The Forensic Analysis of Illicit Methaqualone-Containing Preparations by Gas Chromatography Mass Spectrometry

by

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ABSTRACT

Currently 350 (and steadily increasing) different methaqualone tablet formulations have been received, tested and classed by the Chemistry Unit of the South African Police Service Forensic Science Laboratory (SAPS FSL). In order to help combat the criminal organisations targeting the local market, a National Forensic Drug Intelligence Database (NFDID), also referred to as the Logo Index, was established in March 1999 by the SAPS FSL. Colour photographs, accompanied by the dimensions and chemical identity of the active constituents of methaqualone tablet formulations, amphetamines and lysergic acid diethylamine (LSD) blotter papers are included in this database. The identity of inactive constituents, abundance of active and inactive constituents, precursor or processing chemicals, as well as by-products produced during the synthesis or production of illicit drug samples, are not yet included in the NFDID.

In court in South Africa the Forensic drug analyst is increasingly asked to state whether illicit preparations from different seizures might be originating from the same manufacturer. This creates the need for not only routinely analyzing seized tablets for the purpose of identifying the active ingredients, but also for quantifying the active ingredients, and identifying the precursors and contaminants which might be present. The availability of this data increases the possibility of tracing the origin of different tablets. Establishing whether the performance characteristics of a specific method meet the requirements for the intended analytical applications of the analytical method has thus become essential. This validation process also establishes the limitations of the method, as well as the effects of specified interferences on the performance of the method.

The project has two main aims. The first will be to validate the quantitative determination method of methaqualone in illicit preparations by using gas chromatography mass spectrometry (GC/MS). The same method will be validated in order to identify other active substances in the illicit preparations, for example diazepam and diphenhydramine. Establishing the presence of inactive constituents, the abundance of active and inactive constituents, precursors or processing chemicals and possible by-products produced during the synthesis or production of these illicit drug samples would be included in the validation.

The second main aim is to apply this validated method to determine the presence or absence of certain key compounds in preparations, in order to enable the analytical

chemist to conclude that “real life” illicit preparations received at this laboratory for analysis are the same. The data resulting from this experiment will then be reviewed in order to establish whether the preparations analysed could be traced back to the same manufacturer, or at least the same method of manufacture. This data will also be compared to literature references concerning chemical fingerprinting of other illicitly manufactured drugs such as heroin and the amphetamines.

Should this validated method be deemed competent to achieve these aims, it would be used routinely by the Chemistry Unit of the SAPS FSL in order to generate data for the purpose of expanding the existing NFDID. This will hopefully enable the police to link different seizures, or to link a certain seizure with an illicit manufacturer, suspected to be involved with a certain case.

CHAPTER 1 - INTRODUCTION

1.1 Purpose of the Project

In organized crime, one of the biggest problems currently experienced in South Africa is the illicit manufacture of methaqualone containing preparations or “mandrax” (street name commonly used in South Africa). All drugs, chemicals and/or laboratory equipment seized during raids on illicit laboratories, are sent to the South African Police Service Forensic Science Laboratory (SAPS FSL) for analysis. The Unit responsible for the analysis is the Chemistry Unit, and more specifically, the Drug Section. They mainly utilize gas chromatography-mass spectrometric techniques (GC/MS) for identification purposes. Currently 350 and steadily increasing different methaqualone tablet formulations have been received, tested and classed by the Drug Section. In Chapter 2, current statistics on methaqualone cases received and analysed by this laboratory will be reported.

To assist in combating the criminal organisations targeting the local market, a National Forensic Drug Intelligence Database (NFDID), also referred to as the Logo Index [1], was established in March 1999, by the SAPS FSL. Methaqualone tablet formulations, amphetamines and LSD blotter papers are included in this database. Colour photographs, accompanied by the dimensions and chemical identity of the active constituents, are currently included in this database. The concentration of active constituents, identity and concentration of inactive constituents, precursor or processing chemicals, as well as by-products produced during the synthesis or production of illicit drug formulations, are not currently included in the NFDID.

In South African courts of law, the forensic drug analyst is increasingly required to state whether illicit preparations from different seizures might originate from the same manufacturer or manufacturing batch. The following questions often arise: (1) “Can it be concluded that tablets with the same Logo and thus the same Logo Index code, are of the same origin?”, (2) “Is it sufficient to establish the presence of active constituents such as methaqualone and diphenhydramine in cases suspected to be of the same origin?”, (3) “Is it essential to determine the concentration of the active constituents as well?” and (4) “Does the presence/absence of un-reacted precursors, reaction by-products and contaminants increase the possibility that these tablets are of the same origin?”

These questions create the need for not only routine analysis of seized tablets for the purpose of identifying the active ingredients, but also for quantitative determination of the active ingredients, and identification of the precursors and contaminants that might be present. In order to do just that, an applicable analytical method would have to be sourced or developed. Establishing whether the performance characteristics of such a specific method meet the requirements for the intended applications of the analytical method is essential (method validation). This validation process establishes the limitations of the method, as well as the effects of specified interfering compounds on the performance of the method. Numerous scientific publications were studied to source an applicable analytical method.

One such publication addresses the fact that in the United States of America (USA), illicit methaqualone and/or mecloqualone containing products are also being produced in clandestine laboratories. Their Forensic Chemists are thus often requested to analyse unlabelled reaction mixtures confiscated during laboratory seizures. Identifying precursors and by-products in such mixtures is essential in determining the synthetic route used in the illicit manufacture of these quinazolinones. In this publication Angelos and Meyers [2] present a rapid method of isolation and identification of precursors and products of clandestine mixtures. They made use of gas chromatography (GC) and high performance liquid chromatography (HPLC) for chromatographic separations of methanol solutions of actual clandestine mathaqualone reaction mixtures. Infrared (IR), utilizing the standard potassium bromide (KBr) disk method, nuclear magnetic resonance (NMR) and quadropole mass spectrometry (MS) techniques were used in order to identify the separated compounds.

This publication served as basis for developing a quantitative analysis method of methaqualone in illicit preparations, and the subsequent application thereof to the quantitative analysis of illicitly produced methaqualone containing tablets. The project had two main aims. The first aim of this project was to validate the quantitative analysis of methaqualone in illicit preparations utilizing gas GC/MS. The theory concerning method validation is discussed in Chapter 2. Planning of the validation process, as well as the results of this validation process is reported and discussed in Chapter 3.

The second aim of this project was to apply this validated method to the quantitative analysis of seized illicit preparations received at the SAPS FSL for analysis. In the planning of this experiment (Chapter 3), literature references on the characteristics of methaqualone (Chapter 2), and the extraction and analyses of methaqualone-containing preparations (Chapter 2), were consulted. Literature references concerning chemical fingerprinting of other illicitly manufactured drugs such as heroin and the amphetamines, as referred to in Chapter 2, were also consulted.
Four batches of tablets from past cases were selected for further chemical analysis, two batches had the same logo imprinted on the tablets and the other two batches carried another imprinted logo. The data resulting from the application of the validated method on these illicit preparations is reviewed in Chapter 3 to establish whether the preparations analysed can be traced back to the same manufacturer. The applicability of the validated extraction and GC/MS method in determining the presence/absence of un-reacted precursors, reaction by-products and contaminants, is addressed in Chapter 4.

This method of choice would then be judged by its ability to give satisfactory answers to the four questions raised above. If deemed suitable, this method would be adopted as a routine analytical method forming part of the chemical fingerprinting program of illicitly manufactured methaqualone containing preparations.

As mentioned in literature [3], information of the synthesis method, as well as chemicals and equipment used, can be derived from the identity of the impurities present. The data generated through the application of this method on seized cases would then be used to update and expand the existing National Forensic Drug Intelligence Database.[1] Applying this knowledge to South African conditions could result in the SAPS being able to monitor the production and sale of commercially available precursors by law enforcement, and the subsequent detection of clandestine laboratories. Conspiracy links regarding samples of common origin could be established based on the presence or absence of certain impurities. The value of this method for future use by the Chemistry Unit of the South African Police Service Forensic Science Laboratory is evaluated in the concluding Chapter, namely Chapter 5.

1.2 Abbreviations

dph or DPH	diphenhydramine
g	gram
GC/MS	gas chromatography-mass spectrometry
GLC	Gas-Liquid chromatography
HPLC	high performance liquid chromatography
IR	Infrared
LOD	limit of detection
LOQ	limit of quantitation
LSD	lysergic acid diethylamide
MCT method	methaqualone, cocaine and tetrahidrocannabinol method
mL	milliliter
mtq or MTQ	methaqualone
n/d	not detected
NMR	nuclear magnetic resonance
NPD	nitrogen phosphorous detector
FID	flame ionization detector
µg/g	micrograms per gram
µg/mL	micrograms per milliliter
µL	microliter
SAPS FSL	South African Police Service Forensic Science Laboratory
TIC	total ion chromatogram
TLC	thin layer chromatography
UV	ultra violet

CHAPTER 2 - BACKGROUND

2.1 METHAQUALONE

2.1.1 Characteristics

Methaqualone (2-methyl-3-*o*-tolylquinazolin-4-(3H)-one), is a quinazoline derivative. The structure of methaqualone is shown in Figure 2.1 and an example of the electron impact mass spectrum of methaqualone is shown in Figure 2.2 below.

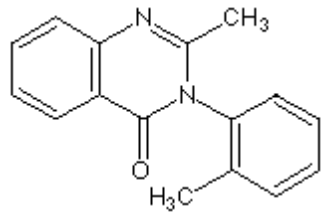

Figure 2.1: Structure of Methaqualone

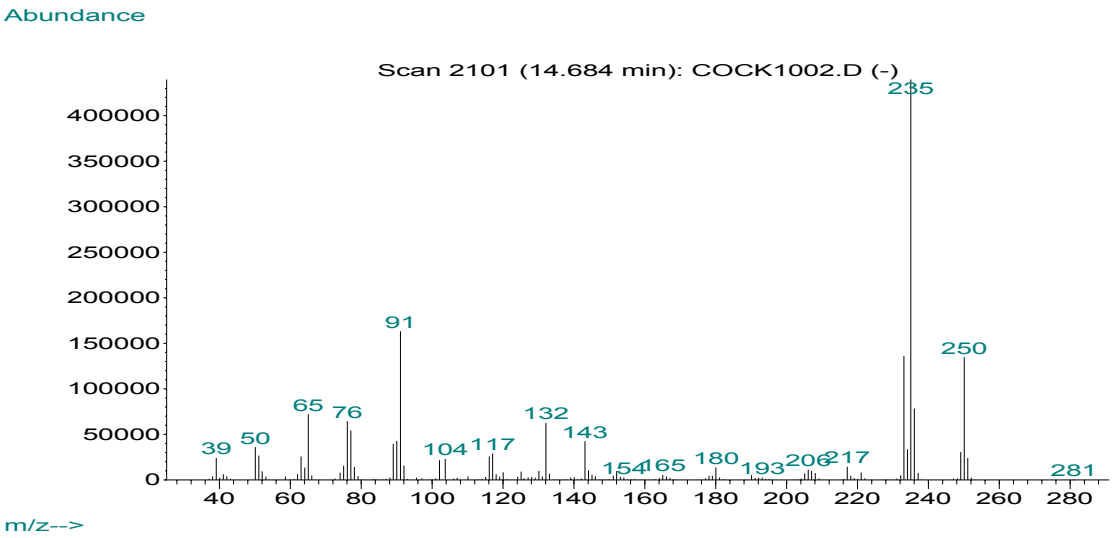

Figure 2.2: Electron Impact Mass Spectrum of Methaqualone

Methaqualone has hypnotic and sedative properties.[4] It has been administered orally for the short-term treatment of insomnia, but found to be less effective than the benzodiazepines.[4] Methaqualone also has anticonvulsant, antitussive and weak antihistaminic properties.[4] It is no longer used for legitimate therapeutic purposes, and is manufactured in clandestine laboratories and widely abused for its mind altering properties.

Methaqualone is a central nervous system depressant. Overdose gives rise to symptoms such as gastrointestinal stress, drowsiness, ataxia, slurred speech, paresthesias to agitation, convulsions and coma. The cough reflex is also decreased. It also selectively depresses spinal reflexes, leading to muscular hyperactivity.[4] This muscular hyperactivity can be treated by administering diazepam. (Can this be the reason why diazepam is sometimes identified in illicit methaqualone preparations?)[5] Gastric lavage and/or activated charcoal should be used for gastric decontamination.[4]

2.1. 2 History and Statistics

Methaqualone has been withdrawn from the market in many countries [6], including South Africa, due to its widespread abuse. In South Africa, methaqualone is listed in Part III, Schedule 2 of the Drug and Drug Trafficking Act, Act 140/1992, and Schedule 8 of the Medicines and Related Substances Control Act, Act 101/1965.

In some preparations, for example “Mandrax”, at that time produced by Roussell, methaqualone hydrochloride and the antihistamine diphenhydramine, were combined. This combination is said to be synergistic in sedative hypnotic activity.[7] In South Africa the term “Mandrax” is used generically to refer to all forms of methaqualone-containing preparations. Instead of dyphenhydramine, diazepam has also been identified in certain preparations and found in two out of the nine formulations examined by M. Sher.[5]

During 1999, 2 400 seizures or occurrences that tested positive for methaqualone were examined by the SAPS FSL Drug Section (Nationally). In 2000, 5 945 seizures were examined by the SAPS FSL Drug Section (Nationally) and tested positive for the presence of methaqualone. In 2001 and 2002, 5 921 and 6 160 seizures were examined by the SAPS FSL Drug Section (Nationally), and tested positive for the presence of methaqualone. In 2003 this number increased to 8 351 entities, which involved 1,3 million tablets and 5 000 kilogram tablet pieces and powder. Figure 2.3 summarizes the trend over five years.

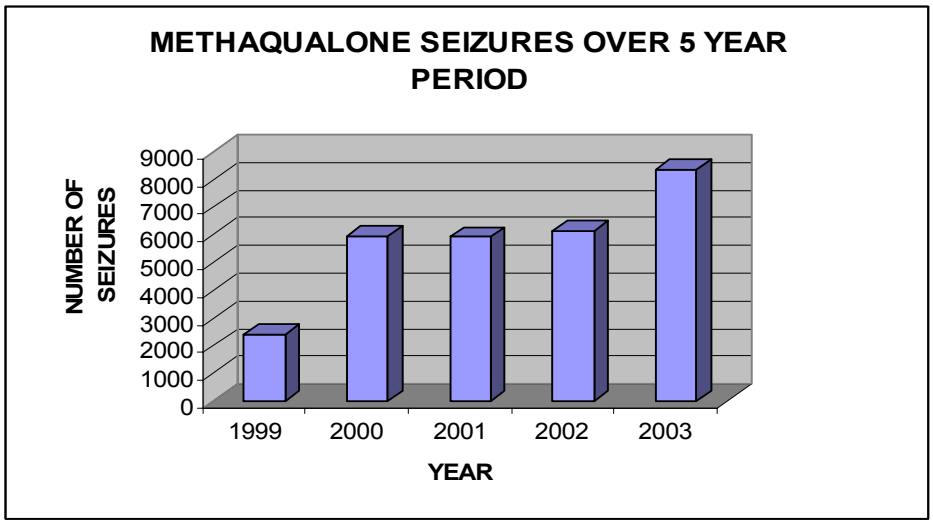

Figure 2.3: **The number of Methaqualone seizures taken to the SAPS FSL for analyses over a five year period.**

2.1.3 Synthesis of Methaqualone

The synthesis of methaqualone is widely documented. Acharya and Chattopadhyay [8] discuss the cyclodehydration to 2,3-disubstituted 4(3H)-quinazolinones of 2-acylamino (N-aryl) benzamides, on refluxing with toluenesulphonic acid (TsOH) in benzene or acetonitrile solution. A convenient procedure for converting isatoic anhydride to 2-amino (N-aryl/alkyl) benzamides is also discussed.

Akazome, Kondo, and Wtanbe [9] described the use of several ruthenium and platinum complexes as catalysts for the reductive N-heterocyclization of N-(2-nitrobenzoyl)amides under carbon monoxide pressure, to afford the corresponding 4(3H)-quinazolinone derivatives. This included some quinazolinone alkaloids in good yields.

In a Czech patent, described by Borovicka [10], methaqualone was prepared by condensation of o-$AcNHC_6H_4CO_2H$ with o-$MeC_6H_4NH_2$ in the presence of $POCl_3$ (phosphorous trichloride). This was however contaminated with o-$AcNHC_6H_4CONHC_6H_4Me$-*o* and o-MeC_6H_4NHAc, making therapeutical application impossible. An aqueous solution of methaqualone-HCl was treated with repeated portions of toluene to extract contaminants. Purified methaqualone base was separated after alkalinization and crystallisation. Methaqualone-HCl is recovered in 85% yield.

Cerini and Allen [11] described the use of 2-methyl-4-(3H)-quinazolinone as starting material in the synthesis of methaqualone. This process involves the 1,3 sigmatropic rearrangement of 2-methyl-4-aryloxyquinazolinone to the nitrogen adduct 2-methyl-3-aryl-4(3H)-quinazolinone. This is a result of the inherent instability of the lactam *versus* the lactim structure.

In a Dutch Patent of 1965 [12], 18 gram of *N*-acetylanthranilic acid and 14.5 gram of toluidine-HCl were heated at 170 degrees Celsius for 5 minutes. The solidified matrix was dissolved in 150

milliliter water and 10 milliliter concentrated hydrochloric acid, and filtered. The hot solution was alkalinized with a 50 % sodium hydroxide solution, and cooled. The precipitate was dissolved in 40 milliliter of ethanol and filtered. 20 Milliliter 20% alcoholic hydrochloric acid was added and left to cool. 60 Milliliter diethyl ether was added. The precipitate was filtered and washed with 60 milliliter 1:1 diethyl ether : ethanol, to yield 19.8 gram 2-methyl-3-(*o*-tolyl)-4-quinazolinone-HCl.

Another Czech patent described by Frantisek Jiri [13], describes the purification of 2-methyl-3-(*o*-tolyl)-quinazolinone-4, as prepared from acetylanthranilic acid and *o*-toluidine, using the cycling agent phosphoroxychloride, and toluene as a medium. After completion of the cyclic reaction, the mixture is decomposed by methanol, (2/3 of volume of toluene), and then by acetone, (the same volume as toluene), where toluene is used as a reaction medium, and afterwards pure product is isolated from the mixture.

The synthesis of 3-*o*-tolyl-4-quinazolinone is discussed in a British Patent December 6, 1967[14]. 10,7g of *o*-toluidine, and with stirring, 4mL of phosphorous trichloride in 25mL toluene, were added to a suspension of 16.5g N-formyl-anthranilic acid in 125mL of toluene. The mixture was refluxed for two hours, cooled and alkalinized with 15% sodium carbonate. After evaporation of the toluene, the residue was dissolved in ethanol. The 3-*o*-tolyl-4-quinazolinone was precipitated with gaseous hydrogen chloride.

In a step-by-step Quaalude synthesis guide [15], the synthesis of Methaqualone from anthranilic acid, N-acetylanthranilic acid and isatoic anhydride is discussed. The synthesis of the precursor anthranilic acid from o-toluidine and phtalic anhydride, as well as that of o-toluidine from acetone and ethylacetate and from toluene, is also discussed.

A description of the synthesis of methaqualone in a clandestine laboratory, with detailed information on how to obtain the chemicals required for the synthesis of methaqualone, is available on the internet.[16] The availability of this type of information highlights the extent of illicit methaqualone-containing preparations, as well as the ease of gaining information on how to operate clandestine operations.

Kiser and Allen [17] developed a direct approach for methaqualone synthesis, using isatoic anhydride. Direct reaction of isatoic anhydride with *o*-toluidine resulted in the opening of the anhydride ring, with simultaneous formation of *N*-(*o*-tolyl)-anthranilamide. Acetylation with acetic anhydride yielded *N*-(*o*-tolyl)-acetylanthranilamide. Ring closure was achieved with $POCl_3$ (phosphorous trichloride), to form methaqualone.

A paper by Manhas, Amin and Rao [18] described a new synthesis of methaqualone and its substituted analogues. A detailed discussion on the procedure for the reaction of *o*-aminoamides with diketones, yielding 2-methyl-4-quinazolone and its pyrimidine derivatives is provided. Two methods of methaqualone synthesis are also described. In the first method a mixture of isatoic

anhydride and *o*-toluidine was heated and triturated with ether after cooling. The resulting solid was collected by suction and recrystallized to yield the *o*-aminoamide. A mixture of the *o*-aminoamide, acetylacetone in ethanol and a few drops of hydrochloric acid were then refluxed. On cooling, methaqualone-HCl is separated (85% yield). In the second method a mixture of isatoic anhydride and *o*-toluidine in toluene, was refluxed. Acetylacetone, containing a few drops of hydrochloric acid, was added, after which the refluxing continued. Evaporation of the solvent yielded methaqualone-HCl (80% yield).

In a United States patent of October 1965, Morgan, Simmons and Simmons [19] described the synthesis of 2-methyl-3-(*o*-tolyl)-4-quinazolone, 2-methyl-3-(*m*-chlorophenyl)-4-quinazolone, 2-ethyl-3-(*m*-tolyl)-4-quinazolone, 2-propyl-3-phenyl-4-quinazolone, 2-ethyl-3-phenyl-4-quinazolone, 2-methyl-3-phenyl-4-quinazolone and 2-n-pentyl-3-phenyl-4-quinazolone.

4(3H)-Quinazolinones have been prepared by Nielsen and Pedersen [20] by heating methyl N-acylanthranilates and the hydrochlorides of primary aliphatic and aromatic amines with phosphorous pentaoxide and N,N-dimethylcyclohexylamine at 180 degrees Celsius. 4-Quinazolinamines and the amidine were isolated as by-products. The carboxamides were believed to be reaction intermediates. By raising the temperature to 250 degrees Celsius, 4-Quinazolinamines were obtained in preparative yield.

A one-step synthesis of methaqualone from *N*-acetylanthranilic acid and *o*-toluidine in bromobenzene, in the absence of a catalyst, was described by Soliman, Shafik and Elnenaey.[21] A rapid diphasic titration (analogues to that applied for the microdetermination of secondary and tertiary organic bases and their salts) of methaqualone was also described. The method was used for methaqualone base and its tablet form, and the results were compared with those obtained from non-aqueous titration procedures.

The synthesis of 2-methyl-3-ortho-tolyl-4-quinazolone and acid addition salts thereof, was described in a British Patent of 1960. [22] The acid addition salts included the hydrochloride, hydrobromide, acetate, propionate, maleate, succinate, citrate, tartrate, phosphate and sulphate, of which the hydrochloride was preferred.

A feasible synthesis of methaqualone hydrochloride was recorded in 1977 by the United News Service. [23] A 5-step synthesis of methaqualone hydrochloride was described. In step one, *o*-nitrotoluene was reduced using tin, iron or zinc, and hydrochloric acid. *o*-Nitrotoluene was subsequently oxidized to *o*-nitrobenzoic acid, which was then reduced to *o*-aminobenzoic acid. *o*-Aminobenzoic acid was acylated to yield 2-acetamidobenzoic acid. In the final step, 2-acetamidobenzoic acid and *o*-aminotoluene reacted to form methaqualone.

Another method for preparing 4(3H)-quinazolinones involved the 20-40 minute reaction of 2-acylaminobenzonitrile with arylamine hydrochlorides, phosphorous pentoxide N,N-dimethylcyclohexylamine hydrochloride and water at 180 degrees Celsius with a 31-53% yield. The hypnotic drugs mecloqualone and methaqualone can also be prepared in this way.[24]

All of the above mentioned procedures for methaqualone synthesis can lead to the possible presence of various un-reacted precursors and by-products. This aspect will be addressed in Section 2.2.

2.2 METHAQUALONE-CONTAINING PREPARATIONS

As mentioned in Chapter 1, the paper by Angelos and Meyers [2] discussed a rapid method of isolation and identification of precursors and products of clandestine mixtures. Despite the information given in this publication, it was still essential to study other relevant/similar publications to gain more information on the subject. Publications on the extraction and analysis of methaqualone, its precursors as well as its intermediates and reaction by-products formed during methaqualone synthesis, are discussed below.

2.2.1 Extraction and Analysis of Methaqualone

Sher [5] quantitatively screened nine different illicitly manufactured Mandrax formulations for the presence of methaqualone and other psychoactive compounds, using high performance liquid chromatography. The methaqualone containing tablets were dissolved in methanol. Methaqualone was detected in all the tablets, and the quantity of methaqualone varied between 181 and 413 milligram for the nine different formulations analysed. Two of the formulations contained diazepam at 2 to 3 milligram per tablet.

Already in 1967 the mass spectrum of 3,4-dihydroquinazolin-4-one was studied by Batterham *et al* [25]. In 1977, Baker [26] presented a study where a gas chromatographic system equipped with dual flame ionisation and nitrogen selective rubidium detectors (FID and NPD respectively) were used in the identification of drugs, including methaqualone. The response index (ratios of FID and NPD peak heights of the drug, compared to the same response ratio of the internal standard) and relative retention times of drugs were recorded. He concluded that the response index was characteristic of a given drug, and had exclusionary value in reducing the possible candidates in the identification of unknowns. He also concluded that the response index of drugs with the same type of nitrogen structure had very similar values. Overlapping response index ranges decreased the possibility of uniquely determining the chemical class of unknowns. Christ *et al* [27] also used the highly selective nitrogen phosphorous detector to determine retention indices in a rapid screen for drugs, including methaqualone. They concluded that their results were comparable to results obtained by using the older methods with the FID and packed or capillary columns.

Baker *et al* [28] making use of high performance liquid chromatography, concluded that using relative retention times alone, only 9% of the drugs tested by them, could be distinguished. When both the retention times and absorbance ratios (absorbance at 254 and 280 nm) were used, 95% of the drugs, could be distinguished. Reuland and Trinler [29] came to the same conclusion. In both these studies methaqualone was included as one of the drugs tested.

Eight laboratories collaboratively studied a procedure for the quantitative determination of methaqualone-HCl in pharmaceutical and clandestine preparations, as described by Hanel.[30] Two commercial products, and four samples prepared by the author were used in the study. Methaqualone was extracted from an aqueous bicarbonate solution into chloroform, and quantified by gas-liquid chromatography (GLC) on a 3% OV-1 column. Tetraphenylethylene was used as an internal standard. The four prepared samples yielded recoveries of 100.0 to 102.6%. Coefficients of variation ranged from 1.58 to 4.15% for all the samples studied. The method was adopted as official first action by all these laboratories.

Note: "Collaborative study" refers to a study performed by different laboratories, employing the same analytical method, to assess the random analytical error.

The gas chromatographic-mass spectrometric analysis of methaqualone, phenmetrazine and phendimetrazine was investigated by Boyd, Moses and Bowman. [31] During this study GC/MS was considered as complementary technique to the standard ultraviolet and infrared methods used at the Louisiana State Police Crime Laboratory. These techniques became less conclusive due to the increasing diversification of street drugs. The inherent dangers of utilizing less specific analytical techniques were emphasized. In Chapter 3 this is also illustrated by the fact that the routine TLC technique was not sufficient for the separation and subsequent identification of methaqualone, its precursors, isomers and reaction by-products.

In 1973 Saferstein and Chao [32] described the forensic application of chemical ionisation mass spectrometry in the identification of drugs, including methaqualone. Isobutane was used as the reaction gas to produce chemical ionisation spectra. The utilization of a dual electron/chemical ionisation source was recommended. This would enable the analyst to control the complexity of the mass spectra produced. For the electron ionisation spectra the ionisation voltage was set at 70 eV and for the chemical ionisation spectra at 300 eV.

Warkentin, Wynne and Wendlandt [33] presented differential scanning calorimetry (d.s.c.) curves of commercial samples of formulations containing methaqualone. New and old formulations could be differentiated by differences in the tablet filler and binder, and could be used to identify samples containing 0.15 milligram of methaqualone or less. This technique could not establish the country of origin, since Quaalude tablets from the USA and Mexico gave similar d.s.c. curves. For this reason, linking illicit preparations with each other by d.s.c. in this project would not be a suitable technique.

Micellar electrokinetic capillary chromatography (MECC) gave significantly greater efficiency, selectivity, peak symmetry and speed, as compared to high performance liquid chromatography (HPLC), for the determination of methaqualone and other illicit drugs.[34] Methaqualone was determined to be an adulterant of an illicit heroin seizure sample. MECC can achieve superior resolution in approximately one third of the analysis time of HPLC. This study was performed by Weinberger and Lurie in 1991.

It is clear that techniques like gas chromatographic systems with dual flame ionization and nitrogen selective bead detectors, GC/MS, HPLC, as well as the standard ultraviolet and infrared methods, are all suitable for the identification and quantification of methaqualone and other drugs. In this project, GC/MS is the method of choice.

2.2.2 Extraction and Analysis of Precursors, Intermediates and Reaction By-products formed during Methaqualone Synthesis

As mentioned earlier, identification of intermediates and by-products in a clandestine preparation yields useful information in identifying the specific synthesis route. It increases the possibility of tracing the illicit product to a specific manufacturer or manufacturing batch, and/or enables the linking of tablets of different cases. The value of identifying the above-mentioned compounds is emphasized by Angelos and Meyers.[2] A rapid method for the isolation and identification of the precursors and reaction products of methaqualone and mecloqualone was described. Chromatographic separations of the reactants and by-products were achieved by both HPLC and GLC, and identification of the compounds was performed by infrared spectroscopy and quadropole mass spectrometric techniques.

Dal Cason, Angelos and Washington [35] described the synthesis of the drug 2-methyl-3-ortho-tolyl-4-quinazolinone (methaqualone) as well as fifteen chemical analogues and positional isomers. Their identification by spectroscopic techniques was also described. The series of analogues studied included the compounds formed through substitution of hydrogen or halogen atoms in place of the methyl group of the 3-tolyl substituent in methaqualone. The substituent's positional orientation of ortho, meta or para was also considered. 2-Methyl-3-*o*-tolyl-4(3H)-quinazolinone, 2-methyl-3-*m*-tolyl-4(3H)-quinazolinone and 2-methyl-3-*p*-tolyl-4(3H)-quinazolinone were among the analogues studied. Infrared, nuclear magnetic resonance, and mass spectra of the compounds were distinctive. GLC and thin-layer chromatographic (TLC) systems for analysis of these compounds, as well as melting point and ultraviolet data were discussed. They concluded that GLC was a good screening method. If the identities of the compounds were known, it would make a good quantitative tool. TLC was found to have no value due to the small differences in Rf values. (See Chapter 3, paragraph 3.1.12.2).

Nitromethaqualone and three positional isomers formed by moving the nitro-group on the phenyl ring containing the methoxy-group, have been synthesised and characterised by Clark.[36] Infrared,

nuclear magnetic resonance, mass spectra and gas-liquid chromatographic behaviour of these compounds are presented. It is shown that the combination of these techniques can differentiate nitromethaqualone from any of the studied isomers, methaqualone, and mecloqualone.

Orphenadrine has been identified in counterfeit Quaalude (LEMMON 714) tablets by Churchill. [37] This was achieved by gas liquid chromatography (isothermal run), infrared spectrophotometry and nuclear magnetic resonance.

The analytical data for the identification of three hypnotics of the quinazolinone series, namely methaqualone, mecloqualone, and nitromethaqualone, was presented by Daenens and Van Boen. [38] A chloroform extraction was performed on crushed tablets dissolved in an alkaline aqeous phase. The analytical data included thin-layer chromatography (TLC), GLC, infrared (IR) spectrophotometry, ultraviolet (UV) spectroscopy, NMR spectroscopy, and MS. It was concluded that combined GC/MS provided the best results. This strengthens the reasons for employing GC/MS as preferred technique for the current study.

2-Ethyl-3-phenyl-4(3H)-quinazolinone was readily prepared by substituting n-propionylanthranilic acid and aniline for N-acetylanthranilic acid and *o*-toluidine in procedures for the synthesis of methaqualone. Mass spectra, infrared spectra, and nuclear magnetic resonance spectra of these compounds were presented by Kiser. [39] This analytical data could help in the recognition of precursors which might be present in illicit preparations.

The presence of 2-ethyl-3-phenyl-4(3H)-quinazolinone, a structural isomer of methaqualone, was established by Lorimer [40] in counterfeit Quaalude (LEMMON 714) tablets. The synthesis of this structural isomer was similar to that of methaqualone, except that propionic anhydride and aniline were used instead of acetic anhydride and *o*-toluidine respectively. Mass spectra, infrared spectra, and nuclear magnetic resonance spectra for the hydrochloride salt and the free base were presented. This work provided valuable information on the synthetic route of compounds detected in clandestine preparations.

A simple and quick micro-crystalline test for anthranilic acid and N-acetylanthranilic acid was presented, as an alternative to GC/MS analysis. [41] The crystals formed with the $AgNO_3$ reagent, could be used to distinguish between anthranilic acid and N-acetylanthranilic acid. This method could be applied to the analysis of compounds in counterfeit Quaalude tablets, which have been found to contain N-acetylanthranilic acid as a major impurity.

The mass spectra of 2-substituted-3-(2-methylphenyl)-4(3H)-quinazolinones and their thio-analogues under electron impact conditions, were studied by Ramana and Kanthara. [42] The mass spectra were recorded on a Finnigan MAT 8230 mass spectrometer, and all the samples were introduced into the mass spectrometer through direct probe insertion.

The United Nations recommend the extraction of methaqualone containing preparations from a 1 M bicarbonate solution, using several portions of chloroform.[43] Sampling, extraction techniques, presumptive colour tests, thin-layer chromatography, gas-liquid chromatography, high performance liquid chromatography and infra-red spectroscopy of methaqualone and mecloqualone, as used by national narcotics laboratories in Vienna, were described.

It is apparent that the possibilities of extracting and detecting methaqualone, its precursors, intermediates and reaction by-products formed during methaqualone synthesis, are well documented. An informed decision can thus be made in the choice of the simplest and most effective options that would best suit the needs of the SAPS FSL. In Chapter 3 the method of choice and the planning of the validation thereof, is described in detail.

2.3 CHEMICAL FINGERPRINTING OF ILLICITLY MANUFACTURED DRUGS OTHER THAN METHAQUALONE

As mentioned in Chapter 1, the SAPS FSL records the physical aspects of methaqualone tablets in the NFDID.[1] The NFDID consists out of a collection of colour photographs of the back and front of tablets seized by the SAPS, and handed in for analysis at the FSL, as well as their dimensions. The tablets are indexed under their main active ingredient, in this instance, methaqualone. Other active ingredients and possible precursors, reaction by-products or contaminants are not currently routinely tested for, and are therefore not included in the NFDID. This database is updated on a yearly basis, and grows at an alarming pace.

Other countries have a similar albeit more sophisticated approach, however, they do not have the extensive problem with methaqualone experienced in South Africa. Despite the fact that methaqualone is mainly a South African problem, it still is essential to be aware of how other countries approach the same problem with different drugs. In the following discussion the difficulties experienced by, for example the Australian authorities with heroin and the Canadian authorities with 3,4-methylenedioxyamphetamine (MDA), amongst others, are highlighted.

2.3.1 Heroin

Myors et al [44] employed a GC/MS procedure for detecting trace organic constituents to aid in the chemical profiling of heroin samples from a variety of opium production areas. Two statistical models for data analysis and identification of parameters capable of indicating sample origins, were then applied and compared. They concluded that the profiling procedure was capable of identifying parameters which are useful for discriminating between samples of different origins. This was possible because the GC/MS method gave reproducible and reliable data. This enabled them to carry out parameter selection and reduction to a level where highly efficient predictive models could be developed to discriminate between samples with different origins. If data for organic compounds could be combined with quantitative trace element data and qualitative data for physical properties

such as colour and packaging, it could lead to an even more powerful profiling method.

In 1991 Weinberger and Lurie [34] performed a study on a complex mixture consisting of acidic and neutral impurities present in an illicit heroin seizure sample. They compared the results obtained by micellar electrokinetic capillary chromatography (MECC) to that obtained by high performance liquid chromatography (HPLC). MECC resolved at least twice as many peaks as HPLC, making MECC an acceptable technique for signature analysis of illicit drug preparations.

The same principle was applied to amphetamines, as discussed below.

2.3.2 The Amphetamines

3,4-Methylenedioxyamphetamine (MDA), is a popular illicit drug in Canada. Chemicals seized from illicit laboratories often contain impurities, precursors and intermediates, in addition to, or in the absence of MDA. Impurities can interfere with some analytical methods, whereas the identification of these impurities can, however, assist in establishing the method of MDA synthesis used. This can be used in the comparison of samples of diverse origin. Identification of precursors and intermediates helps to establish the synthetic routes and the potential quantity of MDA that can be produced. Ultraviolet-, infrared-, nuclear magnetic resonance- and mass spectra, as well as gas-liquid and thin-layer chromatography data were used by Lukaszewski, in the identification of the different substances. [45]

Analytical data from thin-layer chromatography, gas chromatography, gas chromatography-mass spectrometry of the precursors, intermediates and reaction by-products in the synthesis of 3,4-methylenedioxymethylamphetamine (MDMA), was obtained by Renton *et al*. [46] MDMA was also analysed by magnetic resonance spectroscopy, X-ray diffraction, infrared spectroscopy, ultraviolet spectroscopy and high performance liquid chromatography. The results were used as reference data for the identification of MDMA in seized samples, and also to establish the synthetic routes of illicitly prepared MDMA by the study of trace impurities.

Illegal drugs are often contaminated with impurities resulting from inadequate purification procedures, imperfect chemical handling, starting materials, side and subsequent reactions, intermediate products, diluents, laboratory dirt, as well as handling and packaging the drugs. Chromatographic patterns resulting from the analysis of these illegally produced drugs contain valuable information about the drug and its synthesis, even if the identity of each of the chromatographic peaks is not known. Comparison of the peak patterns is generally referred to as signature analysis. Commonly used synthetic methods of amphetamine and methamphetamine were discussed in the paper of Verweij. [47] The isolation of impurities was also discussed. The impurities were analysed with chromatographic and mass spectrometric methods, providing important indications about the type of synthesis used.

Van der Ark, Verweij and Sinnema [48] explained that side reactions and incomplete conversions during the Leuckart caused the presence of impurities in illegal amphetamine. Nuclear magnetic resonance spectra, as well as gas-liquid and thin-layer chromatography data were used in the identification of the different substances. This could be helpful in avoiding possible analytical interferences of the by-products. The detection of these products in soil or water can support the assumption that amphetamine production is taking place in a certain area.

Kram and Kreugel [49] also describe the side reactions and incomplete conversions during the Leuckart synthesis, which cause the presence of impurities in illegal amphetamine. Like Van der Ark, Verweij and Sinnema [48] employed nuclear magnetic resonance spectra, as well as gas chromatography/mass spectrometry data in the identification of the different substances. This provided significant clues towards characterization of the manufacturing process, in the absence of the finished product. [49]

Identifying impurities in illicit Methamphetamine samples could be helpful in avoiding possible analytical interferences of the by-products. Ultraviolet, infrared, nuclear magnetic resonance and mass spectral data were used by Barron *et al* in the identification of the various substances. [50]

Cantrell *et al* [3] concentrated on the fact that knowledge of the impurities resulting from the illicit synthetic of Methamphetamine provides valuable information. Information of the synthesis method, as well as chemicals and equipment used, can be derived from the identity of the impurities present. This leads to the monitoring of production and sale of commercially available precursors by law enforcement, and the subsequent detection of clandestine laboratories. Conspiracy links regarding samples of common origin can also be made by the presence or absence of certain impurities. Possible dangers to Methamphetamine users can also be identified. Identification of impurities could be beneficial in the avoidance of possible analytical interferences of the by-products.

2.4 THE THEORY OF METHOD VALIDATION

2.4.1 Why Method Validation?

Validation is the procedure by which the ability of a specific method to meet the requirements for the intended analytical applications thereof is evaluated.

In the line of Forensic Analytical Chemistry, it is absolutely essential to establish the limitations of extraction and instrumental techniques. Often the Forensic Scientist has to defend his/her experimental work in court. Being able to present documented proof of the abilities and limitations of the techniques applied, increases the credibility of the scientist, as well as of the analytical techniques applied. Validation data enables the scientist to answer questions on, for example, the detection limit of the method for certain compounds, the influence of interferences, or the ability of the method to distinguish between isomers with confidence.

2.4.2 Definitions and Formulas

2.4.2.1 Limit of Detection (LOD), and Limit of Quantification (LOQ) [51]

The limit of detection (LOD) refers to the lowest amount of analyte in a sample that can be detected, not necessarily quantified, under specified experimental conditions. The detection limit is usually expressed as the concentration of analyte in the sample. The LOD may also be determined by calculating the mean value for the blank, and adding three standard deviations (SD) of the blank to this value (LOD = Xm + 3 SD). Xm represents the mean value for the blank. In chromatography the LOD expresses the smallest concentration of analyte in a sample needed to give a peak height three times the noise level of the background signal of a blank sample.

The limit of quantification (LOQ) refers to the lowest amount of analyte in a sample that can be quantitated with acceptable precision and accuracy under the specified experimental conditions. The LOQ may be determined by calculating the mean value for the blank, and adding ten standard deviations (SD) of the blank to this value (LOD = Xm + 10 SD). It is preferable to determine the LOQ experimentally as the lowest concentration for which an acceptable coefficient of variation of the measured value (cv < 20%), can be routinely achieved.

2.4.2.2 Linearity range [51]

Linearity is defined as the method's ability to produce test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. Data with significant scatter can be processed mathematically to obtain a straight line that best fits the data points. This method is referred to as the method of least squares, or linear regression analysis. In practice this refers to a linear curve determined in a calibration graph of measured *versus* actual concentration. The range of an analytical method is the interval (confidence interval) between the upper and lower levels of analyte that can be determined with a suitable level of precision, accuracy, and linearity, applying the method as specified. In other words, the range within which it may be reasonably assumed that a true measurement can be made. The range should be expressed in the same units as the test results obtained by the analytical method.

Applicable formulas:

(i) Correlation coefficient, r, where

$$r = \sum^{n}_{i=1} X_i Y_i / \sqrt{ \left(\sum^{n}_{i=1} X_i^2 \right) \left(\sum^{n}_{i=1} Y_i^2 \right) }$$

X = analyte concentration, and Y = analyte response

(ii) Slope of the best line fit, m, where

$$m = (n\sum X_i Y_i - \sum X_i \sum Y_i) / (n\sum X_i^2 - (\sum X_i)^2)$$

(iii) y-Intercept of the best line fit, b, where

$$b = (\sum X_i^2 \sum Y_i - \sum X_i \sum X_i Y_i) / (n\sum X_i^2 - (\sum X_i)^2)$$

2.4.2.3 Matrix effect [51]

The effect the composition of the matrix has on the performance of the analytical method is known as the matrix effect.

2.4.2.4 Sampling [51]

For the purpose of the validation procedure, each sample analysed was spiked and prepared individually before extraction. Individual 1gram samples (1 tablet weighed 1gram), were extracted for the purpose of GC/MS analysis. When performing analysis on seized tablets for court purposes, the hyper-geometric model of sampling will be used. [52], [53]

2.4.2.5 Selectivity [51]

The selectivity of an analytical method refers to the ability of the method to respond to the analyte of interest in the presence of possible interferences.

2.4.2.6 Precision [51]

The precision or repeatability of an analytical method is normally measured in terms of the standard deviation (coefficient of variation/closeness of results to each other) of a series of measurements.

s = standard deviation of the mean observed value, where

$$s = \sqrt{\sum^{n}_{i} (X_i - \bar{x})}$$

and $\bar{x}$ is the arithmetic mean ($\sum X_i / n$), i.e. the sum of all the measurements, divided by the number of measurements.

2.4.2.7 Accuracy [51], [54], [55]

The accuracy of an analytical method is the extent to which the data or the average value of a set of data obtained with a specific method, matches the true value. It is expressed as the percent recovery of the analyte of interest, ($\bar{x}/\mu$) x 100, or the percent error, (μ - $\bar{x}/\mu$) x 100. μ = true or nominal value at which sample has been spiked, and $\bar{x}$ = mean observed value. The Accuracy for a specific Matrix tested, is also determined by looking at the % Recovery, Standard Deviation, % RSD (RSD = (s / $\bar{x}$) x 100%), the confidence interval, and the t experimental (t_{exp}) and t calculated (t_{calc}) values (degrees of freedom at 95% confidence level, or Student's t-test).

The Student's t-test is a way of comparing two means of two different sets of data to determine the probability that the difference is insignificant (null hypothesis). If t_{exp} exceeds a certain critical value, then the null hypothesis is rejected. This critical value (t_{calc}) for a particular significant level of degrees of freedom can be found in the "t table" as depicted in Appendix J. For example, if working with a sample size of 10, the degrees of freedom will be 9. At the 95% confidence level t_{calc} will be 2.26. If t_{exp} is less than t_{calc}, there is at least a 95% probability that the values for the two portions of the sample are the same. t_{exp} is calculated substituting the experimental values in the following equation:

$$t_{exp} = \frac{(\bar{x} - \bar{y})}{s}\sqrt{\frac{mn}{(m+n)}}$$

where $\bar{x}$ = the mean of the 1st set of results, $\bar{y}$ = the mean of the 2nd set of results, m = the number of replicate analysis for the 1st set of results and n = the number of replicate analysis for the 2nd set of results. [54]

2.4.2.8 Q-Test for Outliers [51], [54]

A great concern is to know whether to reject a certain experimental value that appears to be out of place. The quantitative method for rejecting a result is based on the Q-Test. The experimental aspects of the analysis are firstly reviewed in order to determine whether the value under suspicion can be rejected on the grounds of an experimental error. If an experimental value is not responsible for the seemingly erroneous value, the Q-Test is performed, and the experimental Q value, called Q_{exp}, is determined by using the following formula: $Q_{exp} = \Delta_{dev}/\omega$, where Δ_{dev} = the difference between the discordant result and the value closest to it, and ω = the range, or the difference between the highest and lowest values of a set of experimental results. Q_{exp} is then compared to a

table of critical values of Q, or Q_{crit}. (See Appendix J) If $Q_{exp} < Q_{crit}$ at eg the 90% confidence level, the said value should not be discarded. If $Q_{exp} > Q_{crit}$ the value may be discarded.

CHAPTER 3 – QUANTITATIVE ANALYSIS OF METHAQUALONE-CONTAINING TABLETS

3.1 METHOD DEVELOPMENT AND VALIDATION

The validation was performed by two teams of analysts, each consisting of two members. Each team used the same neutral chloroform extract as detailed in this chapter, to extract methaqualone from a starch and lactose matrix. [56] The matrix was spiked while in powder form, and tablets were pressed from the spiked powder. The tablets were then pulverized, since seized samples are received in tablet form, homogenized to powder, and extracted.

Finally the extracts were run on the GC/MS, using a short routine MCT (methaqualone, cocaine, tetrahidrocannabinol) method, as well as a longer GC/MS method. The aim of testing the short method was to determine if it could separate the methaqualone isomers from potential interferents. The aim of testing the longer method was to determine if it could effectively separate all the methaqualone isomers from each other and from potential interferents.

Parameters such as limit of detection, limit of quantification, linearity range, matrix effects, sampling, selectivity, precision, accuracy, recovery and reproducibility were reviewed. Should these extraction and instrumentation methods prove to be fit for purpose, implementation as routine analysis methods by the Drugs Section of the SAPS FSL would be considered.

3.1.1 Sample Extraction Procedure

3.1.1.1 Chemicals, Reference Standards and Tools

The following chemicals, reference standards and tools were used:

Chloroform (Burdick & Jackson)
Anhydrous Sodium Sulfate (LABCHEM)
All the isomers of methaqualone were synthesized by Senior Superintendent E. F. van Zyl. [57]
All other reference standards were certified reference standards purchased from Industrial Analytical (Vorna Valley, RSA)
Mortar and pestle
10 mL A-grade volumetric flasks, glass pasteur pipettes, Gilson automatic pipettes in the range 0.5 to 5.0 uL, and 0.5 to 1.0 mL respectively, pipette tips (Separations, Randburg, RSA)
GC/MS vials and caps compatible with the Hewlett Packard 6890 GC/MS
GC column: 5% Phenyl Methyl Siloxane HP-5MS

3.1.1.2 Detailed Extraction Procedure

For the detailed extraction procedure see Table 3.1 below.

TABLE 3.1: Extraction Procedure

Negative Control	Sample
	Grind the tablet, and weigh 1 g (whole tablet if less than 1 g) into the same test-tube that was used for the preparation of the negative control.
Add 5 mL $CHCl_3$ to a test-tube.	Add 5 mL $CHCl_3$ to a test-tube.
Vortex for 10 seconds.	Vortex for 10 seconds.
Remove $CHCl_3$ with a glass Pasteur pipette.	Remove $CHCl_3$ with the same glass pasteur pipette that was used for the preparation of the negative control.
Filter $CHCl_3$ through a funnel containing filter paper filled with anhydrous sodium sulfate, into a 10 mL A grade volumetric flask.	Filter $CHCl_3$ through the same funnel containing the same filter paper filled with the same anhydrous sodium sulfate, into the same (now emptied) 10 mL A grade volumetric flask that was used for the preparation of the negative control.
Wash the sodium sulfate with another aliquot of 5 mL chloroform (to 10 mL mark).	Wash the sodium sulfate with another aliquot of 5 mL $CHCl_3$ (to 10 mL mark).
Remove 1 µL of the $CHCl_3$, and transfer to a clean GC/MS vial.	Remove 1 µL of the $CHCl_3$ extract, and transfer to a clean GC/MS vial.
Dry the $CHCl_3$ under a gentle stream of nitrogen.	Dry the $CHCl_3$ under a gentle stream of nitrogen.
Reconstitute with 1 mL $CHCl_3$.	Reconstitute with 1 mL $CHCl_3$.
Cap the vial, and load unto the GC/MS, running the sample on both the short and long methods.	Cap the vial, and load unto the GC/MS, running the sample on both the short and long methods.

3.1.2 Determination of Limit of Detection (LOD), and Limit of Quantification (LOQ)

A range of solvent blanks ($CHCl_3$), and methaqualone standards were analysed. At least 7 determinations of at least 7 concentrations covering the anticipated range were carried out. A maximum concentration of 60 µg/mL was not exceeded for any of the standards, in order not to overload the GC column.

Each of the two analysts per team prepared a range of 10 standards for methaqualone, ranging in concentration from 5 µg/mL to 50 µg/mL. The data generated by analysing the solvent blanks and standards (average of 7 determinations) on the GC/MS were used to set up the quantitation method on the GC/MS software. Linear regression was performed on the resulting data. (See Validation Results in paragraph 3.2 of this Chapter)

3.1.3 Linearity range

Analysis of a range of methaqualone standards was required. At least 7 determinations of at least 7 concentrations covering the anticipated working range - representing approximately 10 to 200% of the target concentration - were carried out.

The linear regression data as obtained from the calculations mentioned in paragraph 3.1.2 above were used. The linearity range was determined by inspection of the correlation coefficient r, and the equation of a straight line, y = mx + c. In this equation, m represents the slope, c the y-intercept of the line, and y and x, the y and x values of a certain point on the line respectively.

3.1.4 Matrix effects

Analysis of matrix blanks, matrix spiked with standards, and standards, was required. The matrix was spiked at three different concentrations. As obtained from literature, the blank matrix consisted of 36% lactose, 8.5% starch, 5% talk and 0.5% magnesium stearate. [56] The methaqualone isomers, mecloqualone (depending on availability), diphenhydramine, diazepam, cocaine and tetrahidrocannabinol were the active ingredients which were considered as interferences. Anthranilic acid, N-acetyl anthranilic acid, o-toluidine and isatoic anhydride, were the precursors which were tested for their possible interference. Acetanthranil, o-methyl acetanilid, as well as the amide of isatoic anhydride, were by-products of the synthesis of methaqualone which were tested for their interference. Mainly publications by Sher [5], Angelos and Meyers [2] and Dal Cason et al [35], provided information on which compounds to select for the validation. Table 3.2 reflects the concentrations at which the various stock solutions were prepared. Table 3.3 indicates the steps taken from which data for matrix effects, selectivity, precision, accuracy and recovery were obtained.

TABLE 3.2: Concentrations of Various Stock Solutions Utilized in the Validation Process

STOCK SOLUTION	**CONCENTRATION (µg/mL)**
Ketamine for reconstitution after extraction only! **This was abandoned after preliminary tests.**	50
Nor-methaqualone	100
13 Methaqualone isomers	100
Methaqualone	100
Precursors (acetanthranil, N-acetyl anthranilic acid, o-methyl acetanilid, 2-amino benzoic acid, amide of isatoic anhydride)	100
Interferences (diazepam, tetrahidrocannabinol, cocaine, diphenhydramine, mecloqualone)	100

TABLE 3.3: Steps that were followed by Team 1 Analyst A and Team 2 Analyst C for injection of the sample extract on the short GC/MS method, as well as Team 1 Analyst B and Team 2 Analyst D for injection on the long GC/MS method

[Final spiked Mtq]	**1 g (1)**	**1 g (2)**	**1 g (3)**	**1 g (4)**
0 µg/mL (Blank)	Ketamine stock: 0 µl Nor-Mtq stock: 0 µl 13 Isomer stock: 0 µl Precursor stock: 0 µl Interferences stock: 0 µl Mtq stock: 0 µl	Ketamine stock: 0 µl Nor-Mtq stock: 0 µl 13 Isomer stock: 0 µl Precursor stock: 0 µl Interferences stock: 0 µl Mtq stock: 0 µl	Ketamine stock: 0 µl Nor-Mtq stock: 0 µl 13 Isomer stock: 0 µl Precursor stock: 0 µl Interferences stock: 0 µl Mtq stock: 0 µl	Ketamine stock: 0 µl Nor-Mtq stock: 0 µl 13 Isomer stock: 0 µl Precursor stock: 0 µl Interferences stock: 0 µl Mtq stock: 0 µl
[Final spiked Mtq]	**1 g (1)**	**1 g (2)**	**1 g (3)**	**1 g (4)**
10 µg/mL	Ketamine stock: 0 µl Nor-Mtq stock: 0 µl 13 Isomer stock: 250 µl Precursor stock: 250 µl Interferences stock: 250 µl Mtq stock: 100 µl	Ketamine stock: 0 µl Nor-Mtq stock: 0 µl 13 Isomer stock: 250 µl Precursor stock: 250 µl Interferences stock: 250 µl Mtq stock: 100 µl	Ketamine stock: 0 µl Nor-Mtq stock: 0 µl 13 Isomer stock: 250 µl Precursor stock: 250 µl Interferences stock: 250 µl Mtq stock: 100 µl	Ketamine stock: 0 µl Nor-Mtq stock: 250 µl 13 Isomer stock: 250 µl Precursor stock: 250 µl Interferences stock: 250 µl Mtq stock: 100 µl
[Final spiked Mtq]	**1 g (1)**	**1 g (2)**	**1 g (3)**	**1 g (4)**
20 µg/mL	Ketamine stock: 0 µl Nor-Mtq stock: 0 µl 13 Isomer stock: 250 µl Precursor stock: 250 µl Interferences stock: 250 µl Mtq stock: 200 µl	Ketamine stock: 0 µl Nor-Mtq stock: 0 µl 13 Isomer stock: 250 µl Precursor stock: 250 µl Interferences stock: 250 µl Mtq stock: 200 µl	Ketamine stock: 0 µl Nor-Mtq stock: 0 µl 13 Isomer stock: 250 µl Precursor stock: 250 µl Interferences stock: 250 µl Mtq stock: 200 µl	Ketamine stock: 0 µl Nor-Mtq stock: 250 µl 13 Isomer stock: 250 µl Precursor stock: 250 µl Interferences stock: 250 µl Mtq stock: 200 µl
[Final spiked Mtq]	**1 g (1)**	**1 g (2)**	**1 g (3)**	**1 g (4)**
30 µg/mL	Ketamine stock: 0 µl Nor-Mtq stock: 0 µl 13 Isomer stock: 250 µl Precursor stock: 250 µl Interferences stock: 250 µl Mtq stock: 300 µl	Ketamine stock: 0 µl Nor-Mtq stock: 0 µl 13 Isomer stock: 250 µl Precursor stock: 250 µl Interferences stock: 250 µl Mtq stock: 300 µl	Ketamine stock: 0 µl Nor-Mtq stock: 0 µl 13 Isomer stock: 250 µl Precursor stock: 250 µl Interferences stock: 250 µl Mtq stock: 300 µl	Ketamine stock: 0 µl Nor-Mtq stock: 250 µl 13 Isomer stock: 250 µl Precursor stock: 250 µl Interferences stock: 250 µl Mtq stock: 300 µl
Notes to the analyst: Each analyst will weigh out 16 individual 1 g amounts of each matrix separately, and spike it as shown above. Reconstitute the dried extracts with 1 mL of Ketamine at a concentration of 50 µg/mL. **After preliminary tests this was changed to 1 mL chloroform only.**				

3.1.5 Sampling

Each sample that had to be analysed was spiked and prepared individually before extraction and subsequent GC/MS analysis.

3.1.6 Selectivity

Analysis of reagent blanks, matrix blanks, matrix spiked with a methaqualone standard within the working range, and standards of methaqualone and its interferences were required. This was achieved by injecting an extract of the matrix spiked with a cocktail of methaqualone and the above-mentioned interferences, on GC/MS.

3.1.7 Precision

Looking at linearity range, three different concentrations of methaqualone representing the upper, middle and lower end of the linear range were selected. At least 3 replicate analyses of each of the duplicate extracts of the matrix containing the methaqualone standard, as well as the interferences, were run on GC/MS. The %RSD was calculated, using the applicable formula. A % RSD of less than 10% for the spiked samples will be considered as acceptable, while the % RSD should be less than 5% for the pure standards analysed. The whole exercise was carried out by 4 analysts, and all the samples were analysed on the same GC/MS.

3.1.8 Accuracy

The matrix spiked with methaqualone standards within the working range, was analysed. At least 3 replicate analyses of each of the duplicate extracts of the matrix containing the methaqualone standard (at 3 different concentrations), as well as the interferences at a fixed concentration, were run on GC/MS. The same range of concentrations as employed in the linearity studies was used. The linearity experiment was thus repeated in the presence of matrix constituents. Recoveries of 70% - 120% were considered to be acceptable.

Each person performed 3 analyses at each of 3 concentrations (10 ug/mL, 20 ug/mL and 30 ug/mL) of the methaqualone standard and interferences mixture, spiked into the sample matrix, in duplicate. Interferences were spiked at a fixed concentration of 50 ug/mL in all the samples. A neutral $CHCl_3$ extract, as described in Table 3.1 was used. Even though the United Nations recorded the use of a basic $CHCl_3$ extract [34], it was decided to perform the neutral $CHCl_3$ extract for this experiment. The same steps were followed in determining matrix effects, selectivity, precision, accuracy and recovery.

3.1.9 Q-Test for Outliers

The quantitative method for rejecting an experimental result was based on the Q-Test. If an experimental value was not obviously responsible for the seemingly erroneous value, the Q-Test was performed. Q_{exp} was compared to the table of critical values of Q, or Q_{crit}. If $Q_{exp} < Q_{crit}$, at eg the 90% confidence level, the said value was not discarded, if $Q_{exp} > Q_{crit}$, the value was discarded. [54]

3.1.10 Preliminary Testing: Results and Discussion

3.1.10.1 Homogenising Step

The homogenising step was tested, and manual grinding with a mortar and pestle was compared to grinding, using an automatic grinding device. The automatic grinding device was not found suitable for grinding one tablet at a time, leading to an uneven texture of ground material after 30 seconds of grinding. Lengthening of the grinding time did not improve the results. Even though cleaning of the device was easy, and contamination was not experienced, this did not seem to be the best option. It was decided to resort to manual grinding, which seemed less time consuming, and more effective. The tablet could be manually ground to a fine homogenous powder, which was not possible with the automatic grinder when grinding only a single tablet.

3.1.10.2 Thin layer chromatography

Thin layer chromatography systems, which are general screening procedures for nitrogenous bases, were evaluated or verified as a pre-screen of tablets submitted for analysis. [58] In the system referred to as TB, cyclohexane : toluene : diethylamine in the ratio 75 : 15 : 10, was used as mobile phase. In the system referred to as TC, chloroform : methanol in the ratio 90 : 10, was used as mobile phase. In both systems ninhydrin, Dragendorff or acidified iodoplatinate spray can be used as visualizing agents. All plates used were coated with silica gel G with 250 µm film thickness.

The Rf values of the compounds of interest were determined for methaqualone, the methaqualone isomers [57] (Table 3.4), mecloqualone, diphenhydramine, diphenhydramine-HCl, diazepam, cocaine and tetrahidrocannabinol, using a mixture of pure standards, as well as the individual standards (Table 3.5). Anthranilic acid, N-acetyl anthranilic acid, o-toluidine and isatoic anhydride were the precursors tested for interference. By-products of the synthesis of methaqualone, namely acetanthranil, o-methyl acetanilid, as well as the amide of isatoic anhydride, were tested for interference (Table 3.5).

TABLE 3.4: Rf Values of the Isomers of methaqualone [57] (System TB: cyclohexane : toluene : diethylamine (75 : 15 : 10) and System TC: chloroform : methanol (90 : 10), silica gel G with 250 µm film thickness)

Compound	Rf System TB	Rf System TC
Mtq Isomer I : 2-methyl-3-*o*-tolyl-4(3H)-quinazolinone	0.33	0.85
Mtq Isomer II : 2-methyl-3-*m*-tolyl-4(3H)-quinazolinone	0.33	0.86
Mtq Isomer III : 2-methyl-3-*p*-tolyl-4(3H)-quinazolinone	0.31	0.86
Mtq Isomer IV : 3-(2,3-dimethylphenyl)-4(3H)-quinazolinone	0.37	0.87
Mtq Isomer V : 3-(2,4-dimethylphenyl)-4(3H)-quinazolinone	0.38	0.87
Mtq Isomer VI : 3-(2,5-dimethylphenyl)-4(3H)-quinazolinone	0.38	0.87
Mtq Isomer VII : 3-(2,6-dimethylphenyl)-4(3H)-quinazolinone	0.37	0.86
Mtq Isomer VIII : 3-(3,4-dimethylphenyl)-4(3H)-quinazolinone	0.36	0.86
Mtq Isomer IX : 3-(3,5-dimethylphenyl)-4(3H)-quinazolinone	0.39	0.87
Mtq Isomer X : 2-Ethyl-3-phenyl-4(3H)-quinazolinone	0.35	0.85
Mtq Isomer XI : 3-*o*-Ethylphenyl-4(3H)-quinazolinone	0.37	0.86
Mtq Isomer XII : 3-*m*-Ethylphenyl-4(3H)-quinazolinone	0.39	0.87
Mtq Isomer XIII : 3-*p*-Ethylphenyl-4(3H)-quinazolinone	0.38	0.87

TABLE 3.5: Rf Values of the Interferents, Precursors and By-products (System TB: cyclohexane : toluene : diethylamine (75 : 15 : 10) and System TC: chloroform : methanol (90 : 10), silica gel G with 250 µm film thickness)

Compound	Rf System TB	Rf System TC
Diazepam	0.25	0.91
Methaqualone	0.35	0.96
Nor-methaqualone	0.28	0.94
Diphenhydramine	0.48	0.28
Cocaine	0.45	0.16
Isotoic anhydride	0.00	0.61
Amide of Isotoic anhydride	0.05 and 0.10	0.92
N-Acetyl anthranilic acid	0.00	0.37
Acetanthranil	0.19	0.31 and 0.94
2-Benzoic acid	0.00	0.54
o-Toluidine	0.31	0.86
o-Methyl acetanilide	0.04	0.67
THC	0.42	0.93

Inspection of the Rf values tabulated above clearly showed that neither system TB nor system TC would be effective enough to separate all the compounds of interest from each other. Experiments trying to separate a cocktail including all the above-mentioned compounds were unsuccessful. For the purpose of separating these compounds, these two systems were not to be considered to be an option. It was decided not to test it on extracts of the cocktail from the matrix. This screening method will also not be employed for routine analysis of seized samples in future.

Dal Cason, Angelos and Washington [35] described the synthesis of the drug 2-methyl-3-*o*-tolyl-4-quinazolinone (methaqualone) and fifteen chemical analogues and positional isomers, as well as their identification by spectroscopic techniques. 2-Methyl-3-*m*-tolyl-4(3H)-quinazolinone and 2-methyl-3-*p*-tolyl-4(3H)-quinazolinone were among the analogues studied. Gas-liquid chromatography (GLC) and TLC systems were *inter alia* used for analysis of the compounds. TLC was found to have no value due to the small differences in Rf values.

3.1.10.3 GC/MS Analysis

A Hewlett Packard 6890 GC/MS (Quadropole) in electron ionization mode was used for the analysis. Firstly, the existing short MCT-method, routinely used for qualitative analyses of seized samples suspected to contain methaqualone, cocaine or tetrahidrocannabinol on GC/MS, was tested. Its ability to separate all the interferences, isomers, reaction by-products and precursors from methaqualone, was evaluated. The longer, non-routine GC/MS method was tested to determine whether it would separate all the interferences, isomers, reaction by-products and precursors from methaqualone and from each other. The GC/MS conditions for the two methods are detailed in Table 3.6 below. The main differences in the two methods were the oven temperature programs.

Retention times were obtained by analysing solutions of the individual components (Table 3.7 and Table 3.8). The ability of the method to separate all compounds was assessed by analysing a mixture of the components (Table 3.9). Figure 3.1 shows the total ion chromatogram (TIC) obtained by analysing the mixture of compounds on the long GC/MS method. The mass spectra of the compounds in the mixture are displayed from Figure 3.1 to Figure 3.24.

TABLE 3.6: GC/MS Conditions for the long and the short method used to analyse solutions of the individual components as well as solutions of a mixture of components. (Hewlett Packard 6890 GC/MS (Quadropole) in electron ionization mode)

	Long method	**Short method**
Oven: Initial temp	100 'C	120 ‘C
Hold time	0.00 min	Same
Rate	10 ‘C/min	30 ‘C/min
Final temp	280 ‘C	290 ‘C
Hold time	0.00 min (changed to 5.00 min)	2.30 min
Run time	19.00 min (changed to 23.00 min)	7.97 min
Inlet: Mode	Splitless (splitter vented @ 1.5 min)	Same
Initial temp	250 ‘C	250 ‘C
Pressure	72.1 kPa	80.3 kPa
Total flow	23.7 mL/min	23.7 mL/min
Gas type	Helium	Same
Capillary column	5% Phenyl Methyl Siloxane HP-5MS	Same
Column dimensions	30 m x 250 µm; d_f 0.25 µm	Same
Constant flow	1.0 mL/min	Same
Injection volume	1.0 uL	Same
MS information		
· Solvent delay	4.00 min	Same
Resulting EM voltage	1341.2	Same
Scan parameters		
· Low mass	40.0	35.0
· High mass	450.0	600.0

TABLE 3.7: Isomers of methaqualone analyzed individually.[57] (Hewlett Packard 6890 GC/MS (Quadropole) in electron ionization mode, GC/MS conditions are listed in Table 3.6). TIC's and mass spectra can be seen in Appendix A.

Compound	Rt in min for Short Method	Rt in min for Long Method
Mtq Isomer I	5.90	14.68
Mtq Isomer II	6.21	15.52
Mtq Isomer III	6.30	15.74
Mtq Isomer IV	6.28	15.68
Mtq Isomer V	6.17	15.42
Mtq Isomer VI	6.12	15.29
Mtq Isomer VII	5.84	14.54
Mtq Isomer VIII	6.69	16.62
Mtq Isomer IX	6.44	16.11
Mtq Isomer X	6.08	15.21
Mtq Isomer XI	5.98	14.92
Mtq Isomer XII	6.41	15.99
Mtq Isomer XIII	6.55	16.30

TABLE 3.8: Interferents, precursors and by-products analysed individually. (Hewlett Packard 6890 GC/MS (Quadropole) in electron ionization mode, GC/MS conditions are listed in Table 3.6). TIC's and mass spectra can be seen in Appendix B.

Compound	Rt in min for Short Method	Rt in min for Long Method
Methaqualone	5.90	14.68
Cocaine	6.01	15.06
Ketamine	N/d	11.67
Diphenhydramine	4.84	11.77
Diazepam	6.94	17.15
THC	6.97	17.33
N-Acetyl anthranilic acid	N/d	10.45
2-Aminobenzoic acid (Anthranilic acid)	N/d	06.82
Isatoic anhydride	N/d	10.04
Amide of Isatoic anhydride	6.19	15.37
Acetanthranil	N/d	06.49
o-Methyl acetanilide	N/d	7.38
o-Toluidine	N/d	5.73

N/d = not detected

TABLE 3.9: Peak Identification Table

Peak Number	Compound	Mass Spectrum Depicted in Figure	Rt in min for Long Method
1	Acetanthranil	3.2	6.552
2	*o*-Toluidine	3.3	6.749
3	N-Acetylanthranilic acid	3.4	9.827
4	Ketamine	3.5	11.664
5	Diphenhydramine	3.6	11.808
6	Isomer VII	3.7	14.520
7	Isomer I (Methaqualone)	3.8	14.684
8	MPQ	3.9	14.713
9	Isomer XI	3.10	14.910
10	Cocaine	3.11	15.064
11	Amide of Isatoicanhydride	3.12	15.131
12	Isomer X	3.13	15.213
13	Isomer VI	3.14	15.295
14	Isomer V	3.15	15.405
15	Isomer II	3.16	15.525
16	Isomer IV	3.17	15.684
17	Isomer III	3.18	15.727
18	Isomer XII	3.19	15.992
19	Isomer IX	3.20	16.093
20	Isomer XIII	3.21	16.304
21	Isomer VIII	3.22	16.603
22	Diazepam	3.23	17.141
23	Tetrahidrocannabinol	3.24	17.324

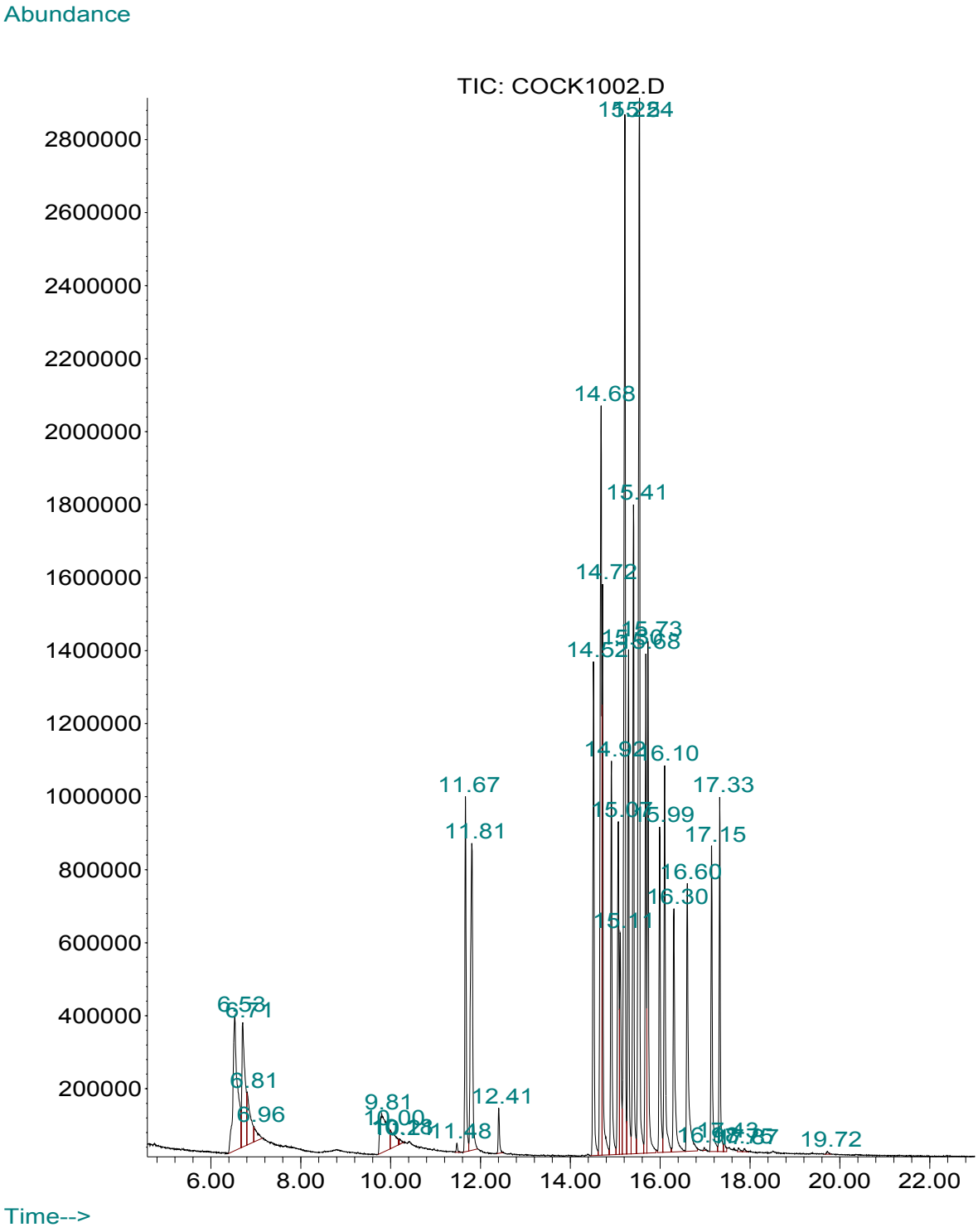

Figure 3.1: Total Ion Chromatogram of the Cocktail/standard mixture Run on the Long GC/MS Method

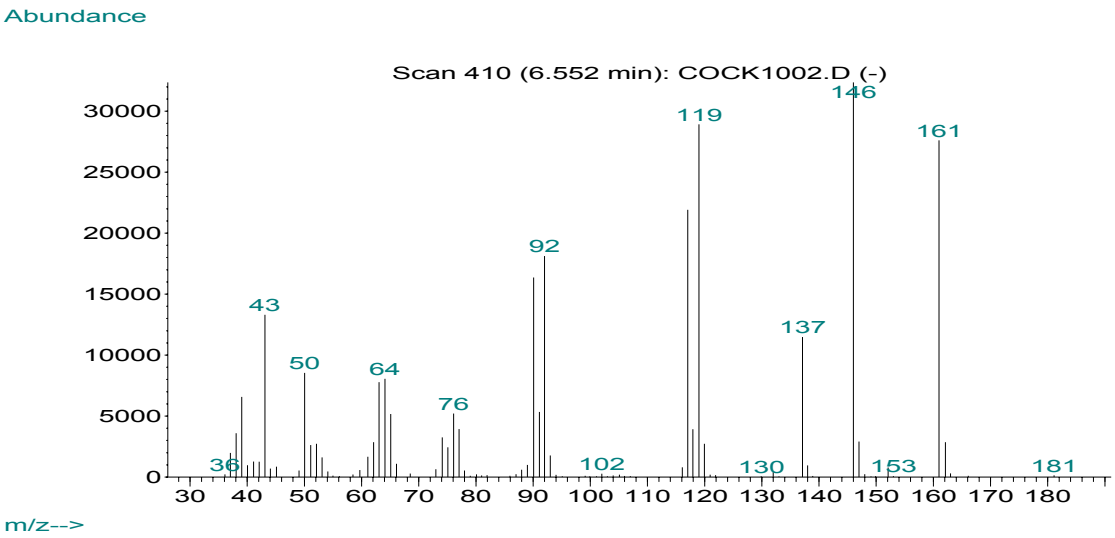

Figure 3.2: Electron Impact Mass Spectrum of Peak 1 (Acetanthranil) at 6.552 minutes

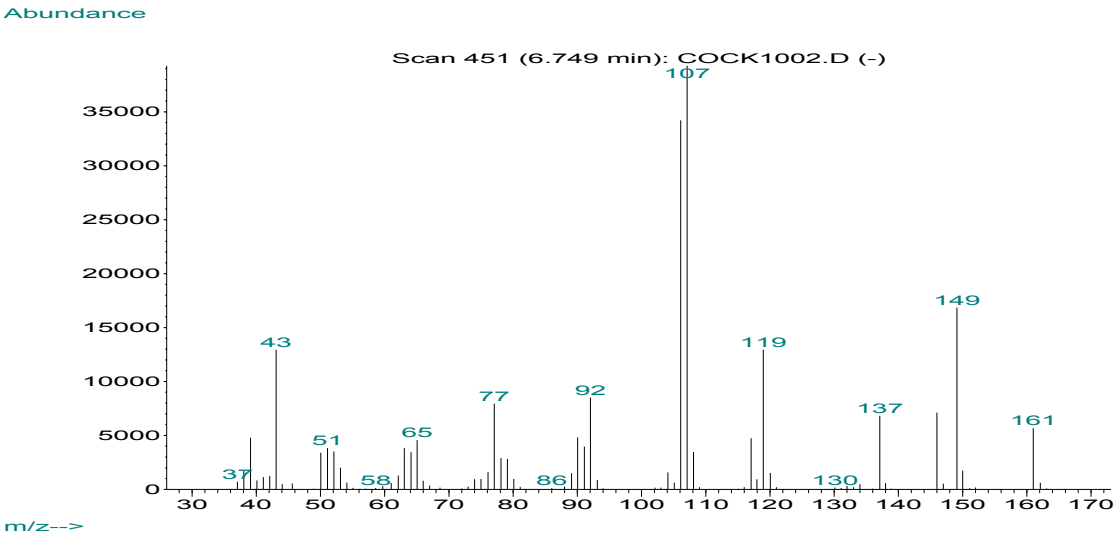

Figure 3.3: Electron Impact Mass Spectrum of Peak 2 (*o*-Toluidine) at 6.749 minutes

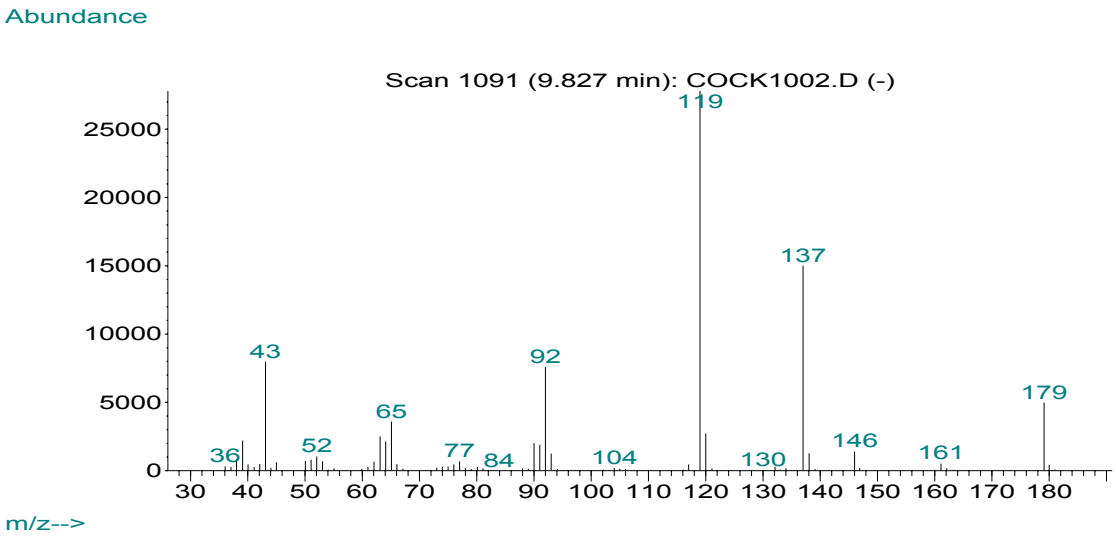

Figure 3.4: **Electron Impact Mass Spectrum of Peak 3 (N-Acetylanthranilic acid) at 9.827 minutes**

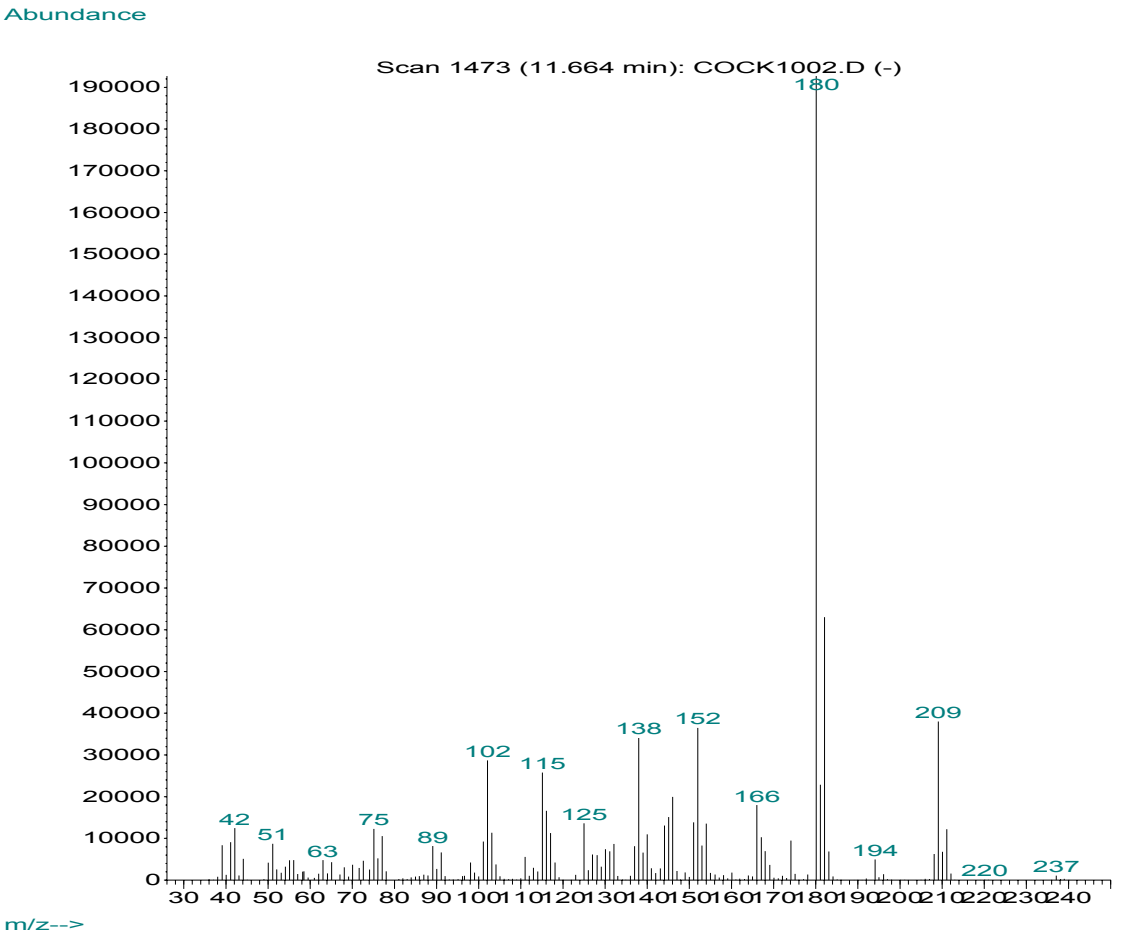

Figure 3.5: **Electron Impact Mass Spectrum of Peak 4 (Ketamine) at 11.664 minutes**

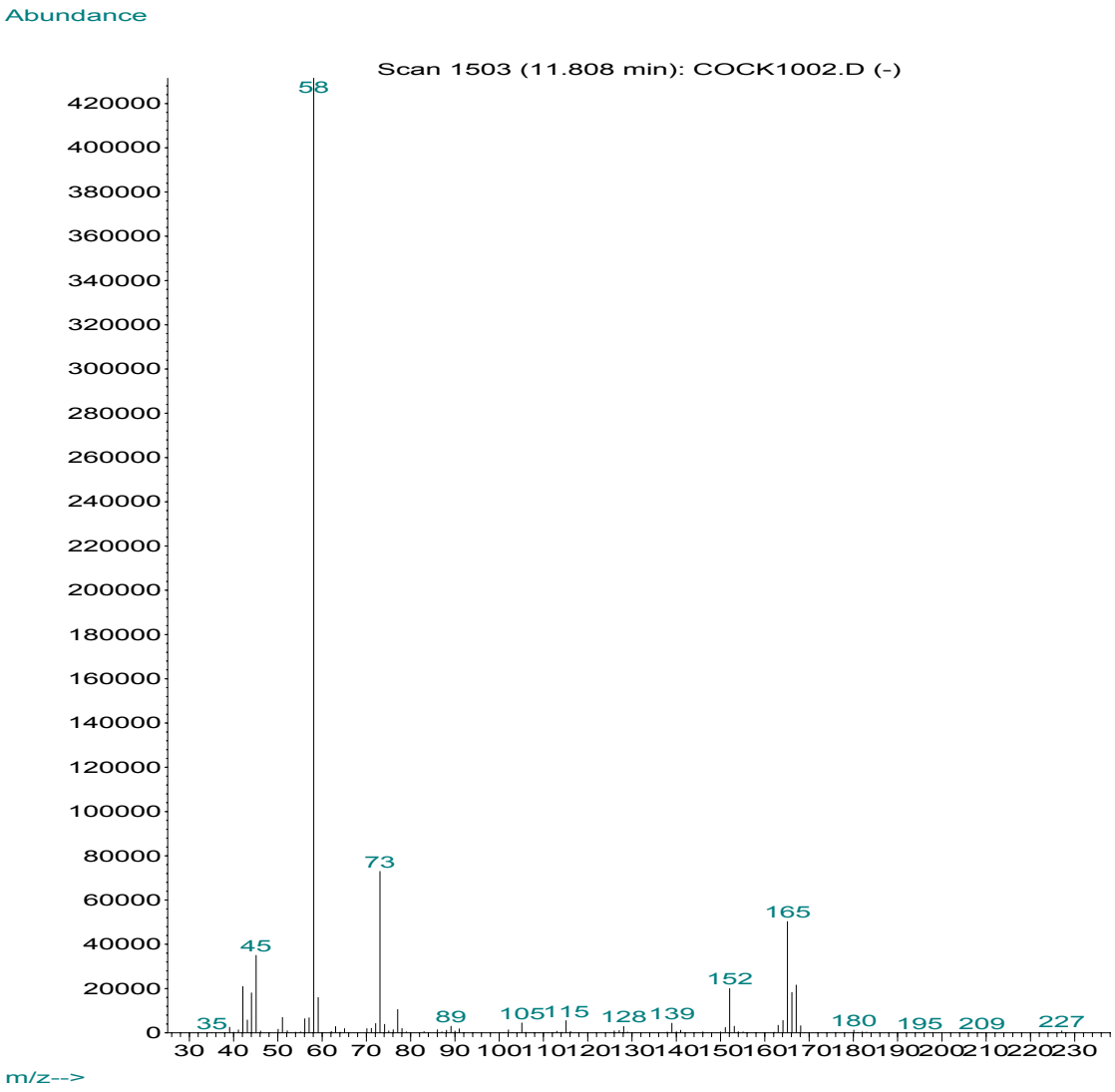

Figure 3.6: **Electron Impact Mass Spectrum of Peak 5 (Diphenhydramine) at 11.808 minutes**

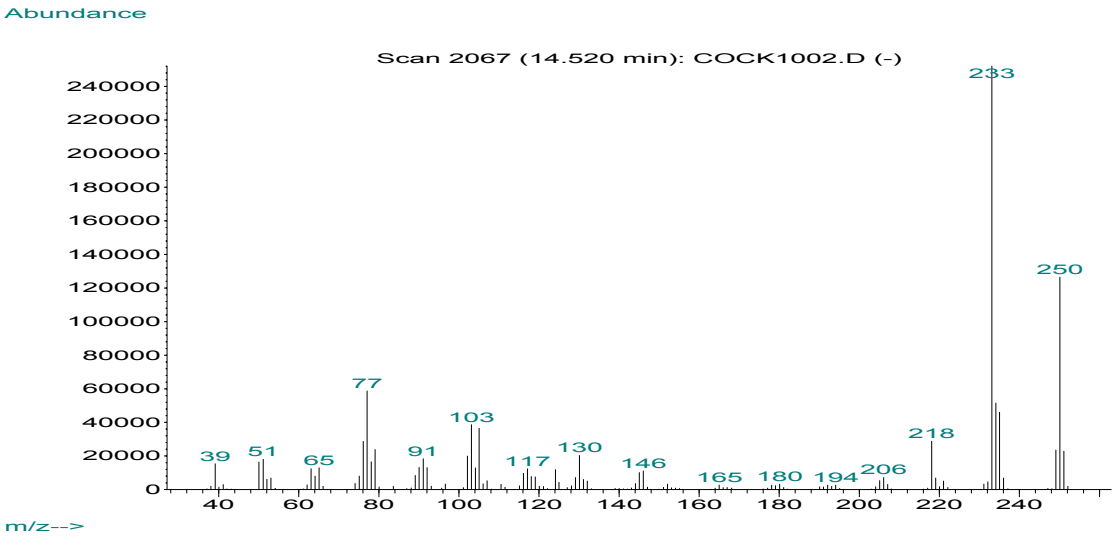

Figure 3.7: Electron Impact Mass Spectrum of Peak 6 (Isomer VII) at 14.520 minutes

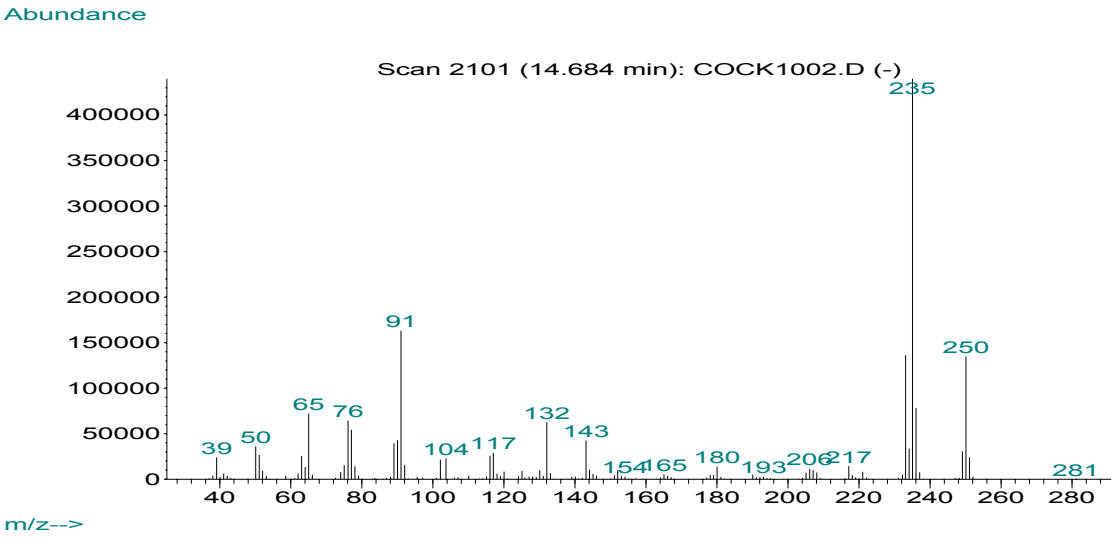

Figure 3.8: Electron Impact Mass Spectrum of Peak 7 (Isomer I) at 14.684 minutes

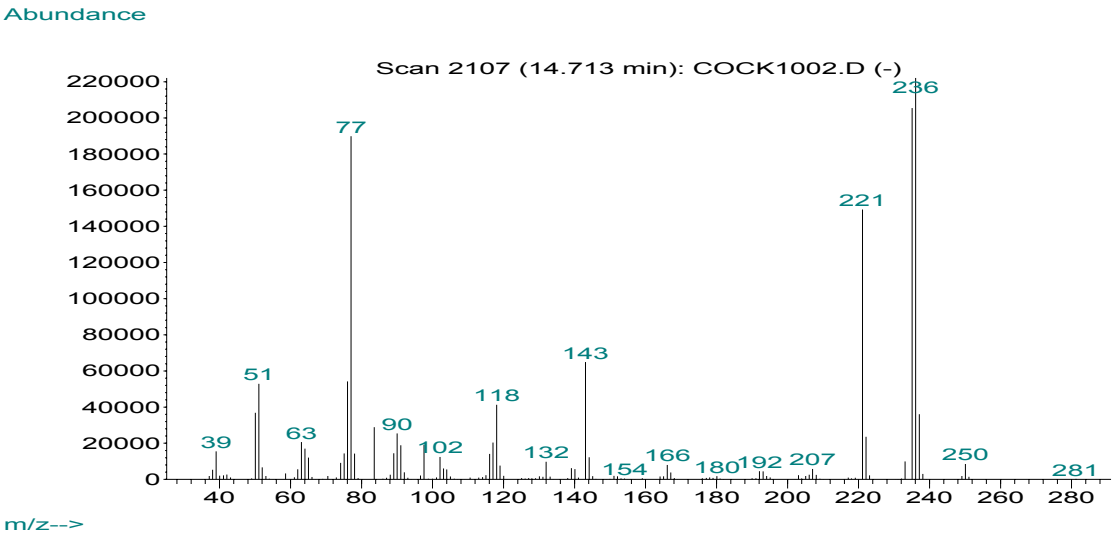

Figure 3.9: Electron Impact Mass Spectrum of Peak 8 (MPQ) at 14.713 minutes

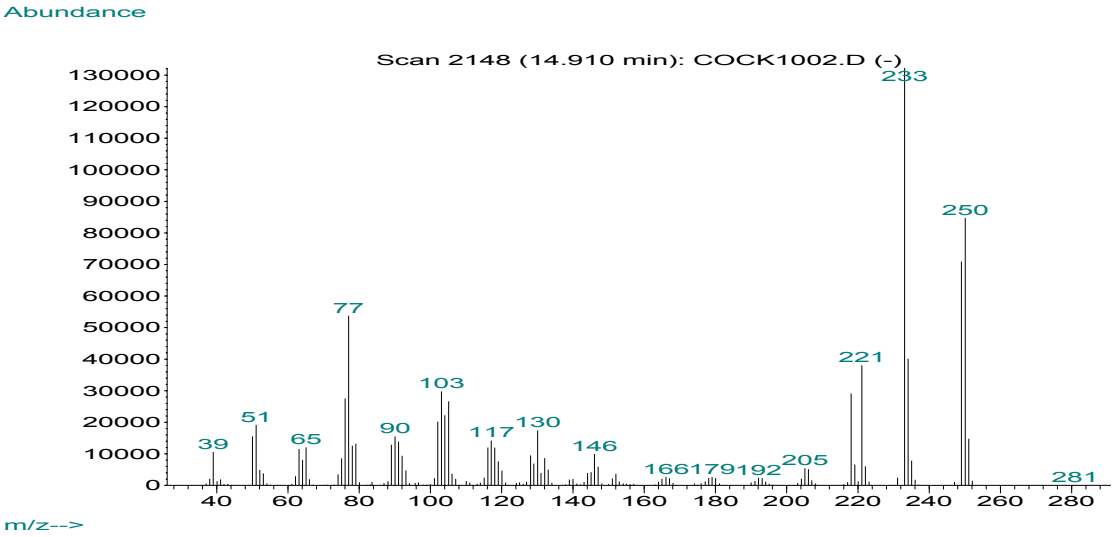

Figure 3.10: Electron Impact Mass Spectrum of Peak 9 (Isomer XI) at 14.910 minutes

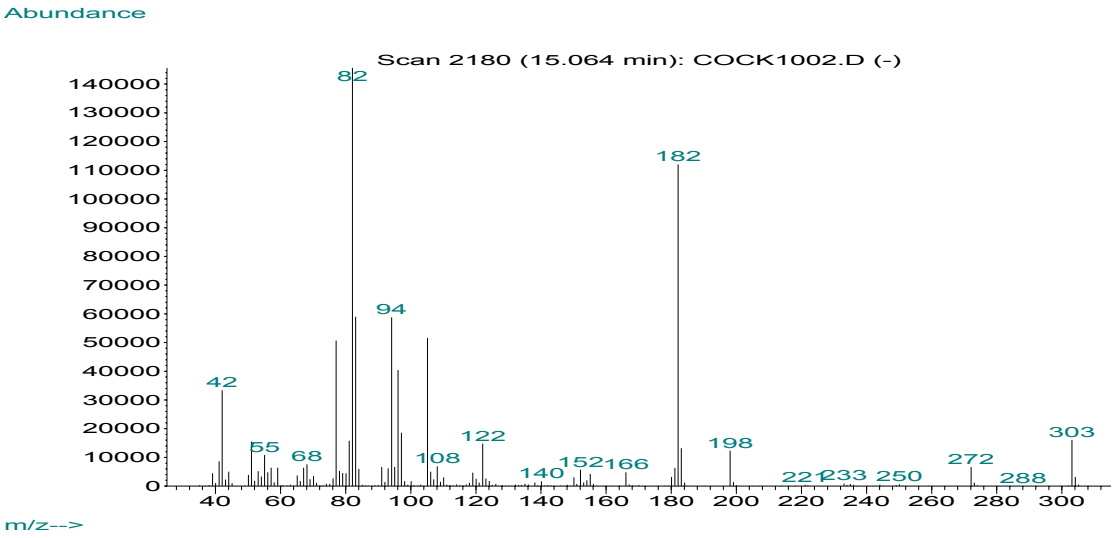

Figure 3.11: Electron Impact Mass Spectrum of Peak 10 (Cocaine) at 15.064 minutes

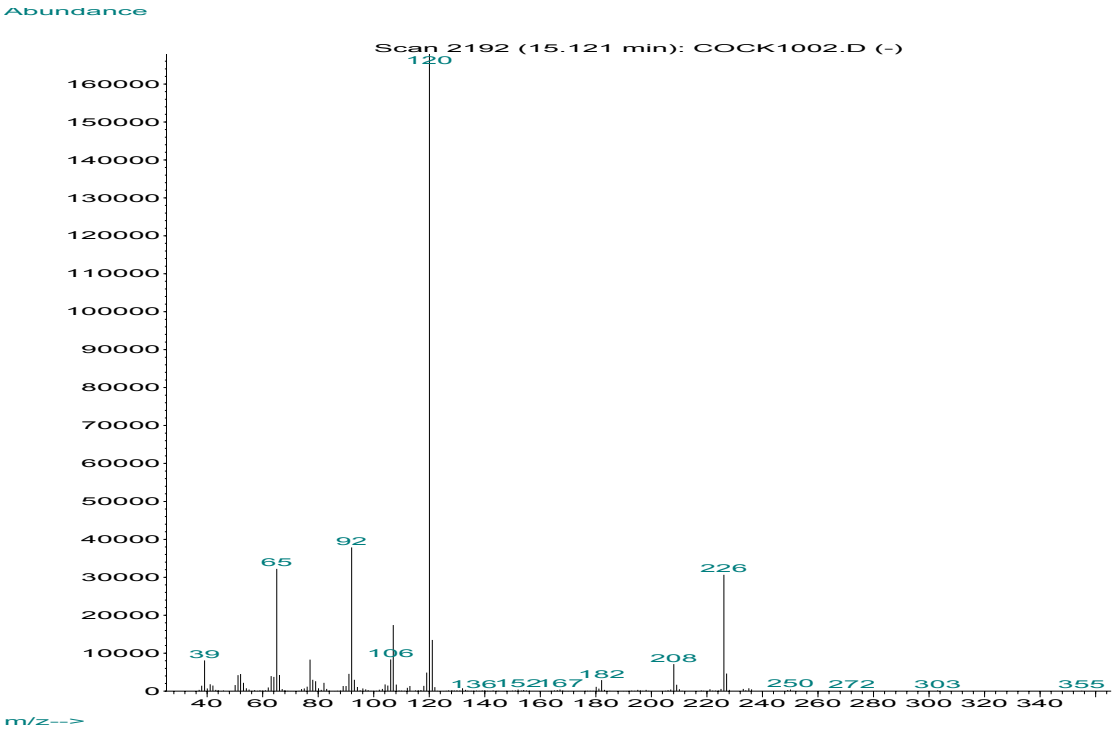

Figure 3.12: Electron Impact Mass Spectrum of Peak 11 (Amide of Isatoicanhydride) at 15.131 minutes

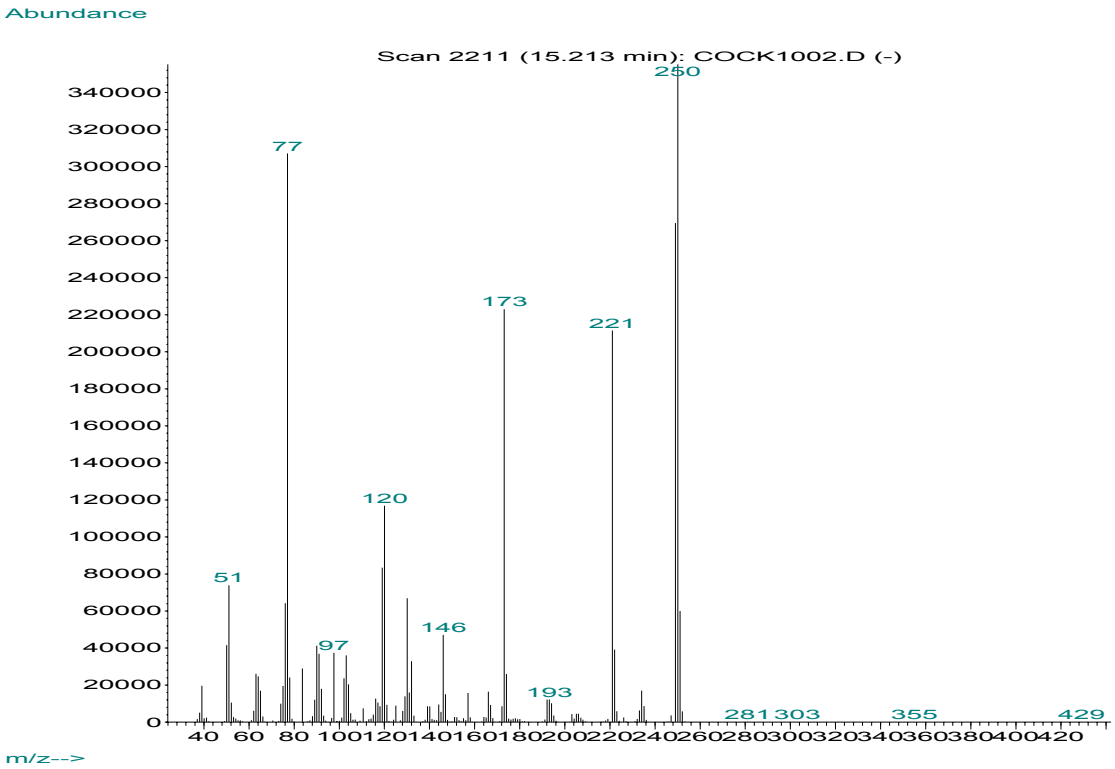

Figure 3.13: Electron Impact Mass Spectrum of Peak 12 (Isomer X) at 15.213 minutes

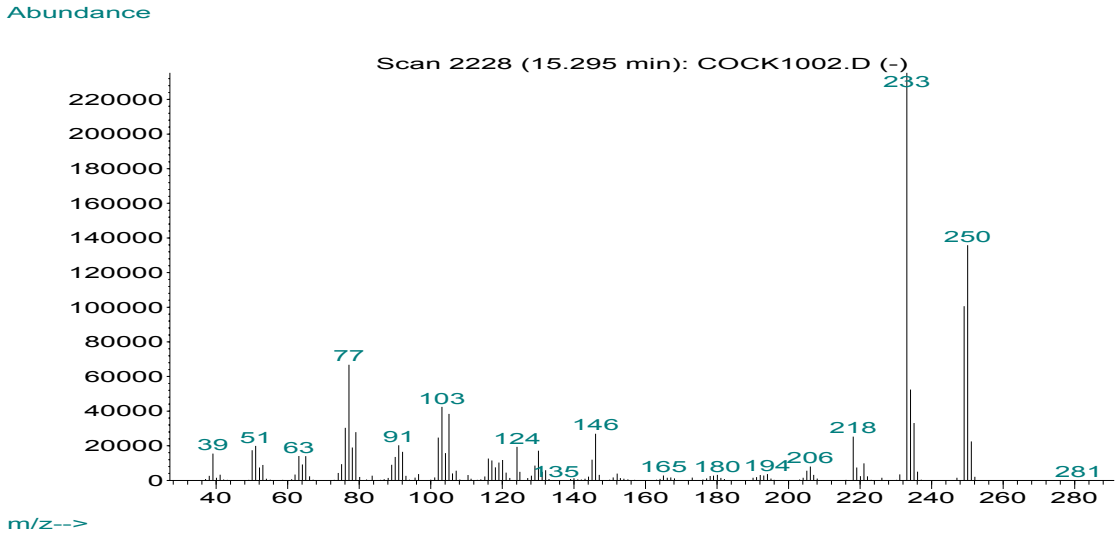

Figure 3.14: Electron Impact Mass Spectrum of Peak 13 (Isomer VI) at 15.295 minutes

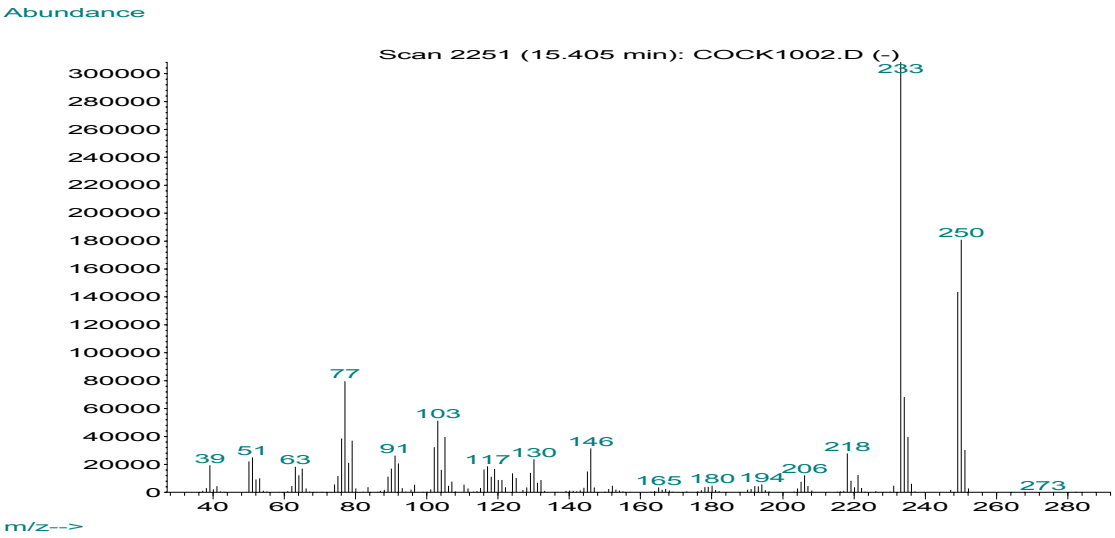

Figure 3.15: Electron Impact Mass Spectrum of Peak 14 (Isomer V) at 15.405 minutes

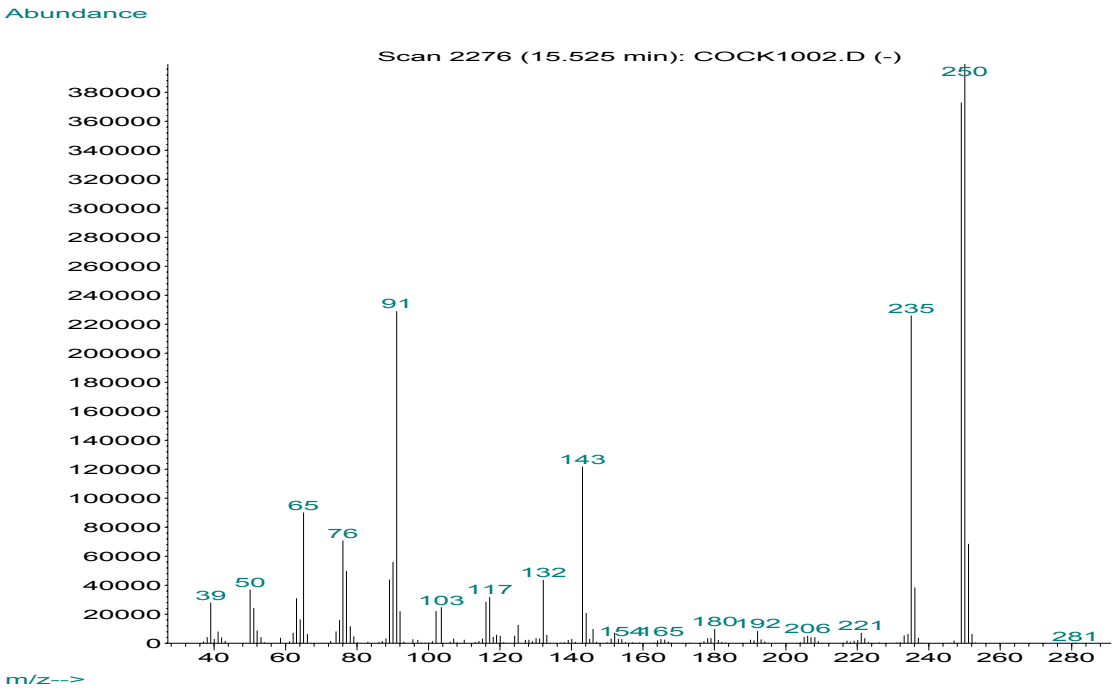

Figure 3.16: Electron Impact Mass Spectrum of Peak 15 (Isomer II) at 15.525 minutes

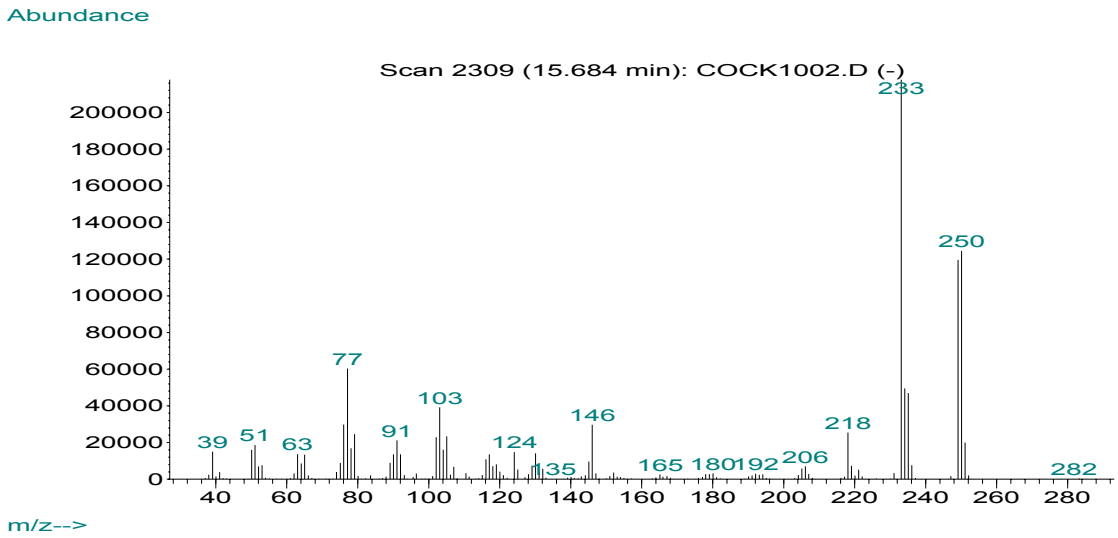

Figure 3.17: Electron Impact Mass Spectrum of Peak 16 (Isomer IV) at 15.684 minutes

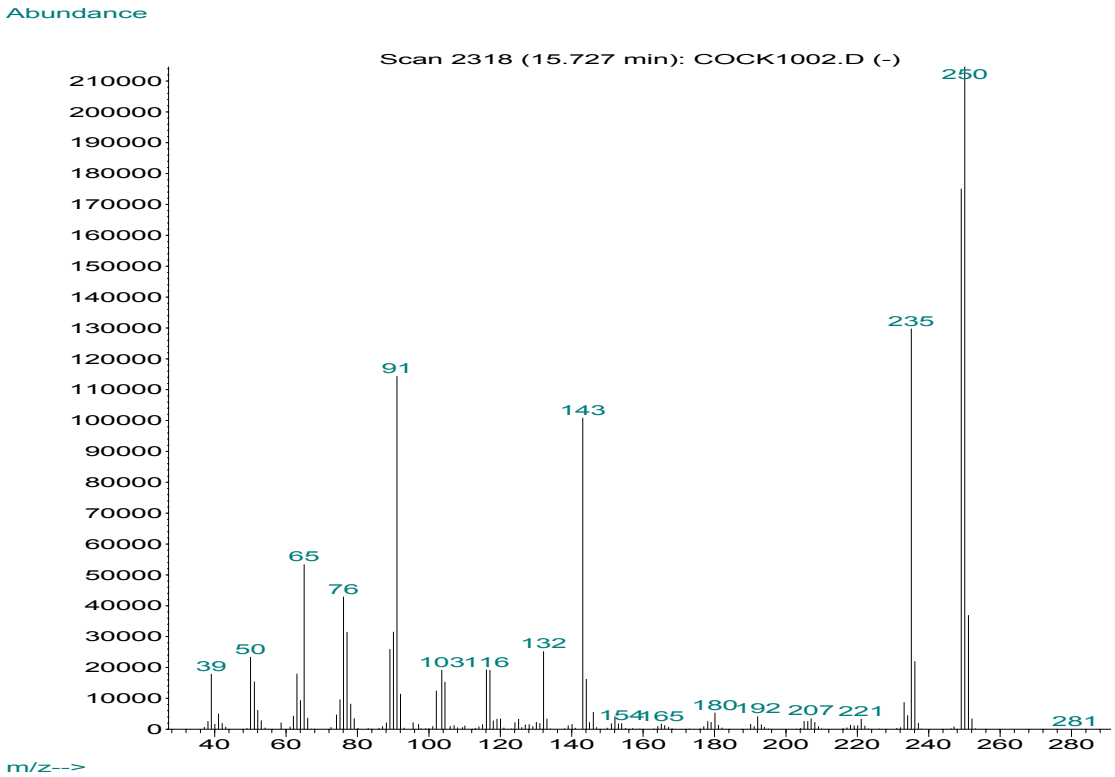

Figure 3.18: Electron Impact Mass Spectrum of Peak 17 (Isomer III) at 15.727 minutes

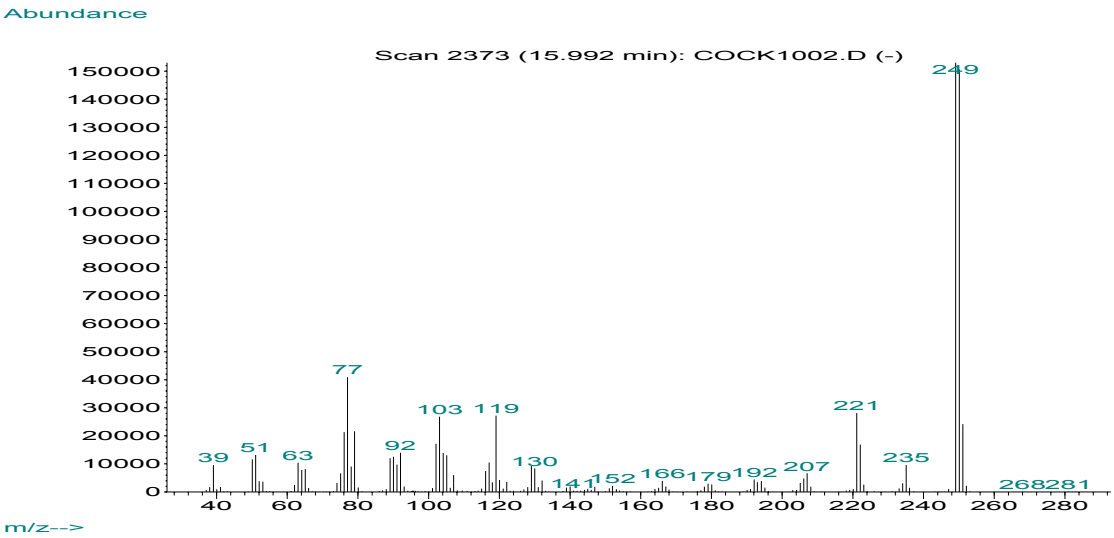

Figure 3.19: Electron Impact Mass Spectrum of Peak 18 (Isomer XII) at 15.992

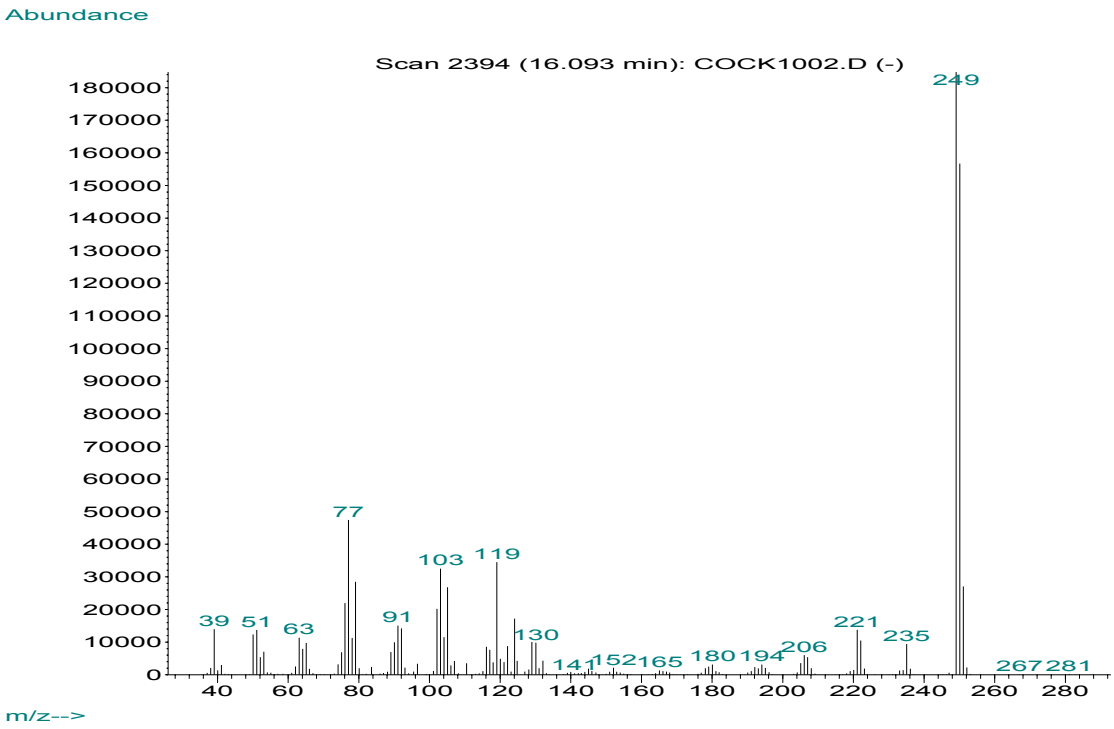

Figure 3.20: Electron Impact Mass Spectrum of Peak 19 (Isomer IX) at 16.093

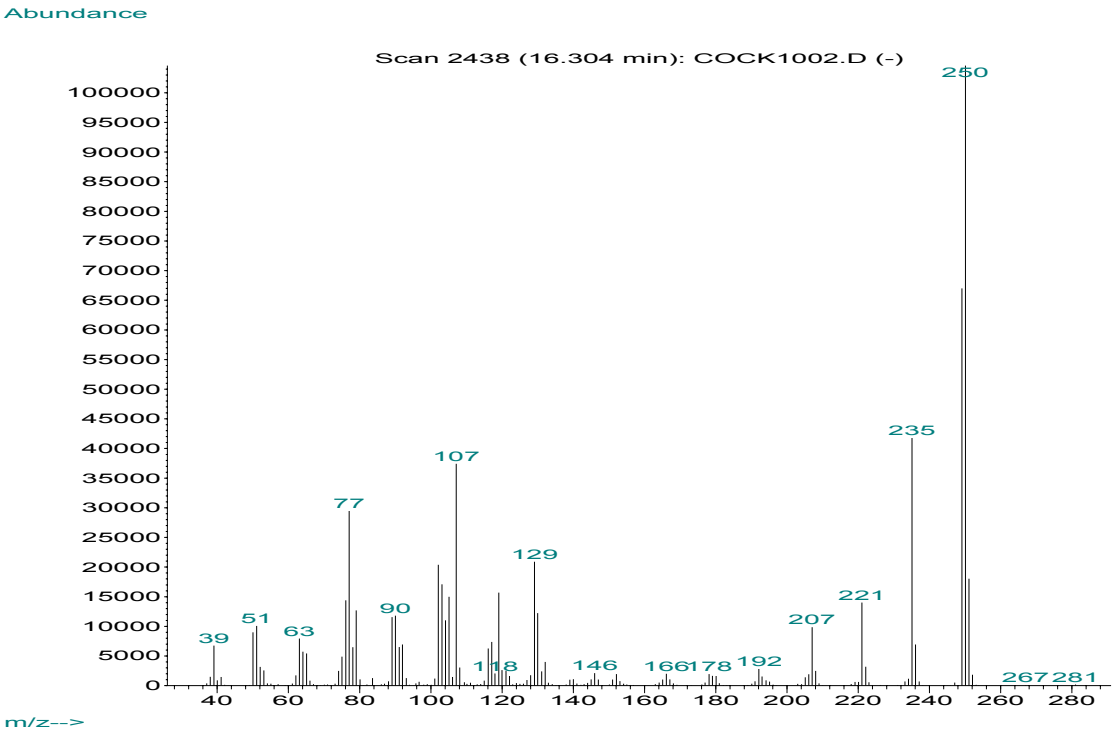

Figure 3.21: Electron Impact Mass Spectrum of Peak 20 (Isomer XIII) at 16.304 minutes

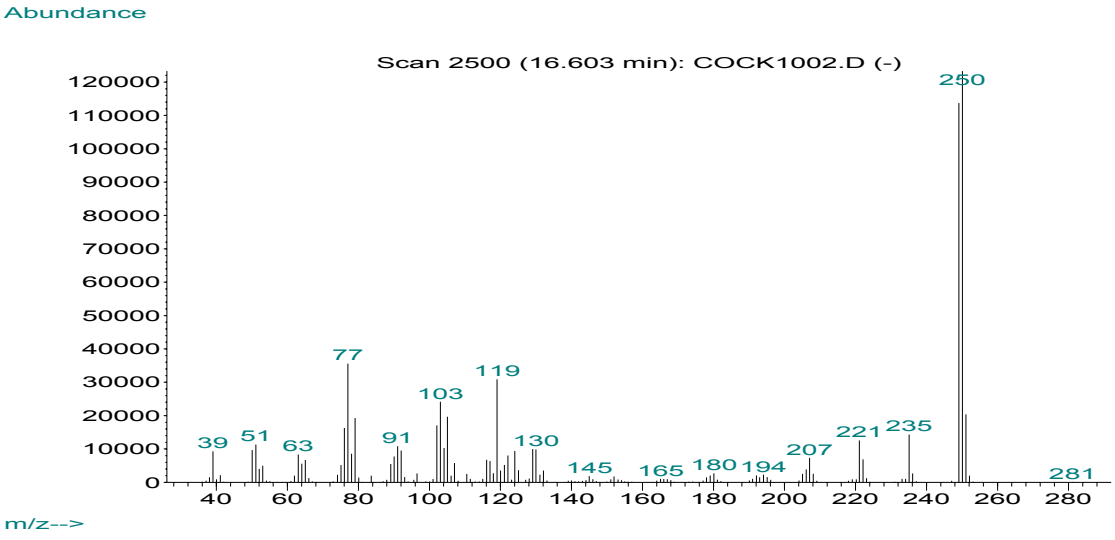

Figure 3.22: Electron Impact Mass Spectrum of Peak 21 (Isomer VIII) at 16.603 minutes

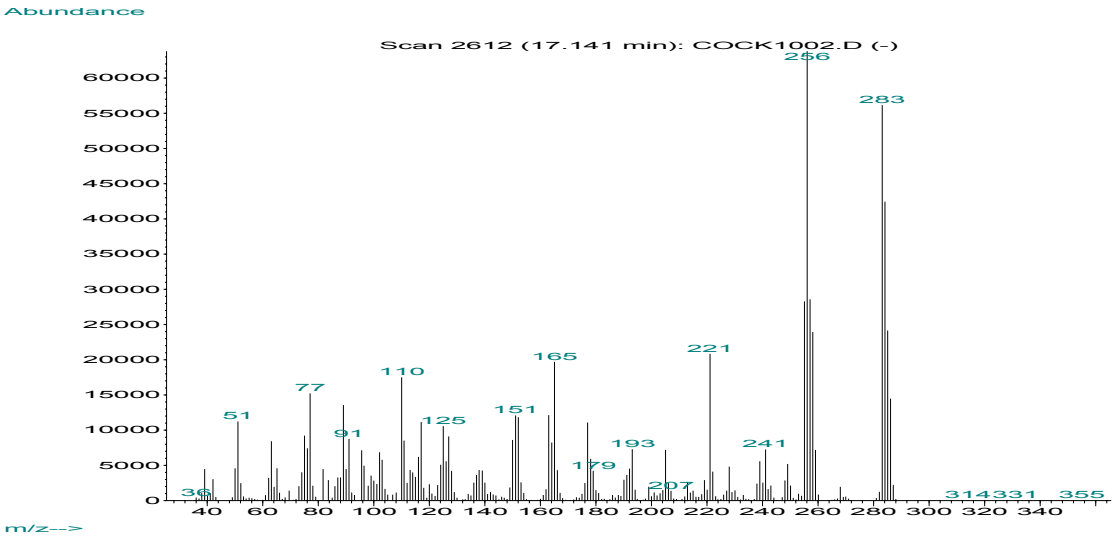

Figure 3.23: Electron Impact Mass Spectrum of Peak 22 (Diazepam) at 17.141 minutes

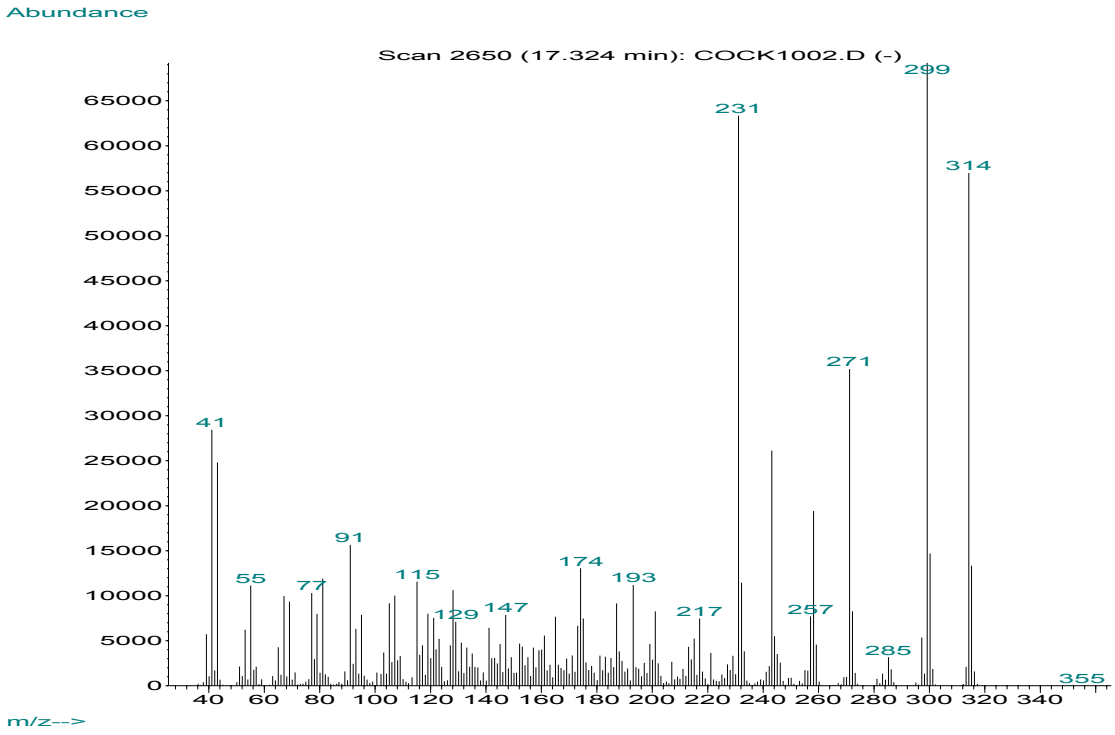

Figure 3.24: Electron Impact Mass Spectrum of Peak 23 (tetrahidrocannabinol) at 17.324 minutes

The mixture of components consisting of all the compounds of interest presented problems in, among others, achieving baseline separation of the proposed internal standard, namely ketamine, from diphenhydramine, one of the main compounds of interest. (Peak 4 and 5 on the TIC in Figure 3.1) Attempting to achieve baseline separation was not considered, and a decision was made to rather use external standards in the quantification of methaqualone.

Also, the hold time of the temperature program at the final temperature of 280 ˚C was increased from 0.00 minutes to 5.00 minutes (Table 3.6 above), to allow diazepam and tetrahidrocannabinol to completely elute. (Peak 22 and 23 on the TIC in Figure 3.1) This resulted in the total run time being extended from nineteen minutes to twenty-three minutes.

The short GC/MS method did achieve separation of all the compounds from methaqualone. It, as expected, failed in separating the individual methaqualone isomers and interferents from each other. The TIC and mass spectra of the individual compounds in the mixture of compounds that was run on the short method can be seen in Appendix C.

The GC/MS conditions were thus finalized, and the validation of the extraction procedure of the spiked matrixes and subsequent GC/MS quantitation of methaqualone, could commence.

3.1.11 Validation Results and Discussion

All chromatograms and integration results obtained during the validation procedure, can be seen in Appendix I. All calculations done in order to create graphs and derive conclusions in the discussions following below can be found in Appendix H.

3.1.11.1 Linearity Results

The calibration lines depicted in Figure 3.25, 3.26, 3.27 and 3.28 were obtained by each of the four analysts respectively. Data from the best line (Figure 3.28) was subsequently used to set up the GC/MS calibration parameters.

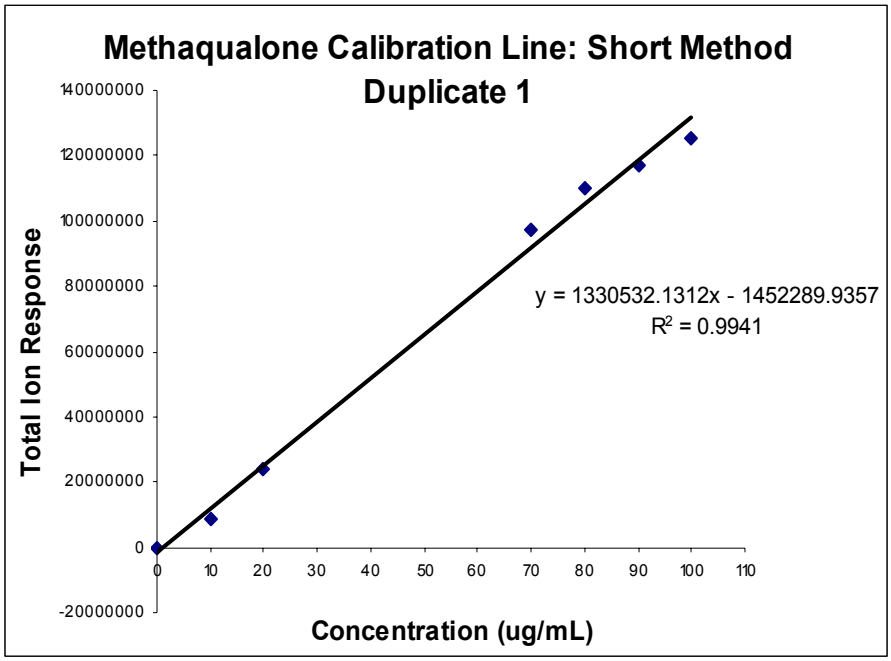

Figure 3.25: Methaqualone Calibration Line of the Short GC/MS Method, Duplicate One

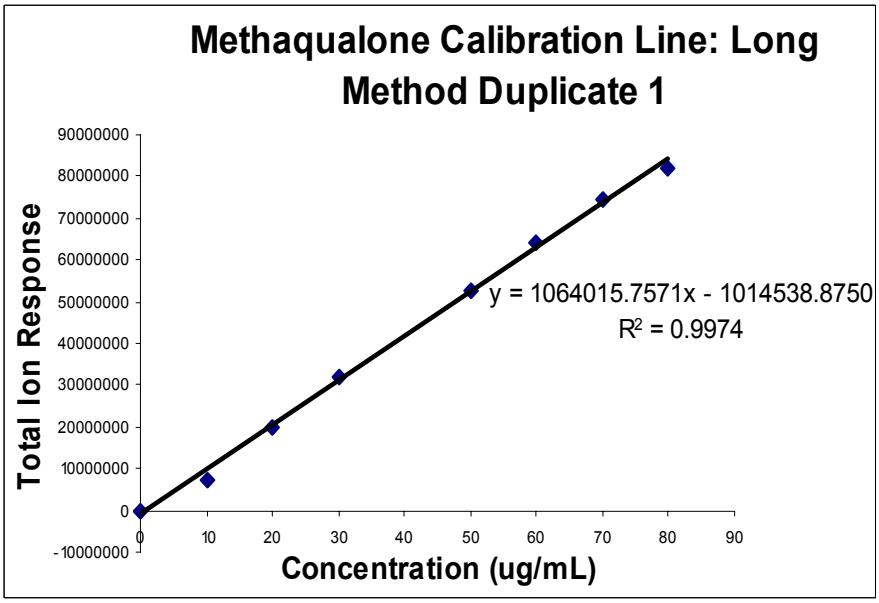

Figure 3.26: Methaqualone Calibration Line of the Long GC/MS Method, Duplicate One

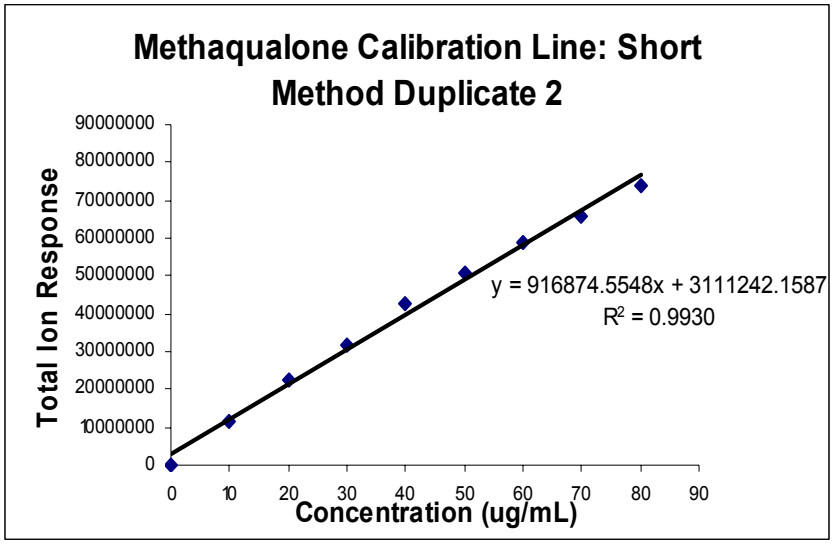

Figure 3.27: Methaqualone Calibration Line of the Short GC/MS Method, Duplicate Two

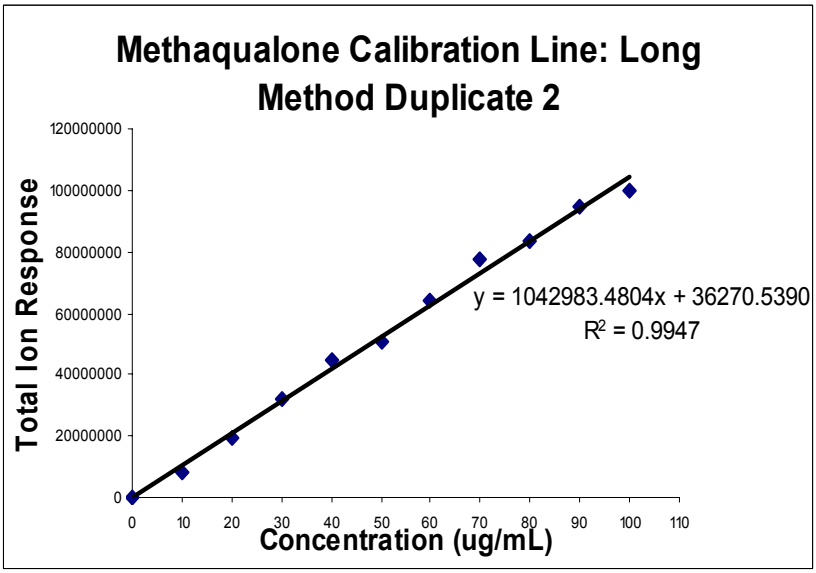

Figure 3.28: Methaqualone Calibration Line of the Long GC/MS Method, Duplicate Two

Table 3.10: Summary of Methaqualone LOD, LOQ and Linearity Range Results

Compound	Equation (y=mx+c)	R^2	LOD (µg/mL)	LOQ (µg/mL)	Linearity Range
Methaqualone, short method, duplicate 1	y=1330532.1312x-1452289.9357	0.9941	1.1516	1.2176	From LOD to 100 µg/mL
Methaqualone, short method, duplicate 2	y=916874.5548x+3111242.1587	0.9930	-3.2030	-3.3071	From LOD to 80 µg/mL
Methaqualone, long method, duplicate 1	y=1064015.7571x-1014538.8750	0.9974	0.9716	0.9985	From LOD to 80 µg/mL
Methaqualone, long method, duplicate 2	y=1042983.4804x-36270.5390	0.9947	0.0018	0.0687	From LOD to 90 µg/mL

The linearity results were obtained by analyzing methaqualone standards ranging from 10 to 100 µg/mL as described in Chapter 3. For the long GC/MS method, the correlation coefficient was 0.9974 and 0.9947 for duplicate 1 and 2 respectively. For the short GC/MS method, the correlation coefficient was 0.9941 and 0.9930 for duplicate 1 and 2 respectively. For the intended application, i.e. quantifying methaqualone in illicit preparations, these values were considered acceptable. The average LOD for the short GC/MS method was of 2.18 µg/mL, and for the long method 0.49 µg/mL. The average LOQ for the short GC/MS method was 2.26 µg/mL and that for the long method 0.53 µg/mL. The anticipated working range would be at concentrations above 20 µg/mL, which are well above the LOD for both the methods. Both methods proved to be linear from the LOD to 90 µg/mL (short method) and 85 µg/mL (long method) methaqualone.

3.1.11.2 Precision, Accuracy and Recovery Results

The precision, accuracy and recovery results were obtained by analyzing matrices spiked with methaqualone standards at 10, 20 and 30 µg/mL. One in ten dilutions of these extracts were analyzed on the GC/MS.

TABLE 3.11: Comparison of the Average Total Ion Response for Methaqualone for the Long and Short GC/MS Methods: Student's t Test Results

Spike (µg/mL)	$t_{experimental}$	$t_{calculated}$ (95% confidence level)
10	0.17	12.7
20	-0.97	12.7
30	-0.98	12.7

In Chapter 2 it is stated that if the t experimental value is less than the t-calculated value, there is at least a 95% probability that the values for the two portions of the sample are the same. The Student's t test results are used to determine whether results from differents sets of data are comparable. As shown in Table 3.11, the comparison between the average total ion response of methaqualone at certain spiked levels of the long and short GC/MS methods yielded t-experimental values which were less than the t-calculated values. It can thus be derived that the two methods gave comparable quantitative results.

TABLE 3.12: Comparison of the Precision, Accuracy and Recovery Results of the Long and Short GC/MS Methods ([ ] = concentration)

	Short GC/MS Method	**Mean Total Ion Response Std**	**Mean % RSD**	**Mean % Recovery**	**Pooled SD**	**[ ] in ug/ Tablet**	**Confidence Limit (95% confidence level)**	**[ ] in ug/ Tablet**
D 1	10 ug/mL Spike	9954752.0	11.74	41.95	39019.79	0.39	35961.09	0.36
D 2	10 ug/mL Spike	9954752.0	2.27	44.97	-	-	-	-
D 1	20 ug/mL Spike	23211691.6	6.90	54.05	151124	0.14	31761.22	0.14
D 2	20 ug/mL Spike	23211691.6	14.79	57.94	-	-	-	-
D 1	30 ug/mL Spike	50400173.8	6.18	56.96	87471.78	0.17	37538.97	0.07
D 2	30 ug/mL Spike	50400173.8	10.25	63.08	-	-	-	-
	Long GC/MS Method	**Mean Response Std**	**Mean % RSD**	**Mean % Recovery**	**Pooled SD**	**[ ] in ug/ Tablet**	**Confidence Limit**	**[ ] in ug/Tablet**
D 1	10 ug/mL Spike	9954752.0	5.77	54.97	24334.56	0.24	22859.41	0.23
D 2	10 ug/mL Spike	9954752.0	1.57	52.11	-	-	-	-
D 1	20 ug/mL Spike	23211691.6	5.68	67.96	55880.87	0.24	24055.36	0.10
D 2	20 ug/mL Spike	23211691.6	10.23	69.10	-	-	-	-
D 1	30 ug/mL Spike	50400173.8	6.40	87.84	46253.13	0.09	43684.64	0.09
D 2	30 ug/mL Spike	50400173.8	3.28	96.10	-	-	-	-

In order to conclude that either one or both of the methods yield acceptable precision, accuracy and recovery results, it is necessary to also evaluate the mean % RSD and mean % recovery data. As explained in Chapter 2, the accuracy of an analytical method is the extent to which the data or the average value of a set of data obtained with a specific method agrees to the true value. In this experiment the true value refers to the spiked concentration of the methaqualone in the matrix. From Table 3.12, it is clear that both the methods performed best at the 20 and 30 µg/mL levels. This is in line with the intended purpose of the routine method, namely to work with dilutions of approximately 20 to 30 µg/mL of methaqualone extracted from illicit preparations.

The short method had an average recovery of 56% at the 20 µg/mL level and an average recovery of 60% at the 30 µg/mL level. The long method had an average recovery of 68.5% at the 20 µg/mL level and an average recovery of 90% at the 30 µg/mL level. Recoveries using the long method were found to be acceptable. Extraction of the samples spiked at a concentration of 10 µg/mL of methaqualone, yielded recoveries that fell below 60% using the long method and below 50% for the short method. This might be due to the increased matrix effect at lower concentrations of analyte. In order to compensate for the matrix effect when quantifying methaqualone in illicit mandrax tablets, a recovery factor has to be taken into consideration (1.1 at the 30 µg/mL level).

The precision of an analytical method is normally referred to as the standard deviation or the closeness of results of a series of measurements. This is also a measure of either the degree of reproducibility or repeatability. The relative standard deviation of the mean, or RSD, is also known as the coefficient of variation. A % RSD of less than 10 % for the extracts of the spiked samples was considered to be acceptable. For the long method the mean % RSD for all the levels of spiked samples fell below 7%, with only one at the 20 µg/mL which was 10.23%. The short method did not perform as well, as can be seen in Table 3.12.

It was concluded that the short method could be effectively utilized only for the qualitative determination of methaqualone in a mixture of compounds, if methaqualone is present at the 20 to 30 µg/mL. The long method proved to be most suitable for analysing illicit preparations allegedly containing methaqualone. Judging from the method development results, the method will also be able to distinguish amongst all the possible interferences as mentioned in the study, and methaqualone. The method also proved to be adequate for the quantification of methaqualone in the presence of the said interferences.

3.2 APPLICATION OF THE VALIDATED METHOD TO THE QUANTITATIVE ANALYSIS OF ILLICIT PREPARATIONS: METHAQUALONE AND DIPHENHYDRAMINE QUANTITATION

Following the validation of the extraction and quantitative GC/MS method, the real test lay in applying the method to seized illicitly produced preparations. The protocol followed, as well as the results obtained and the deductions made, are discussed in this Chapter.

3.2.1 Sampling and Purpose

Four test cases, each having more than one hundred tablets left after the original analyses for court purposes, were re-analysed. These four cases tested positive for Methaqualone and were identified for destruction because the court proceedings were finalized. These cases included two pairs of cases with the same Logo/imprint and the same code in the Logo Index. One case with the code "3.41" was seized in George, while the other case with the same code was seized in Kuilsrivier, both in the Western Cape. One case with the code "35.11" was from Upington in the Northern Cape, and the other case with the same code was from Lenasia in Gauteng.

It was not possible to include more cases in this study, seeing that more examples with the same codes containing more than one hundred tablets after sampling for the original analysis for court purposes were not available. The request from management was also specific in the sense that the most recent cases should be included in the study. Thirty tablets per selected test case were analysed by using the validated extraction method, and quantified on the long GC/MS method. Table 3.13 shows the determinations that formed part of the project and Table 3.14 shows the questions that were considered to decide whether tablets with the same Logos were from a common origin.

TABLE 3.13 Parameters that were Determined during the Project.

	Parameter
1	The mass of each tablet.
2	The presence of methaqualone in each test case.
3	The presence of other illicit substances such as diphenhydramine in each test case.
4	The concentration of methaqualone per tablet in each test case.
5	The concentration of diphenhydramine per tablet in each test case.
6	The ratio of diphenhydramine relative to methaqualone per tablet in each test case.
7	The average methaqualone concentration per test case.
8	The average diphenhydramine concentration per test case.
9	The average methaqualone concentration relative to that of diphenhydramine per test case.

TABLE 3.14 Questions that were Considered to Decide whether Tablets with the Same Logos were from a Common Origin.

	Question
1	Were the analytical results sufficient to conclude on a potential common origin of the tablets with the same Logos?
2	Was the validated method suitable for the specific application?
3	Would there be a need to explore any aspects of this project any further?

3.2.2 Sample Extraction and Analysis

Contrary to the validation study, the application of the validated extraction procedure (Table 3.1) and quantitation using the validated long GC/MS method (Table 3.6) was performed by only one analyst on the four selected seized cases. In order to keep the experimental conditions as comparable as possible, all thirty tablets per test case were extracted on the same day, and subsequently loaded on the GC/MS in one sequence. A range of diphenhydramine and methaquaolone standards in chloroform, ranging from 10 µg/mL to 60 µg/mL was run at the beginning, in the middle and at the end of each sequence. Total ion response was used in the quantitation of the analytes extracted from the illicit preparations. A recovery factor of 1.1 was taken into consideration to compensate for the matrix effect when calculating the methaqualone concentration in each tablet. The average total ion response of the batches of standards was used to construct calibration lines. Calculations were done using Microsoft Excel (See Appendix H). All analytical data and calculations resulting from these analyses can be found in Appendices D, E, F and G.

3.2.3 Quantitation of Methaqualone and Diphenhydramine Present in 4 Test Cases: Results

3.2.3.1 Results: Test Case 1 [Lab number 9471/03]

Logo of Tablet  (front and back of tablet are identical)

Tablet code : 35.11

Other active constituents present : Diphenhydramine

Area of seizure : Upington (Northern Cape)

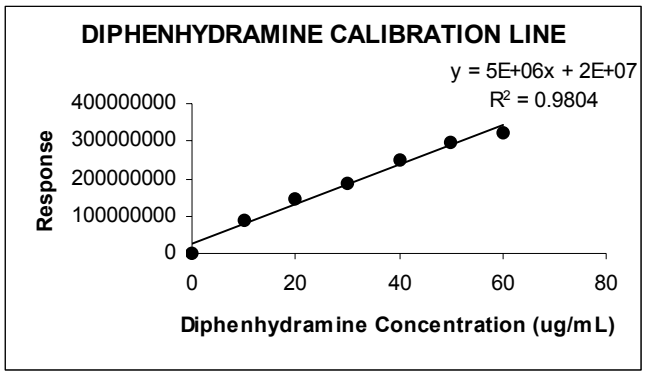

Figure 3.29: Diphenhydramine Calibration Line for Test Case 1

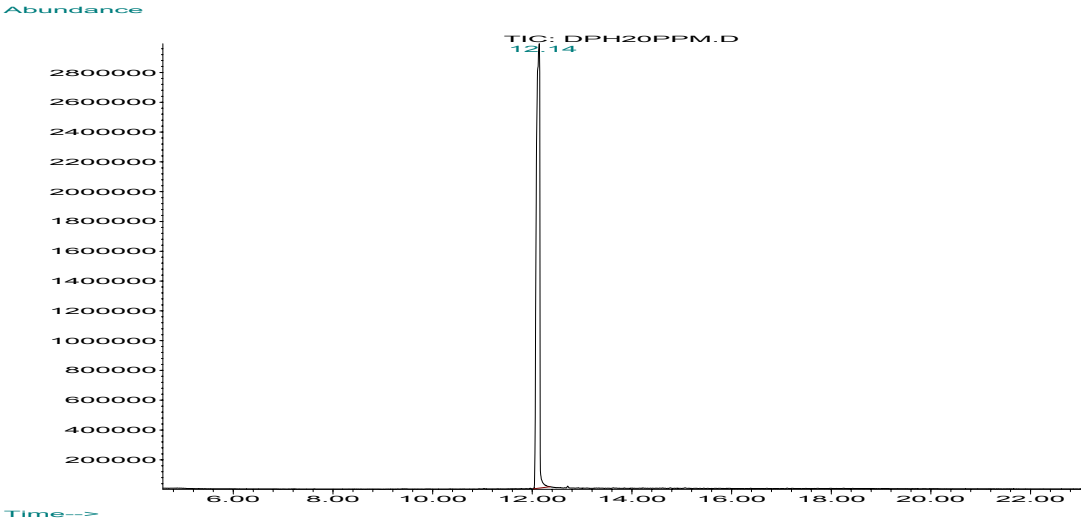

Figure 3.30: An example of the total ion chromatogram of a 20 µg/mL Diphenhydramine standard (12.14 minutes) used to compile the calibration line.

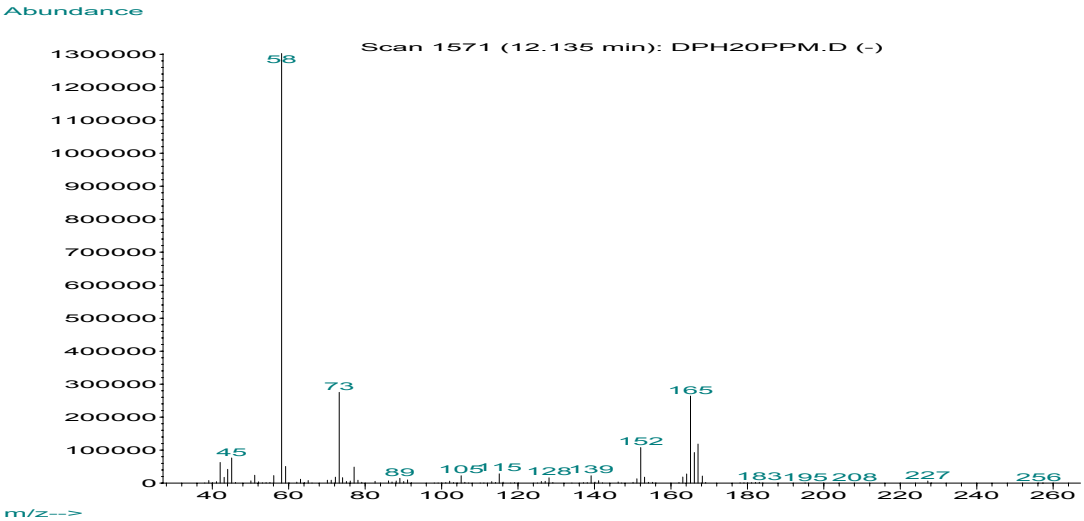

Figure 3.31: An example of the mass spectrum of the Diphenhydramine present at 12.14 minutes.

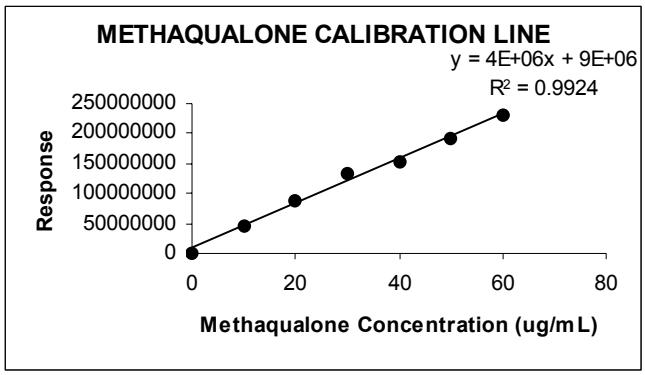

Figure 3.32: Methaqualone Calibration Line for Test Case 1

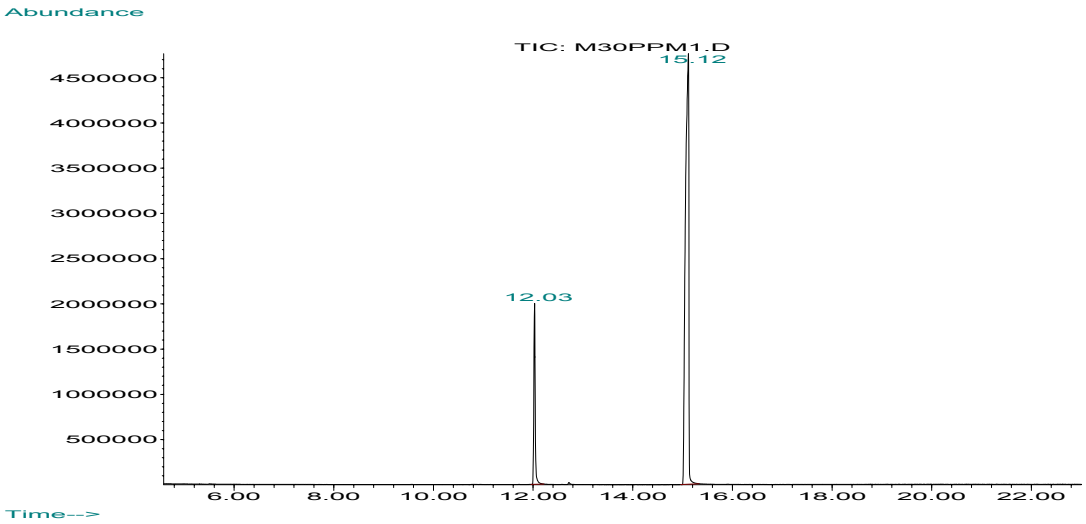

Figure 3.33: An example of the total ion chromatogram of a 30 µg/mL Methaqualone standard (15.12 minutes) used to compile the calibration line.

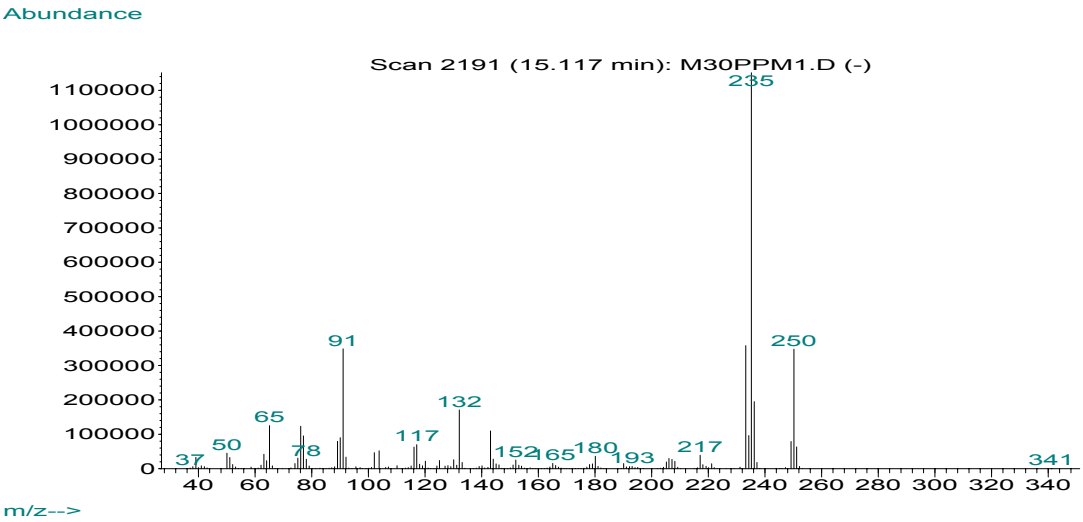

Figure 3.34: An example of the mass spectrum of the Methaqualone present at 15.12 minutes.

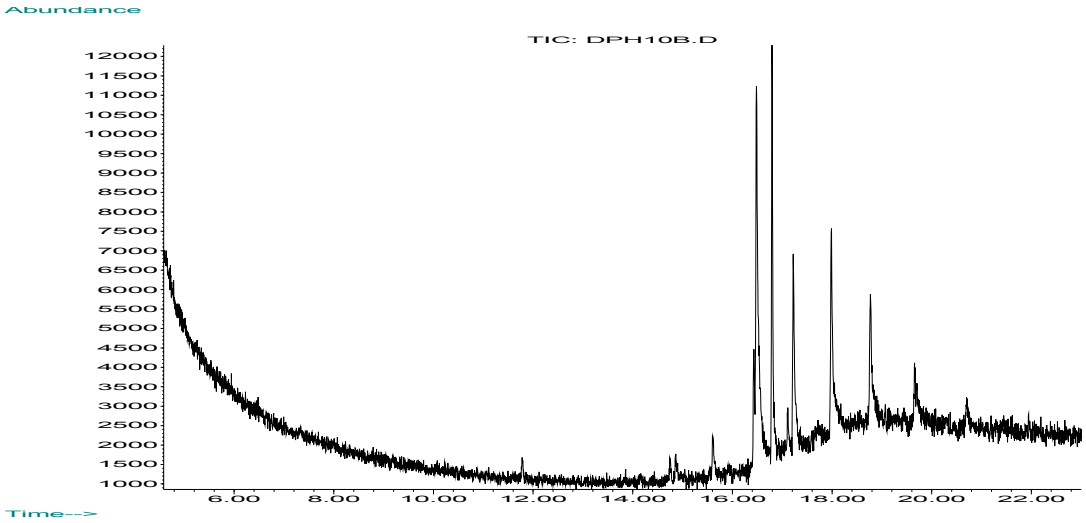

Figure 3.35: **An example of the total ion chromatogram of the chloroform blank for the 10th tablet in Test Case 1.**

Figure 3.36: **An example of the extracted ion chromatogram for Methaqualone (ions 235 and 91) and Diphenhydramine (ions 58, 73 and 165) of the chloroform blank for the 10th tablet in Test Case 1. No methaqualone or diphenhydramine contamination is present.**

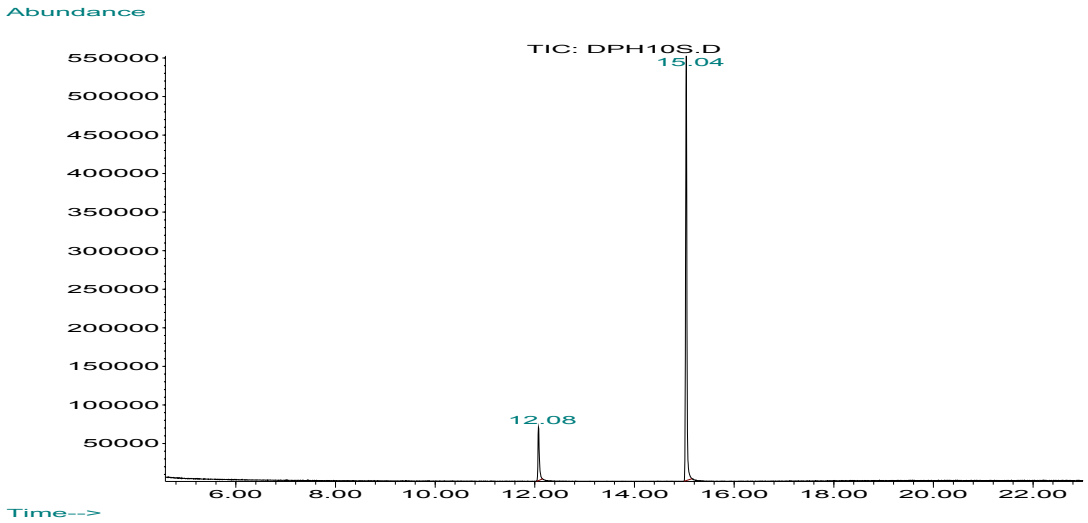

Figure 3.37: **An example of the total ion chromatogram of the chloroform extract of the 10th tablet in Test Case 1. Methaqualone can be seen at 15.04 minutes and Diphenhydramine at 12.08 minutes.**

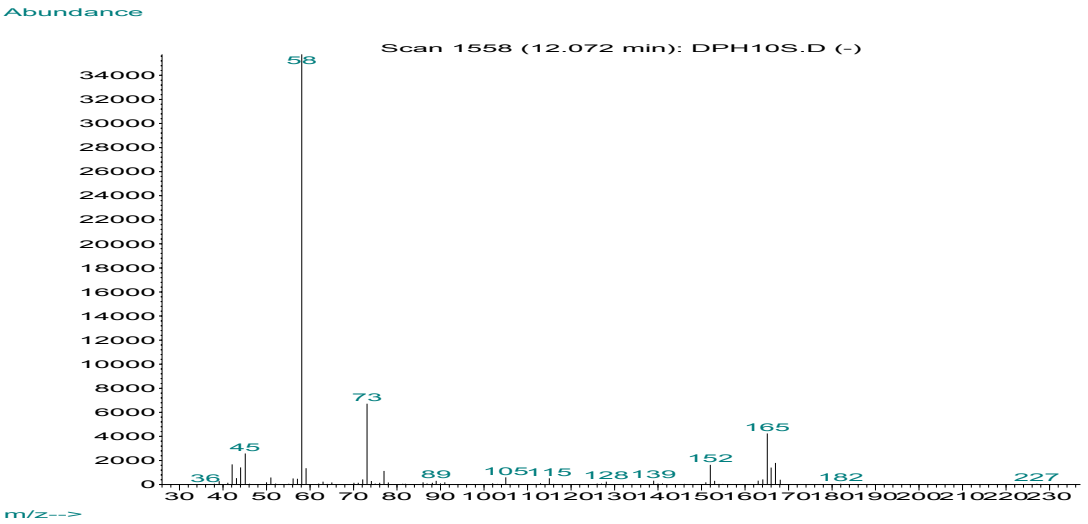

Figure 3.38: **An example of the mass spectrum of the Diphenhydramine present at 12.07 minutes in the chloroform extract of the 10th tablet in Test Case 1.**

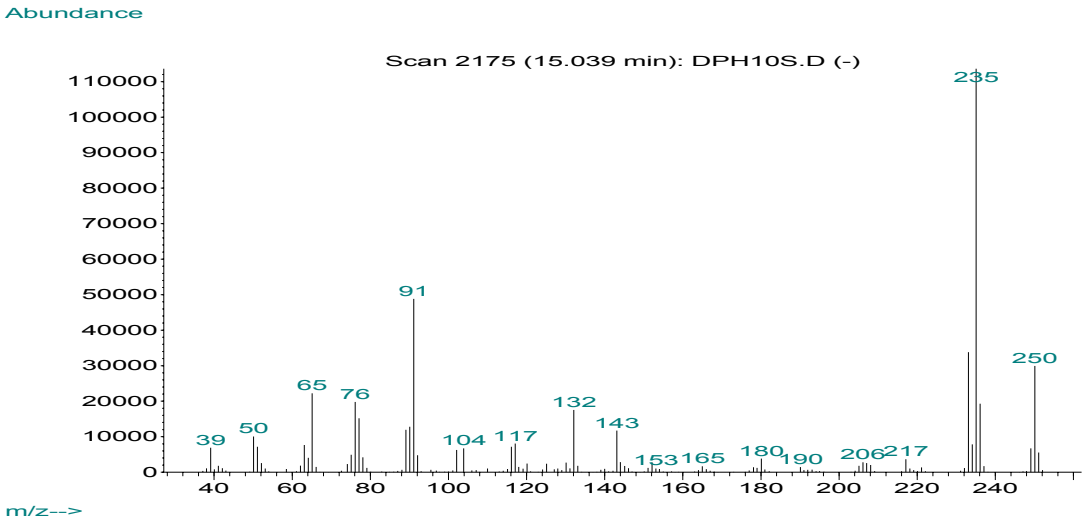

Figure 3.39: An example of the mass spectrum of the methaqualone present at 15.04 minutes in the chloroform extract of the 10th tablet in Test Case 1.

For the chromatograms and mass spectra of all the standards used, as well as that of the individual compounds present as obtained from the analysis of the thirty tablets, see Appendix D.

Table 3.15: The mass of each tablet, the concentration Methaqualone ([MTQ]) and the concentration Diphenhydramine ([DPH]), determined per Tablet for Test Case 1.

TABLET	MASS (g)	[MTQ] in µg/Tablet	[DPH] in µg/ Tablet	TABLET	MASS (g)	[MTQ] in µg/Tablet	[DPH] in µg/ Tablet
1	1.00141	7510.49	1163.05	16	1.09326	4885.38	586.48
2	1.03391	5917.44	655.68	17	1.09966	4293.80	411.23
3	1.00697	2455.03	227.68	18	1.02506	3192.92	297.25
4	1.05556	6409.97	642.81	19	1.02729	3847.80	346.99
5	1.00543	8146.47	752.27	20	1.06744	2084.64	106.16
6	1.03852	7212.09	616.45	21	1.07374	4591.26	456.42
7	1.01128	4233.35	344.81	22	1.09252	6682.91	549.61
8	1.03697	4996.70	385.31	23	1.05528	4191.44	347.85
9	1.0217	323.41	212.65	24	1.04956	5803.69	508.23
10	1.0997	399.77	467.94	25	1.03558	3511.02	316.00
11	1.09785	274.23	472.42	26	1.08357	7224.06	635.54
12	1.08101	1308.10	195.21	27	1.0664	997.63	429.68
13	1.01314	5754.47	689.32	28	1.02229	4992.99	402.58
14	1.05738	184.64	448.75	29	1.07068	6879.61	570.26
15	1.02759	3452.41	260.36	30	1.08501	1375.19	676.97

Table 3.16: Average Abundance (Methaqualone : Diphenhydramine) for Test Case 1

TABLET	[MTQ] : [DPH]	TABLET	[MTQ] : [DPH]
1	6 : 1	**16**	8 : 1
2	9 : 1	**17**	10 : 1
3	11 : 1	**18**	11 : 1
4	10 : 1	**19**	11 : 1
5	11 : 1	**20**	20 : 1
6	12 : 1	**21**	10 : 1
7	12 : 1	**22**	12 : 1
8	13 : 1	**23**	12 : 1
9	15 : 1	**24**	11 : 1
10	1 : 1	**25**	11 : 1
11	0.6: 1	**26**	11 : 1
12	7 : 1	**27**	2 : 1
13	8 : 1	**28**	12 : 1
14	0.4: 1	**29**	12 : 1
15	13 : 1	**30**	2 : 1

3.2.3.2 Results: Test Case 2 [Lab number 2426/03]

Logo of Tablet 

Tablet Code : 3.41

Other active constituents present : Diphenhydramine, Mtq Isomer II

Area of seizure : Kuilsrivier (Western Cape)

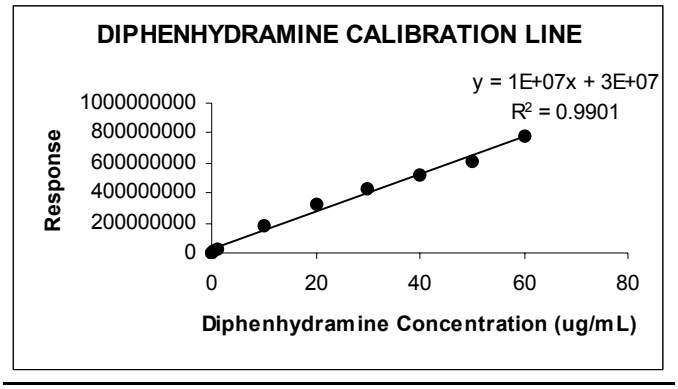

Figure 3.40: **Diphenhydramine Calibration Line for Test Case 2**

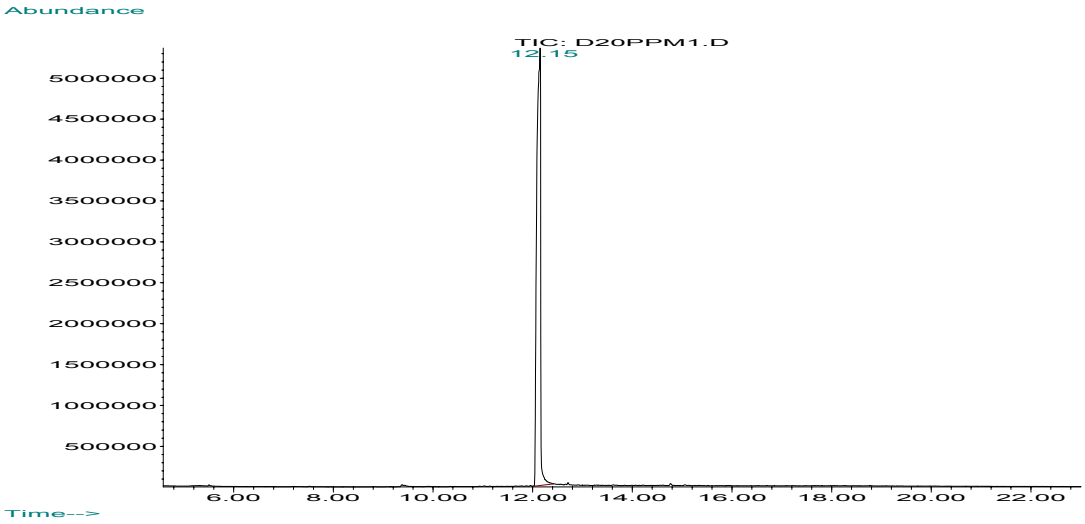

Figure 3.41: **An example of the total ion chromatogram of a 20 μg/mL Diphenhydramine standard (12.15 minutes) used to compile the calibration line.**

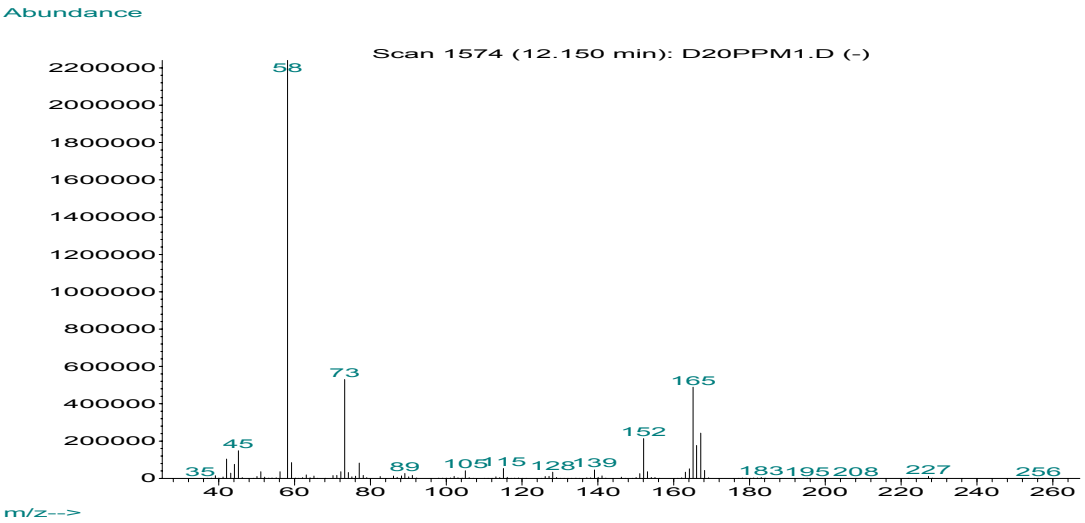

Figure 3.42: **An example of the mass spectrum of the Diphenhydramine standard at 12.15 minutes.**

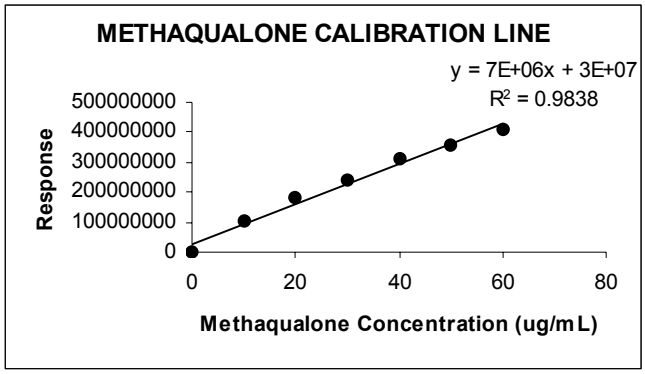

Figure 3.43: **Methaqualone Calibration Line for Test Case 2**

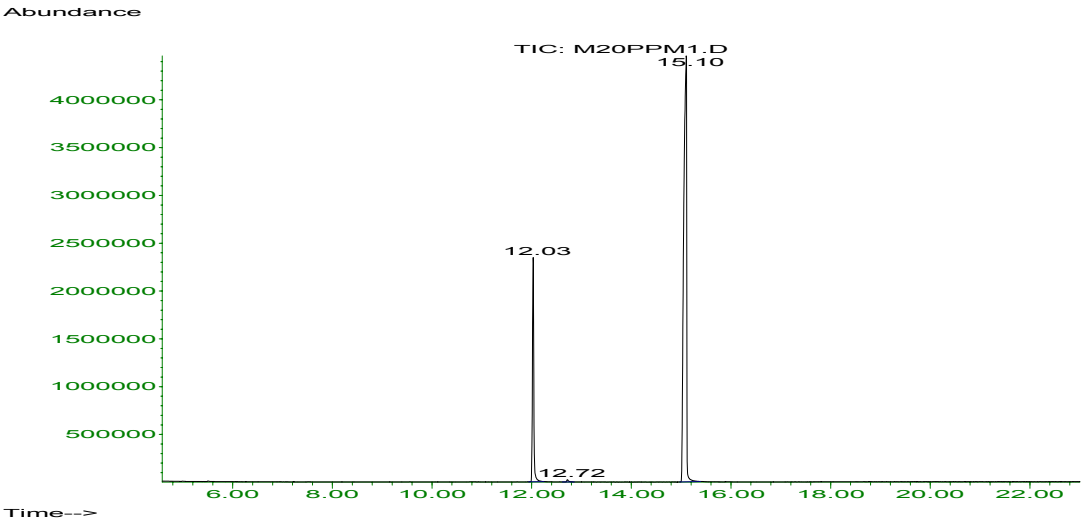

Figure 3.44: **An example of the total ion chromatogram of a 20 µg/mL Methaqualone standard (15.10 minutes) used to compile the calibration line.**

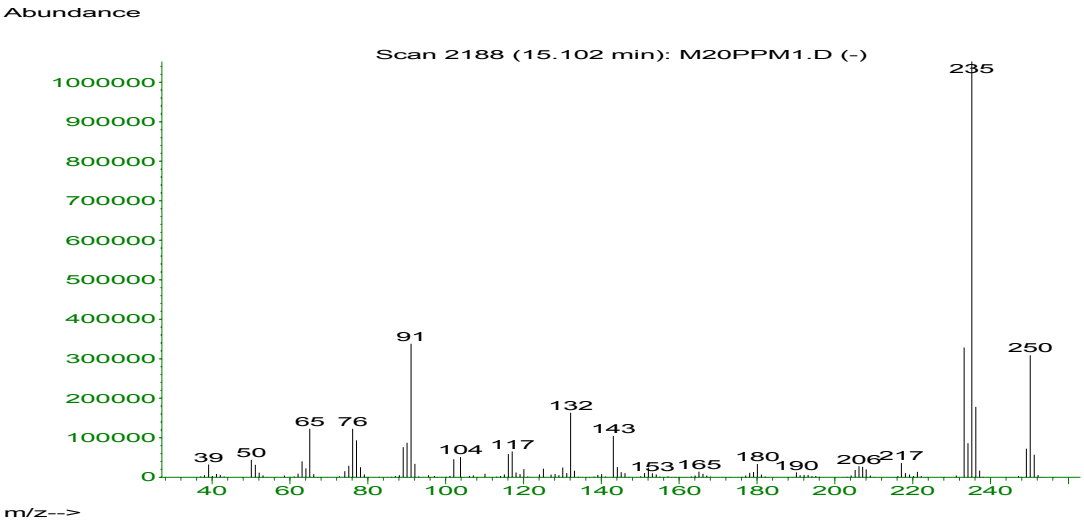

Figure 3.45: **An example of the mass spectrum of the Methaqualone standard at 15.10 minutes.**

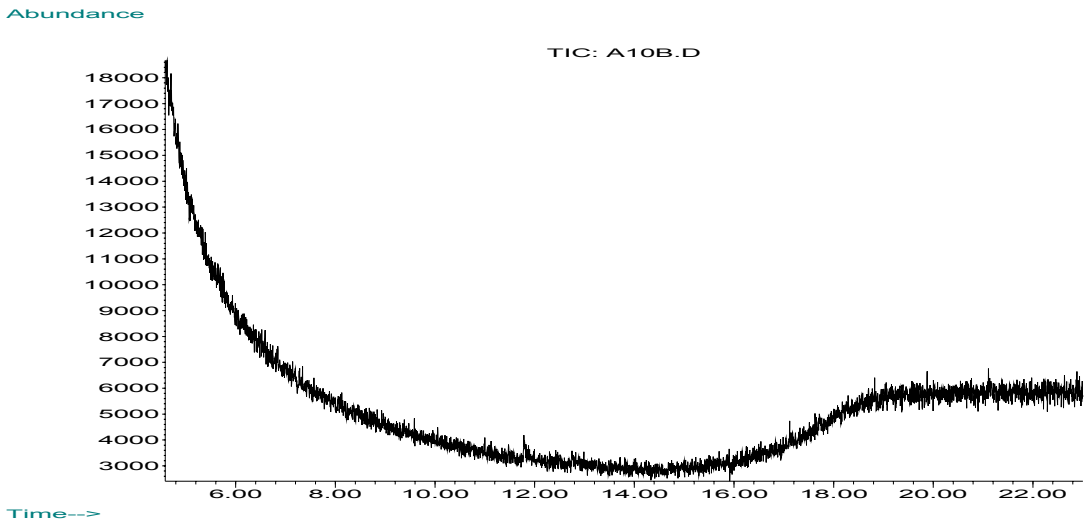

Figure 3.46: **An example of the total ion chromatogram of the chloroform blank for the 10th tablet in Test Case 2.**

Figure 3.47: **An example of the extracted ion chromatogram for Methaqualone (ions 235 and 91) and Diphenhydramine (ions 58, 73 and 165) of the chloroform blank for the 10th tablet in Test Case 2. No methaqualone or diphenhydramine contamination is present.**

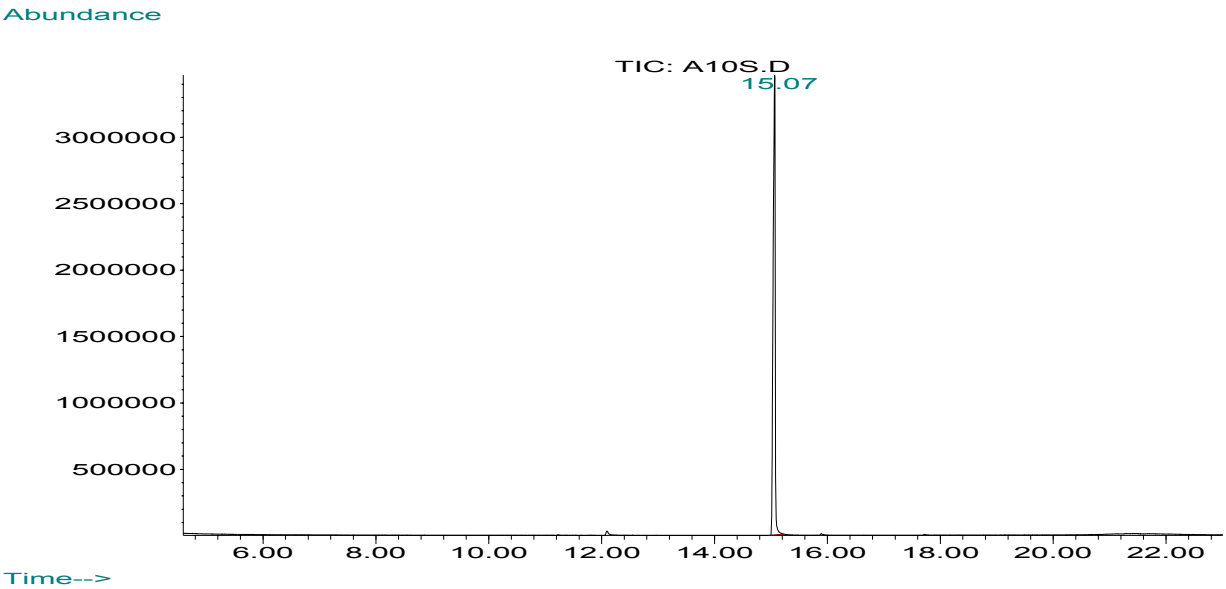

Figure 3.48: **An example of the total ion chromatogram of the chloroform extract of the 10th tablet in Test Case 2. Methaqualone can be seen at 15.07 minutes and Diphenhydramine at 12.10 minutes.**

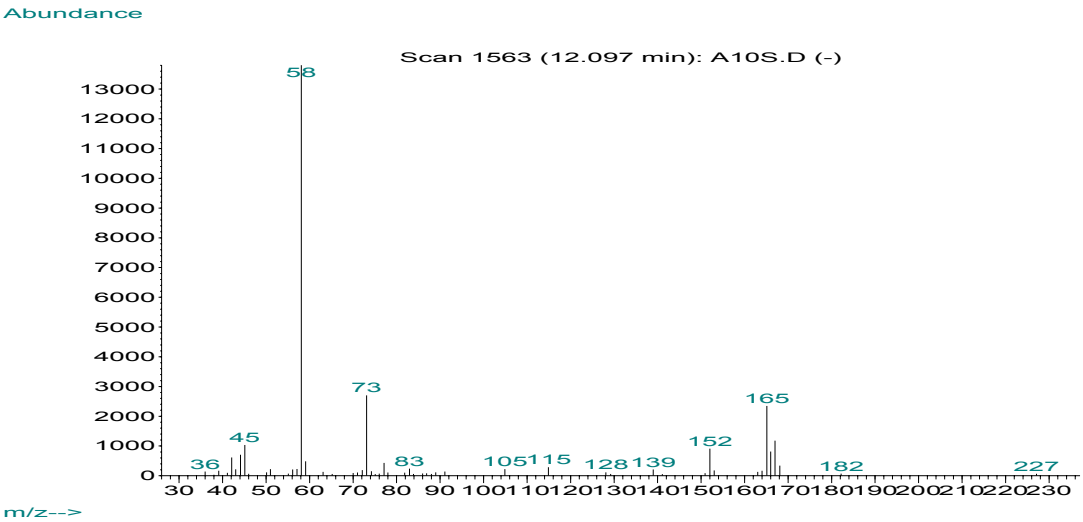

Figure 3.49: **An example of the mass spectrum of the Diphenhydramine present at 12.10 minutes in the chloroform extract of the 10th tablet in Test Case 2.**

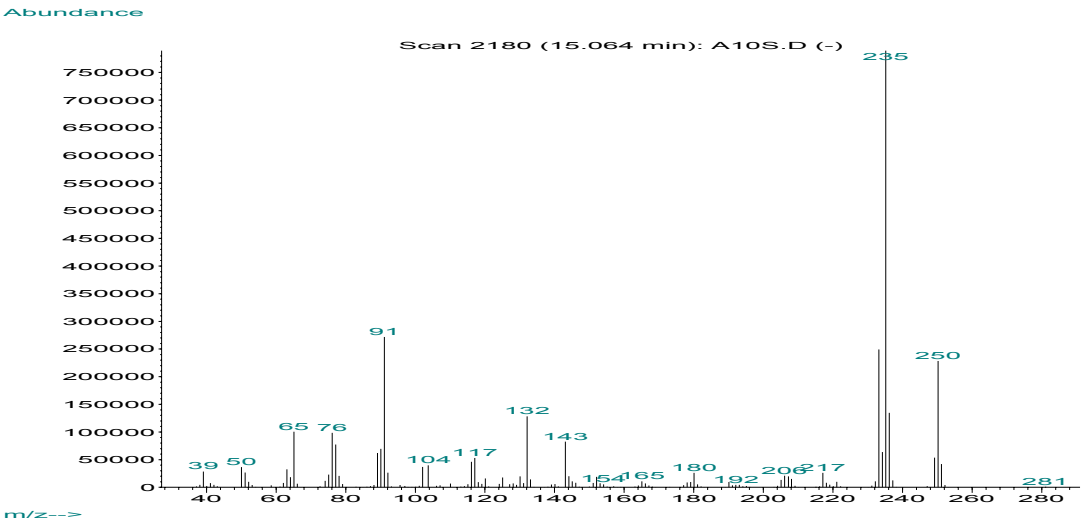

Figure 3.50: **An example of the mass spectrum of the Methaqualone present at 15.07 minutes in the chloroform extract of the 10th tablet in Test Case 2.**

For the chromatograms and mass spectra of the standards used, as well as that of the individual compounds present as obtained from the analysis of the thirty tablets, see Appendix E.

Table 3.17: The mass of each tablet, the concentration Methaqualone ([MTQ]) and the concentration Diphenhydramine ([DPH]), determined per Tablet for Test Case 2.

TABLET	MASS (g)	[MTQ] in µg/Tablet	[DPH] in µg/ Tablet	TABLET	MASS (g)	[MTQ] in µg/ Tablet	[DPH] in µg/ Tablet
1	0.49742	29924.64	243.64	**16**	0.51313	33673.82	311.51
2	0.49422	23956.41	150.34	**17**	0.47666	36784.74	271.01
3	0.48733	30118.35	309.97	**18**	0.49847	33042.28	230.84
4	0.49771	19791.34	88.26	**19**	0.49602	32782.52	221.34
5	0.48181	22597.61	99.68	**20**	0.48624	32075.97	201.11
6	0.49058	33707.06	222.27	**21**	0.49566	36499.79	256.66
7	0.49515	30198.10	154.08	**22**	0.50085	32084.46	202.62
8	0.52344	27906.43	167.01	**23**	0.48266	32950.12	219.74
9	0.50694	30609.48	310.65	**24**	0.49989	26965.70	153.76
10	0.5049	29641.79	169.80	**25**	0.4999	30473.51	168.44
11	0.49817	32416.93	203.08	**26**	0.51172	27374.28	156.07
12	0.49662	28586.42	149.45	**27**	0.47705	34967.56	248.34
13	0.5014	32658.40	195.89	**28**	0.49252	27639.67	136.62
14	0.49341	34920.82	222.45	**29**	0.50606	33135.00	200.11
15	0.49597	22916.78	102.54	**30**	0.49232	29057.67	139.50

Table 3.18: Average Abundance (Methaqualone : Diphenhydramine) for Test Case 2

TABLET	[MTQ] : [DPH]	TABLET	[MTQ] : [DPH]
1	123 : 1	**16**	108 : 1
2	159 : 1	**17**	136 : 1
3	97 : 1	**18**	143 : 1
4	224 : 1	**19**	148 : 1
5	226 : 1	**20**	159 : 1
6	152 : 1	**21**	142 : 1
7	195 : 1	**22**	158 : 1
8	167 : 1	**23**	150 : 1
9	98 : 1	**24**	175 : 1
10	174 : 1	**25**	181 : 1
11	160 : 1	**26**	175 : 1
12	191 : 1	**27**	141 : 1
13	167 : 1	**28**	202 : 1
14	157 : 1	**29**	165 : 1
15	223 : 1	**30**	208 : 1

3.2.3.3 Results: Test Case 3 [Lab number 63171/03]

Logo of Tablet 

Tablet Code	:	3.41
Other active constituents present	:	Diphenhydramine, Mtq Isomer II
Area of seizure	:	George (Western Cape)

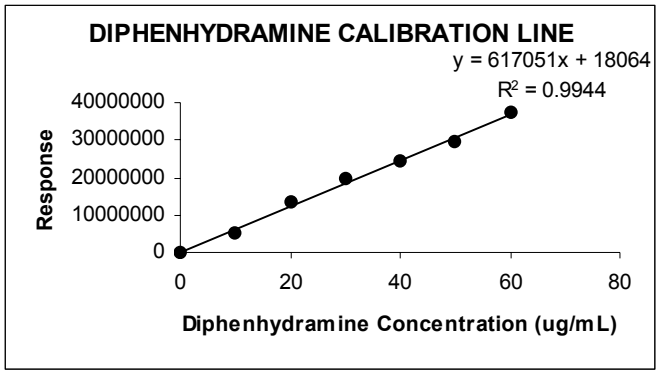

Figure 3.51: Diphenhydramine Calibration Line for Test Case 3

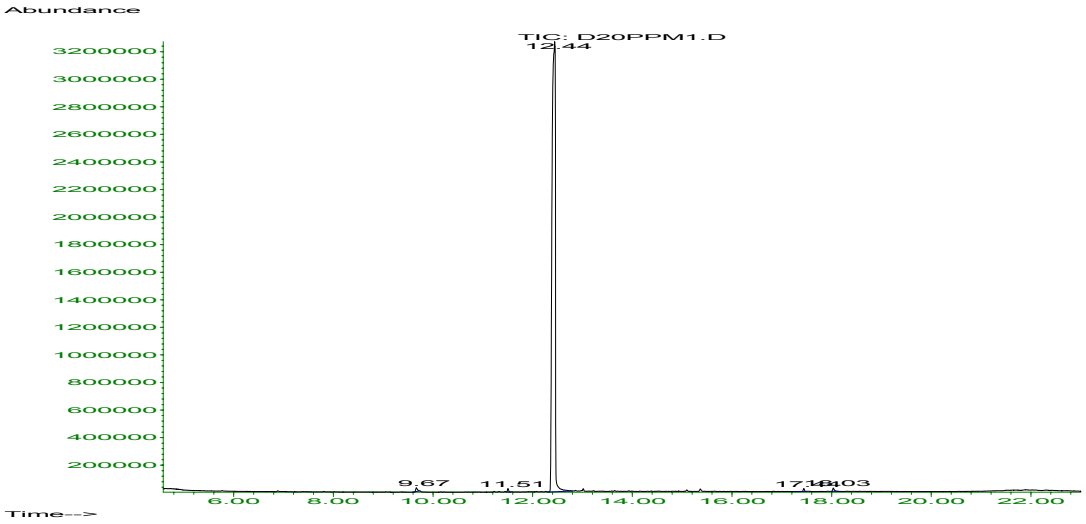

Figure 3.52: An example of the total ion chromatogram of a 20 µg/mL Diphenhydramine standard (12.44 minutes) used to compile the calibration line.

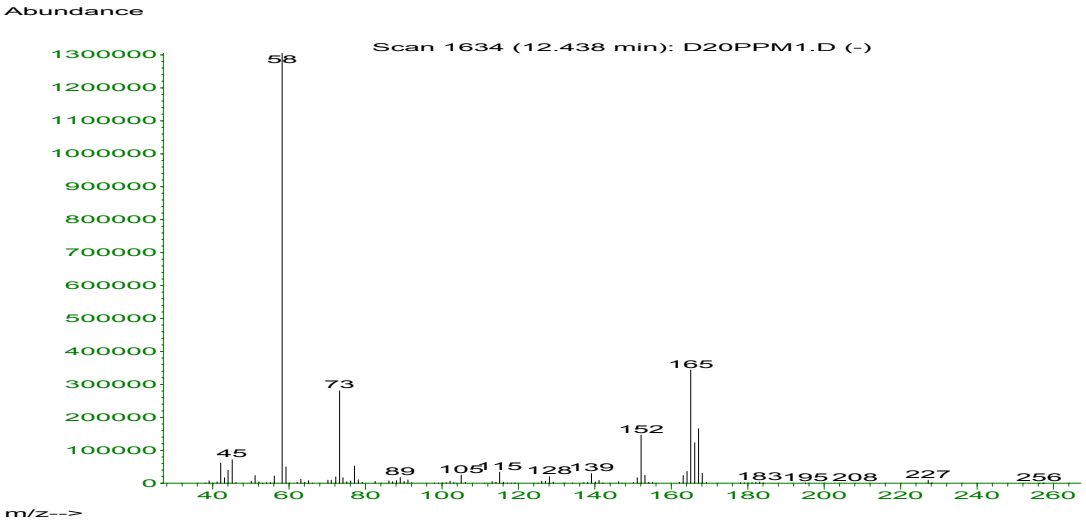

Figure 3.53: **An example of the mass spectrum of the Diphenhydramine standard at 12.44 minutes.**

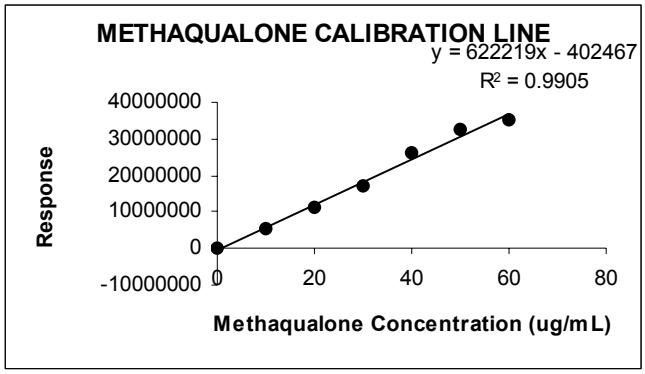

Figure 3.54: **Methaqualone Calibration Line for Test Case 3**

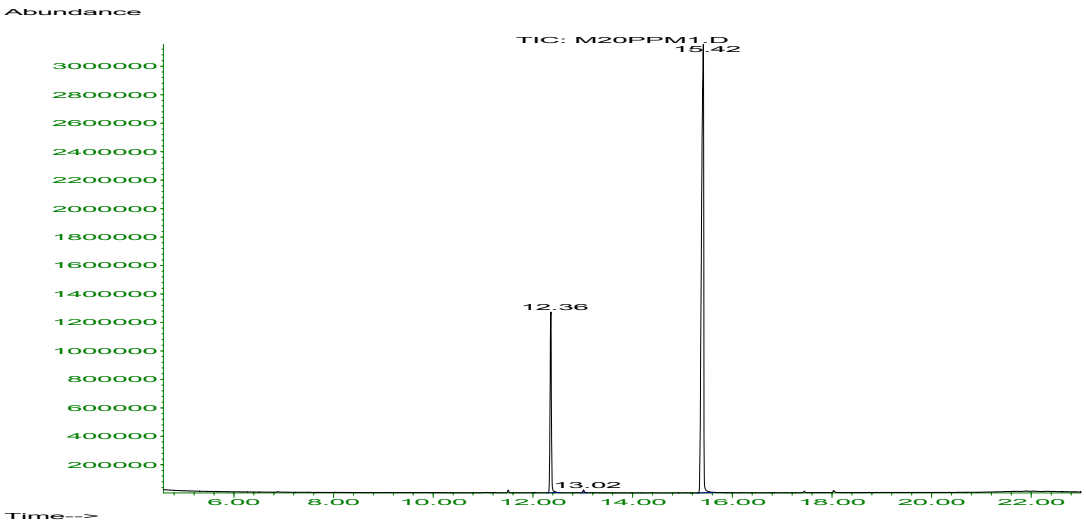

Figure 3.55: **An example of the total ion chromatogram of a 20 μg/mL Methaqualone standard (15.42 minutes) used to compile the calibration line.**

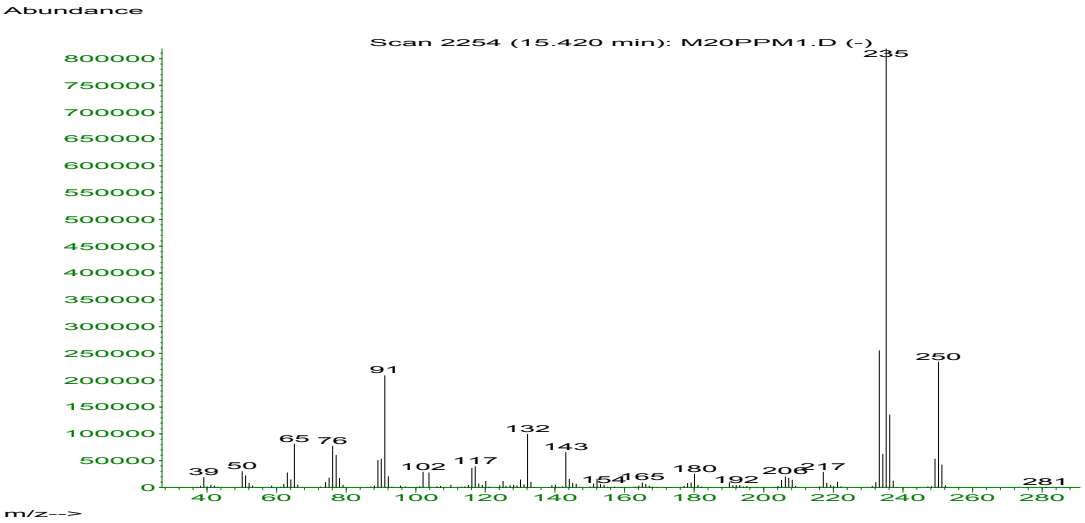

Figure 3.56: **An example of the mass spectrum of the Methaqualone standard 15.42 minutes.**

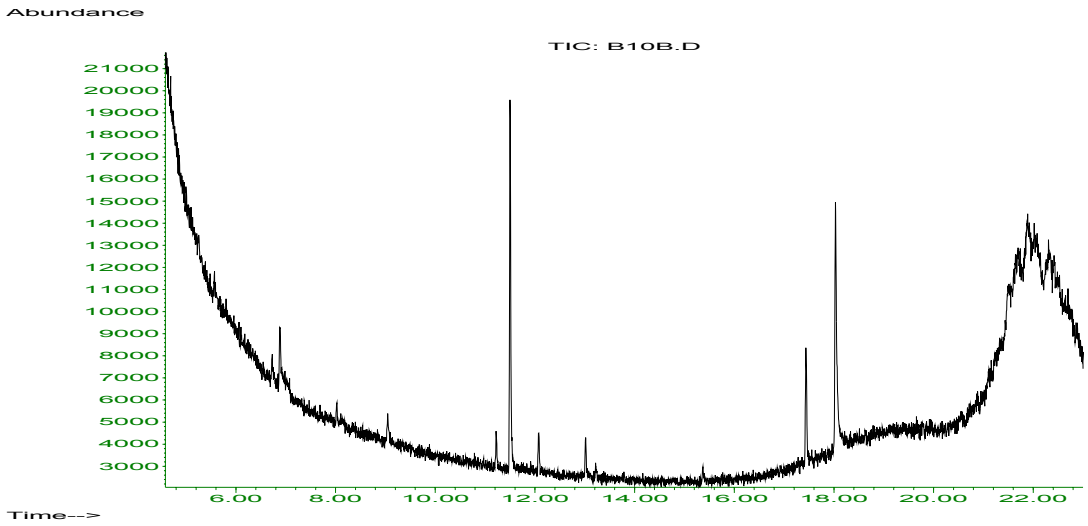

Figure 3.57: **An example of the total ion chromatogram of the chloroform blank for the 10th tablet in Test Case 3.**

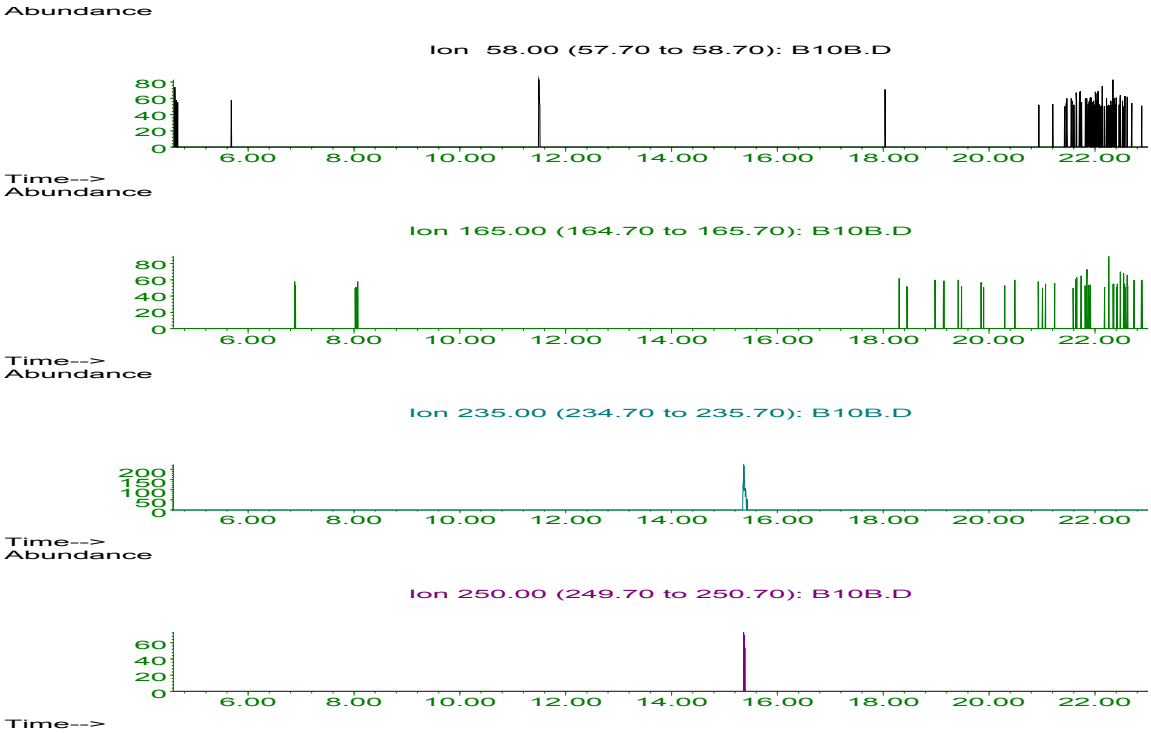

Figure 3.58: **An example of the extracted ion chromatogram for Methaqualone (ions 235 and 250) and Diphenhydramine (ions 58 and 165) of the chloroform blank for the 10th tablet in Test Case 3. No diphenhydramine contamination is present. The abundance of the 235 and 250 ions at 15.37 minutes is approximately 0.25% of that in the chloroform extract of the 10th tablet in Test Case 3 below.**

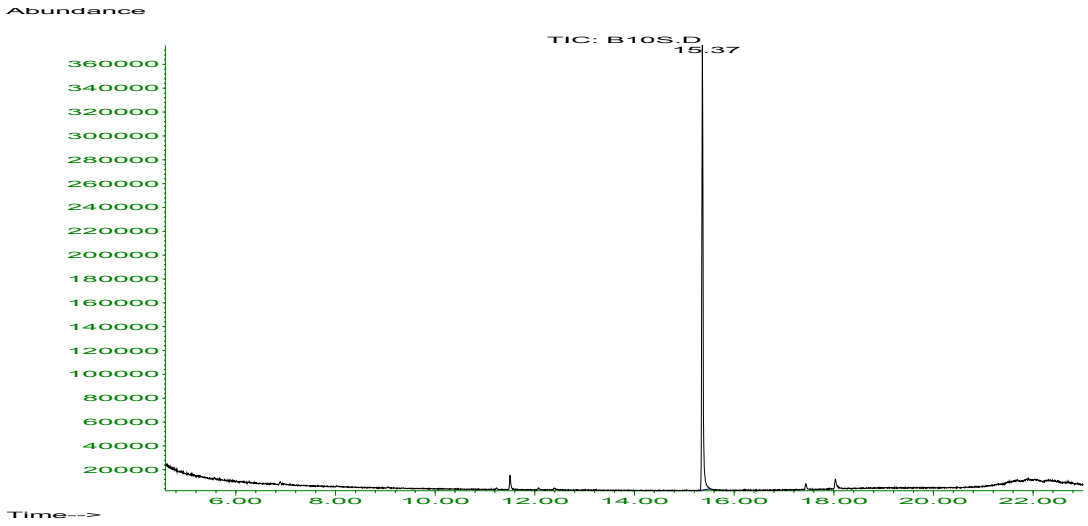

Figure 3.59: **An example of the total ion chromatogram of the chloroform extract of the 10th tablet in Test Case 3. Methaqualone can be seen at 15.37 minutes and Diphenhydramine at 12.34 minutes.**

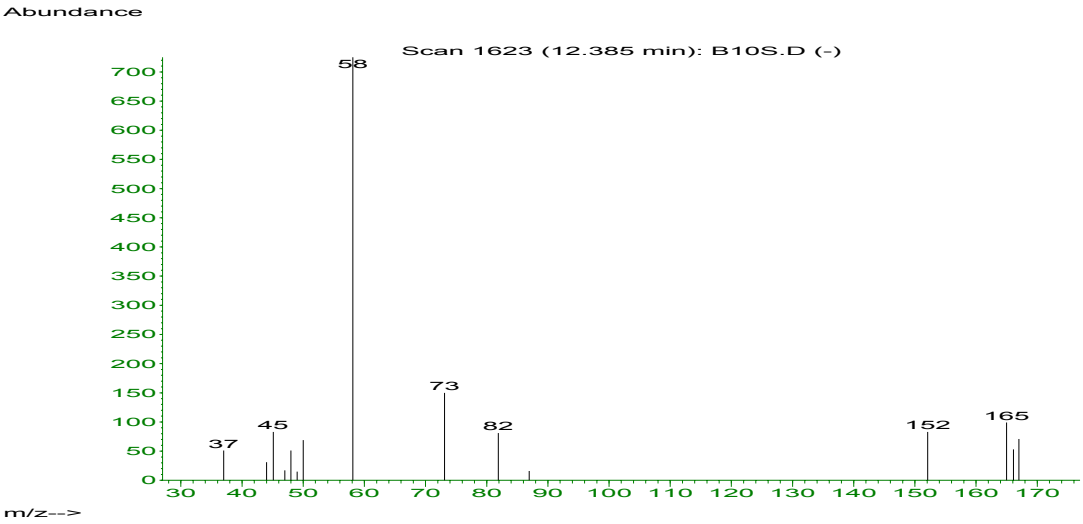

Figure 3.60: An example of the mass spectrum of the diphenhydramine present at 12.40 minutes in the chloroform extract of the 10th tablet in Test Case 3.

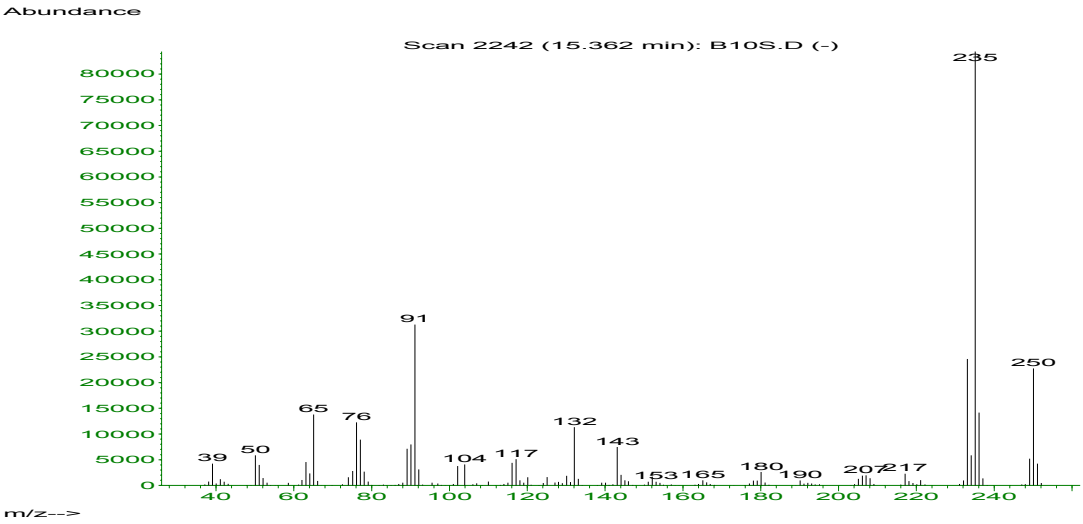

Figure 3.61: An example of the mass spectrum of the methaqualone present at 15.40 minutes in the chloroform extract of the 10th tablet in Test Case 3.

For the chromatograms and mass spectra of the standards used, as well as that of the individual compounds present as obtained from the analysis of the thirty tablets, see Appendix F.

Table 3.19: The mass of each tablet, the concentration Methaqualone ([MTQ]) and the concentration Diphenhydramine ([DPH]), determined per Tablet for Test Case 3.

TABLET	MASS (g)	[MTQ] in µg/Tablet	[DPH] in µg/ Tablet	TABLET	MASS (g)	[MTQ] in µg/ Tablet	[DPH] in µg/ Tablet
1	0.47664	55704.97	1108.88	**16**	0.50217	57191.06	550.47
2	0.49452	34370.62	481.95	**17**	0.49411	59652.89	547.49
3	0.50529	53677.34	694.19	**18**	0.49782	58638.56	575.95
4	0.53526	49113.19	560.20	**19**	0.48507	50554.01	349.73
5	0.4793	41061.72	374.69	**20**	0.48756	59144.91	501.35
6	0.50581	25113.19	153.01	**21**	0.49526	53871.74	348.21
7	0.49936	48046.69	388.15	**22**	0.49614	65452.94	460.65
8	0.52484	56944.36	530.99	**23**	0.48825	60633.53	486.49
9	0.49276	53267.05	444.06	**24**	0.49272	63233.4	341.45
10	0.48747	25066.54	182.63	**25**	0.47138	64412.99	419.47
11	0.49346	47712.4	373.45	**26**	0.48903	53422.42	421.29
12	0.52503	45999.12	384.30	**27**	0.49698	68654.77	475.94
13	0.50048	48647.7	563.34	**28**	0.50493	59707.55	420.15
14	0.50286	62849.56	681.42	**29**	0.49652	49028.36	453.58
15	0.49864	53747.01	617.89	**30**	0.49477	66392.55	531.79

Table 3.20: Average Abundance (Methaqualone : Diphenhydramine) for Test Case 3

TABLET	[MTQ] : [DPH]	TABLET	[MTQ] : [DPH]
1	50 : 1	**16**	104 : 1
2	71 : 1	**17**	109 : 1
3	77 : 1	**18**	102 : 1
4	88 : 1	**19**	144 : 1
5	109 : 1	**20**	118 : 1
6	164 : 1	**21**	155 : 1
7	124 : 1	**22**	142 : 1
8	107 : 1	**23**	125 : 1
9	120 : 1	**24**	185 : 1
10	137 : 1	**25**	153 : 1
11	128 : 1	**26**	127 : 1
12	120 : 1	**27**	144 : 1
13	86 : 1	**28**	142 : 1
14	92 : 1	**29**	108 : 1
15	87 : 1	**30**	125 : 1

3.2.3.4 Results: Test Case 4 [Lab number 1125/01]

Logo of Tablet  (front and back of tablet are identical)

Tablet Code	:	35.11
Other active constituents present	:	None
Area of seizure	:	Lenasia (Gauteng)

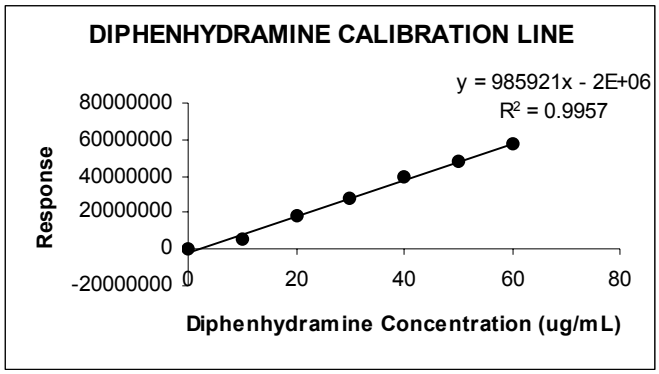

Figure 3.62: Diphenhydramine Calibration Line for Test Case 4

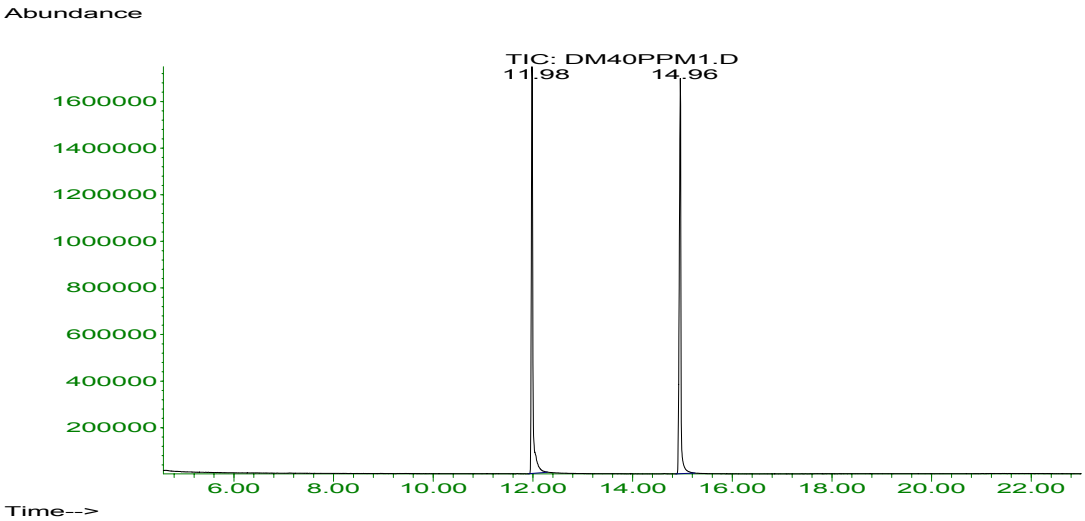

Figure 3.63: An example of the total ion chromatogram of a 40 µg/mL Diphenhydramine and Methaqualone standard used to compile the calibration line. Methaqualone can be seen at 14.96 minutes and Diphenhydramine at 11.98 minutes.

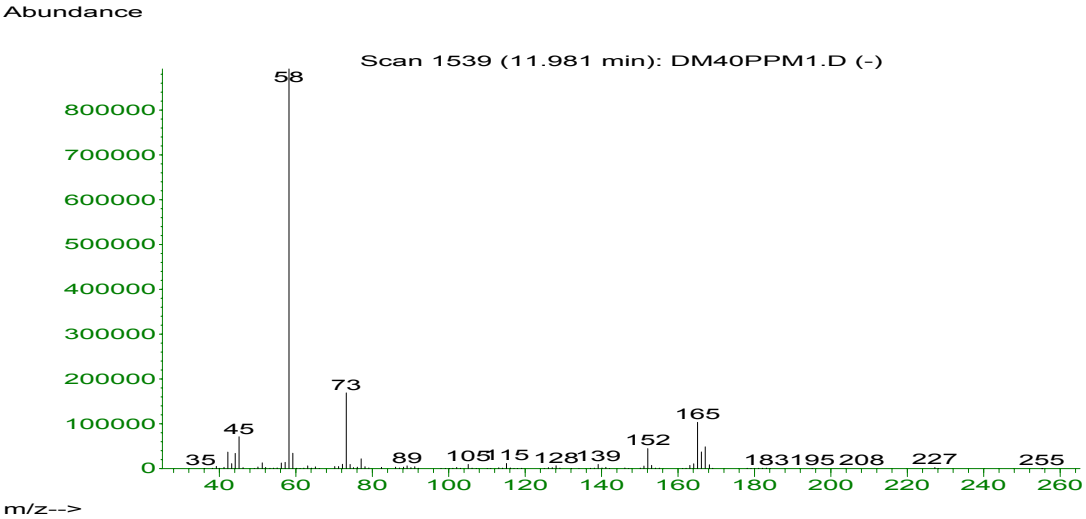

Figure 3.64: **An example of the mass spectrum of the Diphenhydramine standard at 11.98 minutes.**

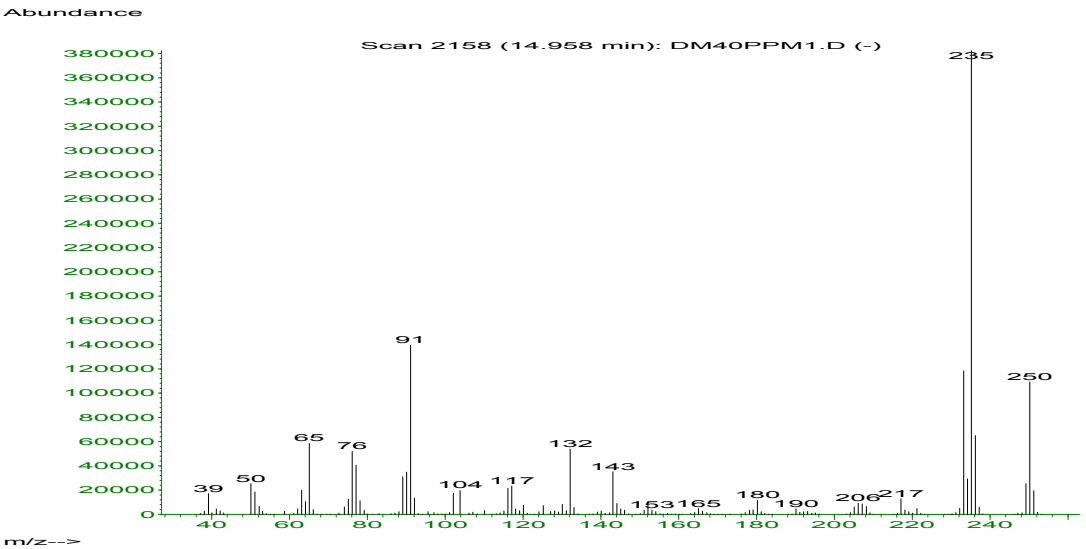

Figure 3.65: **An example of the mass spectrum of the Methaqualone standard at 14.96 minutes.**

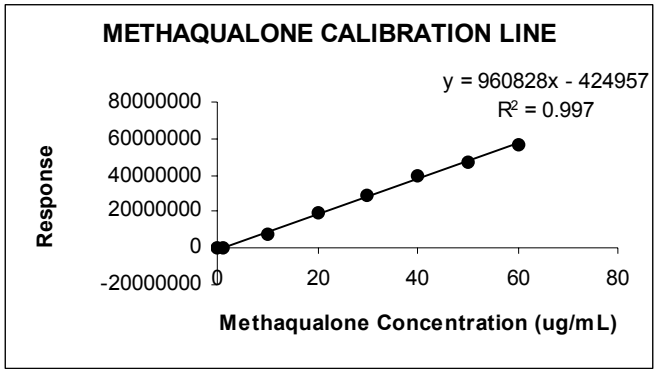

Figure 3.66: **Methaqualone Calibration Line for Test Case 4**

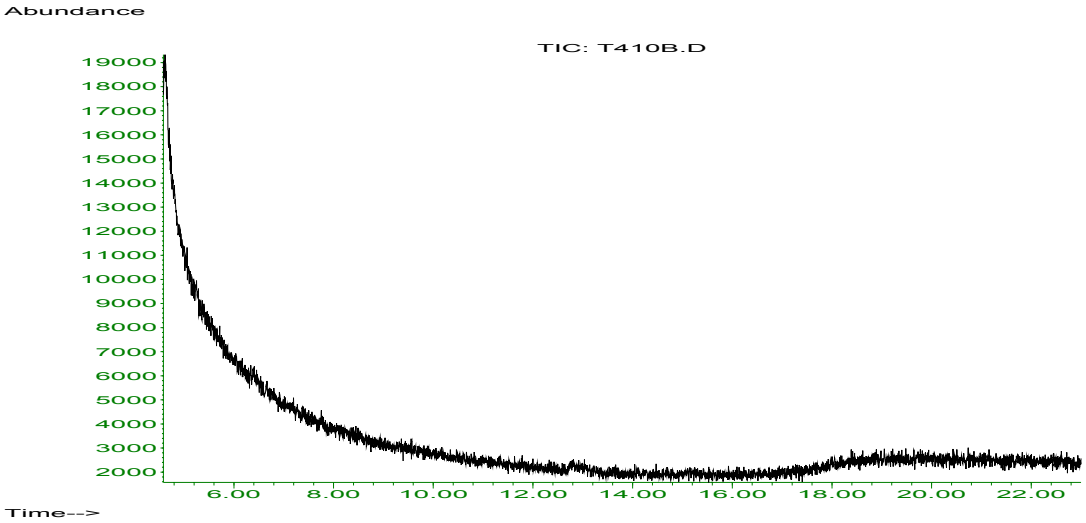

Figure 3.67: **An example of the total ion chromatogram of the chloroform blank for the 10th tablet in Test Case 4.**

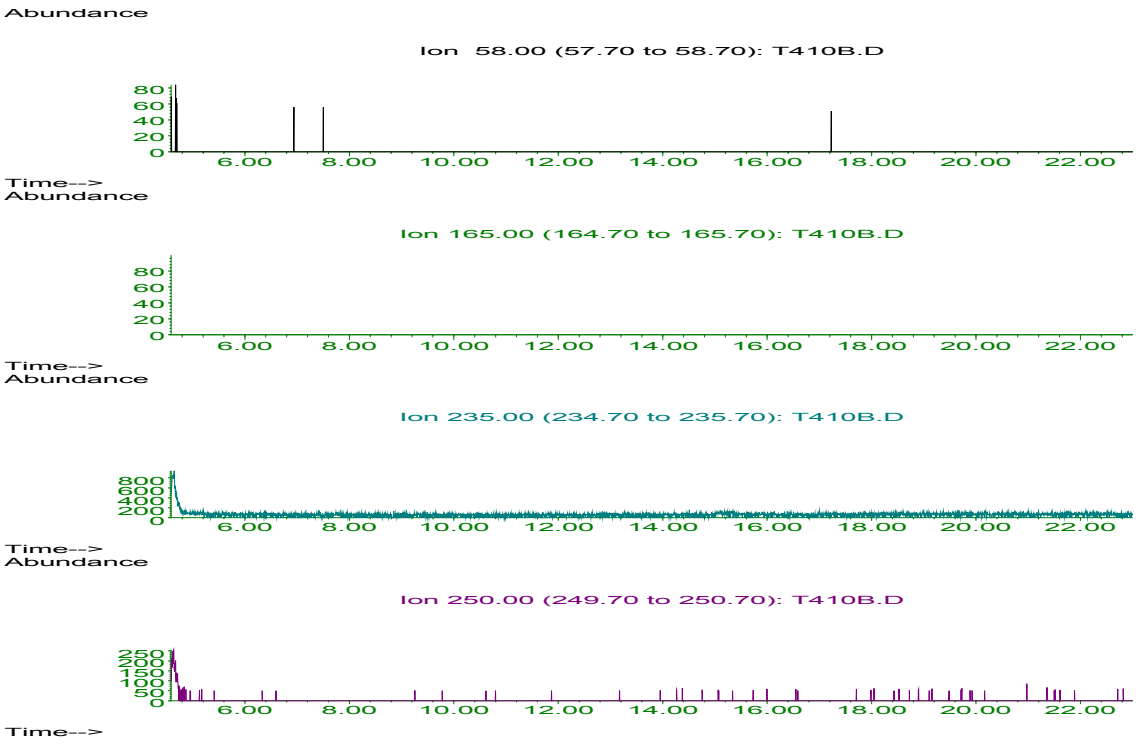

Figure 3.68: **An example of the extracted ion chromatogram for Methaqualone (ions 235 and 250) and Diphenhydramine (ions 58 and 165) of the chloroform blank for the 10th tablet in Test Case 4. No methaqualone or diphenhydramine contamination is present.**

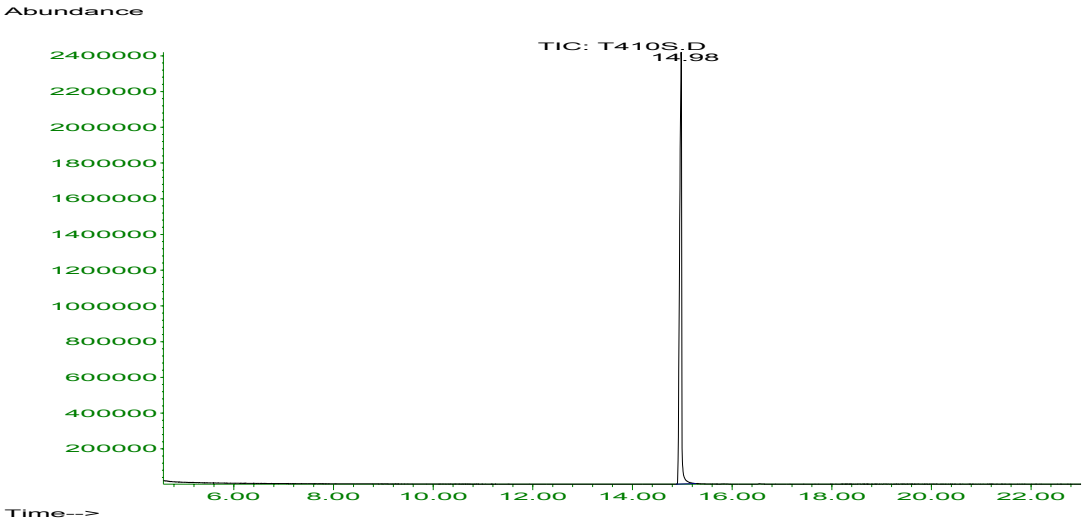

Figure 3.69: **An example of the total ion chromatogram of the chloroform extract of the 10th tablet in Test Case 4. Methaqualone can be seen at 14.98 minutes.**

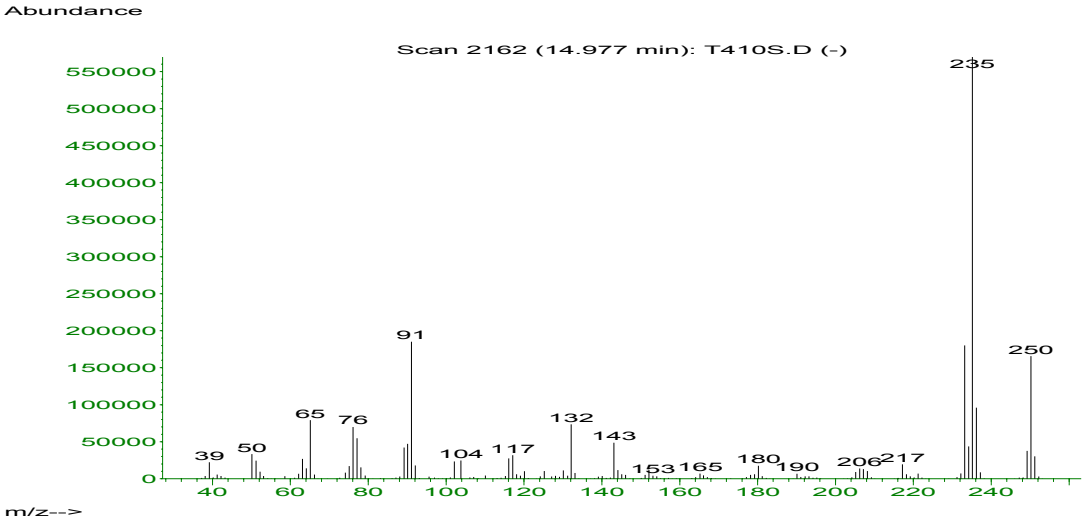

Figure 3.70: **An example of the mass spectrum of the methaqualone present at 14.98 minutes in the chloroform extract of the 10th tablet in Test Case 4.**

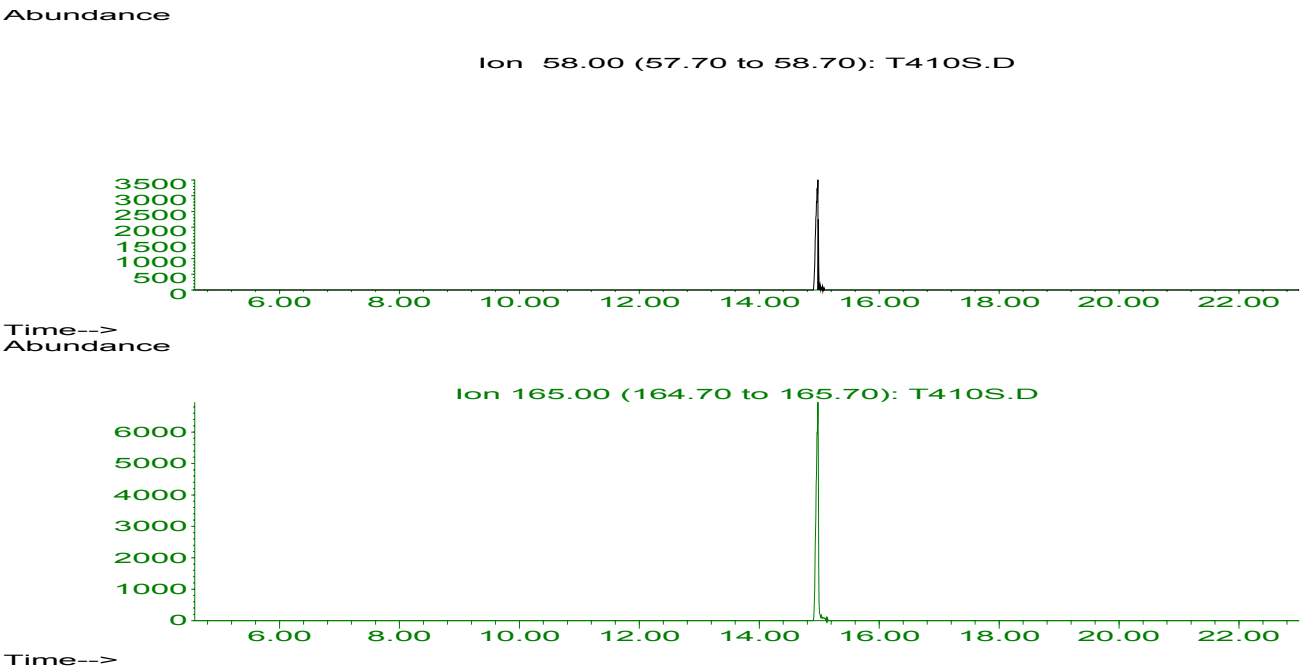

Figure 3.71: An example of the extracted ion chromatogram for Diphenhydramine of the chloroform extract of the 10th tablet in Test Case 4. No diphenhydramine is present.

For the chromatograms and mass spectra of the standards used, as well as that of the individual compounds present as obtained from the analysis of the thirty tablets, see Appendix G.

Table 3.21: The mass of each tablet, the concentration Methaqualone ([MTQ]) and the concentration Diphenhydramine ([DPH]), determined per Tablet for Test Case 4.

TABLET	MASS (g)	[MTQ] in µg/g	[DPH] in µg/g	TABLET	MASS (g)	[MTQ] in µg/g	[DPH] in µg/g
1	0.99049	136319.2	n/d	16	1.01336	155459.8	n/d
2	0.9708	138210.1	n/d	17	1.03592	165494.3	n/d
3	0.9839	116758.3	n/d	18	1.0233	159915.7	n/d
4	0.98678	150346.4	n/d	19	0.9782	168074.8	n/d
5	0.9217	177289.0	n/d	20	1.03598	169502.6	n/d
6	1.01083	148157.1	n/d	21	1.03031	184055.0	n/d
7	0.93415	185693.2	n/d	22	1.01935	107528.5	n/d
8	1.02467	142014.4	n/d	23	0.93926	172666.2	n/d
9	0.9892	188874.4	n/d	24	1.01029	134875.7	n/d
10	1.0175	156240.6	n/d	25	1.02413	146524.5	n/d
11	0.98975	166792.8	n/d	26	1.03401	92954.86	n/d
12	1.03467	194432.2	n/d	27	1.03384	180598.8	n/d
13	0.95092	176783.5	n/d	28	1.05502	140435.5	n/d
14	1.04203	99075.86	n/d	29	1.03202	107126.8	n/d
15	0.97175	138174.5	n/d	30	0.95791	161497.2	n/d

3.2.3.5 Summarized Results

In Table 3.13, a summary of the nine parameters that were determined during the project were listed. The first parameter required the determination of the mass of each individual tablet in each test case. Figure 3.72 below shows a summary of the mass of each of the thirty tablets determined in each test case.

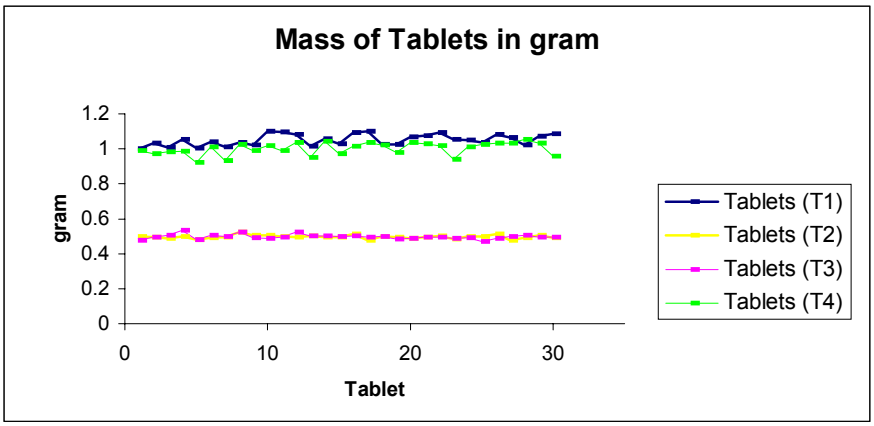

Figure 3.72: Mass of Tablets in gram. (T1 = Test Case 1, T2 = Test Case 2, T3 = Test Case 3 and T4 = Test Case 4)

The second parameter required the determination of the presence of methaqualone, and the third parameter that of diphenhydramine in each tablet in each test case. Parameters four and five required the determination of the concentration of methaqualone and diphenhydramine per tablet in each test case.

The presence and concentration of Methaqualone were determined in all thirty tablets in each of the four test cases. The results are summarized in Figure 3.73 below.

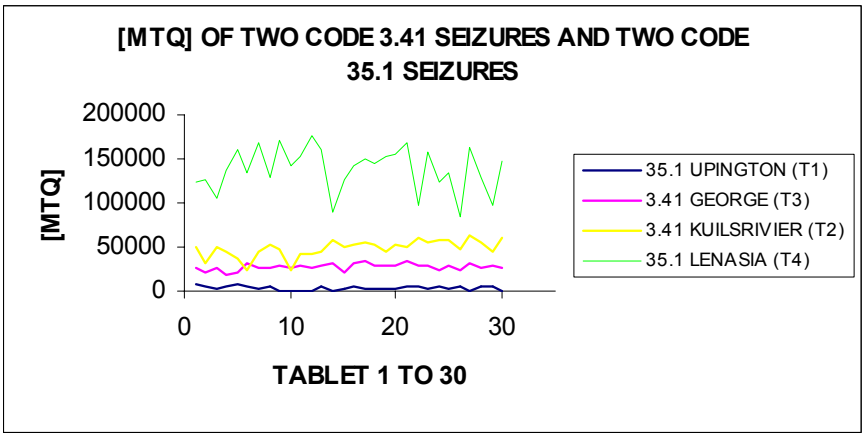

Figure 3.73: Methaqualone Concentration ([MTQ]) of Two Code 3.41 Seizures and Two 35.1 Seizures

The presence and concentration of diphenhydramine were determined in three of the four test cases. As can be seen in Figure 3.74 below, test case 4 did not contain diphenhydramine.

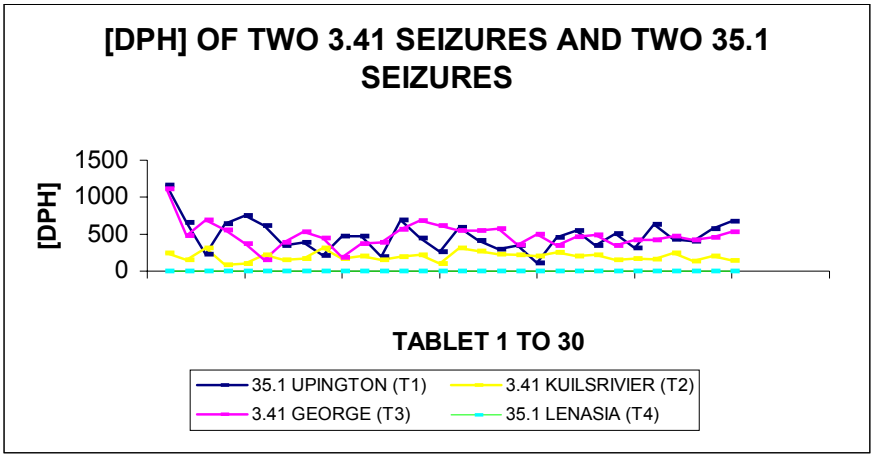

Figure 3.74: Diphenhydramine Concentration of Two Code 3.41 Seizures and Two 35.1 Seizures

Parameter 6 required the determination of the ratio of diphenhydramine relative to Methaqualone per tablet in each test case, whereas parameters 7 and 8 required the determination of the average methaqualone and diphenhydramine concentrations respectively per test case. The average methaqualone concentration relative to that of diphenhydramine per test case was required in parameter nine. Table 3.22 below shows a summary of the average methaqualone and diphenhydramine concentrations obtained in each of the test cases. The ratio of methaqualone concentration to diphenhydramine concentration is also shown.

Table 3.22: The average concentrations of methaqualone and diphenhydramine and the average abundance of methaqualone relative to that of diphenhydramine detected in each of the four test cases.

Code	**35.11**		**3.41**	
Compound	**Average [ ] in Test Case 1 (in µg/g)**	**Average [ ] in Test Case 4 (in µg/g)**	**Average [ ] in Test Case 2 (in µg/g)**	**Average [ ] in Test Case 3 (in µg/g)**
Mtq	4 104	152 062	30 315	53 044
Dph	472	n/d	197	481
Compound	**Average Abundance (Mtq : Dph) Test Case 1**	**Average Abundance (Mtq : Dph) Test Case 4**	**Average Abundance (Mtq : Dph) Test Case 2**	**Average Abundance (Mtq : Dph) Test Case 3**
Mtq:Dph	9 : 1	n/a	154 : 1	110 : 1

Figures 3.75 to 3.82 show the range of concentrations of methaqualone and diphenhydramine obtained in the tablets of the four test cases.

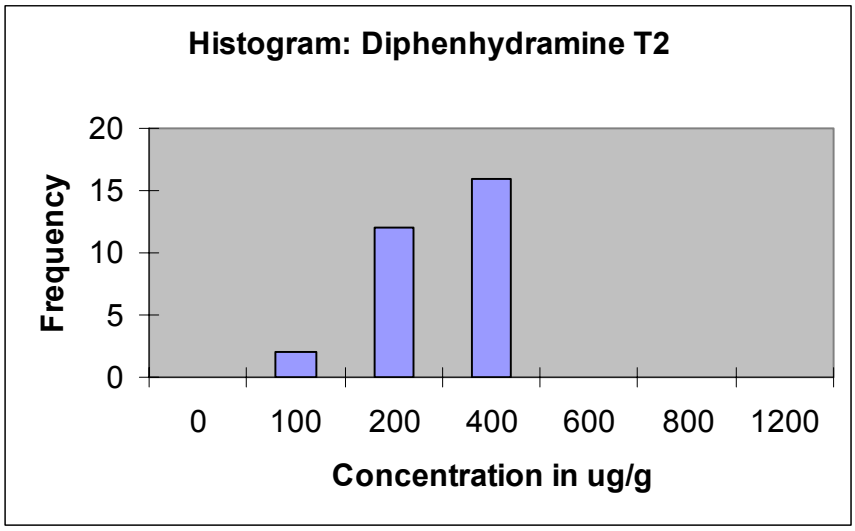

Figure 3.75: The distribution of diphenhydramine concentration obtained in 30 tablets from Test Case 2.

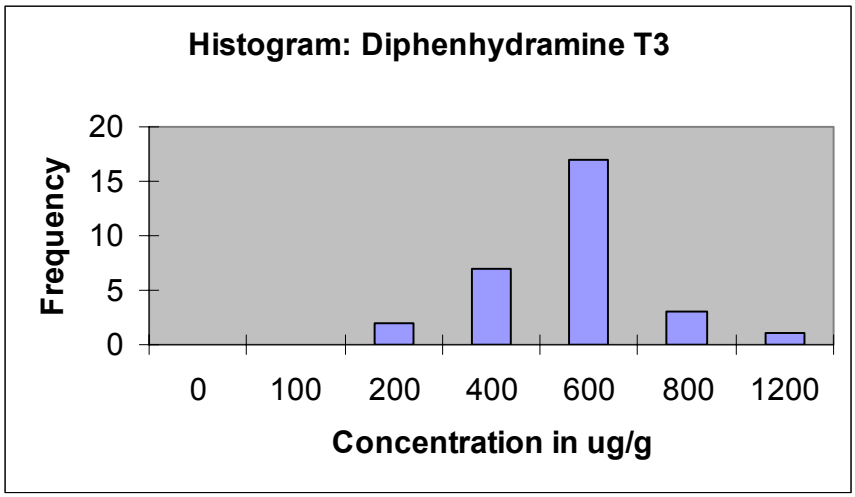

Figure 3.76: **The distribution of diphenhydramine concentration obtained in 30 tablets from Test Case 3.**

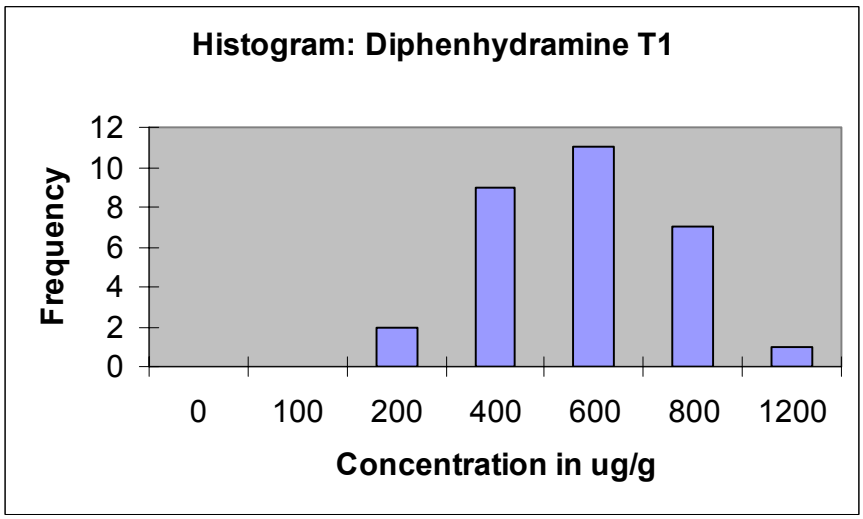

Figure 3.77: **The distribution of diphenhydramine concentration obtained in 30 tablets from Test Case 1.**

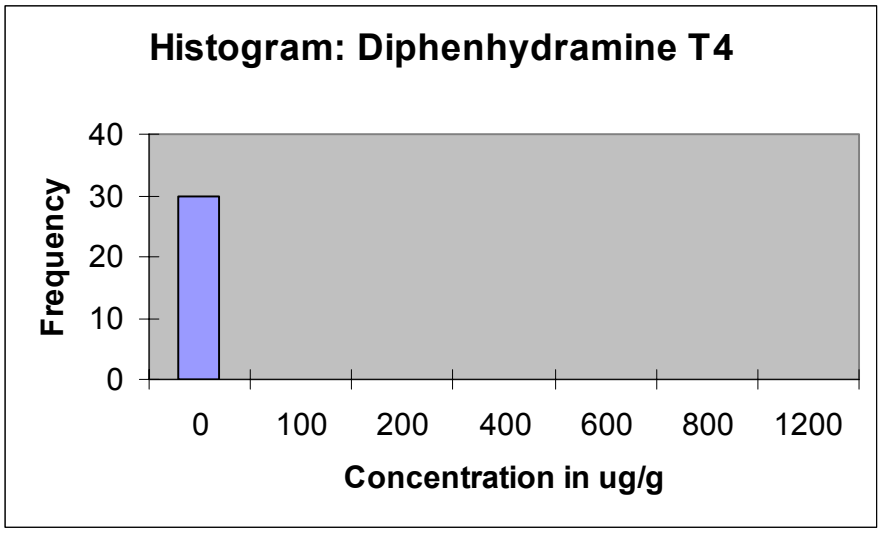

Figure 3.78: **The distribution of diphenhydramine concentration obtained in 30 tablets from Test Case 4.**

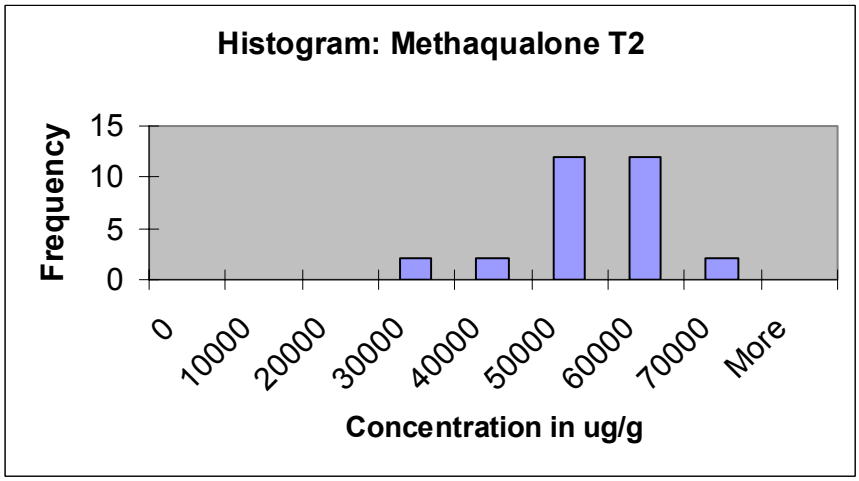

Figure 3.79: **The distribution of methaqualone concentration obtained in 30 tablets from Test Case 2.**

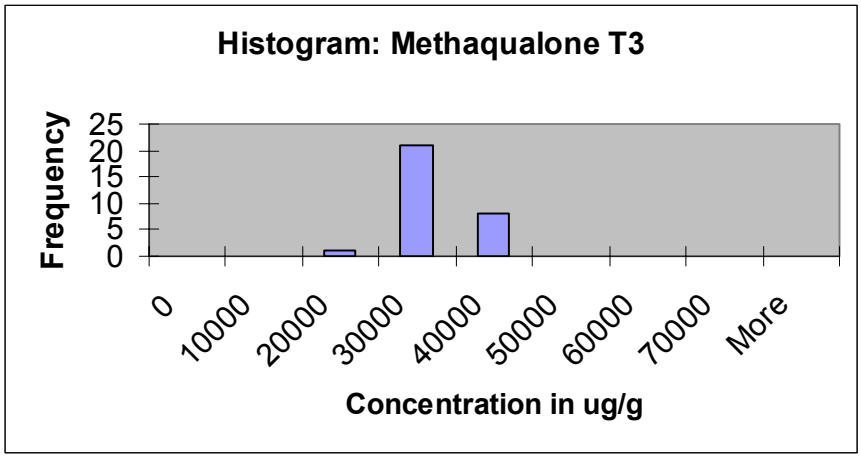

Figure 3.80: **The distribution of methaqualone concentration obtained in 30 tablets from Test Case 3.**

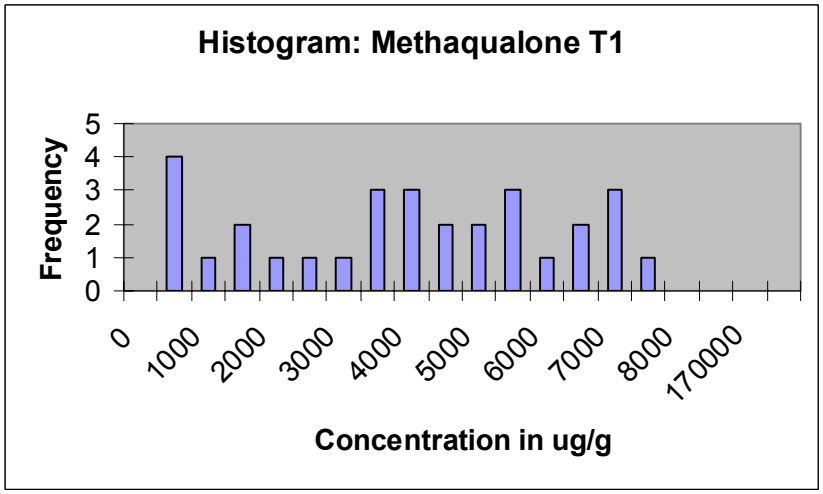

Figure 3.81: **The distribution of methaqualone concentration obtained in 30 tablets from Test Case 1.**

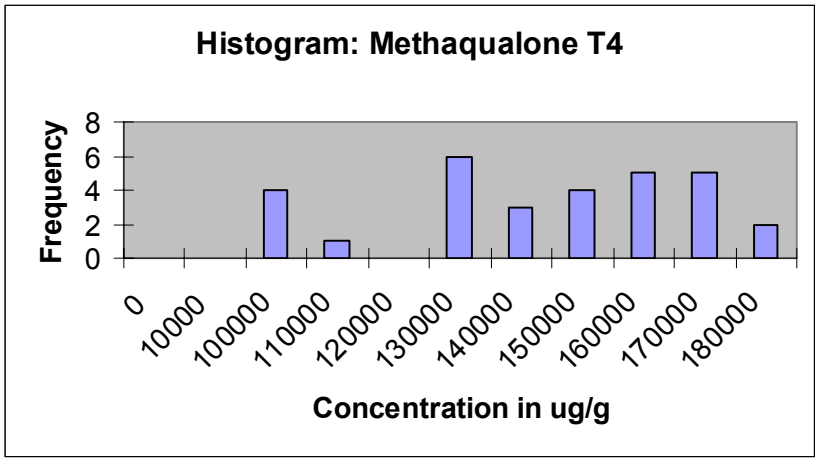

Figure 3.82: The distribution of methaqualone concentration obtained in 30 tablets from Test Case 4.

3.2.4 Quantitation of Methaqualone and Diphenhydramine Present in 4 Test Cases: Discussion

From the photos of the tablets it can be seen that the tablets from test cases 1 and 4 are similar, and those from test cases 2 and 3 are similar. This does not however imply that the two sets of "similar" tablets are in fact originating from the same illicit manufacturer or manufacturing batch. It is known from seized cases analysed at the SAPS FSL that different manufacturers use the same Logo that may be fashionable at the time.

Methaqualone and Diphenhydramine have been detected and quantified in three of the four test cases, while the fourth test case only contained methaqualone. Table 3.22 above summarizes the average concentrations of the methaqualone and diphenhydramine detected in the thirty tablets analysed in each of the four test cases. It also shows the average abundance of methaqualone relative to that of diphenhydramine in each case, excluding of course test case four (Table 3.21).

The quantitative results obtained, are reviewed below in an attempt to link the respective batches with similar logos.

3.2.4.1 Test Cases 2 and 3, Code "3.41"

Figure 3.83: The Logo associated with Tablet Code "3.41" from Test Cases 2 and 3

The average mass of the tablets in both cases was 0.5 gram, and the Logos were the same. Looking at the average methaqualone concentration in test cases 2 and 3 as shown in Figure 3.73, it is clear that the average methaqualone concentration in test case 3, was nearly double that of the average methaqualone concentration in test case 2. For diphenhydramine, as shown in Figure 3.74, the average concentration in test case 3 was more than double the average diphenhydramine concentration in test case 2. The average abundance of methaqualone relative to diphenhydramine in test case 2 was 154 : 1, and in test case 3 it was 110 : 1 (Table 3.22).

It was also noted that it was virtually impossible to compare even two tablets from the same case. In both test cases 2 and 3, a fluctuation in concentration of methaqualone and diphenhydramine could be seen. In test case 3, the highest and lowest concentration of methaqualone detected were 68 655 µg/Tablet and 25 066 µg/Tablet in Tablets 27 and 10 respectively. The highest and lowest concentrations of diphenhydramine detected were 1 108 µg/ Tablet and 153 µg/ Tablet in Tablets 1 and 6 respectively. The highest methaqualone value and the highest diphenhydramine value were not detected in the same tablet. The same applies to the lowest concentrations of these compounds (Table 3.19 above).

In test case 2, the highest and lowest concentration of methaqualone detected were 36 785 µg/Tablet and 19 791 µg/Tablet in Tablets 17 and 4 respectively. The highest and lowest concentrations of diphenhydramine detected were 310 µg/Tablet and 88 µg/Tablet in Tablets 9 and 4 respectively. Again, the highest methaqualone value and the highest diphenhydramine value were not detected in the same tablet. The same does not apply to the lowest concentrations of these compounds, as they both occurred in Tablet 4 (Table 3.17 above).

A common deduction that can be made when looking at the variation in concentration from tablet to tablet in both test cases is that homogenization was a problem during the manufacturing process. Looking at the variation in the average abundance of methaqualone to diphenhydramine in both cases, the same deduction can be made. In any reputable pharmaceutical laboratory, homogenization is one of the most critical steps in the manufacturing process. It is also clear that comparing the methaqualone

concentration in a single tablet from one seizure to that of a single tablet or a batch from another seizure will not be enough proof that they are from a common origin.

Considering all of the above, it can be concluded that, even though the Logos are the same, the analytical results obtained are not sufficient to conclude that these two test cases come from the same illicit manufacturer. The distribution of diphenhydramine concentrations obtained, (Figure 3.75 and 3.76), suggest that these two batches do not originate from the same manufacturing batch. Strangely the distribution of diphenhydramine concentrations obtained in test case 2, is very similar to that in test case one, which has a different Logo (Figure 3.77). That may suggest the same supplier of matrix, already containing diphenhydramine for two manufacturers, or just coincidence. The distribution of methaqualone concentrations in test cases 2 and 3 are not comparable (Figure 3.79 and 3.80). It would be safe to assume that test cases 2 and 3 did not originate from the same manufacturing batch.

3.2.4.2 Test Cases 1 and 4, Code "35.11"

Figure 3.84: The Logo associated with Tablet Code "35.11" from Test Cases 1 and 4.

The average mass of the tablets in both cases was 1 gram, and the Logos were the same. The average methaqualone concentration in test case 4 is approximately 37 times more than the average methaqualone concentration in test case 1 (Figure 3.73 and Table 3.22). For diphenhydramine, as shown in Figure 3.74, the average concentration in test case 1 cannot be compared to that in test case 4, seeing that diphenhydramine could not be detected in test case 4. This fact alone suggests that the two cases, even though they have identical Logos, probably do not originate from the same manufacturing batch. This suggestion is strengthened by the big difference in average methaqualone concentration in the two cases.

For test case 1 it has also been noted that it is virtually impossible to compare even two tablets from the same case. For example, in test case 1, the highest and lowest concentrations of methaqualone detected were 8 146 µg/Tablet and 323 µg/Tablet in Tablets 5 and 9 respectively. The highest and lowest concentrations of diphenhydramine detected were 1163 µg/Tablet and 106 µg/Tablet in Tablets 1 and 20 respectively. The highest methaqualone value and the highest diphenhydramine value were not detected in the same tablet. The same applies to the lowest concentrations of these compounds (Table 3.15 above).

In test case 4, the highest and lowest concentration of Methaqualone detected, were 194 432 µg/Tablet and 92 955 µg/Tablet in Tablets 12 and 26 respectively (Table 3.21 above).

Considering all of the above, it can be concluded that it is highly likely that tablets from these two test cases do not come from the same illicit manufacturing batch. This conclusion is supported by the distribution of methaqualone concentrations obtained (Figure 3.81 and 3.82).

3.2.4.3 Overview

Sher [5] screened nine different illicitly manufactured Mandrax formulations for the presence of methaqualone and other psychoactive compounds, using high performance liquid chromatography. Like in the four test cases analysed in this project, Sher also found significant variation in methaqualone concentration between tablets of the same series. Methaqualone was detected in all the tablets, and the concentration varied between 181 milligram (181 000 microgram) and 413 milligram (413 000 microgram) for the different formulations analysed.

The above is mirrored in the variation in average methaqualone concentration determined in the four test cases respectively. Another disturbing fact which came to light, was the tablet to tablet variation of methaqualone concentration in all four the test cases. The implications thereof are that drug abusers might unknowingly overdose on methaqualone if they purchase a tablet with the same Logo from two different manufacturers, and even from the same batch from the same manufacturer. This variation can most likely be contributed to hurried and ineffective mixing during the illicit manufacture of methaqualone containing tablets. Ineffective mixing leads to an uneven distribution of the active compounds in individual tablets before the tablets are pressed. This is emphasized by the variation of the average abundance of methaqualone and diphenhydramine detected in each of the 30 tablets analysed in test cases 2 and 3 (Table 3.22). Undoubtedly, the concentrations of the main active compounds and their relative abundance to each other in illicitly manufactured Methaqualone containing tablets alone, do not present enough evidence to safely conclude that the origin of two tablets or two batches of tablets with the same Logo, are the same.

A classification system for distinguishing counterfeit Quaalude tablets from the legal tablets has been developed by J Price of the Charlotte Mecklenburg Crime Laboratory in North Carolina, in order to aid field agents. They investigated eighteen different counterfeit LEMMON 714, and four different RORER 714 tablets and presses used in their manufacture. This enabled them to trace the counterfeit tablets back to a specific tablet press. Another fact discovered, was that the drug content of tablets from an individual press seldom changed.[59] The author did not, however, report the concentrations of the compounds which were detected.

This same phenomenon can be seen in test cases 1 and 4. Both these cases had the same Logo, but no Diphenhydramine was present in test case 4. There is, however, no proof that the same tablet press was not used, and it can only be deducted that the tablets did not originate from the same manufacturing batch.

Looking at the various concentrations obtained in more depth shed more light on the possible origin of these test cases. Histograms (Figure 3.75 to 3.82) were drawn up of the frequency of occurrence of certain concentrations of methaqualone and diphenhydramine in all the cases. These test cases had enough dissimilarities, as supported by the histograms, to assume that all these cases had a different origin or manufacturing batch.

But what if the cases were more similar in nature? If two batches of methaqualone containing tablets had to be compared to possibly link them to the same manufacturer, and both contained diphenhydramine and methaqualone at concentrations within the same range, the situation would become more complicated. Only looking at the concentrations of the active constituents and the distribution thereof would not be sufficient to conclude that these batches are from a common origin. It would then be necessary to investigate the presence of possible precursors and/or reaction by-products or other contaminants.

In Chapter four the applicability of the validated extraction and GC/MS method to the determination of the presence of possible precursors and/or reaction by-products or other contaminants in the same four test cases will be discussed.

CHAPTER 4 – QUALITATIVE GC-MS ANALYSIS OF TRACE IMPURITIES FOR FINGERPRINTING METHAQUALONE-CONTAINING TABLETS

4.1 Establishing the presence of possible precursors and by-products present in 4 test cases: Results

The validated extraction method did not give satisfactory qualitative results on the presence of possible precursors and/or reaction by-products. During method development the precursors, by-products and possible interferences were spiked at a concentration of 50 µg/g to achieve the maximum interference. All the precursors and by-products could be detected and successfully separated from methaqualone and diphenhydramine. In the test cases however, the precursors, by-products and possible interferences were present at much lower levels. To be able to detect these compounds, the extracts would have had to be concentrated, leading to methaqualone peak broadening and potential problems with quantitation of the active compound. It was thus decided to extract five more tablets related to each of the four cases as depicted above, using diethyl ether as extraction solvent.

Samples were extracted using 5 milliliter of diethyl ether. 1-Milliliter portions of the extracts were capped in GC/MS vials, and injected on the GC/MS. Due to the small quantity of these compounds present, the Selected Ion Monitoring (SIM) facility of the GC/MS was used instead of the scanning mode of the validated quantification method. The ions that were monitored are indicated in Table 4.1, the rest of the GC/MS conditions would remain the same as detailed in Table 3.6 in Chapter 3.

TABLE 4.1: Ions Monitored using Selected Ion Monitoring

Compound	Ions	Rt in min	SIM Method
N-Acetyl anthranilic acid	119, 137, 43, 92, 65	10.45	1
2-Aminobenzoic acid (Anthranilic acid)	119, 137	06.82	1
Isatoic anhydride	119, 136, 92	10.04	1
Amide of Isatoic anhydride	120, 92, 65, 226	15.37	1
Acetanthranil	146, 161, 117	06.49	2
o-Methyl acetanilide	107, 106, 43, 149	7.38	2
o-Toluidine	106, 107, 77	5.73	2

Examples of the total selected ion chromatograms of one of the five tablets extracted with ether, and analysed on the two different SIM methods, as well as the mass spectra of the compounds identified in each of the test cases are shown in the Figures below.

4.1.1 Test Case 1

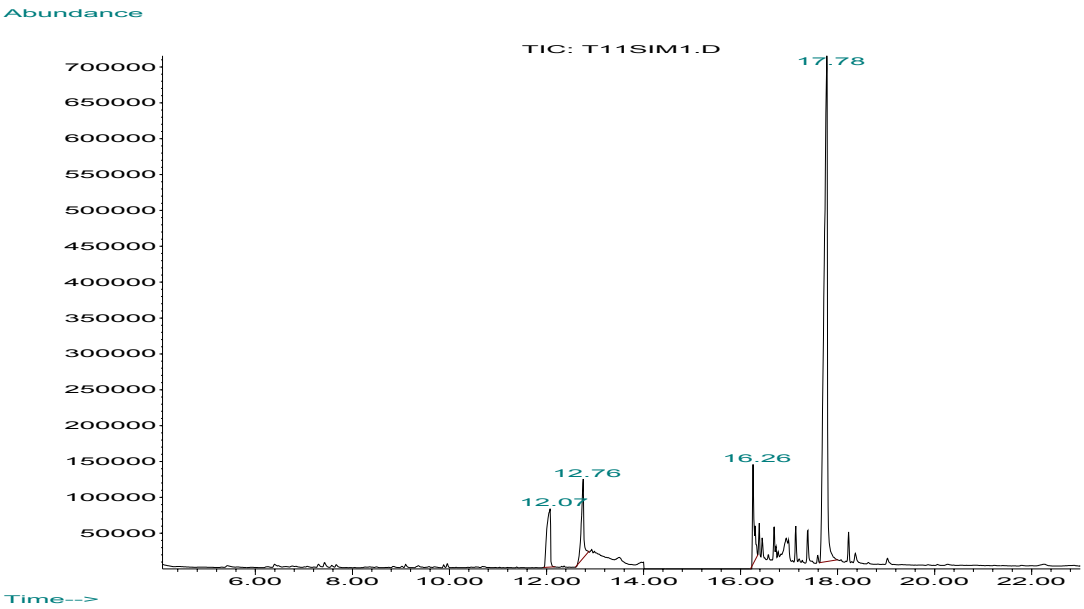

Figure 4.1: **The Total Selected Ion Chromatogram of the Diethyl Ether Extract of one of the five Tablets extracted in Test Case 1, run on the SIM 1 method.**

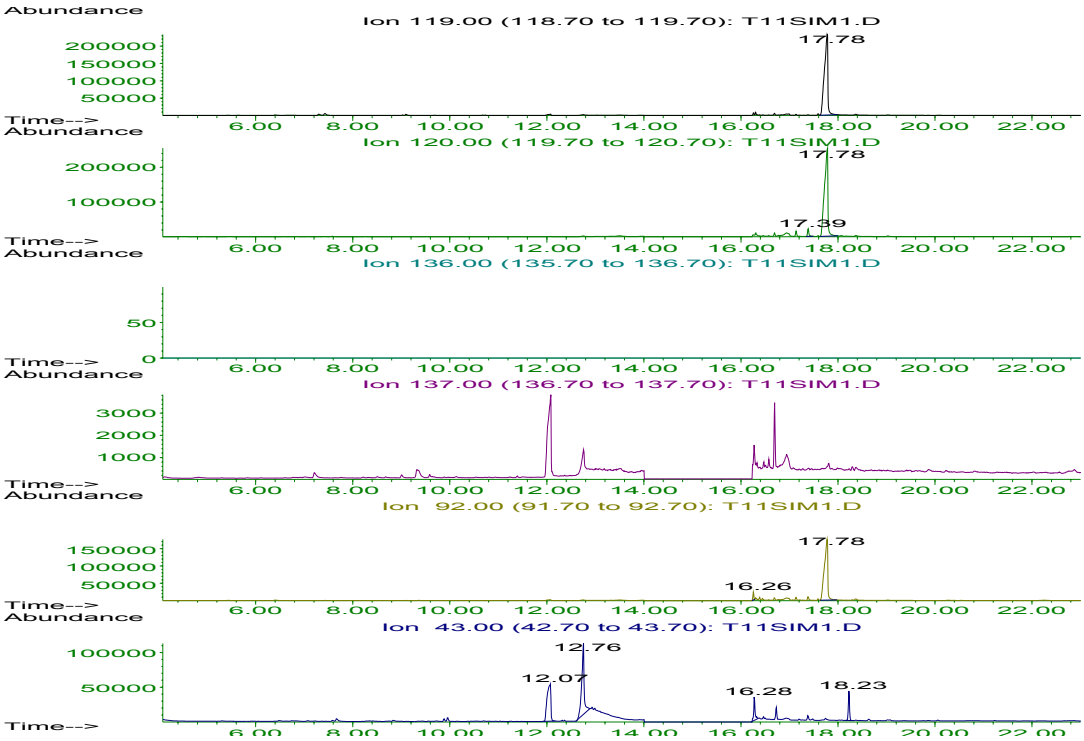

Figure 4.2: **The Extracted Ion Chromatogram of the Diethyl Ether Extract of one of the five Tablets extracted in Test Case 1, run on the SIM 1 method. The following masses were extracted: 119, 120, 136, 137, 92 and 43.**

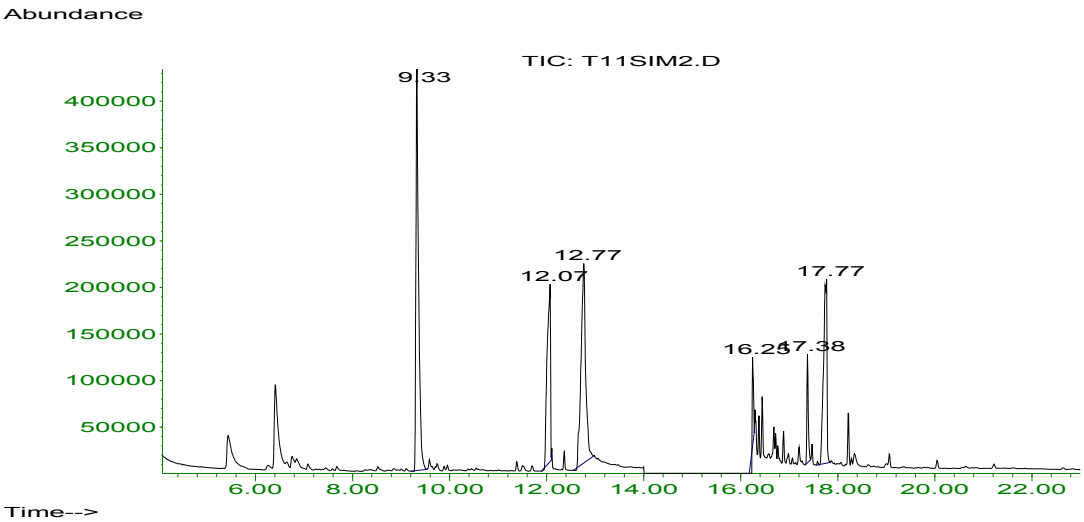

Figure 4.3: **The Total Selected Ion Chromatogram of the Diethyl Ether Extract of one of the five Tablets extracted in Test Case 1, run on the SIM 2 method.**

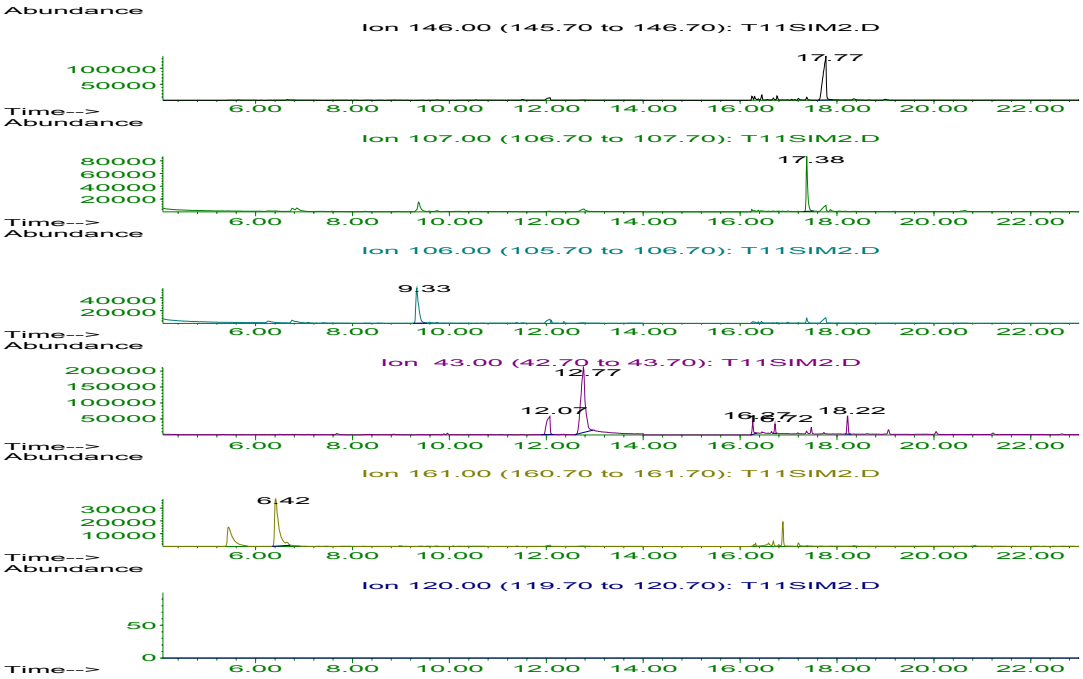

Figure 4.4: **The Extracted Ion Chromatogram of the Diethyl Ether Extract of one of the five Tablets extracted in Test Case 1, run on the SIM 2 method. The following masses were extracted: 146, 107, 106, 161, 43 and 120.**

For the chromatograms and mass spectra of the individual compounds present, as obtained from the analysis of the five tablets for Test Case 1, see Appendix D.

4.1.2 Test Case 2

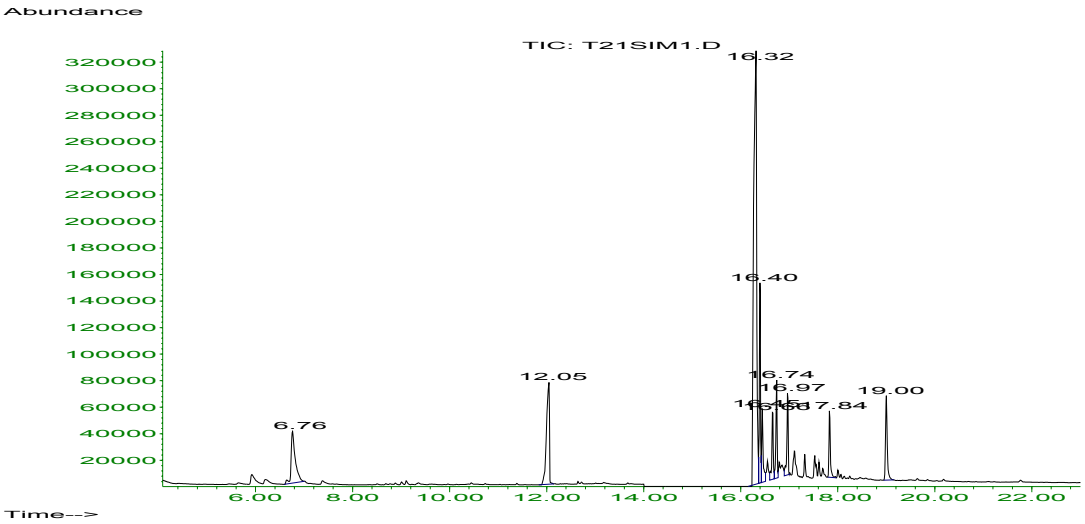

Figure 4.5: **The Total Selected Ion Chromatogram of the Diethyl Ether Extract of one of the five Tablets extracted in Test Case 2, run on the SIM 1 method.**

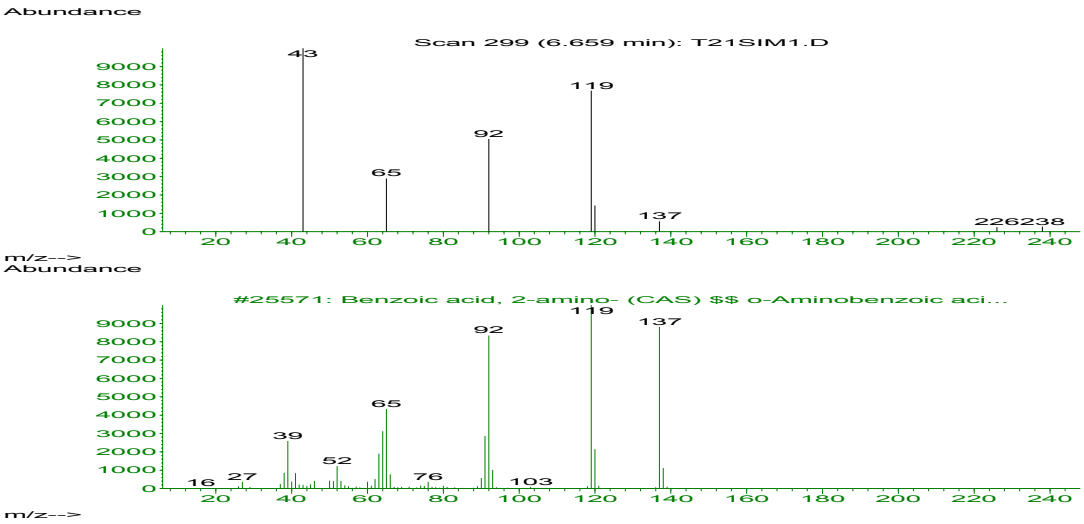

Figure 4.6: **The relative abundance of selected ions at 6.659 minutes of anthranilic acid in the Diethyl Ether Extract of one of the five Tablets extracted in Test Case 2, run on the SIM 1 method. The spectrum at the top is the spectrum of the unknown, and the spectrum at the bottom is the standard spectrum from the electronic library.**

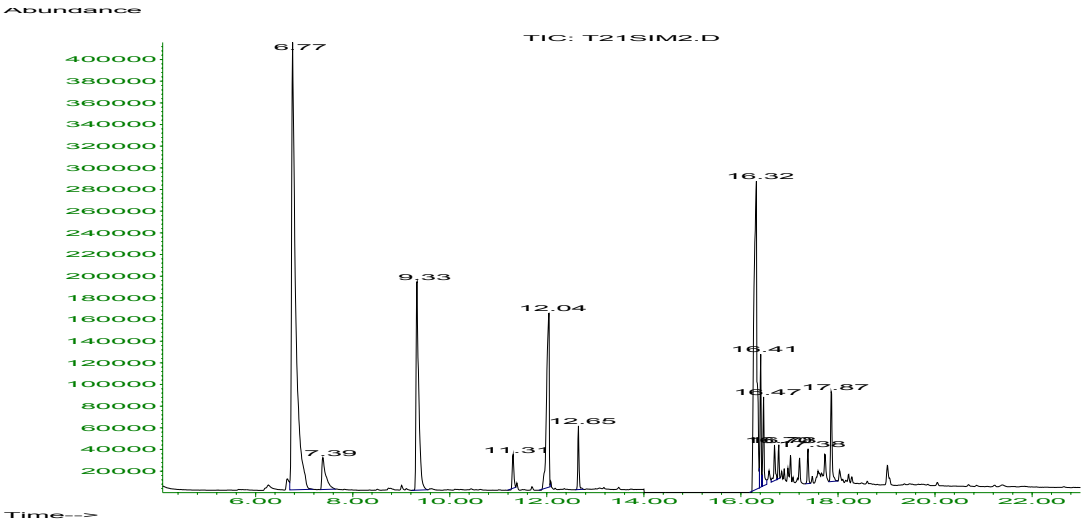

Figure 4.7: **The Total Selected Ion Chromatogram of the Diethyl Ether Extract of one of the five Tablets extracted in Test Case 2, run on the SIM 2 method.**

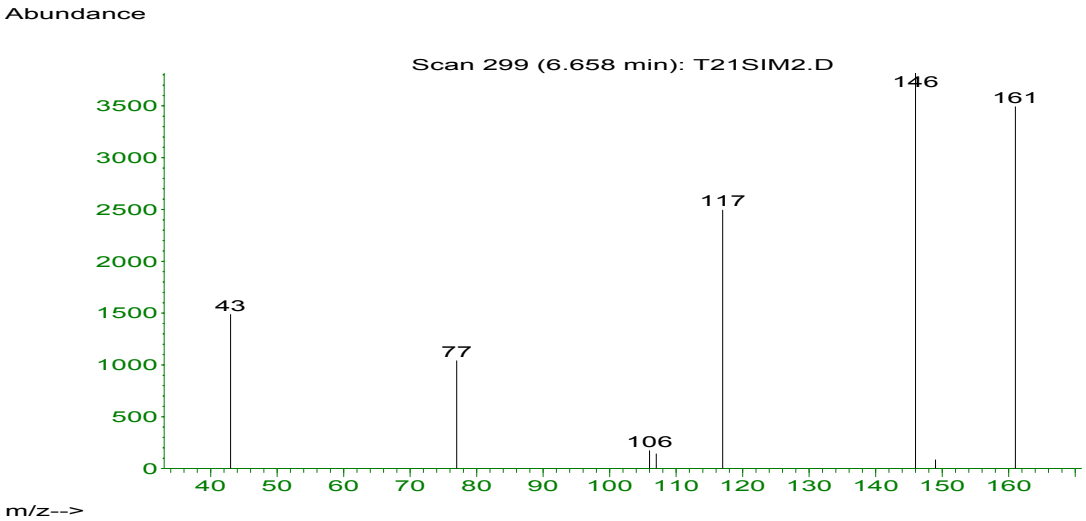

Figure 4.8: **The relative abundance of selected ions at 6.658 minutes of Acetanthranil in the Ether Extract of one of the five Tablets extracted in Test Case 2, run on the SIM 2 method.**

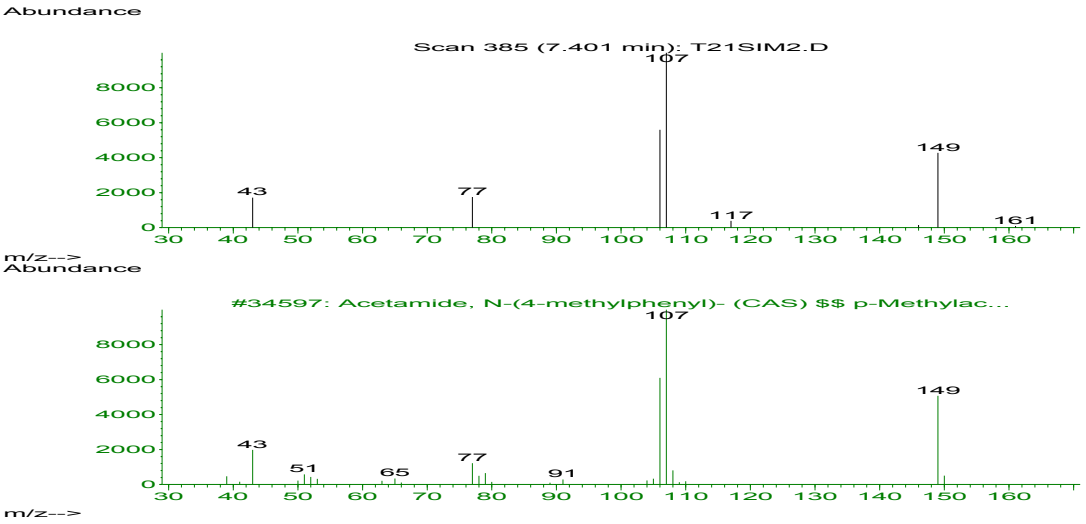

Figure 4.9: **The relative abundance of selected ions at 7.401 minutes of o-Methyl acetanilid in the Diethyl Ether Extract of one of the five Tablets extracted in Test Case 2, run on the SIM 2 method. The spectrum at the top is the spectrum of the unknown, and the spectrum at the bottom is the standard spectrum from the electronic library.**

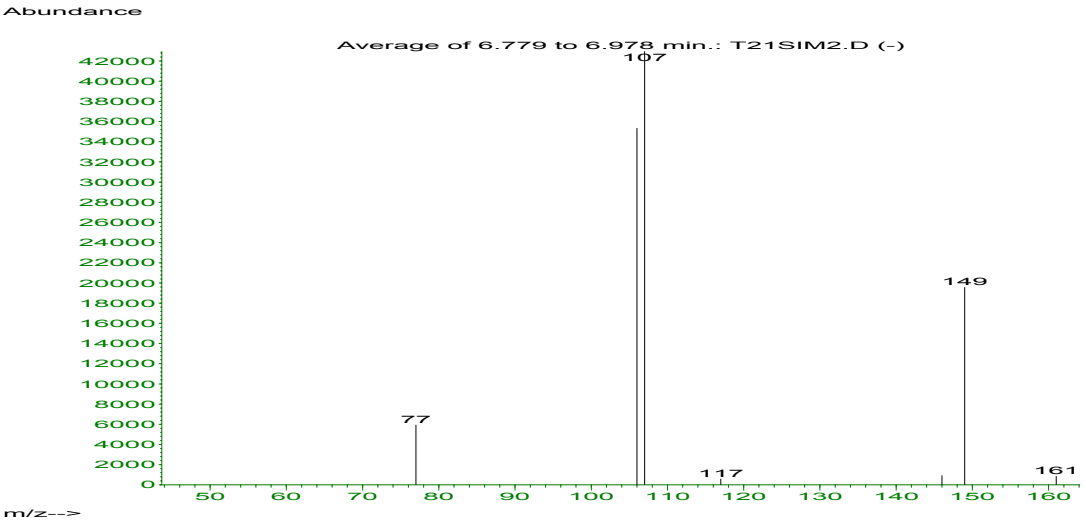

Figure 4.10: **The relative abundance of selected ions at 6.978 minutes of p-Toluidine in the Diethyl Ether Extract of one of the five Tablets extracted in test Case 2, run on the SIM 2 method.**

For the chromatograms and mass spectra of the individual compounds present, as obtained from the analysis of the five tablets for Test Case 2, see Appendix E.

4.1.3 Test Case 3

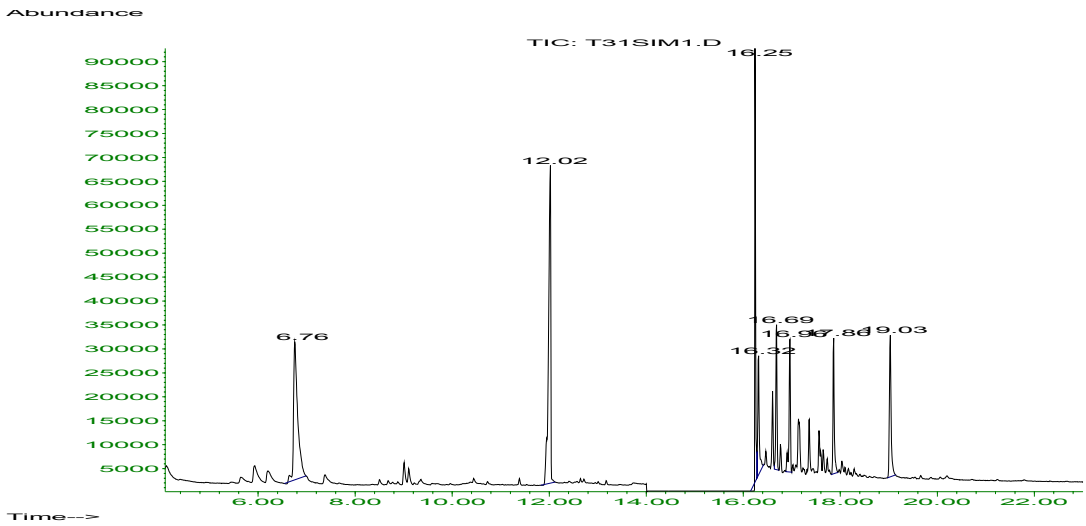

Figure 4.11: **The Total Selected Ion Chromatogram of the Diethyl Ether Extract of one of the five Tablets extracted in Test Case 3, run on the SIM 1 method.**

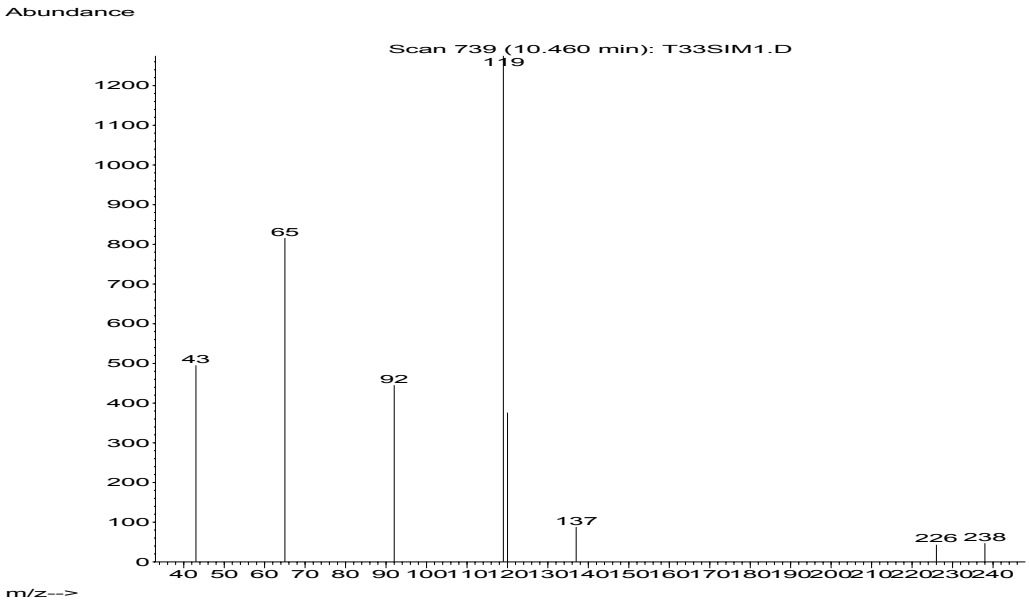

Figure 4.12: **The relative abundance of selected ions at 10.46 minutes of N-Acetyl anthranilic acid in the Diethyl Ether Extract of one of the five Tablets extracted in Test Case 3, run on the SIM 1 method.**

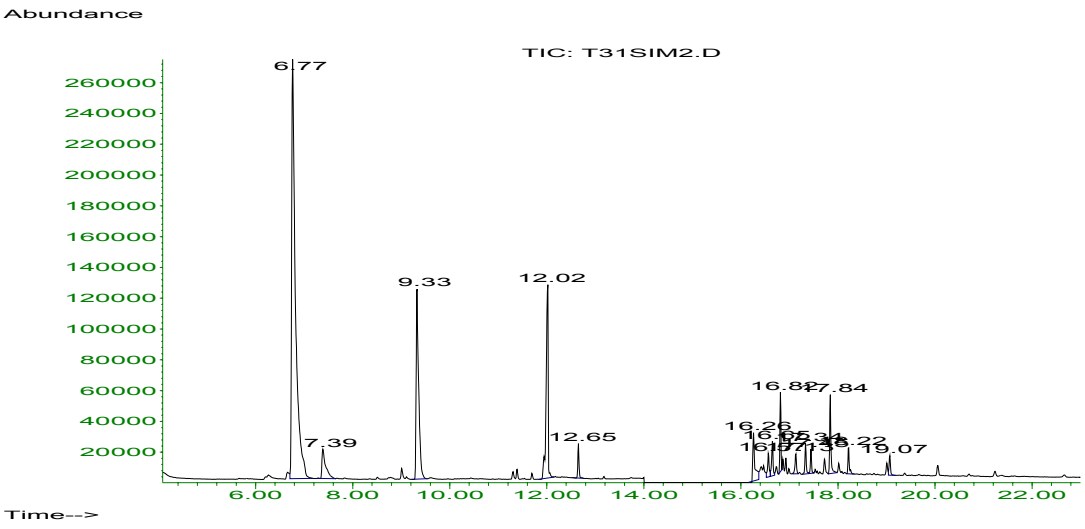

Figure 4.13: **The Total Selected Ion Chromatogram of the Diethyl Ether Extract of one of the five Tablets extracted in Test Case 3, run on the SIM 2 method.**

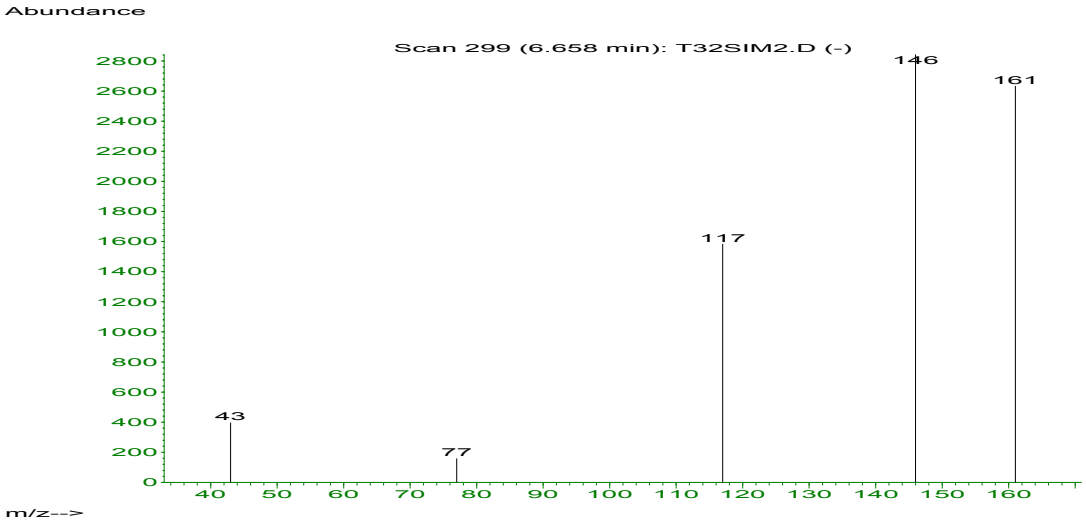

Figure 4.14: **The relative abundance of selected ions at 6.658 minutes of Acetanthranil in the Diethyl Ether Extract of one of the five Tablets extracted in Test Case 3, run on the SIM 2 method.**

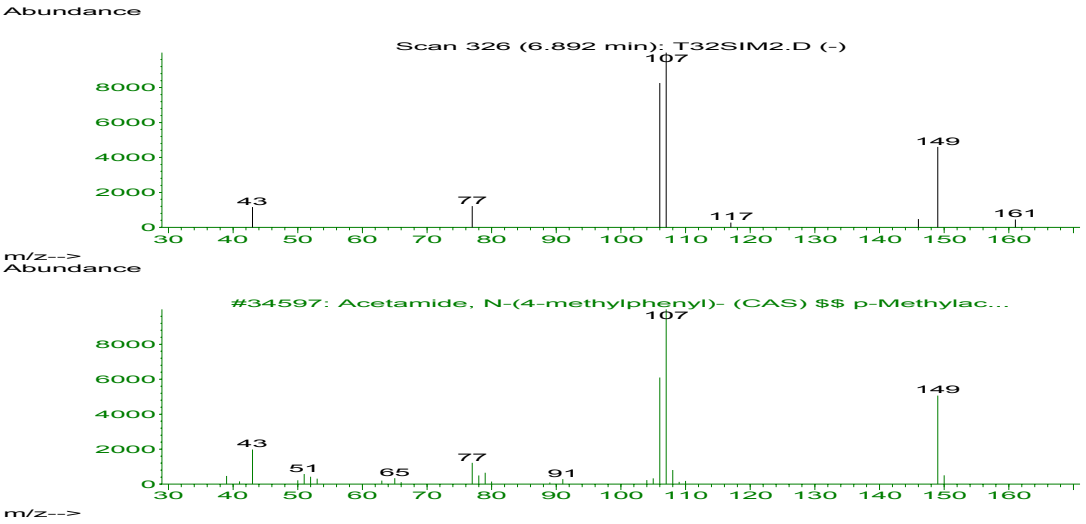

Figure 4.15: **The relative abundance of selected ions at 6.892 minutes of o-Methyl acetanilid in the Diethyl Ether Extract of one of the five Tablets extracted in Test Case 3, run on the SIM 2 method. The spectrum at the top is the spectrum of the unknown, and the spectrum at the bottom is the standard spectrum from the electronic library.**

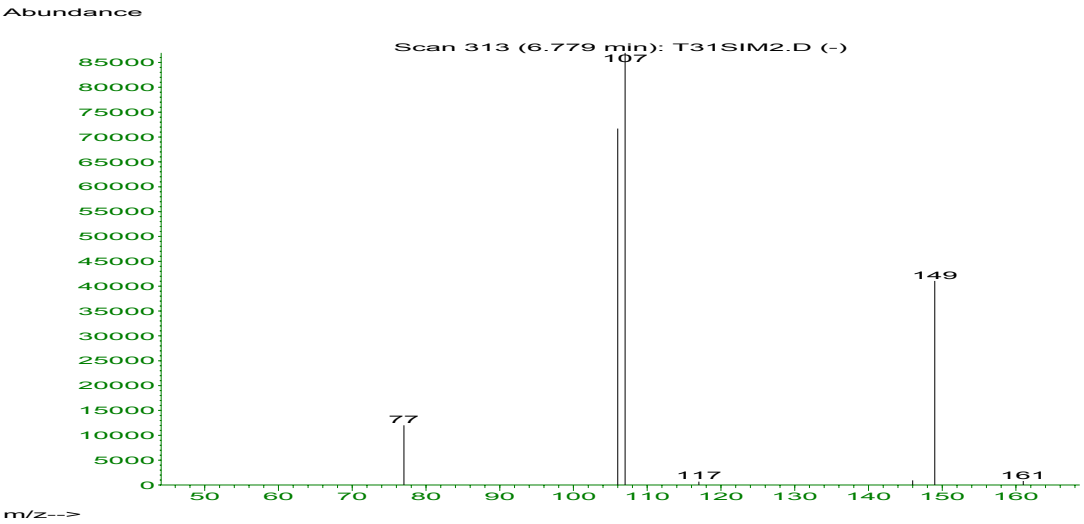

Figure 4.16: **The relative abundance of selected ions at 6.779 minutes of p-Toluidine in the Diethyl Ether Extract of one of the five Tablets extracted in Test Case 3, run on the SIM 2 method.**

For the chromatograms and mass spectra of the individual compounds present, as obtained from the analysis of the five tablets for Test Case 3, see Appendix F.

4.1.4 Test Case 4

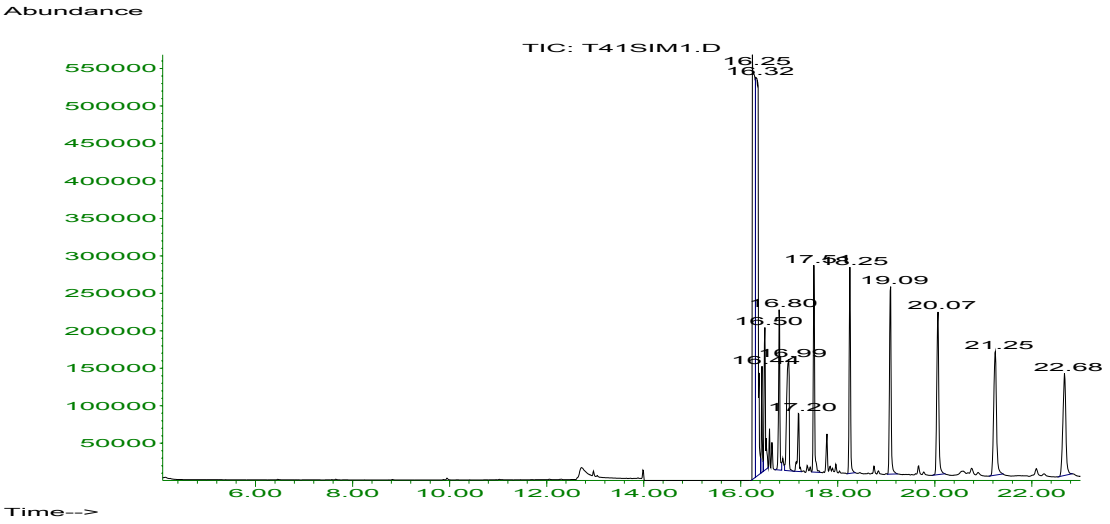

Figure 4.17: **The Total Selected Ion Chromatogram of the Diethyl Ether Extract of one of the five Tablets extracted in Test Case 4, run on the SIM 1 method.**

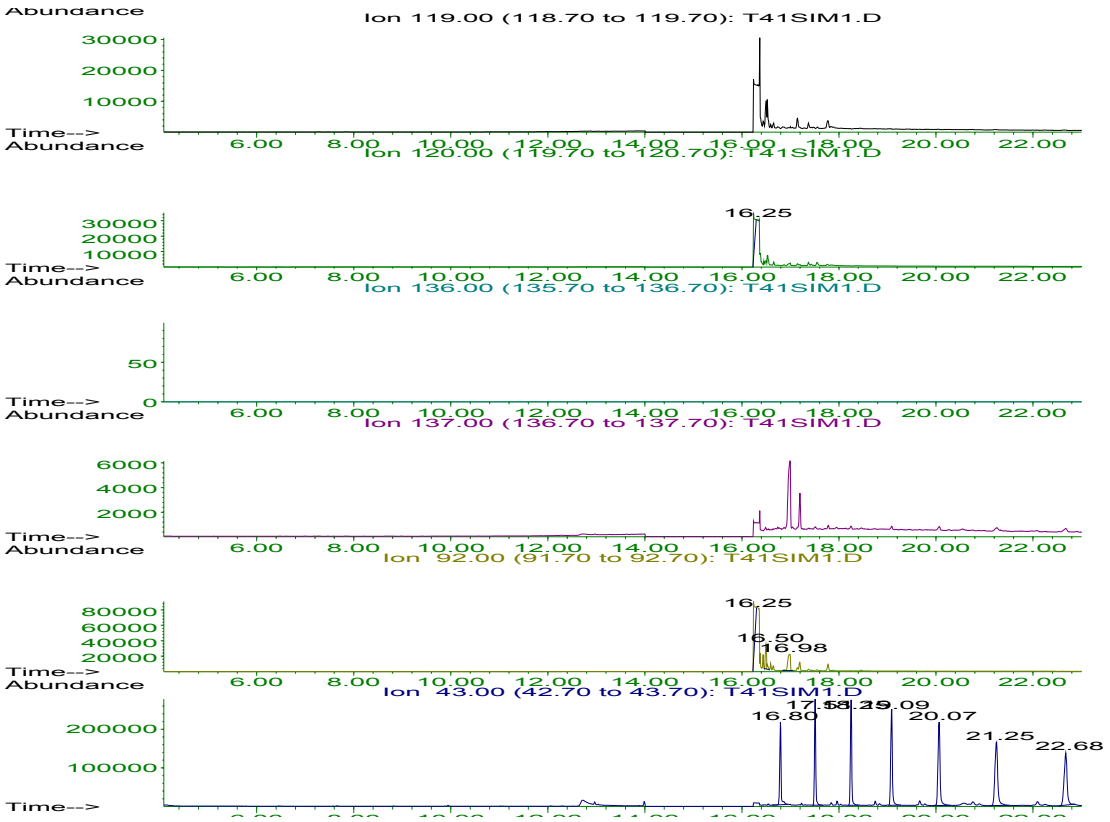

Figure 4.18: **The Extracted Ion Chromatogram of the Diethyl Ether Extract of one of the five Tablets extracted in Test Case 4, run on the SIM 1 method. The following masses were extracted: 119, 120, 136, 137, 92 and 43.**

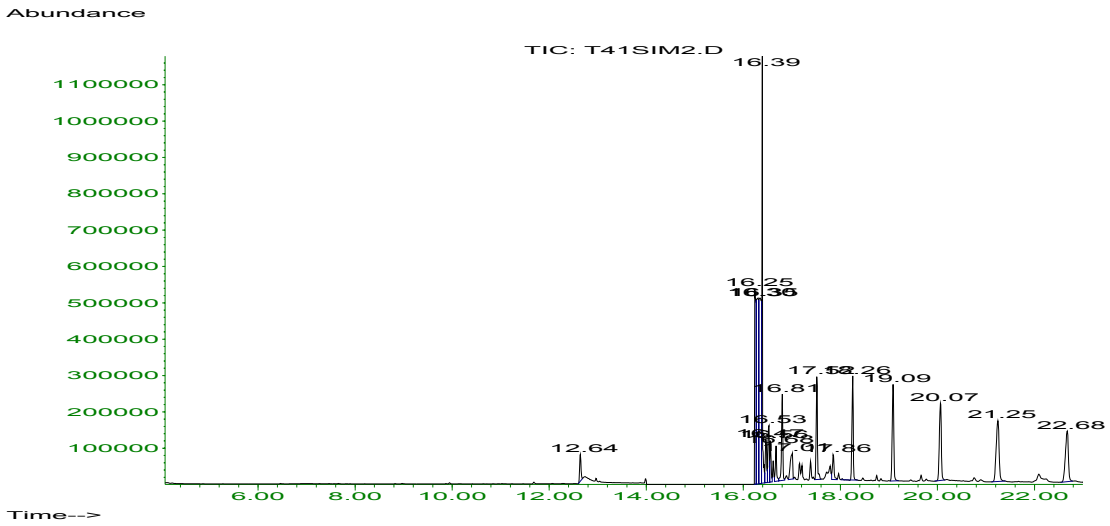

Figure 4.19: **The Total Selected Ion Chromatogram of the Diethyl Ether Extract of one of the five Tablets extracted in Test Case 4, run on the SIM 2 method.**

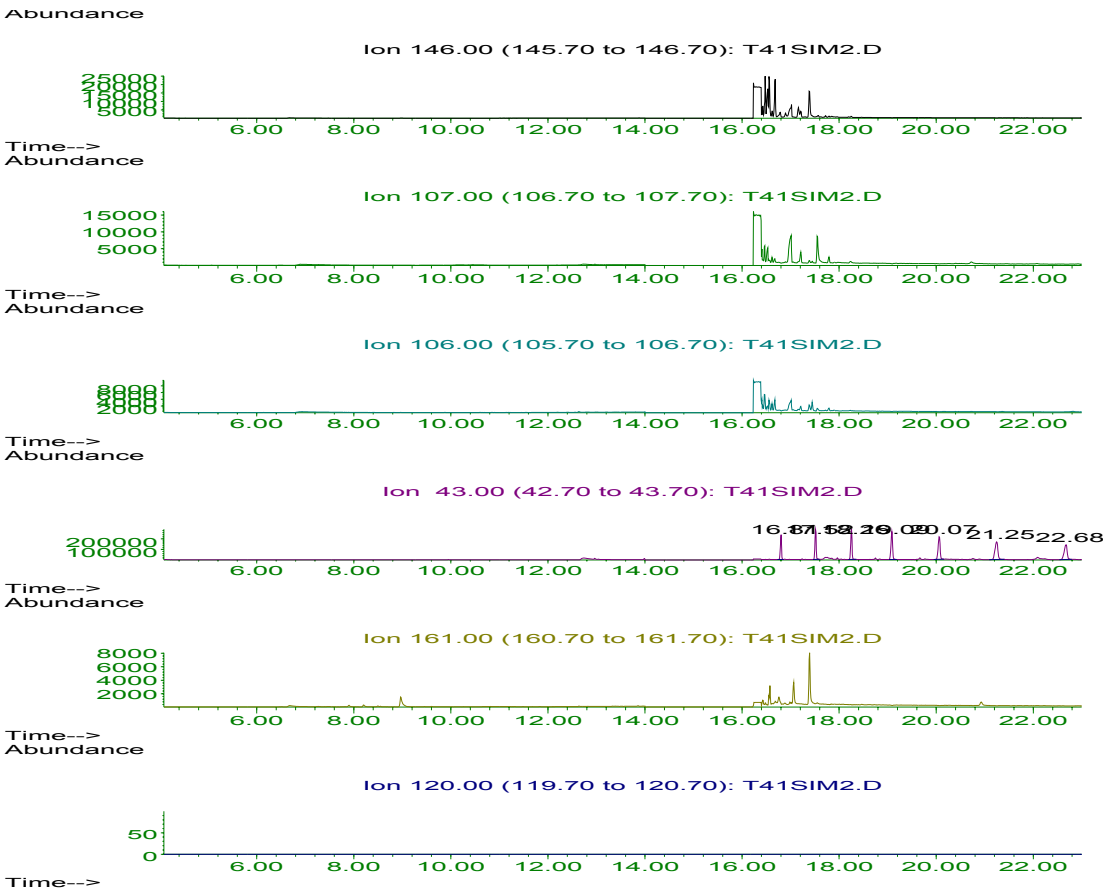

Figure 4.20: **The Extracted Ion Chromatogram of the Diethyl Ether Extract of one of the five Tablets extracted in Test Case 1, run on the SIM 2 method. The following masses were extracted: 146, 107, 106, 161, 43 and 120.**

For the chromatograms and mass spectra of the individual compounds present, as obtained from the analysis of the five tablets for Test Case 4, see Appendix G.

Results yielded by using the ether extract are summarized in Table 4.2 below.

Table 4.2: Active compounds, isomers, precursors, by-products and possible Interferences detected in the four test cases. = Present, = Not Present

Other	Isomers	Precursors	By-products	Test Case 1	Test Case 4	Test Case 2	Test Case 3
	Isomer I (Mtq)						
	Isomer II						
	Isomer III						
	Isomer IV						
	Isomer V						
	Isomer VI						
	Isomer VII						
	Isomer VIII						
	Isomer IX						
	Isomer X						
	Isomer XI						
	Isomer XII						
	Isomer XIII						
		Anthranilic acid					
		N-acetyl anthranilic acid					
		0-Toluidine					
		p-Toluidine					
		Isatoic anhydride					
			Acetanthranil				
			o-Methyl acetanilid				
			Amide of Isatoic anhydride				
Diphenhydramine							
Methyl cinnamate							

4.2 Establishing the presence of possible precursors and by-products present in 4 test cases: Discussion

The synthesis of methaqualone is widely documented. (Chapter 2, paragraph 2.1.3) Methaqualone can be synthesized using different synthesis routes. These different routes make use of different precursors, and would thus lead to different contaminants, precursors and reaction by-products being present in the final product. Identifying these contaminants, precursors and by-products, albeit in trace amounts, might assist in pinpointing the analysis route followed in the illicit synthesis of methaqualone containing products. This information could then be used to trace the origin of the final product, by comparing precursors and chemicals confiscated during, for example, a raid on an illicit laboratory, with that identified in a specific product.

The value of identifying the above-mentioned compounds, was emphasized by Angelos and Meyers.[2] They also described a rapid method for the isolation and identification of the precursors and reaction by-products of methaqualone and mecloqualone. Chromatographic separations of the reactants and by-products dissolved in methanol were achieved by both HPLC and GLC. Identification of the compound was done by using infrared spectroscopy and quadropole mass spectrometry techniques. This study tested components such as anthranilic acid, N-acetylanthranilic acid, *o*-toluidine and *o*-methyl acetanilide, which gave an indication of which types of components to include in this study.

The precursors and reaction by-products that have been identified in the four selected test cases analysed by a quadropole mass spectrometric technique, are illustrated in Table 4.2, above.

As mentioned in Chapter 3, two cases containing the same identification code in the Logo Index,[1] and thus identical logos, as well as two cases with a different code, and thus a different logo, were chosen for comprehensive analysis. The reasoning behind choosing two test cases having different logos, was to illustrate that tablets with different logos would in fact give different analytical results.

From the ether extracts not much information regarding the known precursors and by-products was obtained pertaining to the five tablets each from test cases 1 and 4. Their total selected ion chromatograms differed significantly for both the SIM 1 and 2 methods. The unknown components were not identified though. Both extracts are not likely to come from the same manufacturer, seeing that test case 1 contained methyl cinnamate and diphenhydramine, and test case 4 did not. From the chloroform extracts performed for the purpose of quantifying methaqualone, it was evident that test case 1 contained both methaqualone and diphenhydramine, where-as test case 4 contained only methaqualone. The deduction that these two test cases definitely did not come from the same batch of illicit tablets can be made with confidence. The same synthesis method was definitely not used in the manufacturing of these preparations, seeing that when comparing the total selected ion chromatograms for both the SIM 1 and 2 methods, the trace components present seemed to differ.

As mentioned earlier, the intermediates and by-products identified in a clandestine preparation are useful in identifying the synthesis route. Both test cases 2 and 3 contained methaqualone, diphenhydramine, 2-methyl-3-*m*-tolyl-4 (3H)-quinazolinone (methaqualone isomer II), *p*-toluidine, N-acetylanthranilic acid and *o*-methyl acetanilid. These compounds indicate that at least the same synthesis method was used for these two batches. In Chapter 3 it was preliminarily concluded that there was a likelihood that these two test cases did not originate from the same batch. Looking at the evidence collected during the further analysis of these cases, the only deduction that can be made is that the synthesis routes used were most likely the same.

CHAPTER 5 – CONCLUSION

The first aim of this project was the validation of the extraction and quantitative determination method for methaqualone in illicit preparations by using Gas Chromatography Mass Spectrometry (GC/MS). This method was then applied to the analysis of four batches of illicit preparations. Methaqualone and diphenhydramine were quantified and the results used to investigate a link between logo and manufacturer. The same method was applied to the qualitative analysis of the four test cases to determine whether the presence of precursors and reaction by-products could be established and used to link manufacturer and logo.

5.1 VALIDATION OF THE ANALYTICAL METHOD FOR QUANTITATIVE ANALYSIS OF METHAQUALONE

Judging by the validation results reported and discussed in Chapter 3, paragraph 3.1, it was clear that the 1st aim of this project was reached. An extraction and quantitative analytical method for methaqualone in illicit preparations using Gas Chromatography Mass Spectrometry (GC/MS) was successfully validated. The short method was able to separate methaqualone from potential interferents, whereas the longer method could, additionally, separate all the methaqualone isomers. The validation results obtained were acceptable for the intended application of the method to the extraction and subsequent quantitative analysis of methaqualone in illicit preparations. The long GC/MS method gave the best results and was thus the method of choice in the quantitation of methaqualone in the four test cases. It gave acceptable linearity (r^2 = 0.9947), % recovery (60 to 90%), precision and accuracy results (% RSD < 10%).

5.2 APPLICATION OF THE VALIDATED ANALYTICAL METHOD TO THE QUANTIFICATION OF METHAQUALONE

The second aim of this project was to apply the validated method to the quantitative analysis of seized illicit preparations received at the SAPS FSL for analysis. The validated method was indeed applied to four true illicit batches consisting of methaqualone-containing tablets as detailed in Chapter 3, paragraph 3.2. The extraction of methaqualone from illicit preparations and quantitation of methaqualone by GC/MS proved to be a success. Valuable data was compiled relating to the average concentration of methaqualone and diphenhydramine present in the different batches as well as to the distribution of methaqualone and diphenhydramine concentrations in each batch.

This quantitative data alone was not sufficient to conclude that specifically test cases 2 and 3 (carrying the same logo) were of the same origin, synthesis route or manufacturer. It could however be suggested that test cases 1 and 4 (carrying the same logo) were not of the same origin, due to the presence of diphenhydramine in test case 1, and its absence in test case 4. Clearly, more than just quantitative results for methaqualone, and if applicable, diphenhydramine, would be needed to link two batches of methaqualone containing tablets with each other.

5.3 APPLICATION OF THE VALIDATED ANALYTICAL METHOD TO THE QUALITATIVE GC-MS ANALYSIS OF TRACE IMPURITIES FOR FINGERPRINTING METHAQUALONE CONTAINING TABLETS

The validated extraction and GC/MS quantitation method for methaqualone in illicit preparations was used to determine the presence of precursors and reaction by-products in illicit preparations. Using this method, no precursors and reaction by-products could however be detected in the four test cases. (During method development the precursors, by-products and possible interferences were spiked at high concentrations of 50 µg/g to achieve maximum interference – levels at which all the precursors and by-products could be detected and successfully separated from methaqualone and diphenhydramine.) In the test cases however, the precursors, by-products and possible interferences were present at much lower levels, rendering the method ineffective for this application.

Extraction of the test cases was thus repeated with diethyl ether and the qualitative analysis on the extracts was done using the Selected Ion Monitoring (SIM) facility of the GC/MS. Isomer II, the precursor N-acetyl anthranilic acid, as well as the by-products acetanthranil and o-methyl acetanilide were detected in both test cases 2 and 3. This strengthened the suspicion that at least the same synthesis route was followed. Extraction of test cases 1 and 4 were also repeated and analysis done using the SIM facility of the GC/MS. It was concluded that both extracts are not likely to come from the same manufacturer, seeing that test case 1 contained methyl cinnamate and diphenhydramine (quantitative study), and test case 4 did not. Also, comparing the selected ion chromatograms for both the SIM 1 and 2 methods, the trace components present seemed to differ, although none of the target impurities were found.

5.4 GENERAL OBSERVATIONS AND RECOMMENDATIONS

Myors et al [44] employed a GC/MS procedure for detecting trace organic constituents to aid in the chemical profiling of heroin samples from a variety of opium production areas (chapter 2). Reproducible and reliable GC/MS data enabled them to carry out parameter selection and reduction to a level where highly efficient predictive statistical models could be developed to discriminate between samples of different origin. If data for organic compounds could be combined with quantitative trace element data and qualitative data for physical properties such as colour and packaging, it could lead to an even more powerful profiling method. They concluded that the profiling procedure was capable of identifying parameters which are useful for discriminating between samples of different origin.

As described earlier [59], the imprints on illicitly produced counterfeit methaqualone-containing tablets were compared to tablet presses confiscated from clandestine laboratories. Another parameter investigated by them was the drug content of the different tablets. They did however not look at

parameters such as origin, port of entry, means of entry, concealment method, wrapping, packaging, drug form, markings or logos, shape, colour, contour, tool marks and mass, to assist them in their conclusions. For their application though, it was sufficient to only look at imprints, tablet presses and drug content.

It is clear that the analysis results as discussed in Chapters 3 and 4, are only part of the information needed to prove that the tablets of different seizures are from the same batch, synthesis route or manufacturer. More work has to be done to design a foolproof chemical fingerprinting program at the SAPS FSL. However, the necessity of detailed chemical analysis to complement the physical appearance of illicit tablets could clearly be illustrated. Two batches of tablets with similar external features were shown to be probably of the same origin, whereas another two batches with the same logos were found to be probably of different origin. Complicating the interpretation of results is the absence of data to estimate how much a manufacturing batch can vary from another of the same origin. Given the tablet to tablet variation within one batch, this could be quite appreciable.

The disturbing variation (highest to lowest dose per tablet) in tablet to tablet dose found even in this limited survey, highlights the extreme danger of drug abuse. This information should be made available to reach for example school children via the police drug awareness programmes.

It is recommended that the validation study be repeated using more realistic spiked concentrations of possible contaminants, methaqualone precursors and by-products. The fixed concentration of 50 ug/mL was clearly too high. A new method should include splitless injection and a single ion monitoring for detection of the trace impurities and full-scan operation and a low gradient during elution of the methaqualone isomers to ensure separation and correct identification of the latter. Such an integrated method should provide all the information of the separate methods used during this study, with analysis times about the same as the “short” quantitation method.

It should also be considered adding information such as concealment method, wrapping, packaging and tool marks to the existing NFDID, in order to render it more effective. It would also be advisable to include the microscopic examination of the imprints on tablets with the same logos in a next project. In order to ensure the success of the chemical “fingerprinting” procedure of the Drug Section of the SAPS FSL, it would be essential to combine the qualitative and quantitative methods of this project, with the parameters mentioned below. Only then can it be concluded that two tablets, or two batches of tablets, are likely to originate from the same manufacturer.

1. Perform a microscopic investigation of imprint/logo.
2. Compare imprint/logo with tablet press if available.
3. Document concealment method if any and add information to the existing NFDID.
4. Document wrapping/packaging if any and add information to the existing NFDID.
5. Document “area confiscated” and compare the information to that in the existing NFDID for the same imprint/logo.
6. Compare logo/imprint, colour and size to that already documented in the existing Logo Index.
7. Analyse illicit preparation for the presence of volatiles by doing headspace GC/MS analysis, and add information to the existing NFDID.
8. Follow validated extraction procedure to quantify methaqualone and possibly diphenhydramine, and add information to the existing NFDID.
9. Do an ether extract on the illicit preparation, and use the Selected Ion Monitoring (SIM) facility of the GC/MS in order to detect the possible presence of contaminants, precursors and reaction by-products. Add information to the existing NFDID.
10. Perform ICP-AES analysis on the illicit preparation for the presence of trace metals, and add information to the existing NFDID.
11. Combine the results from all above tests on a given preparation for comparison with archived information in the expanded NFDID.

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APPENDICES

LIST OF FILENAMES ON CD

APPENDIX A

FILE NAME	DESCRIPTION	PROGRAM
ISOMI	GC/MS Data MTQ Isomer I	CHEMSTATION Version C
ISOMII	GC/MS Data MTQ Isomer II	CHEMSTATION Version C
ISOMIII	GC/MS Data MTQ Isomer III	CHEMSTATION Version C
ISOMIV	GC/MS Data MTQ Isomer IV	CHEMSTATION Version C
ISOMV	GC/MS Data MTQ Isomer V	CHEMSTATION Version C
ISOMVI	GC/MS Data MTQ Isomer VI	CHEMSTATION Version C
ISOMVII	GC/MS Data MTQ Isomer VII	CHEMSTATION Version C
ISOMVIII	GC/MS Data MTQ Isomer VIII	CHEMSTATION Version C
ISOMIX	GC/MS Data MTQ Isomer IX	CHEMSTATION Version C
ISOMX	GC/MS Data MTQ Isomer X	CHEMSTATION Version C
ISOMXI	GC/MS Data MTQ Isomer XI	CHEMSTATION Version C
ISOMXII	GC/MS Data MTQ Isomer XII	CHEMSTATION Version C
ISOMXIII	GC/MS Data MTQ Isomer XIII	CHEMSTATION Version C
ISOI and ISOIL	GC/MS Data MTQ Isomer I	CHEMSTATION Version C
ISOII and ISOIIL	GC/MS Data MTQ Isomer II	CHEMSTATION Version C
ISOIII and ISOIIIL	GC/MS Data MTQ Isomer III	CHEMSTATION Version C
ISOIV and ISOIVL	GC/MS Data MTQ Isomer IV	CHEMSTATION Version C
ISOV and ISOVL	GC/MS Data MTQ Isomer V	CHEMSTATION Version C
ISOVI and ISOVIL	GC/MS Data MTQ Isomer VI	CHEMSTATION Version C
ISOVII and ISOVIIL	GC/MS Data MTQ Isomer VII	CHEMSTATION Version C
ISOVIII and ISOVIIIL	GC/MS Data MTQ Isomer VIII	CHEMSTATION Version C
ISOIX and ISOIXL	GC/MS Data MTQ Isomer IX	CHEMSTATION Version C
ISOX and ISOXL	GC/MS Data MTQ Isomer X	CHEMSTATION Version C
ISOXI and ISOXIL	GC/MS Data MTQ Isomer XI	CHEMSTATION Version C
ISOXII and ISOXIIL	GC/MS Data MTQ Isomer XII	CHEMSTATION Version C
ISOXIII and ISOXIIIL	GC/MS Data MTQ Isomer XIII	CHEMSTATION Version C

APPENDIX B

FILE NAME	DESCRIPTION	PROGRAM
MTQ1001	GC/MS Data MTQ	CHEMSTATION Version C
MPQ1001	GC/MS Data	CHEMSTATION Version C
DPH1001	GC/MS Data	CHEMSTATION Version C
COC1001	GC/MS Data	CHEMSTATION Version C
KET1001	GC/MS Data	CHEMSTATION Version C
ACE1001	GC/MS Data	CHEMSTATION Version C
THC1001	GC/MS Data	CHEMSTATION Version C
DIA1001	GC/MS Data	CHEMSTATION Version C
2AM1002	GC/MS Data	CHEMSTATION Version C
ISA1002	GC/MS Data	CHEMSTATION Version C
AMI1002	GC/MS Data	CHEMSTATION Version C
NAC1002	GC/MS Data	CHEMSTATION Version C
NAC0911	GC/MS Data	CHEMSTATION Version C
NAC0911L	GC/MS Data	CHEMSTATION Version C
MTQ1001	GC/MS Data	CHEMSTATION Version C
MPQ1001	GC/MS Data	CHEMSTATION Version C
DPH1001	GC/MS Data	CHEMSTATION Version C
COC0905	GC/MS Data	CHEMSTATION Version C
COC0905L	GC/MS Data	CHEMSTATION Version C
DPH0904	GC/MS Data	CHEMSTATION Version C
DPH0904L	GC/MS Data	CHEMSTATION Version C
DIA0904	GC/MS Data	CHEMSTATION Version C
DIA0904L	GC/MS Data	CHEMSTATION Version C
MPQ0904	GC/MS Data	CHEMSTATION Version C
MPQ0904L	GC/MS Data	CHEMSTATION Version C
AMI0910	GC/MS Data	CHEMSTATION Version C
AMI0910L	GC/MS Data	CHEMSTATION Version C
MTQ0905	GC/MS Data	CHEMSTATION Version C
MTQ0905L	GC/MS Data	CHEMSTATION Version C
THC0904	GC/MS Data	CHEMSTATION Version C
THC0904L	GC/MS Data	CHEMSTATION Version C
2AM0910	GC/MS Data	CHEMSTATION Version C
2AM0910L	GC/MS Data	CHEMSTATION Version C
ACE0904	GC/MS Data	CHEMSTATION Version C
ACE0904L	GC/MS Data	CHEMSTATION Version C

COCKTAI	GC/MS Data	CHEMSTATION Version C
COCKTAIL	GC/MS Data	CHEMSTATION Version C
ISA0910	GC/MS Data	CHEMSTATION Version C
ISA0910L	GC/MS Data	CHEMSTATION Version C

APPENDIX C

FILE NAME	DESCRIPTION	PROGRAM
COCK1002	GC/MS Data: Cocktail of all Compounds	CHEMSTATION Version C
COC1008A	GC/MS Data: Cocktail of all Compounds	CHEMSTATION Version C

APPENDIX D

FILE NAME	DESCRIPTION	PROGRAM
DPH10PPM	GC/MS Data: 10 ppm DPH Std	CHEMSTATION Version C
DPH20PPM	GC/MS Data: 20 ppm DPH Std	CHEMSTATION Version C
DPH30PPM	GC/MS Data: 30 ppm DPH Std	CHEMSTATION Version C
DPH40PPM	GC/MS Data: 40 ppm DPH Std	CHEMSTATION Version C
DPH50PPM	GC/MS Data: 50 ppm DPH Std	CHEMSTATION Version C
DPH60PPM	GC/MS Data: 60 ppm DPH Std	CHEMSTATION Version C
MTQ10PPM	GC/MS Data: 10 ppm MTQ Std	CHEMSTATION Version C
MTQ20PPM	GC/MS Data: 20 ppm MTQ Std	CHEMSTATION Version C
MTQ30PPM	GC/MS Data: 30 ppm MTQ Std	CHEMSTATION Version C
MTQ40PPM	GC/MS Data: 40 ppm MTQ Std	CHEMSTATION Version C
MTQ50PPM	GC/MS Data: 50 ppm MTQ Std	CHEMSTATION Version C
MTQ60PPM	GC/MS Data: 60 ppm MTQ Std	CHEMSTATION Version C
DPH1S to DPH30S	GC/MS Data: Tablet 1 to 30	CHEMSTATION Version C
DPH1B to DPH30B	GC/MS Data: Blank 1 to 30	CHEMSTATION Version C
T11SIM1	GC/MS Data: DE Extract SIM Scan T1	CHEMSTATION Version C
T12SIM1	GC/MS Data: DE Extract SIM Scan T1	CHEMSTATION Version C
T13SIM1	GC/MS Data: DE Extract SIM Scan T1	CHEMSTATION Version C
T14SIM1	GC/MS Data: DE Extract SIM Scan T1	CHEMSTATION Version C
T15SIM1	GC/MS Data: DE Extract SIM Scan T1	CHEMSTATION Version C
T11SIM2	GC/MS Data: DE Extract SIM Scan T1	CHEMSTATION Version C
T12SIM2	GC/MS Data: DE Extract SIM Scan T1	CHEMSTATION Version C
T13SIM2	GC/MS Data: DE Extract SIM Scan T1	CHEMSTATION Version C

T14SIM2	GC/MS Data: DE Extract SIM Scan T1	CHEMSTATION Version C
T15SIM2	GC/MS Data: DE Extract SIM Scan T1	CHEMSTATION Version C

APPENDIX E

FILE NAME	DESCRIPTION	PROGRAM
D05PPM1 to 3	GC/MS Data: 0.5 ppm DPH Std, 3 Injections	CHEMSTATION Version C
D1PPM1 to 3	GC/MS Data: 1.0 ppm DPH Std, 3 Injections	CHEMSTATION Version C
D10PPM1 to 3	GC/MS Data: 10 ppm DPH Std, 3 Injections	CHEMSTATION Version C
D20PPM1 to 3	GC/MS Data: 20 ppm DPH Std, 3 Injections	CHEMSTATION Version C
D30PPM1 to 3	GC/MS Data: 30 ppm DPH Std, 3 Injections	CHEMSTATION Version C
D40PPM1 to 3	GC/MS Data: 40 ppm DPH Std, 3 Injections	CHEMSTATION Version C
D50PPM1 to 3	GC/MS Data: 50 ppm DPH Std, 3 Injections	CHEMSTATION Version C
D60PPM1 to 3	GC/MS Data: 60 ppm DPH Std, 3 Injections	CHEMSTATION Version C
DBLANK1 to 3	GC/MS Data: Chloroform Blank 1 to 3	CHEMSTATION Version C
M10PPM1 to 3	GC/MS Data: 10 ppm MTQ Std, 3 Injections	CHEMSTATION Version C
M20PPM1 to 3	GC/MS Data: 20 ppm MTQ Std, 3 Injections	CHEMSTATION Version C
M30PPM1 to 3	GC/MS Data: 30 ppm MTQ Std, 3 Injections	CHEMSTATION Version C
M40PPM1 to 3	GC/MS Data: 40 ppm MTQ Std, 3 Injections	CHEMSTATION Version C
M50PPM1 to 3	GC/MS Data: 50 ppm MTQ Std, 3 Injections	CHEMSTATION Version C
M60PPM1 to 3	GC/MS Data: 60 ppm MTQ Std, 3 Injections	CHEMSTATION Version C
MBLANK1 to 3	GC/MS Data: Chloroform Blank 1 to 3	CHEMSTATION Version C
A1S to A30S	GC/MS Data: Tablet 1 to 30	CHEMSTATION Version C
A1B to A30B	GC/MS Data: Blank 1 to 30	CHEMSTATION Version C
T21SIM1	GC/MS Data: DE Extract SIM Scan T2	CHEMSTATION Version C
T22SIM1	GC/MS Data: DE Extract SIM Scan T2	CHEMSTATION Version C
T23SIM1	GC/MS Data: DE Extract SIM Scan T2	CHEMSTATION Version C
T24SIM1	GC/MS Data: DE Extract SIM Scan T2	CHEMSTATION Version C
T25SIM1	GC/MS Data: DE Extract SIM Scan T2	CHEMSTATION Version C
T21SIM2	GC/MS Data: DE Extract SIM Scan T2	CHEMSTATION Version C
T22SIM2	GC/MS Data: DE Extract SIM Scan T2	CHEMSTATION Version C
T23SIM2	GC/MS Data: DE Extract SIM Scan T2	CHEMSTATION Version C
T24SIM2	GC/MS Data: DE Extract SIM Scan T2	CHEMSTATION Version C
T25SIM2	GC/MS Data: DE Extract SIM Scan T2	CHEMSTATION Version C

APPENDIX F

FILE NAME	DESCRIPTION	PROGRAM
D05PPM1 to 3	GC/MS Data: 0.5 ppm DPH Std, 3 Injections	CHEMSTATION Version C
D1PPM1 to 3	GC/MS Data: 1.0 ppm DPH Std, 3 Injections	CHEMSTATION Version C
D10PPM1 to 3	GC/MS Data: 10 ppm DPH Std, 3 Injections	CHEMSTATION Version C
D20PPM1 to 3	GC/MS Data: 20 ppm DPH Std, 3 Injections	CHEMSTATION Version C
D30PPM1 to 3	GC/MS Data: 30 ppm DPH Std, 3 Injections	CHEMSTATION Version C
D40PPM1 to 3	GC/MS Data: 40 ppm DPH Std, 3 Injections	CHEMSTATION Version C
D50PPM1 to 3	GC/MS Data: 50 ppm DPH Std, 3 Injections	CHEMSTATION Version C
D60PPM1 to 3	GC/MS Data: 60 ppm DPH Std, 3 Injections	CHEMSTATION Version C
DBLANK1 to 3	GC/MS Data: Chloroform Blank 1 to 3	CHEMSTATION Version C
M10PPM1 to 3	GC/MS Data: 10 ppm MTQ Std, 3 Injections	CHEMSTATION Version C
M20PPM1to 3	GC/MS Data: 20 ppm MTQ Std, 3 Injections	CHEMSTATION Version C
M30PPM1 to 3	GC/MS Data: 30 ppm MTQ Std, 3 Injections	CHEMSTATION Version C
M40PPM1 to 3	GC/MS Data: 40 ppm MTQ Std, 3 Injections	CHEMSTATION Version C
M50PPM1 to 3	GC/MS Data:50 ppm MTQ Std, 3 Injections	CHEMSTATION Version C
M60PPM1 to 3	GC/MS Data: 60 ppm MTQ Std, 3 Injections	CHEMSTATION Version C
MBLANK1 to 3	GC/MS Data: Chloroform Blank 1 to 3	CHEMSTATION Version C
B1S to B30S	GC/MS Data: Tablet 1 to 30	CHEMSTATION Version C
B1B to B30B	GC/MS Data: Blank 1 to 30	CHEMSTATION Version C
T31SIM1	GC/MS Data: DE Extract SIM Scan T3	CHEMSTATION Version C
T32SIM1	GC/MS Data: DE Extract SIM Scan T3	CHEMSTATION Version C
T33SIM1	GC/MS Data: DE Extract SIM Scan T3	CHEMSTATION Version C
T34SIM1	GC/MS Data: DE Extract SIM Scan T3	CHEMSTATION Version C
T35SIM1	GC/MS Data: DE Extract SIM Scan T3	CHEMSTATION Version C
T31SIM2	GC/MS Data: DE Extract SIM Scan T3	CHEMSTATION Version C
T32SIM2	GC/MS Data: DE Extract SIM Scan T3	CHEMSTATION Version C
T33SIM2	GC/MS Data: DE Extract SIM Scan T3	CHEMSTATION Version C
T34SIM2	GC/MS Data: DE Extract SIM Scan T3	CHEMSTATION Version C
T35SIM2	GC/MS Data: DE Extract SIM Scan T3	CHEMSTATION Version C

APPENDIX G

FILE NAME	DESCRIPTION	PROGRAM
DM05PPM1 to 3	GC/MS Data: 0.5 ppm DPH and MTQ Std, 3 Injections	CHEMSTATION Version C
DM1PPM1 to 3	GC/MS Data: 1.0 ppm DPH and MTQ Std, 3 Injections	CHEMSTATION Version C
DM10PPM1 to 3	GC/MS Data: 10 ppm DPH and MTQ Std, 3 Injections	CHEMSTATION Version C
DM20PPM1 to 3	GC/MS Data: 20 ppm DPH and MTQ Std, 3 Injections	CHEMSTATION Version C
DM30PPM1 to 3	GC/MS Data: 30 ppm DPH and MTQ Std, 3 Injections	CHEMSTATION Version C
DM40PPM1 to 3	GC/MS Data: 40 ppm DPH and MTQ Std, 3 Injections	CHEMSTATION Version C
DM50PPM1 to 3	GC/MS Data: 50 ppm DPH and MTQ Std, 3 Injections	CHEMSTATION Version C
DM60PPM1 to 3	GC/MS Data: 60 ppm DPH and MTQ Std, 3 Injections	CHEMSTATION Version C
DMBLANK 1 to 3	GC/MS Data: Chloroform Blank 1 to 3	CHEMSTATION Version C
T4B1 to T4B30	GC/MS Data: Blank 1 to 30	CHEMSTATION Version C
T4S1 to T4S30	GC/MS Data: Tablet 1 to 30	CHEMSTATION Version C
T41SIM1	GC/MS Data: DE Extract SIM Scan T4	CHEMSTATION Version C
T42SIM1	GC/MS Data: DE Extract SIM Scan T4	CHEMSTATION Version C
T43SIM1	GC/MS Data: DE Extract SIM Scan T4	CHEMSTATION Version C
T44SIM1	GC/MS Data: DE Extract SIM Scan T4	CHEMSTATION Version C
T45SIM2	GC/MS Data: DE Extract SIM Scan T4	CHEMSTATION Version C
T41SIM2	GC/MS Data: DE Extract SIM Scan T4	CHEMSTATION Version C
T42SIM2	GC/MS Data: DE Extract SIM Scan T4	CHEMSTATION Version C
T43SIM2	GC/MS Data: DE Extract SIM Scan T4	CHEMSTATION Version C
T443SIM2	GC/MS Data: DE Extract SIM Scan T4	CHEMSTATION Version C
T423SIM2	GC/MS Data: DE Extract SIM Scan T4	CHEMSTATION Version C

APPENDIX H

FILE NAME	DESCRIPTION	PROGRAM
DPHLINE	Diphenhydramine Line for Quantification: Test Case 1	Microsoft Excel
DPHLINE2	Diphenhydramine Line for Quantification: Test Case 2	
DPHLINE3	Diphenhydramine Line for Quantification: Test Case 3	
DPHLINE4	Diphenhydramine Line for Quantification: Test Case 4	
DPHCONCGRAPH	Diphenhydramine Concentration of Two code 3.41 Seizures and Two Code 35.1 Seizures	
MTQ val0101	Methaqualone Calibration Line for Validation: Analyst 1	
MTQval0102	Methaqualone Calibration Line for Validation: Analyst 2	
MTQ val0103	Methaqualone Calibration Line for Validation: Analyst 3	
MTQ val0104	Methaqualone Calibration Line for Validation: Analyst 4	
MTQCORRECTEDLINE	Methaqualone Line for Quantification: Test Case 1	
MTQCORRECTEDLINE2	Methaqualone Line for Quantification: Test Case 2	
MTQCORRECTEDLINE3	Methaqualone Line for Quantification: Test Case 3	
MTQCORRECTEDLINE4	Methaqualone Line for Quantification: Test Case 4	
MTQTODPHGRAPH	Methaqualone Concentration Relative to Diphenhydramine Concentration Per Tablet in Each Test Case	
MTQCORRECTEDLINE3. rondspeel	Methaqualone Concentration of Two code 3.41 Seizures and Two Code 35.1 Seizures	
Tabletmass	Mass of Tablets in Gram	

APPENDIX I

FILE NAME	DESCRIPTION	PROGRAM
1MV01 TO 1MV48	GC/MS Data: Method Validation Study	CHEMSTATION Version C
2MV01 TO 2MV48	GC/MS Data: Method Validation Study	CHEMSTATION Version C
5MV01 TO 5MV48	GC/MS Data: Method Validation Study	CHEMSTATION Version C
6MV01 TO 6MV48	GC/MS Data: Method Validation Study	CHEMSTATION Version C
7MV01 TO 7MV48	GC/MS Data: Method Validation Study	CHEMSTATION Version C
STD01 TO STD06	GC/MS Data: Method Validation Study	CHEMSTATION Version C
BLANK1 TO BLANK 9	GC/MS Data: Method Validation Study	CHEMSTATION Version C

APPENDIX J

The following t-table was taken from Quantitative Chemical Analysis [11], page 72.

Values of t for estimating confidence limits					
Degrees of freedom	**Factor for confidence interval, %**				
	80	**90**	**95**	**99**	**99.9**
1	3.08	6.31	12.7	63.7	637
2	1.89	2.92	4.30	9.92	31.6
3	1.64	2.35	3.18	5.84	12.9
4	1.53	2.13	2.78	4.60	8.60
5	1.48	2.02	2.57	4.03	6.86
6	1.44	1.94	2.45	3.71	5.96
7	1.42	1.90	2.56	3.50	5.40
8	1.40	1.86	2.31	3.36	5.04
9	1.38	1.83	2.26	3.25	4.78
10	1.37	1.81	2.23	3.17	4.59
11	1.36	1.80	2.20	3.11	4.44
12	1.36	1.78	2.18	3.06	4.32
13	1.35	1.77	2.16	3.01	4.22
14	1.34	1.76	2.14	2.98	4.14
∞	1.29	1.64	1.96	2.58	3.29

The following table containing critical Q values, was taken from Quantitative Chemical Analysis [11], page 75.

Critical Q values at three probability levels			
Number of observations	**Q_{crit} (reject if $Q_{exp} > Q_{crit}$)**		
	90% confidence	**96% confidence**	**99% confidence**
3	0.94	0.98	0.99
4	0.76	0.85	0.93
5	0.64	0.73	0.82
6	0.56	0.64	0.74
7	0.51	0.59	0.68
8	0.47	0.54	0.63
9	0.44	0.51	0.60
10	0.41	0.48	0.57

OTHER

DEFINITIONS AND BASIC PRINCIPLES APPLIED

Isomers (Structural Isomers)

Different compounds that have the same molecular formula, but different molecular structures giving rise to different chemical and physical properties. An example is ethyl alcohol and dimethyl ether, or 2-ethyl-3-phenyl-4(3H)-quinazolinone, which is a structural isomer of methaqualone (2-methyl-3-*o*-tolylquinazolin-4-(3H)- one).

Gas chromatography (GC)

The operational principle of gas chromatography is based on the partitioning of a mixture of volatile components. These compounds are transported by a carrier gas through a column containing absorbing or adsorbing material coated on solid material (stationary phase). Each component is partitioned between the carrier gas and the stationary phase. The time required by the component to travel through the column depends on its degree of retention by the stationary phase, and can be measured by a suitable detector. Various degrees of retention by the stationary phase lead to components emerging from the column at different times. The quantity of the component can also be measured by the detector. Detectors commonly used in combination with chromatography are mass selective detectors, thermal conductivity detectors, flame ionization detectors, electron capture detectors, flame photometric detectors, etc.

Electron Ionization (EI) Mass Spectrometry (MS)

When mass spectrometry is preceded by gas chromatographic separation of a complex sample, pure compounds ideally enter the mass spectrometer. In the mass spectrometer, molecules are bombarded with a beam of energetic electrons. The molecules are then ionized and fragmentation into positive ions takes place. Each kind of ion has a specific mass to charge ratio, or *m/e* value. For most ions the charge is one, so that *m/e* is simply the mass of the ion. The set of ions is analysed in such a way that that a signal is obtained for each value of *m/e* that is represented. The intensity of each signal reflects the abundance of the ion producing the signal. The largest peak is called the base peak, with an intensity of 100 allocated to it. The intensities of all other peaks are expressed relative to that of the base peak. A plot depicting the relative intensities of signals at the various *m/e* values is called a mass spectrum. This mass spectrum is highly characteristic for a specific compound. Mass spectra can be used to either prove/confirm the identity of a target compound, or help establish the structure of an unknown compound.

Validation

Validation refers to the process by which it is established that the performance characteristics of a specific method meet the requirements for the intended analytical applications of the method. This process, through practical experiments, also establishes the limitations of the method, as well as the effects of specified interfering compounds on the performance of the method.

Chemical Fingerprinting

Chemical fingerprinting can be defined as the determination of the presence or absence of identical key compounds in preparations, enabling the analytical chemist to derive that the preparations are the same/not the same.

Cocktail

"Cocktail" refers to a mixture of compounds. In this project the cocktail consists out of the following compounds:

Methaqualone Isomer I : 2-methyl-3-o-tolyl-4(3H)-quinazolinone
Methaqualone Isomer II : 2-methyl-3-m-tolyl-4(3H)-quinazolinone
Methaqualone Isomer III : 2-methyl-3-p-tolyl-4(3H)-quinazolinone
Methaqualone Isomer IV : 3-(2,3-dimethylphenyl)-4(3H)-quinazolinone
Methaqualone Isomer V : 3-(2,4-dimethylphenyl)-4(3H)-quinazolinone
Methaqualone Isomer VI : 3-(2,5-dimethylphenyl)-4(3H)-quinazolinone
Methaqualone Isomer VII : 3-(2,6-dimethylphenyl)-4(3H)-quinazolinone
Methaqualone Isomer VIII : 3-(3,4-dimethylphenyl)-4(3H)-quinazolinone
Methaqualone Isomer IX : 3-(3,5-dimethylphenyl)-4(3H)-quinazolinone
Methaqualone Isomer X : 2-Ethyl-3-phenyl-4(3H)-quinazolinone
Methaqualone Isomer XI : 3-o-Ethylphenyl-4(3H)-quinazolinone
Methaqualone Isomer XII : 3-m-Ethylphenyl-4(3H)-quinazolinone
Methaqualone Isomer XIII : 3-p-Ethylphenyl-4(3H)-quinazolinone
Acetanthranil
o-Toluidine
N-Acetylanthranilic acid
Ketamine
Diphenhydramine
p-methaqualone (MPQ)
Cocaine
Amide of Isatoicanhydride
Diazepam
Tetrahidrocannabinol