	4	122.16	122.44	122.42	122.34	122.35	122.46	122.16	122.83	122.39	122.82
Mean temperature	1	121.76	121.95	121.93	121.87	121.99	121.98	121.73	121.82	121.81	121.89
	2	121.84	121.97	121.94	121.89	122.06	122.02	121.72	121.85	122.03	121.92
	3	121.88	122.05	122.03	121.96	122.15	122.18	121.77	121.85	121.93	121.98
	4	122.00	122.21	122.19	122.11	122.12	122.22	121.89	121.94	122.01	122.08
Mean of the four runs		121.87	122.05	122.02	121.96	122.09	122.10	121.84	121.84	121.95	
Standard deviation for the means	1	0.10	0.12	0.12	0.11	0.06	0.11	0.12	0.08	0.09	0.09
	2										0.10
	3										0.13
	4										0.12
t value	1	—	0.07	0.10	0.28	0.01	0.02	0.71	0.69	0.30	—
	2										0.59
	3										0.15
	4										0.002

One thermocouple must always be located next to the autoclave temperature probe, which is usually placed in the chamber drain. The reason for this practice is that, theoretically, the “cold spot” of a chamber is in the drain. Depending on various chambers’ geometry, sometimes the location of the cold spot in the chamber drain is not confirmed in practice.

The OQ phase consists of a verification of the equipment functionality. A series of checks and tests should be performed after the unit is installed. The proper operation of alarms and safety devices also must be tested. For a prevacuum cycle involving an air-removal phase, the attainability, effectiveness, and consistency in time of vacuum conditions must be tested.

Chamber mapping consists of heat distribution studies throughout the chamber for each sterilizing temperature used. Heat distribution studies determine during processing whether the temperature is uniform and reproducible throughout the empty chamber and localize the cold spots within the chamber. These studies also confirm that the system works within the specified limits throughout the entire range of operational parameters.

The sterilizing temperatures are programmed into the autoclave controls. For each sterilizing temperature being validated, the empty chamber should be monitored three times during a period of one hour, and the reproducibility of the results obtained must be compared. The comparative readings of the reference temperature sensor, the thermocouple near the temperature sensor, and the temperature displayed by the autoclave recorder should be $\pm 1^\circ\text{C}$. During the exposure phase, a difference no greater than $\pm 2.5^\circ\text{C}$ between the temperature of

the chamber’s coolest spot and the mean chamber temperature is acceptable (8).

Performance qualification (PQ) phase. PQ represents the confirmatory phase of the validation program and consists of tests performed with the autoclave chamber under loaded conditions. During the PQ phase, which is sometimes referred to as *process validation*, the following objectives must be attained (1):

- demonstration of the uniformity and effectiveness of the process in inactivating or removing microorganisms to the required safety level
- demonstration of the reproducibility of the process—through the use of sufficient cycles
- demonstration of the compatibility of the process with the items to be sterilized—through the assessment of the influence of the sterilization process on the products.

The PQ phase consists of studies that use thermocouples inserted into the articles being sterilized and studies that use BIs in operationally fully loaded autoclave conditions (2).

Heat penetration studies are considered the most critical component of the entire validation program. These studies are intended to find areas in the loads that are difficult to penetrate or heat. When selecting the monitoring sites, one must take into account the cold spots previously found during the monitoring of the empty chamber.

Heat penetration studies must demonstrate the reproducibility of a cycle in relation to the loads and the effectiveness of the killing effect throughout the chamber and load. Selecting loading configurations must be performed very carefully and should be based on worst-case situations. The maxi-

Table II: Temperature data interpretation for a heat penetration study during exposure phase.

Function	Run Number	Values within the Run										Values across the Run
		Thermocouple Numbers for Heat Penetration										
		T ₁₀	T ₁₁	T ₁₂	T ₁₃	T ₁₄	T ₁₅	T ₁₆	T ₁₇	T ₁₈	T ₁₉	
Minimum temperature	1	120.26	120.91	121.05	120.76	120.69	120.86	120.57	120.67	120.91	121.15	120.26
	2	120.84	121.35	121.04	120.80	121.08	120.73	120.80	120.87	121.07	121.14	120.73
	3	120.62	120.95	121.18	120.96	120.86	120.92	120.45	121.15	120.22	120.87	120.21
	4	120.81	121.48	121.31	121.33	121.19	121.38	121.27	121.20	121.14	121.23	120.81
Maximum temperature	1	123.10	122.33	122.20	122.31	122.66	122.34	122.49	122.52	122.28	121.92	123.10
	2	122.35	121.92	122.11	122.31	121.99	122.40	122.10	122.17	122.04	121.98	122.40
	3	122.83	122.27	122.01	122.04	121.91	122.09	121.85	121.71	122.40	121.84	122.83
	4	122.91	122.14	122.26	122.19	122.32	122.20	122.37	122.27	122.30	122.25	122.90
Mean temperature	1	121.67	121.68	121.69	121.61	121.64	121.60	121.53	121.55	121.54	121.56	121.61
	2	121.53	121.63	121.55	121.53	121.56	121.57	121.56	121.63	121.64	121.63	121.58
	3	121.63	121.62	121.54	121.53	121.45	121.54	121.33	121.52	121.33	121.39	121.49
	4	121.58	121.77	121.81	121.81	121.84	121.83	121.82	121.84	121.81	121.82	121.79
Mean of the four runs		121.60	121.67	121.64	121.62	121.62	121.63	121.56	121.63	121.58	121.60	
Standard deviation for the means	1	0.06	0.06	0.12	0.13	1.66	0.13	0.2	0.14	0.19	0.17	1.04
	2											0.04
	3											0.10
	4											0.07
t value	1	—	0.17	0.57	0.83	0.4	0.69	0.71	0.69	0.83	0.98	—
	2											0.35
	3											0.007
	4											0.00002

imum number of bottles in the loads for the most-critical or used products should be decided. Ideally, three consecutive validation runs should be performed per loading configuration. During testing, if some configurations cannot be successfully sterilized because they are too big, the worst case in regard to the size of the loading configuration should be redesigned. If the results are still unsatisfactory, then the cycle should be re-designed, which usually can be achieved by modifying the exposure time.

Heat penetration results must show the slowest heating points in the load and must ensure that the minimum time and temperature requirements are met. The success of a qualified cycle depends on the determination of the F_0 value measured inside the item located at the coldest spot. It should be confirmed that a minimum prescribed F_0 value is delivered consistently throughout the autoclave chamber.

Microbiological validation, which consists of tests involving BIs, should be performed concomitant with heat penetration studies to verify independent of the temperature data results that the minimum F_0 value is met at the coldest spot of the load. For terminal moist heat sterilization, heat penetration studies must demonstrate an SAL of 10^{-6} or higher. The BIs should be placed in operational, maximum loads at the locations presumed to be the least accessible to the sterilizing agent. One should place the BIs next to the thermocouples and inside the item being tested. They must not be placed directly on surfaces or outside the loads because the heating process is rapid in those areas and the BIs may get killed faster. The number of BIs used per load depends on the size and the complexity of the load.

For statistical significance, tests with BIs that are performed during the PQ phase should be conducted at least three times per loading configuration. After sterilization, the BIs should be incubated for seven days and checked daily for growth. As a part of the documentation, information provided by the manufacturer concerning the spore lot number (i.e., D and z values for the current lot) must be available.

Demonstrating the integrity of the product after sterilization represents another aspect of process validation. The product must be tested for possible physical and chemical changes that may have occurred during sterilization. In this regard, the pH of solutions and the physical condition and appearance of goods before and after sterilization must be verified. For culture media, tests for growth promotion that are recommended by USP should prove whether the culture media show growth following the sterilization process.

Qualification-validation report. The final stage of the validation program requires the documentation of all acquired data. The qualification-validation report summarizes the overall results of validation. It includes the calibration certificates for calibrating instrumentation, calibration records, and methods for calibrating the measuring instruments, gauges, and recorders as well as the accuracy verification data of thermocouples (4). Test data such as high-low temperature ranges, average temperatures during exposure time, minimum and maximum F_0 values achieved for every load configuration, run date and time, and autoclave records must be included.

The qualification-validation report is not complete unless it contains evidence checks for the availability of an instrument

Table III: Accumulated F_0 value interpretation for a heat penetration study.

Function	Run No.	Thermocouple Number										Mean F_0 per Run	Std. Dev. per Run	Std. Error of the Mean	95% Confidence Interval		t Values across the Run
		T_{10}	T_{11}	T_{12}	T_{13}	T_{14}	T_{15}	T_{16}	T_{17}	T_{18}	T_{19}						
F_0	1	25.82	25.04	24.92	23.86	24.50	24.35	23.76	24.42	24.42	24.51	24.56	0.59	0.18	24.14*	24.98*	—
	2	23.95	24.81	24.01	23.50	24.20	23.91	24.25	24.80	24.90	25.12	24.35	0.53	0.16	23.97*	24.73*	0.4
	3	23.85	24.55	24.26	23.96	23.03	24.42	22.02	24.08	22.03	22.69	23.49	0.96	0.31	22.80*	24.17*	0.009
	4	24.68	26.05	26.13	26.14	26.05	26.43	25.65	26.32	25.15	26.06	25.87	0.55	0.17	25.47*	26.26*	0.00008
Mean of the four runs		24.58	25.11	24.83	24.36	24.45	24.78	23.92	24.91	24.13	24.60	24.57	1.08		24.23**	24.90**	
Standard deviation for the means		0.90	0.66	0.95	1.20	1.24	1.12	1.50	0.99	1.43	1.42						
t value		—	0.38	0.71	0.78	0.87	0.79	0.49	0.64	0.62	0.98						

* The confidence interval per run was calculated considering t for 0.05 (95%) confidence level = 2.262 (N-1 = 9 degrees of freedom).

** The confidence interval across runs was calculated considering the critical value for 95% level of confidence = 1.96.

logbook, the standard operating procedures (SOPs) used with the autoclave, procedures for preventive and unscheduled maintenance, and recalibration programs. The validation report also should include the user's training records. Training should begin only after the validation has been accomplished to ensure that users will implement already-validated loading configurations.

The qualification-validation report must include the drawings of all loads tested. The location of the thermocouples and BIs must be specified in each drawing. Any specification deviations that were encountered during validation activities and the procedures that followed their discovery should be reviewed. After the validation activities are completed, all data and documents that were accumulated must be revised, approved, and certified by both the owner of the autoclave and the contractor in cases when the validation was performed with third parties or by the manufacturer.

Once the autoclave is qualified and the loading configurations are validated, the following documents must be present in the laboratory at all times:

- diagrams of various loading configurations stamped with their effective dates
- a list of the determined lag times for each loading configuration
- temperature-pressure recorder printouts

- user, calibration, and maintenance SOPs.

When requalification-revalidation activities are required. Once a unit has been installed and qualified, it does not need requalification, unless the unit is reinstalled in another location or has undergone major modifications. Only revalidation is performed periodically. Generally, the extent of requalification-revalidation depends on the nature of the changes that occur and how they affect the already-validated sterilization cycles. The responsibility for deciding which validation activities should be reperformed must be assigned. Validation frequency must be stipulated in the validation documents. The need for possible revalidation should be assessed every 12–24 months, and revalidation should occur whenever major repairs have been performed. Any modifications to control systems should be evaluated to confirm that process conditions delivered to the load are comparable with those originally validated. However, the validation should be repeated whenever new sterilization cycles, loading configurations, or major changes in the sterilization procedure are introduced.

Change control. A system for change control should be implemented that establishes when the qualification-validation process should be repeated (1). The nature of the change must be assessed to determine the potential effects on the cycles and load. Changes that could affect sterilization conditions are not allowed to be introduced without documentation. The change-control procedure must follow a change-control SOP.

Changes in any of the following items can invalidate an autoclave validation: pressure gauge, temperature gauge, control panel programming, temperature-control valve, steam trap, and steam source. Changes introduced by preventive or unplanned maintenance procedures must be evaluated and documented in regard to a decision for revalidation.

Table IV: Sterility assurance levels (SALs) attained for each run.

Run	F_0 Values Representing the Calculated Lower Limit of the 95% Confidence Interval	SAL
1	24.14	$10^{-10.09}$
2	23.97	$10^{-9.98}$
3	22.80	$10^{-9.2}$
4	25.47	$10^{-10.98}$

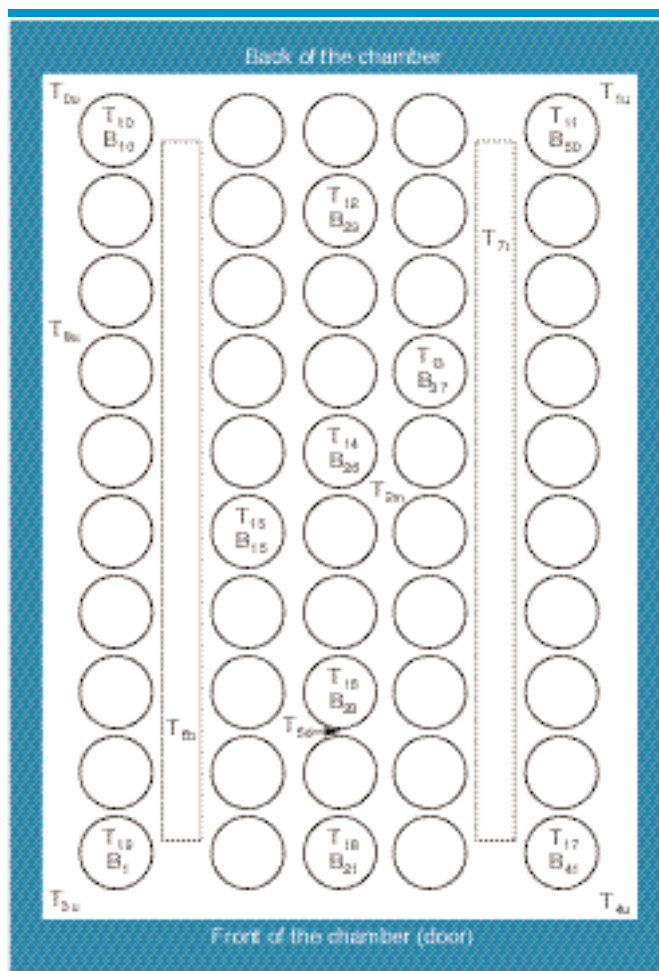


Figure 1: Representation of the autoclave chamber (overhead view). B is the number of the bioindicator ampul, and T is the thermocouple number. Heat-distribution thermocouples are marked with a number and letter indicating their position in the chamber: u = upper position, m = middle position, b = bottom position, r = under the rail, d = in the chamber drain. T_8 was placed in the piping heading to the pressure gauge. The dotted rectangles represent the two rails of the autoclave chamber. The circles represent the 50 bottles, some of them containing thermocouples and bioindicator ampuls.

During routine use, personnel should ensure that the process specifications established during qualification testing are followed and remain valid. The critical parameters governing the process must be monitored routinely to confirm that previously determined conditions are achieved.

Estimation of probability of nonsterility of a load during validation of the steam sterilization process

Qualification-validation activities were performed for a steam sterilizer, model 2020, from Amsco AHSC-South Africa (Pty) Ltd. The following example demonstrates the validation of a saturated steam-vented cycle used with a load containing 50 250-mL bottles filled with 200-mL trypticase soy broth (TSB). TSB is a heat-sensitive medium that is prone to alteration during the sterilization process. Therefore, the parameters for sterilization had to be carefully chosen. The microbiology labora-

tory referred to in this article uses the sterilized broth for microbiological testing of nonsterile products and stab cultures.

Materials and methods. As recommended by pharmacopoeias (2,9,10), the reference sterilization conditions for liquid media (121 °C for an exposure of 15 min) were tested. A preliminary experiment was performed to determine the lag-time characteristic for this loading configuration. A simulation load comprising 50 bottles filled with 200 mL of tap water was used first.

To monitor the temperatures attained in the chamber under loaded conditions, 9 thermocouples were placed in various locations within the chamber, and another 10 were placed in the middle of the liquid contained in the bottles. The placement of the thermocouples in the chamber and the loading configuration are presented in Figure 1, which shows the overhead view of the autoclave chamber.

Timing the lag time began when the thermocouple placed in the drain next to the sterilizer temperature probe reached the sterilizing temperature. Timing stopped when the last thermocouple placed in the load reached the sterilizing temperature.

The heat penetration studies were conducted concomitant with the BI studies. An ampul containing a population of $\sim 10^6$ spores of *Bacillus stearothermophilus* was placed next to each thermocouple inserted into the load. In the loading diagram (see Figure 1) the positioning of the thermocouples and BIs is indicated by the letters T and B. For the ampuls, the number marked on each of the 50 bottles was assigned. After insertion into the thermocouples, the sterilized ampuls were incubated with the positive controls (nonsterilized ampuls) for seven days at 55–60 °C and were checked daily for growth. As a means to verify the sterilization efficiency, 5 of the 50 bottles containing TSB were incubated after sterilization for five days at 32 °C. The broth was checked daily for growth.

To check the effectiveness of the culture medium after its sterilization, the TSB was tested in accordance with the USP Growth Promotion Test (11). The broth was tested for growth in the presence of *Bacillus subtilis*, ATCC 6633, spores and incubated at 37 °C for 48 h.

Another means for verifying the integrity of the physical properties of the broths and buffers is to measure their pH values before and after sterilization. For TSB a pH of 7.3 ± 0.2 should be obtained after its preparation.

Results. For the simulation load, a lag time of ~ 13 min was measured. The TSB has a slightly higher viscosity than tap water. A lag time of 15 min was considered sufficient to be added to the required exposure time of 15 min.

Temperature profiles recorded by using thermocouples inserted into a simulation load have indicated that during the exposure phase the temperature of the load is slightly below the set temperature of 121 °C. To achieve the prescribed sterilization temperature of 121 °C inside the load, the set temperature of the PLC was raised to 122 °C. Therefore the experiments for the validation loads were performed with the new set parameters of 122 °C and 30 min. As an example, Figure 2 shows the middle portion of the temperature profiles achieved within the autoclave chamber, in the chamber drain, and inside the load during the second run.

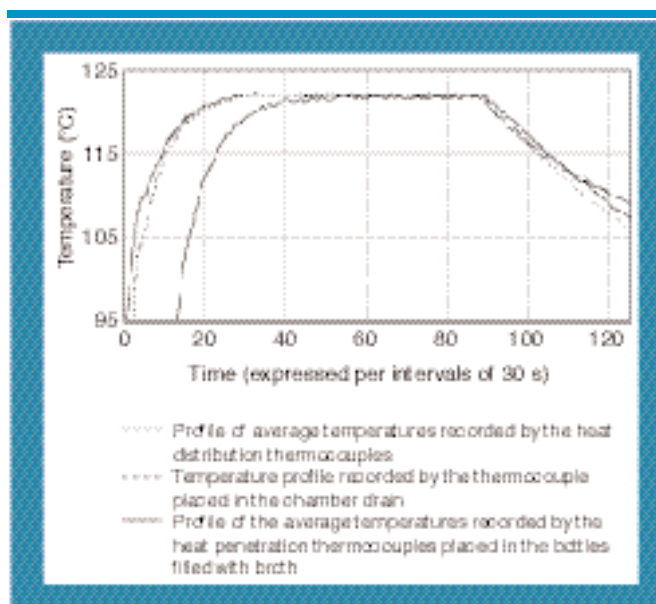


Figure 2: Representation of the middle portion of the temperature profiles in the chamber recorded during the second run.

The data acquired by heat distribution thermocouples during four runs are summarized in Table I. The data acquired by heat penetration thermocouples introduced into the bottles filled with broth during four runs are summarized in Table II.

When observing the mean temperature values per run calculated for heat distribution (see Table I), one will note that position 4 is the hottest spot in the chamber, and positions 6 and 7, situated under the rail, can be considered as the coldest spots within the chamber. Analysis of the data with the t test indicated no statistically significant differences in mean temperature values at a 95% level of confidence between the drain location and any of the arbitrarily chosen points in the chamber, with the exception of thermocouple T_3 , which was situated on the top corner of the chamber farther from the steam inlet. This fact is not critical because loads will not be placed in the top corner.

The heat-penetration temperature data (see Table II) indicate positions 11 and 17 as the hottest spots and positions 14 and 19 as the coldest spots in the chamber. The temperature data recorded at various points in the load proved not to be statistically different from each other at a 95% level of confidence.

Temperature readings taken every 30 s were used for the computation of the F_0 value (8). The value was calculated with

$$F_0 = \Delta t \sum_{i=1}^n F_{\Delta t} = \Delta t \sum_{i=1}^n \left(10^{\frac{T_i - 121.1}{z}} \right) \quad [1]$$

in which $\Delta t = (t_n - t_0)$. The sterilizing effects for temperatures $>100^\circ\text{C}$ attained during the total set time of the cycle, including the entire exposure phase and portions of the heating and cooling phases, were considered in the calculations. The time at which the autoclave temperature probe reached the sterilizing temperature was considered as t_0 , and therefore the timing the cycle started. The lethality values (see Table III) were analyzed to determine the 95% confidence interval for the mean F_0 value per individual run.

The SAL achieved was determined for each of the four runs. The calculated lower limit of the 95% confidence interval was introduced in Equation 4 of Part I of this article indicated by EP (9) for the calculation of the F_0 value. In rearranging that equation, one can define a probabilistic term P as the absolute value of N_t expressed as

$$P = N_t = \text{antilog} \left(\log N_0 - \frac{F_0}{D_{121}} \right) \quad [2]$$

To calculate the probabilities of nonsterility of the product, the equations obtained were solved for N_t .

As indicated in the certificate of conformity provided by the manufacturer for the *B. stearothermophilus* spores, D_{121} was considered 1.5 min and N_0 as 10^6 spores per vial. The results of the Nt calculated at the lower limit of the 95% confidence interval are shown in Table IV. It can be concluded that, when reproducible sterilization conditions are applied to the studied load, a 95% confidence level exists that the probability of a single organism's survival will be $10^{-9.2}$ or better.

Discussion

The previously described approach is based on a worst-case assumption that a population of 10^6 spores per item exists and must be sterilized and that the microorganisms in the bioburden have the same heat resistance as the spores of *B. stearothermophilus*. However, achieving an SAL of 10^{-12} is not necessary for this situation because the raw materials used for the preparation of the broth are pure and possess a low bioburden.

An adequate SAL also was indicated by the results of the experiment using BIs. After sterilization, which was followed by seven days of incubation at $55\text{--}60^\circ\text{C}$, all the spores contained in the ampuls were killed. The positive controls showed growth after the incubation period.

The visual examination of the broth after five days of incubation indicated no spore growth. A bottle containing unsterilized TSB was incubated as a positive control, and the medium turned turbid after the incubation period. This confirmed the conclusion that the sterilization process was efficient.

The effectiveness of the culture medium after its sterilization was also confirmed by the growth promotion test. After sterilization, the broth, spiked with *B. subtilis* and incubated for 48 h at 37°C , showed a positive spore growth. The fact that the medium turned turbid after the prescribed incubation period confirmed that its stability and chemical composition were not altered through the sterilization process and that the medium still supports growth when spiked with a suitable microorganism.

Four pH measurements of the sterilized broth were performed. All pH values ranged between 7.15 and 7.17, which confirmed the preservation of the initial physical and chemical properties of the broth after sterilization. Also, a visual test performed by three independent observers confirmed no color changes or precipitation phenomena in the broth after sterilization.

It can be concluded that, for the studied load, the study's objectives for the PQ phase have been fulfilled. Monitoring the physical parameters attained during the sterilization phase revealed an even distribution of temperatures in the chamber dur-

ing the run, even penetration at all points within the load, and reproducible conditions across three of the four runs. The second run was significantly different from the first, but an SAL of $10^{-9.98}$ was attained. This ensured compliance with the BP, which states that an SAL equal to 10^{-6} or higher must be achieved (10).

The conditions used to sterilize the load are halfway between the overkill approach and the bioburden approach, which were described in Part I of this article. They provide an optimum balance between an acceptable degree of sterilization and an acceptable stability of the broth after sterilization. The authors concluded that for this step of qualification the autoclave worked properly within its functional parameters in four consecutive runs and provided an SAL of $10^{-9.2}$ or higher.

Conclusion

Although the validation approach follows several standard steps, exact methods for validation cannot be offered because various autoclave types exist and each unit has specific operating parameters. Practical examples and requirements for validation are offered in this article for readers who may be inexperienced at qualifying steam sterilizers. Some of the principles presented here can be applied to other sterilization procedures.

As long as the strategy for validation is based on a logical, systematic approach, and meaningful tests are performed to prove the suitability of the autoclave for its intended use, the validation can only be successful. A key factor for a compre-

hensive validation is the meticulous documentation of all activities performed during the process. Documentation provides a comprehensive review of validation results that is essential for acceptance by the quality control unit and also serves to satisfy the scrutiny of regulatory bodies.

Acknowledgment

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A validated HPLC method for determining residues of a dual active ingredient anti-malarial drug on manufacturing equipment surfaces

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Abstract

Analytical method validation, determining the recovery rate from the equipment surface and the stability of a potential contaminant are important steps of a cleaning validation process. A rapid, sensitive and reproducible reversed-phase high-performance liquid chromatographic method was developed for the determination of pyrimethamine (PYR) and sulfadoxine (SUL) in cleaning validation swab samples. The active compounds can be selectively quantified in a sample matrix containing detergent and swab material as low as 0.12 µg/ml. The swabbing procedure used on stainless steel coupons was validated and the stability of PYR and SUL in the swab samples was assessed. The calculated limit of contamination values for PYR (4.99 µg/cm²) and SUL (19.14 µg/cm²) were not exceeded during four consecutive equipment cleaning trials. This confirms that the desired level of cleanliness is achieved with the current cleaning procedures, which are consequently validated.

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Keywords: Cleaning validation; Cross-contamination; Anti-malarial drug; Pyrimethamine; Sulfadoxine; Reversed-phase liquid chromatography

1. Introduction

In the pharmaceutical industry it is of critical importance to establish documented evidence that cleaning procedures applied to the manufacturing equipment are able to remove residues to predetermined levels of safety. The validation of cleaning ensures that subsequent product batches are not contaminated by previously manufactured products or by the cleaning process itself. Much literature has been published on cleaning validation in the past 10 years [1–5] and official organizations (e.g., Pharmaceutical Inspection Convention and Food and Drug Administration) have regulated the subject through guidelines [6,7]. Still, there is limited practical guidance available. This article reports an approach for cleaning validation exemplified for a potent drug.

Challenges for cleaning validation are encountered especially when developing sensitive analytical methods capable of detecting traces of active pharmaceutical ingredients (APIs), which are likely to remain on the surface of the pharmaceutical equipment after cleaning [8,9]. The fact that some residuals are not detected by a method could mean that either the method is not sensitive enough for the residual in question or the sampling procedure is inadequate.

HPLC coupled with UV detection is widely used to monitor the efficiency of the cleaning methods due to its high sensitivity, selectivity and automation characteristics. All the compounds presenting chromophores (e.g., APIs, impurities and degradation products, placebo components, cleaning agents) whether they are hydrophilic or hydrophobic can be detected through HPLC-UV.

In non-dedicated manufacturing facilities, where there is a potential risk for cross-contamination, cleaning validation forms an integral part of the manufacturing process. One of the products that could cause the cross-contamination of

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other products manufactured with the same equipment is the anti-malarial drug Fansidar[®]. Fansidar tablets (Hoffman-La Roche, Basle, Switzerland) contain as active ingredients pyrimethamine (PYR) and sulfadoxine (SUL) in a weight ratio of 1:20. Both compounds present low water solubilities (PYR < 1 mg/ml and SUL < 0.1 mg/ml at 20 °C). PYR is more toxic than SUL (acute toxicity values: LD₅₀ = 128 mg/kg oral, rat for PYR; LD₅₀ = 5200 mg/kg oral, mouse for SUL). Due to its low water solubility and high toxicity, Fansidar[®] was chosen as the worst case among a range of products sharing several equipment pieces (a granulator, a wet mill, a fluid bed drier, a vacuum transfer system, a blender, a compression and a packaging machine).

Pyrimethamine is a dihydrofolate reductase inhibitor, which is indicated in the treatment and prevention of parasitic diseases like malaria caused by *Plasmodium falciparum*, congenital toxoplasmosis caused by *Toxoplasma gondii*, meningeal leukaemia and against coccidiosis [10]. In malaria chemotherapy, pyrimethamine is combined in synergistic effect with sulfadoxine, a long-acting sulfonamide used for the treatment of bacterial infections. Fansidar acts by reciprocal potentiation of its two actives, producing a sequential blockade of two enzymes, namely dihydrofolate reductase and dihydropteroate synthetase, involved in the biosynthesis of folic acid within the parasites [11].

The independent determination of SUL through GC [12] and supercritical fluid chromatography [13] has been reported. Determination of PYR was achieved by fluorimetry [14], GC [15] and HPLC [16–18] in biological fluids, animal tissue and animal feeds. A variety of analytical methods for the simultaneous determination of both drugs in pharmaceutical preparations through spectrophotometry [19], in biological fluids through HPLC [10,20–25] and micellar electrokinetic chromatography in veterinary preparations [26] have been reported.

The simultaneous determination of PYR and SUL is impeded by the differences in their chemical properties. PYR is a weak base (pK_a = 7.3) and SUL is an ampholyte (pK₁ = 1.8; pK₂ = 6.1) [20]. The analysis is even more complicated by the presence in the sample of the detergent components. The detergent contains sodium xylene sulfonate (SXS) with acidic properties (acute toxicity value: LD₅₀ = 1000 mg/kg oral, rat). A method for analyzing all three components has not yet been reported. The chemical structures of these compounds are shown in Fig. 1.

The aim of this study was to validate the cleaning procedures used for the manufacturing equipment involved in Fansidar manufacture. Three tasks were identified:

- development and validation of a sensitive chromatographic method capable of detecting traces of PYR, SUL and SXS likely to remain on pharmaceutical manufacturing equipment surfaces after cleaning;
- development and validation of a suitable sampling method;
- testing the efficiency of the current cleaning procedures.

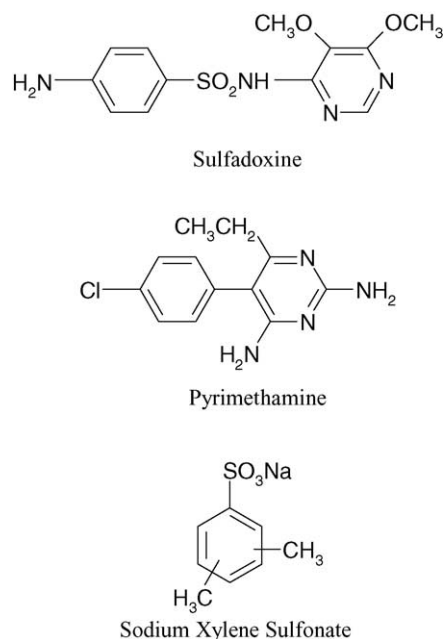


Fig. 1. Chemical structures of sulfadoxine, pyrimethamine and sodium xylene sulfonate.

2. Materials and methods

2.1. Equipment

The HPLC system was an Alliance Waters 2690 (Mildford, MA, US) equipped with Waters 996 photodiode array detector and a detection cell of 8 µl. Waters Millenium software (Version 3.20) was used for data acquisition and processing.

2.2. Chemicals and reagents

All chemicals were of analytical grade. HPLC grade methanol was purchased from Lab-Scan (Dublin, Ireland). *ortho*-Phosphoric acid 85% (v/v) was purchased from Merck (Darmstadt, Germany). Sodium hydroxide (1 M) Fixanal[®] (Riedel-de-Häen), and SXS (Aldrich) were purchased from Sigma–Aldrich (Johannesburg, South Africa). Sulfadoxine (*N'*-(5,6-dimethoxy-4-pyrimidinyl)sulfanilamide) and pyrimethamine (5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidine-diamine) certified standards were supplied by Roche Diagnostics (Mannheim, Germany). Detergent was purchased from Health & Hygiene (Johannesburg, South Africa). Solutions were prepared with deionized water, produced by a MilliQ water purification system from Milipore (Bedford, MA, US). The sampling was performed with Bemcott M₃ wipers purchased from Asahi Chemical Industry (Japan). Samples were filtered through PVDF Millex HV-25 syringe filters purchased from Microsep (Johannesburg, South Africa).

2.3. Chromatographic conditions

Chromatographic separations were performed on a Waters 150 mm $\times$ 4.6 mm Symmetry 5 μ m, C18 analytical column, 100 Å pore diameter.

The chromatographic experiments were performed under isocratic elution. The mobile phase consisted of methanol and 0.06 M *ortho*-phosphoric acid (35:65 v/v). The mixture was allowed to cool down to 20 °C in a water bath and it was adjusted to pH* 3.0 with 1 M sodium hydroxide. The mobile phase was degassed by sonication under low vacuum prior to use. The flow-rate was set to 1.0 ml/min. The autosampler temperature was 15 °C and the column temperature was set at 26 °C. The injection volume was 50 μ l. The detector was programmed to record the absorbance of the samples between 190 and 300 nm with a sampling frequency of 1 spectrum/s. The raw data acquired with the photodiode array detector were processed at 270 nm.

2.4. Preparation of calibration solutions

A stock solution containing approximately 250 μ g/ml PYR and 250 μ g/ml SUL was prepared in methanol. Intermediate stock solutions with concentrations ranging between 5 and 200 μ g/ml were prepared in methanol at seven concentration levels. The final dilutions were prepared as follows: 1 ml of 0.1 M urea solution was spiked with 0.2 ml methanolic intermediate stock solution and the solution was made up to 10 ml with mobile phase. The concentrations of the calibration solutions are: 0.10, 0.20, 0.50, 1.01, 2.02, 3.03, 4.03 μ g/ml for PYR and 0.10, 0.20, 0.51, 1.02, 2.03, 3.05 and 4.07 μ g/ml for SUL.

2.5. Sample preparation

Bemcott M₃, a non-woven, entangled fabric made of continuous filaments of 100% cellulose, fabricated without any adhesive binders was selected for its highly absorbent properties. Three swabs cut in pieces of 2 cm $\times$ 2 cm were placed into 15 ml test tubes. The swabs were spiked with various quantities of active compounds resulting in final solutions with the same theoretical concentrations as for the standard calibration solutions when diluted with 10 ml of solvent. The swabs were left to dry. Then, 1 ml of 0.1 M urea solution and 9 ml of mobile phase was added. The test tubes were vortexed for ca. 30 s and then the solution was filtered through PVDF (0.45 μ m) syringe filters, discarding the first 2 ml of the solution. Four replicates per level were prepared as described above.

Three replicate blank samples for the swab material were prepared as above.

A detergent sample was prepared by diluting 200 mg of detergent concentrate into 100 ml MilliQ water and further diluting 5 ml of this solution with mobile phase in a 20 ml volumetric flask. The final detergent solution has a concentration of 12.5 μ g/ml SXS. An independent standard solution

for SXS was prepared in order to identify the SXS peaks obtained for the detergent solution.

3. Results and discussion

3.1. Method optimization

A proprietary method, which was developed, validated and approved for Roche's affiliates, was re-evaluated. This initial method, which is used to assay PYR and SUL in pharmaceutical formulations at relative high concentrations, was modified in order to accommodate the requirements of trace analysis specific for a cleaning validation study.

A Symmetry[®] column was used to improve the peak symmetry and obtain the best overall chromatographic separation of the cleaning validation samples.

The initial method detects PYR and SUL at 227 nm. For tablet content assay, an optimum simultaneous detection of both components is ensured at this wavelength where the much lower absorption of SUL is compensated by the high content of SUL per tablet. The UV spectra of PYR and SUL in the mobile phase show stronger absorption maxima for both compounds around 270–273 nm.

For the cleaning validation assay, the detection was performed at 270 nm in order to improve the method sensitivity towards SUL which is less soluble than PYR and therefore likely to remain in higher quantities on the pharmaceutical equipment after cleaning. At this wavelength possible interferences from the detergent and other sample compounds are minimized.

The injection volume was increased from 20 μ l, in the initial method, to 50 μ l in order to enhance the method sensitivity.

3.2. Selectivity

The solutions for detergent and for the active components were chromatographed on the Symmetry column but maintaining the mobile phase from the initial method. It was observed that the detergent components are interfering with the active components of the drug. A peak was also observed when solutions prepared for the swab blank were injected.

Several parameters of the mobile phase were simultaneously optimized in such a way until baseline resolution was obtained for all the peaks. The final optimum separation conditions were achieved when the mobile phase composition was methanol and 0.06 M *ortho*-phosphoric acid (35:65 v/v) adjusted to pH* 3.0 with 1 M sodium hydroxide.

With the optimized separation conditions, HPLC runs with detergent solution and swab blanks indicated that no swab or detergent component is detected at the retention time of SUL and PYR. Fig. 2 shows separation profiles of the samples injected to assess the selectivity of the method.

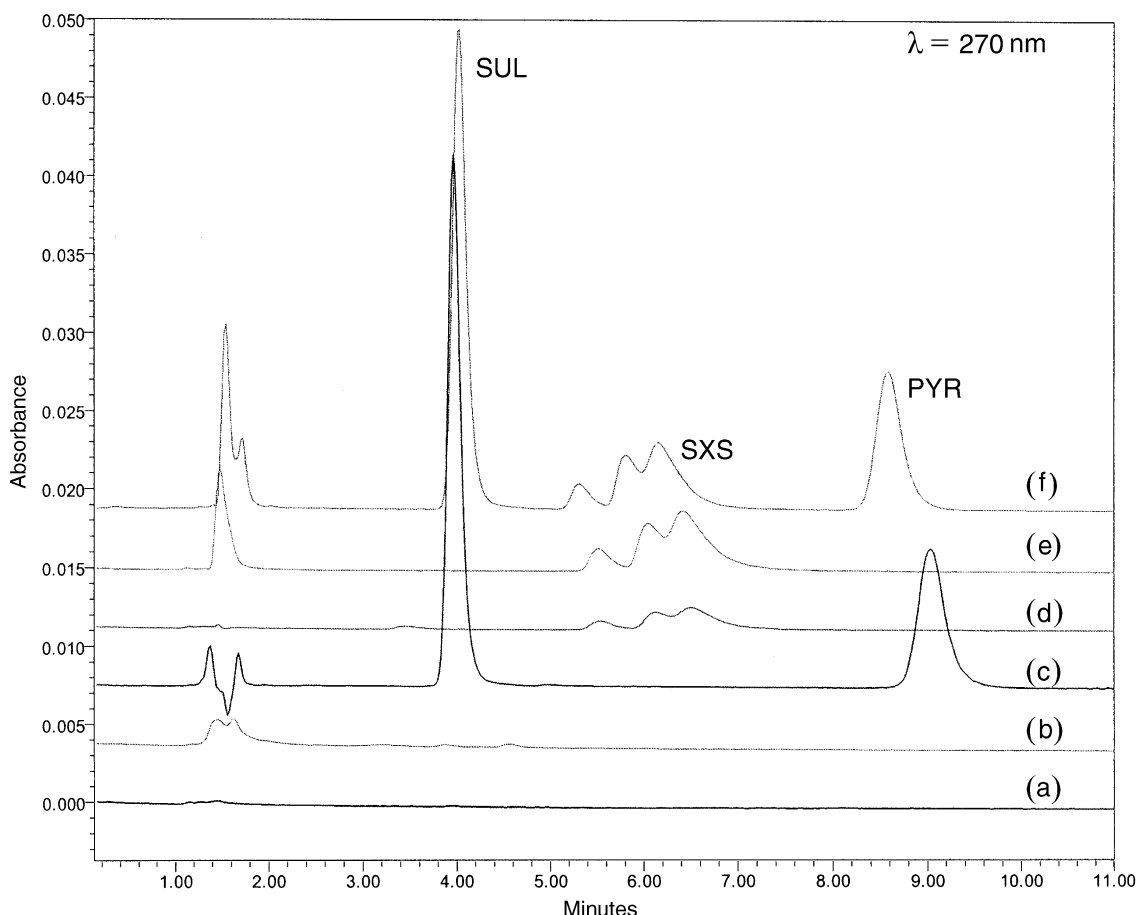


Fig. 2. Typical chromatograms for: mobile phase (a), an extracted swab blank (b), the chromatogram obtained for the 100% level (c), an SXS solution (d), a detergent solution (e) and a combined sample containing detergent, swab and the drug actives (f).

SXS is a mixture of five constitutional isomers. With the current separation conditions it elutes as a group of three peaks in the time interval from 5 to 7 min. SUL and PYR are eluting at 4.2 and 8.6 min, respectively. The retention time of PYR in Fig. 2f was slightly earlier than in Fig. 2c probably due to matrix differences. The identity of PYR in Fig. 2c was confirmed by the UV spectrum. The complete sample separation was achieved within 10 min.

3.3. Method range

The new method was developed for the concentration range of interest for cleaning validation, unlike the case of the methods, which are validated for APIs assay on a relatively small concentration range from 80 to 120% of the expected concentration. For cleaning validation, a wider concentration range with at least five concentration levels should be validated. This is due to the fact that it is difficult to predict the actual level of contaminants found on the production equipment and to avoid re-diluting and re-injecting too concentrated samples.

3.4. Linearity

Linearity was studied for swab samples (seven concentration levels; four replicates per level). Linearity data were obtained by plotting the peak area of PYR and SUL expressed in area units against the concentration of PYR and SUL expressed as $\mu\text{g/ml}$. A linear regression least square analysis was performed in order to determine the slope, intercept and correlation coefficient of the standard curve.

Standard curves were linear from 0.129 to 4.02 $\mu\text{g/ml}$ PYR and from 0.120 to 4.06 $\mu\text{g/ml}$ SUL. The coefficients of correlation (r) are higher than 0.999 for both PYR and SUL indicating a good relationship between the peak area and the concentration of PYR and SUL, respectively in these concentration ranges.

The intercepts of the regression lines are situated for both PYR and SUL within the 95% confidence band of $\pm 10.0\%$ of $\langle Y \rangle_{\text{Ref}}$ indicating that there are no constant systematic errors.

The limits of detection and quantification were calculated with the following formulas:

$$\text{LOD} = \frac{3.3s}{S}, \quad \text{LOQ} = \frac{10s}{S} \quad (1)$$

Table 1
Parameters of linearity for pyrimethamine and sulfadoxine

Analyte	Calibration range (µg/ml)	y-intercept	Slope	r^2	LOD (µg/ml)	LOQ	
						µg/ml	µg/swab
PYR	0.042–4.020	−2.78E+03	1.030E+05	0.9987	0.042	0.129	1.29
SUL	0.040–4.060	−3.391E+03	2.198E+05	0.9989	0.040	0.120	1.20

Table 2
Intra-assay precision (1 day)

Concentration level (%)	%RSD ($n = 4$)	
	PYR	SUL
5	2.1	1.5
10	1.3	1.9
25	2.4	2.2
50	1.6	1.4
100	2.4	2.7
150	1.8	1.0
200	3.3	3.2
Average	2.1	2.0

where S is the slope of the calibration curve and s the standard deviation. For the estimation of the standard deviation s , the standard deviation of the y -intercept was used. The linearity data are presented in Table 1.

3.5. Precision (by repeatability)

The intra-assay precision of the chromatographic method, reported as relative standard deviation, was assessed by measuring the repeatability of the results obtained for four replicate swab samples at seven concentration levels. Swabs were spiked with various quantities of analyte, allowed to dry and then extracted in mobile phase. The %RSD values obtained per level are presented in Table 2.

The overall precision expressed as %RSD is 2.1 for PYR and 2.0 for SUL.

3.6. Precision (by reproducibility)

The inter-assay precision was assessed for three concentration levels (50%, 100%, 150%) with six replicate swab samples per level (see Table 3). The assays were carried out over 3 days on the same instrument by one operator ($n = 18$).

These values demonstrate that the precision of the method is adequate over the range of concentrations expected in cleaning validation samples.

Table 3
Inter-assay precision (3 days)

Concentration level (%)	%RSD ($n = 18$); 3 days	
	PYR	SUL
50	3.2	4.8
100	4.3	1.7
150	2.9	2.3
Average	3.5	2.9

3.7. Accuracy (by recovery)

The method accuracy was determined on spiked and dried swabs that were then extracted in mobile phase. The accuracy of the procedure was assessed by comparing the analyte amount recovered from swabs versus the known amount in the standard injection solutions at seven concentration levels with four replicates ($n = 4$) for each concentration level investigated.

Recoveries from swabs higher than 100% were obtained especially for SUL (Table 4). This was seen as a positive systematic error caused by the low SUL solubility. Various methods were used in order to increase the solubility of SUL (data not shown). It was observed that a solution of 0.1 M urea, when added to the samples, increases the SUL solubility. It is assumed that urea neutralizes the hydrogen bonds between the SUL molecules, therefore preventing their association and precipitation phenomena.

In the case of spiking swabs with SUL it is believed that the cotton acts as a “buffer” medium between the methanolic standard solution containing SUL and the aqueous mobile phase, therefore preventing SUL precipitation. However, precipitation is more readily to occur for the samples prepared without cotton swabs.

3.8. Establishing limits for the cross-contamination level permitted on clean equipment

It is to be remarked that contamination refers to any chemical, microbiological or particulate contaminant likely to remain on the equipment surface after its cleaning, whereas cross-contamination refers to the contamination of a product by a previously manufactured product.

The maximum permitted quantities (R) of PYR and SUL as potential cross-contaminants were calculated through several methods [27] (see Table 5). The total surface area of the equipment chain in direct contact with the product was ac-

Table 4
Accuracy by recovery

Concentration level (%)	Recovery (%) ($n = 4$)	
	PYR	SUL
5	96.6	127.6
10	103.2	136.9
25	108.2	124.3
50	115.1	126.4
100	102.7	106.9
150	99.9	103.6
200	100.2	102.7

Table 5

Maximum permitted levels of cross-contamination obtained through two different calculation methods

Criterion	Permitted cross-contamination ($\mu\text{g}/\text{cm}^2$)	
	PYR	SUL
0.1% dose limit	4.99 (LOC)	39.88
10 ppm	19.14	19.14 (LOC)

counted for in the calculations. The lowest obtained values were selected as limit of contamination (LOC) for this study.

A lowest calculated value of $4.99 \mu\text{g PYR}/\text{cm}^2$ was obtained when the 0.1% dose limit criterion was used for the total equipment chain. The 0.1% dose limit criterion is justified by the principle that an API at a concentration of 1/1000 of its lowest therapeutic dose will not produce any adverse effects to one's health. This criterion as explained by [27] accounts also for the maximum daily intake of a following product and for the batch size of the product that will be manufactured next with the same equipment (see Eq. (2))

$$R = \frac{DS}{IF} \frac{1}{A} (\mu\text{g}/\text{cm}^2) \quad (2)$$

For SUL, the lowest calculated value was obtained when the 10 ppm acceptance criterion was applied. When not more than 10 ppm of SUL were allowed into the next manufactured product, a limit of $19.14 \mu\text{g SUL}/\text{cm}^2$ was determined as LOC (see Eq. (3))

$$R = 10 \times S \frac{1}{A} (\mu\text{g}/\text{cm}^2) \quad (3)$$

where R is the maximum residue of API permitted after cleaning, allowed into the next product; it is assumed that the total amount of residue is distributed homogeneously into the following product; D the lowest daily therapeutic dose of the contaminant; S the lowest batch size of the product to follow; I the maximum daily intake of the product to follow; F the safety factor (can vary from 10 to 100 000 depending on the product nature, e.g., topical, oral or injectable preparations); A the total surface area of equipment in direct contact with the products, calculated based on the assumption that all the products come into contact with all the equipment pieces of the chain.

3.9. Determination of recovery rate of contaminants from stainless steel

Recovery studies were performed in order to determine to what extent the residue could be retrieved from the production equipment with the sampling procedure chosen. The recovery experiment was performed at the LOC level of PYR and SUL on the equipment.

PYR and SUL were independently spiked on 316 stainless steel coupons (10 cm $\times$ 10 cm). The spiked coupons were allowed to dry (ca. 2 h) at room temperature. Since the swabbing is an operator dependant technique, each analyte was recovered six times from coupons in order to obtain a rep-

resentative mean of the recovery rate. The swab sampling simulation was performed by one operator.

A pair of tweezers was used to perform the swabbing. Various proportions of water–methanol were considered as swabbing solvents. A mixture of methanol–MilliQ water 70:30 (v/v) swabbing solvent provided the highest recovery rate. Better recovery results were obtained when the spiked analytes were recovered by using three swabs per sample rather than two. The first swab was wetted with 0.2 ml of swabbing solvent. The coupon was swabbed horizontally with one side of the swab and vertically with the other side. The procedure was repeated two more times with two more wetted swabs. All three swabs were collected into the same test tube. The swab samples were prepared as described above. Appropriate dilutions were performed in order to bring the concentration of the samples within the validated range of the analytical method. Table 6 presents the recovery results.

The recovery experiment provided a good indication regarding the reproducibility of the swabbing procedure. RSD values of 7.4% for PYR and 5.1% for SUL were obtained ($n=6$).

3.10. Sample stability

The stability of the APIs in the swab matrix was tested. Several series of samples were prepared by spiking swabs with a quantity of analyte that would result in the nominal concentration level when diluted with 10 ml solvent. The first lot ($n=4$) of samples were stored in a moistened state. The second lot ($n=4$) was kept in a dry state. All samples were stored in amber test tubes, in the refrigerator (4°C) for 7 days. It is known that a methanolic stock solution containing 5.0 mg SUL/ml and 0.5 mg PYR/ml which is stored in amber glassware is stable at 4°C for over 6 months [28]. Before analysis the samples were allowed to reach room temperature. Urea solution 0.1 M (1 ml) and 9 ml of mobile phase was added to the dry stored samples. A fresh sample was prepared on the day of the analysis. All the samples were then vortexed, filtered through PVDF ($0.45 \mu\text{m}$) syringe filters and analyzed.

Table 6

Recovery of PYR and SUL from stainless steel coupons

Quantity of contaminant spiked	Recovery rate (%)	Mean recovery (%) $\pm$ IC (%)	%RSD ($n=6$)
Pyrimethamine, 498.5 μg	96.4	90.5 ± 6.5	7.4
	81.2		
	84.8		
	93.9		
	97.9		
	88.8		
Sulfadoxine, 1880.7 μg	84.8	83.9 ± 4.2	5.1
	84.4		
	83.2		
	80.7		
	91.3		
	79.0		

Table 7

Sample stability (samples kept at 4 °C in the dark for 7 days)

Contaminant	Concentration (µg/ml)	Sample storage conditions	Recovery (%)	%RSD (n = 4)
PYR	2.01	Dry	95.8	1.3
		Soaked	96.7	1.7
SUL	2.02	Dry	97.9	1.4
		Soaked	98.0	1.8

Table 8

Results obtained for the determination of PYR and SUL in actual swab samples collected from the manufacturing equipment chain

Processing step	Contaminant (µg/cm ²)									
	Pyrimethamine (LOC = 4.99 µg/cm ²)					Sulfadoxine (LOC = 19.14 µg/cm ²)				
	Run 1	Run 2	Run 3	Run 4	Mean + 2s	Run 1	Run 2	Run 3	Run 4	Mean + 2s
Granulation	0.197	2.038	0.302	0.164	2.496	0.583	4.724	0.286	0.179	5.831
Milling	0.020	0.057	0.063	0.059	0.090	0.355	0.173	0.634	0.136	0.779
Drying	0.476	0.250	0.218	0.162	0.552	11.164	1.264	4.543	0.882	13.983
Blending	0.004	0.055	0.056	0.055	0.094	0.131	0.085	0.166	0.084	0.196
Vacuum transfer	0.007	0.041	0.059	0.040	0.080	0.098	0.252	0.166	0.157	0.295
Compression	0.010	0.050	0.081	0.050	0.106	0.167	0.174	1.287	0.128	1.570
Packing	0.077	0.327	0.087	0.072	0.389	3.275	0.837	0.481	0.113	4.036

No changes in the chromatography of the stored samples were found and no additional peaks appeared when compared with chromatograms of freshly prepared samples. It was observed that SUL is slightly more stable than PYR in swab samples. With each compound, similar recoveries were obtained for both dry and moistened stored samples and it was decided that samples should be stored in a dry state in order to avoid the double preparation of the mobile phase (see Table 7).

3.11. Assay of swab samples collected from the equipment train

Swab samples collected from different locations of the manufacturing equipment train were analyzed with the new method. The results found are presented in Table 8.

Since the hand cleaning is generally less reproducible than the automated cleaning, the validation results obtained for manual cleaning procedures must be consistent and well below the LOC. This is to ensure that residues will be removed efficiently and below the acceptance levels in the conditions of routine cleaning of the manufacturing equipment.

For the current study it was observed that all data obtained lie within 2s of the sample mean and well below the LOC. This gives the confidence that the manual cleaning procedures tested do provide sufficient removal of the residues from the equipment chain.

3.12. The place of the analytical method within a cleaning validation strategy

A successfully validated analytical method, sensitive enough for the level of contamination determined by calculating acceptance criteria, and supported by an appropriate sampling method for which validated recovery studies are

established constitute the answers to the question often encountered in the cleaning validation literature of “How clean is clean” [29,30]. The analytical method measures the level of cleanliness of the equipment at the time of validation and monitors any trends and deviations from the validated status of a cleaning process susceptible to develop in time.

4. Conclusion

An accurate, reproducible, sensitive and selective HPLC method for the simultaneous determination of PYR and SUL residuals on pharmaceutical manufacturing production surfaces has been validated to control the efficiency of the equipment cleaning. The chromatographic separation is achieved within 10 min facilitating a high sample throughput. The level of contamination found after equipment cleaning was monitored during several consecutive runs. The results obtained confirm that the cleaning procedures used are able to remove residues from equipment surfaces well below the calculated limit of contamination.

Acknowledgments

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Cleaning Validation

Subject Cleaning Validation Plan for

(Insert Product Name and Number)

(Insert Equipment Name /Identification /Location)

**Document
Information**

Associate documents	
Key words	

Distribution

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Review

Review	Name	Dept	Signature	Date
Author				
Tech. review				
Compliance review				

Approval

Approved by	Name	Dept	Signature	Date
Line Manager				
Line Manager				
QA Manager				

Document No.		Effective Date	Page 1 of 9
--------------	--	----------------	-------------

Att-XX-XX To SOP-XX-XX

Cleaning Validation

Subject Cleaning Validation Plan for

(Insert Product Name and Number)

(Insert Equipment Name /Identification /Location)

Table of Contents	Page
1. Purpose	3
2. Summary of equipment/ product and cleaning procedures selected for validation.....	3
3. Product information	3
4. Field of testing	3
5. Defining acceptance criteria.....	3
5.1 Acceptance criteria for product residues.....	3
5.2 Acceptance criteria for detergent residues.....	3
5.3 Microbiological residues	3
6. Sampling methods and sampling plan	3
7. Analytical method validation, recovery rate and stability study	3
8. Pending Items, deviations, OOS results, investigations and changes to test items and acceptance criteria	3
9. Final assessment of the cleaning validation plan.....	3
10. CVP Subsections	3

Document No.		Effective Date	Page 2 of 9
--------------	--	----------------	-------------

Att-XX-XX To SOP-XX-XX

Cleaning Validation

Subject Cleaning Validation Plan for

(Insert Product Name and Number)

(Insert Equipment Name /Identification /Location)

1. Purpose

This document presents the steps that are followed during the planning stages of the current cleaning validation project and summarizes the relevant information and data gathered during this process.

(This document in its original format is a working template and constitutes Attachment XX of SOP-XX-XX. For every cleaning validation project undertaken this template becomes a validation document).

2. Summary of equipment/ product and cleaning procedures selected for validation

Table 1 contains the summary of cleaning procedures, equipment and products selected for cleaning validation within the current project.

Table 1.

(List either equipment or product, depending on the type of cleaning validation approach undertaken).

Cleaning Procedure		Equipment Identification / Product Name
SOP No. and version	SOP name	

For each cleaning procedure and equipment/ product tested, the Subsection 1.1 of the CVP contains the following information:

- “The Description of Cleaning Procedure” (see Attachment 1 of SOP-XX-XX, General procedure for cleaning validation).
- The copies of training records of the operators performing the cleaning.

Document No.		Effective Date	Page 3 of 9
--------------	--	----------------	-------------

Att-XX-XX To SOP-XX-XX

Cleaning Validation

Subject Cleaning Validation Plan for

(Insert Product Name and Number)

(Insert Equipment Name /Identification /Location)

3. Product information

3.1 The following product was selected for cleaning validation:

(Insert product name, number, formulation, strength and master manufacturing batch record No. and version).

3.2 The reasons for product selection are presented below:

(Provide information regarding product physical form, formulation, activity, potency, toxicity).

3.3 The number of batches after which the effectiveness of the cleaning procedure will be tested is:

(Explain whether the product is manufactured one batch at a time or in campaigns. For products which are manufactured in campaigns, establish the maximum number of batches after which the cleaning effectiveness tested).

4. Field of testing

The cleaning procedures will be evaluated with respect to their capability of removing from the manufacturing equipment potential microbiological and chemical residues.

The testing activities will be carried out as follows:

Microbiological testing will be performed as described in the SOP-XX-XX, Microbiological monitoring in productions area. Chemical testing will be performed as described by the SOP-XX-XX, Analysis of cleaning validation samples, and according to product specific method of analysis validated and approved as described in the SOP-XX-XX, Analytical method validation, recovery rate from coupons and stability studies for cleaning validation.

Table 2 presents the testing fields relevant for this project.

Document No.		Effective Date	Page 4 of 9
--------------	--	----------------	-------------

Att-XX-XX To SOP-XX-XX

Cleaning Validation

Subject Cleaning Validation Plan for

(Insert Product Name and Number)

(Insert Equipment Name /Identification /Location)

Table 2.

(Mark in the table below the items selected for the testing of cleaning procedure(s). For chemical testing fill in the names of the compounds tested).

Chemical Testing	API drug residue ☐ (insert drug compound name(s))
	Detergent residue ☐ (insert detergent name and detergent compound to be analyzed)
Microbiological Testing	After equipment: - Cleaning ☐ - Disinfection ☐ - Sterilization ☐
	Monitoring for the determination of the maximum hold time of - Used equipment ☐ - Clean equipment ☐
Visual testing	No visible stains, spots, dust or foreign matters observed ☐

5. Defining acceptance criteria

The acceptance criteria for the maximum permitted level of chemical residues are presented in detail for every residual compound that will be tested:

5.1 Acceptance criteria for product residues

(Calculate in the space below the acceptance criteria established for the product residue component(s). Select as acceptance criteria the lowest value obtained by applying different calculation methods and document the final result in Table 3).

Document No.		Effective Date	Page 5 of 9
--------------	--	----------------	-------------

Att-XX-XX To SOP-XX-XX

Cleaning Validation

Subject Cleaning Validation Plan for

(Insert Product Name and Number)

(Insert Equipment Name /Identification /Location)

5.2 Acceptance criteria for detergent residues

(Calculate in the space below the acceptance criteria established for the detergent residue component(s). Select as acceptance criteria the lowest value obtained by applying different calculation methods and document the final result in Table 3).

Table 3 presents the summary of the acceptance criteria used for this project.

Table 3.

(Transfer in the table below the final acceptance criteria calculated for each equipment/processing step).

Processing step or equipment identification	Maximum allowable contamination with (g substance/ equipment or processing step/	
	_____	_____
	(Insert Compound Name)	(Insert Compound Name)

5.3 Microbiological residues

For the monitoring of internal surfaces of equipment in direct contact with product the following acceptance criteria will be used:

- Alert Limit: 20 CFU/plate
- Action Limit: 100 CFU/plate

(These limits will be used as a starting point for equipment surfaces in direct contact with the product for which no previous data are available. Once historical data will be available the limits can be revised and modified accordingly).

Document No.		Effective Date	Page 6 of 9
--------------	--	----------------	-------------

Att-XX-XX To SOP-XX-XX

Cleaning Validation

Subject Cleaning Validation Plan for

(Insert Product Name and Number)

(Insert Equipment Name /Identification /Location)

6. Sampling methods and sampling plan

The sampling plan provides the scientific rationale for each sampling point selected. The sampling plan was created based on the template available in Attachment XX of the SOP-XX-XX. Each sampling location was assigned with a unique number on the Sampling Plan. This number will be retained through the entire project. The sampling plan and its versions, when applicable, are filed in Subsection 1.2 of this CVP. The document identification is:

(Insert the identification of the sampling plan: document numbers, names and versions).

At the time of validation use the sampling record to document the sampling activities. The Sampling record form is available in Attachment 01 of the SOP-XX-XX. Fill in the relevant information as indicated by this form and file all the forms on completion in Subsection 2.1 of the CVR.

7. Analytical method validation, recovery rate and stability study

The summary of the data acquired for analytical method validation, recovery rate from material coupons and sample stability are presented in Table 4. The documents generated for the Method Validation Plan (MVP) and Method Validation Report (MVR) and the raw data accumulated are filed in the Subsection 1.3 of this CVP. The MVP and MVR were created based on the templates available in the Attachments XX and XX of SOP-XX-XX.

The Method of Analysis (MOA) – product specific is filed in Subsection 1.4 of this CVP.

Document No.		Effective Date	Page 7 of 9
--------------	--	----------------	-------------

Att-XX-XX To SOP-XX-XX

Cleaning Validation

Subject Cleaning Validation Plan for

(Insert Product Name and Number)

(Insert Equipment Name /Identification /Location)

Table 4.

(Transfer in the table below the summary of the analytical data for method validation, recovery rate study and sample stability.

Validation parameter	Result	Acceptance criteria met (Yes/No)
Analytical method validation (trace determination)		
Linearity range for: (ppm)		
LOD (ppm): LOQ (ppm):		
Precision by repeatability (% RSD):		
Precision by reproducibility (% RSD):		
Accuracy by recovery (%):		
Validation of recovery rate from material coupons		
Material type for the coupon: (List the data obtained for all material types studied:, stainless steel 316, silicone, rubber, plexiglass.)	% Recovery rate = % RSD = Correction factor used for data processing =	
Validation of sample stability		
Storage conditions tested ☐ (describe the storage conditions tested)	% Recovery rate = % RSD = Correction factor used for data processing =	

Document No.		Effective Date	Page 8 of 9
--------------	--	----------------	-------------

Att-XX-XX To SOP-XX-XX

Cleaning Validation

Subject Cleaning Validation Plan for

(Insert Product Name and Number)

(Insert Equipment Name /Identification /Location)

8. Pending Items, deviations, OOS results, investigations and changes to test items and acceptance criteria

Pending items, deviations, OOS results and Investigation encountered while performing the cleaning validation activities are treated as explained in the CVMP and are filed in Subsection 2.7 of the CVR.

Changes to test items and acceptance criteria are to be documented and attached in Section 1 of the folder together with this CVP.

9. Final assessment of the cleaning validation plan

(State whether the activities listed in this plan have been thoroughly and correctly completed and documented).

10. CVP Subsections

Subsection 1.1	Description of cleaning procedure(s)
Subsection 1.2	Sampling plan
Subsection 1.3	Method validation plan and method validation report
Subsection 1.4	Method of analysis

Document No.		Effective Date	Page 9 of 9
--------------	--	----------------	-------------

Att-XX-XX To SOP-XX-XX

Cleaning Validation

Subject **Cleaning Validation Report for**

(Insert Product Name and Number)

(Insert Equipment Name /Identification /Location)

Document Information	Associate documents				
	Key words				
Distribution					
Review	Review	Name	Dept	Signature	Date
	Author				
	Tech. review				
	Compliance review				
Approval	Approved by	Name	Dept	Signature	Date
	Line Manager				
	Line Manager				
	QA Manager				

Cleaning Validation

Subject Cleaning Validation Report for

(Insert Product Name and Number)

(Insert Equipment Name /Identification /Location)

Table of Contents	Page
1. Purpose	3
2. Documentation of sampling activities.....	3
2.1 Summary of sampling for the cleaning trials	3
2.2 Summary of sampling for the validation of maximum equipment hold time	3
3. Summary of results	3
3.1 Results for microbiological testing after equipment cleaning.....	3
3.2 Results for microbiological monitoring of equipment hold times	3
3.3 Results for chemical testing.....	3
3.4 Results for visual testing.....	3
4. Pending Items, deviations, OOS results, investigations and changes to test items and acceptance criteria	3
5. Discussion	3
6. Conclusions	3
7. CVR Subsections.....	3

Document No.		Effective Date	Page 2 of 8
--------------	--	----------------	-------------

Att-XX-XX To SOP-XX-XX

Cleaning Validation

Subject Cleaning Validation Report for

(Insert Product Name and Number)

(Insert Equipment Name /Identification /Location)

1. Purpose

This document presents the details of the sampling activities undertaken, summarizes all the data collected during the chemical, microbiological and visual testing and presents the interpretation of the results. Conclusions and observations with regards to the effectiveness and ruggedness of the cleaning procedures tested are also presented together with the final verdict of the validated or non-validated.

(This document in its original format is a working template and constitutes Attachment XX of SOP-XX-XX. For every cleaning validation project undertaken this template becomes a validation document).

2. Documentation of sampling activities

The sampling for microbiological and chemical testing, and the visual examination of the manufacturing equipment was performed as described in the SOP-XX-XX Sampling for cleaning validation.

2.1 Summary of sampling for the cleaning trials

Samples were collected after the production equipment was cleaned in accordance to the procedures listed in the CVP for the current project. The batch numbers for the product processed in the equipment as well as the document numbers of the sampling plan used are listed in Table 1.

Table 1.

Trial No.	Sampling plan Identification	Batch number	Product Name

The sampling activities were documented on Sampling Records filed in Subsection 2.1 of this CVR.

Each sampling location was assigned with a unique number on the Sampling Plan. This number was consistently used during all cleaning trials and it corresponds with the number recorded on the Sampling Record.

Document No.		Effective Date	Page 3 of 8
--------------	--	----------------	-------------

Att-XX-XX To SOP-XX-XX

Cleaning Validation

Subject Cleaning Validation Report for

(Insert Product Name and Number)

(Insert Equipment Name /Identification /Location)

2.2 Summary of sampling for the validation of maximum equipment hold time

Table 2 contains the summary of the sampling activities undertaken to assess the maximum hold times for clean and dirty equipment.

Table 2.

Equipment ID and sampling points	Cleaning SOP No.	Product Lot No.	Period of monitoring		Total No. of samples collected
			Starting Date	Finishing Date	
Clean equipment hold time					
Dirty equipment hold time					

(Explain in the space below the sampling methods used for the assessment of used equipment hold time. File the documents in Subsection 2.4 of the CVR).

3. Summary of results

3.1 Results for microbiological testing after equipment cleaning

The microbiological samples were collected, treated and interpreted as described by the SOP-XX-XX Microbiological monitoring in productions area. The actions taken thereof are according to the same SOP and according to the procedure outlined by the Doc. No. CVMP-XX-XX.

After each cleaning trial the results were documented on the form Results of Microbiological Testing” (see Attachment XX of SOP-XX-XX, General procedure for cleaning validation) and are filled in Subsection 2.2 of this CVR.

Table 3 contains the summary of microbiological results obtained after sampling of cleaned equipment.

Document No.		Effective Date	Page 4 of 8
--------------	--	----------------	-------------

Att-XX-XX To SOP-XX-XX

Cleaning Validation

Subject Cleaning Validation Report for

(Insert Product Name and Number)

(Insert Equipment Name /Identification /Location)

Table 3.

Trial No.	Cleaning SOP No.	Equipment Identification	Results below the limits (Y/N)	
			Action Limit	Alert Limit

(For cleaning procedures with results above the limits, provide explanations in the space below).

Comments:

3.2 Results for microbiological monitoring of equipment hold times

The results for microbiological monitoring of clean equipment hold times are documented on the form “Microbiological Monitoring for Maximum Standing Time of Clean Equipment” (see Attachment XX of SOP-XX-XX, General procedure for cleaning validation) and are filled in Subsection 2.3 of this CVR.

The results for the microbiological monitoring of used equipment hold times are filled in Subsection 2.4 of this CVR.

Table 4 contains the summary of microbiological results collected to assess the maximum hold times for clean and used equipment.

Table 4.

Cleaning SOP No.	Equipment Identification	Maximum standing time established (Days)
<i>Clean equipment hold time</i>		
<i>Used equipment hold time</i>		

Document No.		Effective Date	Page 5 of 8
--------------	--	----------------	-------------

Att-XX-XX To SOP-XX-XX

Cleaning Validation

Subject Cleaning Validation Report for

(Insert Product Name and Number)

(Insert Equipment Name /Identification /Location)

3.4 Results for visual testing

Table 6 contains the results for the visual testing of some difficult to reach sampling points as indicated by the Sampling Plan.

Table 6.

Cleaning Trial No.	Cleaning SOP No.	Equipment ID	Sampling point	Visually clean (Y/N)

(For cleaning procedures with less than three successful validation runs, provide explanations in the space below).

Comments:

4. Pending Items, deviations, OOS results, investigations and changes to test items and acceptance criteria

All pending items, deviations, out of specification results, investigations and changes to test items and acceptance criteria were handled and documented as described in the CVMP.

All the documentation pertaining to pending items, deviations, out of specification results, investigations are filed in Subsection 2.7 of this CVR.

The changes to test items and acceptance criteria were documented and are attached in the Section 1 of the validation folder under the CVP.

5. Discussion

(Discuss all the results generated during cleaning validation activities and their significance. Relate them to the acceptance criteria previously established).

Document No.		Effective Date	Page 7 of 8
--------------	--	----------------	-------------

Att-XX-XX To SOP-XX-XX

Cleaning Validation

Subject Cleaning Validation Report for

(Insert Product Name and Number)

(Insert Equipment Name /Identification /Location)

6. Conclusions

Based on the results of this study, the cleaning procedures listed in Table 6 can be considered validated/not validated:

Table 7.

Cleaning Procedure SOP No., title and version	Validation Status (Validated/ Not validated)

7. CVR Subsections

Subsection 2.1	Sampling records
Subsection 2.2	Results of microbiological testing
Subsection 2.3	Results of micro-monitoring for maximum hold time of clean equipment
Subsection 2.4	Results of micro-monitoring for maximum hold time of used equipment
Subsection 2.5	Results of chemical testing
Subsection 2.6	Data interpretation for chemical testing
Subsection 2.7	Pending items, deviations, OOS results, investigations

Document No.		Effective Date	Page 8 of 8
---------------------	--	-----------------------	-------------

Att-XX-XX To SOP-XX-XX

Sampling Record

(Insert equipment identification)

(Upon completion, file this form in Subsection 2.1 of the Cleaning Validation Report)

Product Name:	Product Batch No:
Cleaning SOP No:	Location:
Cleaning trial No:	Cleaning agent type and batch No:
Cleaning date:	
Sampling Date:	Sampling Plan No:

Pre-requisites for sampling		Yes/ No		
The equipment was inspected for visual cleanliness before sampling, and it is visually clean (no spots or visible stains) and dry:				
The operator(s) have been trained for the cleaning procedure being tested* <i>(Sign & date):</i> - -				
The sampler have been trained for sample collection* <i>(Sign & date):</i> -				
Remarks: <i>(Justify below the items marked with No, if any.)</i> *See training records attached into Subsection 1.1 of the CVP.				
Sampling details: Swabbing solvent Swabbing procedure For total aerobic counts, record the batch number of the Rodac plate used: ☐				
Sampling details				
Sample No.	Test for	Sample collected (Y/N)	Sampling date	Equipment cleaning date

Comments: *(Justify below why some samples have not been collected, if necessary.)*

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Sign and date for collecting the samples: _____

Results of microbiological testing for

(Insert equipment identification)

(Upon completion, file this form in Subsection 2.2 of the Cleaning Validation Report)

Product name: ☐	Product Batch No:
Cleaning SOP No:	Location:
Cleaning trial No:	Cleaning agent type and batch No: ☐
Cleaning date:	
Sampling date:	CVR No:

Sample No.	Sampling point	CFU/plate	Observations

Limits

Alert Limit: 20 CFU/plate

Action Limit: 100 CFU/plate

If the limits are exceeded, follow the procedure outlined in SOP-XX-XX Microbiological monitoring in production area.

Comments: (Make a statement whether the results are within the limit or not. When deviations occur, assign numbers for them.)

Sign and date for sampling and analysis by: Microbiologist:

Sign and date for reviewing the results by: QC Manager

Sign and date for acknowledging results by: Pharmacist:

Results of chemical testing for*(Upon completion, file this form in Subsection 2.5 of the Cleaning Validation Report)*

Product Name:	Compound analyzed:
Batch No:	Analytical Method No:
Cleaning trial No.	Date of analysis:

Instrument type, calibration interval and working conditions:

(attach the calibration record of the analytical instrument)

(Fill in the requested information and attach to this document the analytical raw data acquired).

Data Interpretation				
A	B	C	D	E
Sample No.	Concentration (µg/ml)	Dilution Factor	Recovery Factor*	Corrected Qty of Residue per sampling point (µg)

*Include both factors calculated for recovery rate from coupon material and sample stability

Calculation formula: $E = B \times C \times D$

Comments:

Sign and date for data interpretation: Lab Analyst _____

Sign and date for results review: QC Manager: _____

Data interpretation for chemical testing

(insert equipment identification)

(Upon completion, file this form in Subsection 2.5 of the Cleaning Validation Report)

Product Name:	Product Batch No:
Cleaning SOP No:	Location:
Cleaning trial No:	Cleaning agent type and batch No:
Cleaning date:	
Sampling Date:	CVR No:

Compound analyzed:

Sample No.	Sampling Point	Sampled Area (cm ²)	Corrected Qty of Residue per sampling point (µg)	Area compensation factor	Final Qty of residue (µg)
A	--	--	E	F	G

Calculation formula: $G = E \times F$

Total Qty of residue per piece of equipment/ processing step (µg) (sum up all the values contained in column G)	
Maximum allowable Qty of Residue per piece of equipment/ processing step (µg)	
Cleaning Validation Result (OK, not OK)	

Comments:

(Make a statement whether the results are within the limit or not. When deviations occur, assign numbers for them.)

Sign and date for data interpretation:

Sign and date for results review:
