

**Increased-rate stability studies for St John's wort
(*Hypericum perforatum*), *Ginkgo biloba* and Kava Kava
(*Piper methysticum*) under unfavourable environmental
conditions.**

Andre Marais

**Dissertation submitted to the faculty of Medicine
(Department of Pharmacology)
University of Pretoria**

In partial fulfillment of the requirements of the degree

Magister Scientiae

Supervisor: Dr J.N. Eloff
Co-supervisor: Dr R van Brummelen

Date of submission: July 2001



TABLE OF CONTENTS

PREFACE	I
ACKNOWLEDGEMENTS	II
INDEX	III
SUMMARY	IX
OPSOMMING	XI
LIST OF ABBREVIATIONS	XII
LIST OF FIGURES	XIV
LIST OF TABLES	XX

ZENITH

AKADEMIESE INLIGTINGSDIENS UNIVERSITEIT VAN PRETORIA
Klasnommer: ZAPR 65.321
Aanwinstnommer: 15918956

MARAS

PREFACE

I hereby confirm that this is my own work, and that it has not been submitted to any other institution.



Andre Marais

ACKNOWLEDGEMENTS

I would like to extend a warm word of thanks to the following:

- Dr Kobus Eloff without whose support and wisdom I would not have been able to complete this project.
- Dr Roy van Brummelen, for his creative ideas, valuable guidance, commitment and motivation, and all those long sleepless nights evaluating and improving this dissertation.
- The Department of Pharmacology, University of Pretoria, for making their facilities available at all hours throughout the two and a half years of this research.
- Biomox Pharmaceuticals for the use of their manufacturing equipment, analytical instruments, and the supplied herbal raw material.
- Marie Murphy and the laboratory staff of Biomox Pharmaceuticals for their valuable, and much appreciated technical assistance.
- My parents for their love and encouragement.
- Tanya for her patience and understanding.
- My Creator for His abundant blessings.

INDEX

CHAPTER 1.	Background and Literature review.	1
1.1	INTRODUCTION AND PROBLEM STATEMENT	1
1.2	ST JOHN'S WORT (<i>Hypericum perforatum</i>)	4
1.2.1	History	4
1.2.2	Chemistry	5
1.2.3	Mechanism of therapeutic action	6
1.2.4	Clinical studies	6
1.2.5	Clinical indications	7
1.2.6	Adverse effects and toxicity	8
1.3	<i>GINKGO BILOBA</i>	8
1.3.1	History	8
1.3.2	Chemistry	9
1.3.3	Mechanism of therapeutic action	10
1.3.4	Clinical studies	11
1.3.5	Clinical indications	12
1.3.6	Adverse effects and toxicity	12
1.4	KAVA KAVA (<i>Piper methysticum</i>)	13
1.4.1	History	13
1.4.2	Chemistry	14
1.4.3	Mechanism of therapeutic action	14
1.4.4	Clinical studies	15
1.4.5	Clinical indications	15
1.4.6	Adverse effects and toxicity	16
1.5	AIM OF THE STUDY	17
CHAPTER 2.	Materials and methods	18
2.1	MATERIALS	18
2.2	METHODS	19
2.2.1	Manufacturing of tablets	19

2.2.1.1	Hypericum herbal tablets	19
2.2.1.2	<i>Ginkgo biloba</i> herbal tablets	20
2.2.1.3	Kava Kava herbal tablets	20
2.2.2	Manufacturing of Capsules	21
2.2.2.1	Extract capsules	21
2.2.2.1.1	Hypericum extract capsules	21
2.2.2.1.2	Ginkgo extract capsules	22
2.2.2.1.3	Kava Kava extract capsules	22
2.2.2.2	Dried herb capsules	22
2.2.2.2.1	Hypericum herbal capsules	23
2.2.2.2.2	Ginkgo herbal capsules	23
2.2.2.2.3	Kava Kava herbal capsules	23
2.2.3	Liquid extracts	23
2.3	STORAGE CONDITIONS OF SAMPLES	24
2.3.1	Radiated samples	24
2.4	EXTRACTION	25
2.4.1	Extraction of raw materials	25
2.4.2	Extractions according to the British Herbal Pharmacopoeia	25
2.4.2.1	<i>Hypericum perforatum</i>	25
2.4.2.2	<i>Ginkgo biloba</i>	26
2.4.2.3	<i>Piper methysticum</i>	26
2.4.3	Extraction of dosage forms	26
2.5	THIN LAYER CHROMATOGRAPHY (TLC)	27
2.5.1	Thin layer chromatography (TLC) analysis of the extracts	27
2.5.1.1	Preparation of the vanillin spray reagent	28
2.5.1.2	Preparation of the <i>p</i> -Anisaldehyde spray reagent	28
2.5.2	Identification (TLC) according to the British Herbal Pharmacopoeia	28
2.5.2.1	<i>Hypericum perforatum</i>	28
2.5.2.2	<i>Ginkgo biloba</i>	29
2.5.2.3	<i>Piper methysticum</i>	29

2.5.3	Thin layer chromatography (TLC) analysis of the sample dosage forms	29
2.6	SPECTROPHOTOMETRY	31
2.6.1	Hypericin assay by Spectrophotometry	31
2.6.1.1	Standard Preparation	31
2.6.1.2	Sample preparation	32
2.6.1.2.1	Herbal capsules	32
2.6.1.2.2	Herbal tablets	32
2.6.1.2.3	Extract capsules	32
2.6.1.2.4	Radiated samples	32
2.6.2	Validation of method	33
2.7	HIGH PRESSURE LIQUID CHROMATOGRAPHY (HPLC)	33
2.7.1	Ginkgo Flavonol Glycoside (quercetin) Assay by HPLC	34
2.7.1.1	Chromatographic Conditions	34
2.7.1.2	Standard Preparation	34
2.7.1.3	Sample Preparation	35
2.7.1.3.1	Herbal Capsules	35
2.7.1.3.2	Herbal Tablets	35
2.7.1.3.3	Extract Capsules	35
2.7.1.3.4	Radiated samples	36
2.7.1.4	Validation of method	36
2.7.2	Kava lactone assay by HPLC	36
2.7.2.1	Chromatographic Conditions	36
2.7.2.2	Standard Preparation	37
2.7.2.3	Sample Preparation	37
2.7.2.3.1	Herbal Capsules	37
2.7.2.3.2	Herbal Tablets	38
2.7.2.3.3	Extract Capsules	38
2.7.2.3.4	Radiated samples	38
2.7.2.4	Standard Deviation on Methods	38

CHAPTER 3. Results and discussion

3.1	EXTRACTION	39
3.1.1	Discussion	39
3.2	<i>HYPERICUM PERFORATUM</i>	40
3.2.1	Results	40
3.2.1.1	Thin layer chromatography	40
3.2.1.1.1	Hypericum samples at 25°C	42
3.2.1.1.2	Hypericum samples at 40°C	43
3.2.1.1.3	Hypericum samples at 60°C	44
3.2.1.1.4	Hypericum samples at 80°C	45
3.2.1.1.5	Hypericum samples at direct sunlight	45
3.2.1.1.6	Hypericum samples at high humidity and direct sunlight	47
3.2.1.2	Spectrophotometry	54
3.2.1.2.1	Hypericum herbal tablets	57
3.2.1.2.2	Hypericum herbal capsules	59
3.2.1.2.3	Hypericum extract capsules	60
3.2.1.2.4	Hypericum root powder adiated with Cobalt-60 source	61
3.2.1.3	Summary of TLC and Spectrophotometry results	64
3.2.1.3.1	Herbal tablets	64
3.2.1.3.2	Herbal capsules	65
3.2.1.3.3	Extract capsules	66
3.2.1.3.4	Liquid extract	67
3.2.1.3.5	Radiated dried herb	67
3.2.2	BACKGROUND OF STABILITY PRINCIPLES	67
3.2.2.1	Example	69
3.2.2.2	Effect of temperature on the Rate constant	69
3.2.3	DISCUSSION	73
3.2.4	SUMMARY	78



3.3	<i>GINKGO BILOBA</i>	79
3.3.1	Results	79
3.3.1.1	Thin layer Chromatography	79
3.3.1.1.1	Ginkgo samples at 25°C	81
3.3.1.1.2	Ginkgo at samples 40°C	82
3.3.1.1.3	Ginkgo at samples 60°C	83
3.3.1.1.4	Ginkgo samples at 80°C	83
3.3.1.1.5	Ginkgo samples at direct sunlight	84
3.3.1.1.6	Ginkgo samples at high humidity and direct sunlight	85
3.3.1.2	High Pressure Liquid Chromatography (HPLC)	93
3.3.1.2.1	Ginkgo herbal tablets	96
3.3.1.2.2	Ginkgo herbal capsules	97
3.3.1.2.3	Ginkgo extract capsules	98
3.3.1.2.4	Ginkgo leaf powder radiated with Cobalt-60 Source	99
3.3.1.3	Summary of TLC and HPLC results	102
3.3.1.3.1	Herbal tablets	102
3.3.1.3.2	Herbal capsules	103
3.3.1.3.3	Extract capsules	104
3.3.1.3.4	Liquid extracts	105
3.3.1.3.5	Radiated dried leaf powder	105
3.3.2	KINETICS OF QUERCETIN	105
3.3.3	DISCUSSION	107
3.3.4	SUMMARY	110
3.4	<i>PIPER METHYSTICUM</i>	111
3.4.1	Results	111
3.4.1.1	Thin layer chromatography	111
3.4.1.1.1	Kava Kava samples at 25°C	113

SUMMARY

This was a chemical laboratory study. The main focus was to evaluate the chemical stability of *Hypericum perforatum* (St John's wort), *Ginkgo biloba* and *Piper methysticum* (Kava Kava) under unfavourable environmental conditions. Different dosage forms representing the same amount of active ingredients for each were used. Some of the dosage forms were self manufactured according to Good Manufacturing Practice. Samples of the dried powder of each plant was also exposed to a series of gamma-radiation.

Acetone was used as an extractant for all three plants, after evaluating and discarding the extraction method stipulated in the British Herbal Pharmacopoeia. Identification of the different plants were carried out by means of Thin Layer Chromatography. The in-house developed mobile phases EMW, BEA and CEF, showed better separation and visibility compared to the mobile phases used in the British Herbal Pharmacopoeia. The plates were sprayed with either vanillin or *p*-anisaldehyde for optimal visualization of the separated compounds.

After the specified period of 6-months, comparative TLC was performed on all samples. This was achieved for each plant by applying all samples stored at a specific condition i.e.25°C, on the same plate. The samples were stored at low temperature after exposure to the specific time interval.

Quantitative analysis was performed by spectrophotometry, and high pressure liquid chromatography. The data obtained from these analytical methods, were used to evaluate the relative chemical stability of each dosage form. The relationship between the quantitative data and the qualitative changes in the TLC fingerprints, were compared, hoping to achieve a common pattern relating to the stability.

The order of the reaction as well as the reaction rate constant (*k*) for each dosage form was calculated, except for kava kava. The shelf-life (t_{90}) was calculated using the analyzed data obtained by spectrophotometry or HPLC. The relevance of conventional pharmaceutical calculations in the prediction of shelf-life, by means of accelerated stability tests, was investigated for the possible application to herbal products.

The effects of gamma radiation on the degradation of the chemical compounds present in each plant, was evaluated.

After an evaluation of all the relevant data, it seemed that the tablet-dosage forms were equally effective regarding stability, compared to the capsules. Liquid extracts appeared to be less stable than the extract capsules. The extract capsules seemed to degrade more rapidly than the herbal tablets or herbal capsules. Exposure to low dose radiation (4.4 kGy) did not seem to have an influence on the stability. It was evident that some herbs were more sensitive to sunlight or heat than others.

In general, all three of the chosen plants seemed to be relatively stable if stored in the specified conditions. It seemed valid for the shelf-life to be expressed as two years.

OPSOMMING

Die hoof klem van hierdie projek was om die chemiese stabiliteit van drie van die mees algemeen gebruikte natuurlike medisyne in Suid-Afrika te ondersoek. Verskillende doseervorme van St John's wort (*Hypericum perforatum*), *Ginkgo biloba*, en Kava Kava (*Piper methysticum*), is vir die ondersoek gebruik. Uitsluitend Kava Kava, het die verskillende doseervorme elkeen oor dieselfde hoeveelheid aktiewe plant materiaal beskik. Waar geskikte doseervorme nie beskikbaar was nie, is dit self vervaardig. Alle vervaardiging het geskied onder sogenaamde Goeie Vervaardigings Praktyk (GMP), 'n vereiste gestel deur die medisyne-beheer-raad tydens vervaardiging van alle etiese produkte. Monsters van die gedroogde poeier van elke plant is ook blootgestel aan verskillende dosisse van gamma-bestraling.

In al drie plante was asetoon die gekose ekstraheermiddel, nadat daar besluit is om nie die ekstraksie metode, soos vervat in die British Herbal Pharmacopoeia, te implementeer nie. Identifikasie van die verskillende plante is ook uitgevoer deur middel van dunlaag chromatografie. Met die gebruik van ons eie ontwikkelde mobiele fases, EMW, CEF en BEA in die plek van die mobiele fases soos vermeld in die BHP. Die skeiding en visualisering van die bande in die verskillende plante was meer duidelik waameembaar met ons eie metodes. Die verskillende dunlaagplate is gesproei met vanillien of anysaldehid vir die optimale visualisering van die geskeide komponente.

Na die verstryk van die gespesifiseerde 6-maande, is die verskillende dunlaagplate met mekaar vergelyk. 'n Goeie vergelyking kon getref word deur elke monster wat by dieselfde kondisie onderworpe was, op dieselfde plaat aan te wend bv. Al die monsters van kava kava wat by 25°C gestoor was, is op dieselfde plaat aangewend. Na die onttrekking van die monsters by elke gekose tydsinterval, is it by 'n lae temperatuur gestoor totdat analises daarop gedoen kon word.

Kwantitatiewe analise is uitgevoer deur gebruik te maak van spektrofotometrie sowel as hoë-druk vloeistof chromatografie. Die data wat deur hierdie analitiese metodes verkry was, is gebruik om die chemiese stabiliteit in elke doseerform te evalueer. Die verwantskap tussen die ge-analiseerde data en die dunlaag-identifikasie profiele is ondersoek, met die hoop dat daar 'n sekere mate van ooreenstemming getoon kon word, of 'n waarneembare patroon wat 'n moontlike toepassing op die stabiliteit kon hê.

Die orde van die chemiese afbraak, sowel as die reaksie snelheids-konstante (k) is ook vir elke produk, behalwe Kava Kava bepaal. Die rakleef tyd (t_{90}) is ook vir elke produk bepaal deur die waardes uit die analyses verkry uit spektrofotometrie en hoë-druk vloeistof chromatografie te gebruik. Die toepaslikheid van konvensionele farmaseutiese vergelykings in die skatting van 'n rakleef tyd, deur gebruik te maak van versnelde stabiliteitstoetse, is ook ondersoek. Dit is uitgevoer met die hoop van 'n moonlike toepassing in natuurlike medisyne.

Na evaluering van al die relevante data het dit geblyk dat die tablette net so effektief, betreffende die stabiliteit, is in vergelyking met die kapsules. Verder het dit ook geblyk dat die vloeistof ekstrakte minder stabiel was as die ekstrak kapsules. Die ekstrak kapsules toon 'n vinniger afbraak as die tablette of die fyn-kruie kapsules. Blootstelling aan 'n lae dosis bestraling (4,4 kGy) het geen noemenswaardige invloed op die stabiliteit getoon nie.

Dit was duidelik dat sekere produkte meer sensitief teenoor blootstelling aan sonlig en hoë temperature was, as ander. Oor die algemeen het dit geblyk dat al drie hierdie plante oor 'n aanvaarbare stabiliteit beskik, tensy dit onder die regte bewaringstoestand gestoor word. 'n Vervaldatum van twee jaar op hierdie produkte blyk aanvaarbaar te wees.

LIST OF ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
BEA	Benzene/Ethanol/Ammonium hydroxide [18/2/0.2 v/v/v]
BHP	British Herbal Pharmacopoeia
BP	British Pharmacopoeia
CEF	Chloroform/ethyl acetate/formic acid [10/8/2 v/v/v]
DI water	De-Ionized water
EC	Extract capsule
EMW	Ethylacetate/methanol/water [10/1/35 v/v/v]
GABA	Gamma Amino Butyric Acid
GMP	Good Manufacturing Practice
G-protein	Glucoprotein
HC	Herbal capsule
HIV	Human Immune deficiency Virus
HPLC	High Pressure Liquid Chromatography
HT	Herbal Tablet
LIQ	Liquid Extract
MCC	Medicine Control Council
MAO	Monoamine oxidase
MeOH	Methanol
OTC	Over the Counter
PAF	Platelet activation factor
Rf	Retention factor
TLC	Thin layer Chromatography

LIST OF FIGURES

CHAPTER 1

Figure 1.1	Lead Herbal sales in the United States for 1998	1
Figure 1.2	<i>Hypericum perforatum</i> flowering plant	4
Figure 1.3	Chemical structure for hypericin and pseudohypericin	6
Figure 1.4	<i>Ginkgo biloba</i> leaves	8
Figure 1.5	Chemical structure for quercetin	10
Figure 1.6	Chemical structure for ginkgolide A,B,C and bilobalide	10
Figure 1.7	Leaves of <i>Piper methysticum</i>	13
Figure 1.8	Chemical structure for kawain, di-hydrokawain, methysticin, and di-hydromethysticin	14

CHAPTER 3

Figure 3.1	Total quantity of compounds extracted with 3 different extractants	39
Figure 3.2.1	TLC of <i>Hypericum</i> root powder with mobile phase EMW (top), BEA (center) and CEF (bottom) and sprayed with <i>p</i> -anisaldehyde (left) and vanillin (right). Each plate shows extraction with Methanol (left), Acetone (center) and n-Hexane (right). R_f values for hypericin are indicated	41
Figure 3.2.2	<i>Hypericum perforatum</i> identification according to the British Herbal Pharmacopoeia. (<i>Hypericum</i> left and Rutin right)	42

Figure 3.2.3 Hypericum samples stored at 25°C. [Sprayed with *p*-Anisaldehyde with mobile phase EMW (top), BEA (center) and CEF (bottom). From left :Herbal Tablets (HT) 0,3,6 months. Herbal Capsules (HC) 0,3,6 months. Extract Capsules (EC) 0,3,6 months. Liquid extract (LIQ) 0,3,6 months]

48

Figure 3.2.4 Hypericum samples stored at 40°C. [Sprayed with *p*-Anisaldehyde with mobile phase EMW (top), BEA (center) and CEF (bottom). From left :Herbal Tablets (HT) 0,3,6 months. Herbal Capsules (HC) 0,3,6 months. Extract Capsules (EC) 0,3,6 months. Liquid extract (LIQ) 0,3,6 months]

49

Figure 3.2.5 Hypericum samples stored at 60°C. [Sprayed with *p*-Anisaldehyde with mobile phase EMW (top), BEA (center) and CEF (bottom). From left :Herbal Tablets (HT) 0,2,4,6 weeks. Herbal Capsules (HC) 0,2,4,6 weeks. Extract Capsules (EC) 0,2,4,6 weeks.]

50

Figure 3.2.6 Hypericum samples stored at 80°C. [Sprayed with *p*-Anisaldehyde with mobile phase EMW (top), BEA (center) and CEF (bottom). From left :Herbal Tablets (HT) 0,2,4,6 weeks. Herbal Capsules (HC) 0,2,4,6 weeks. Extract Capsules (EC) 0,2,4,6 weeks.]

51

Figure 3.2.7 Hypericum stored at direct sunlight. [Sprayed with *p*-Anisaldehyde with mobile phase EMW (top), BEA (center) and CEF (bottom). From left :Herbal Tablets (HT) 0,3,6 months. Herbal Capsules (HC) 0,3,6 months. Extract Capsules (EC) 0,3,6 months. Liquid extract (LIQ)0,3,6months.]

52

Figure 3.2.8 Hypericum samples stored at direct sunlight and high humidity. [Sprayed with *p*-Anisaldehyde with mobile phase EMW (top), BEA (center) and CEF (bottom). From left :Herbal tablets (HT) 0,3,6 months. Herbal capsules (HC) 0,3,6 months. Ectract capsules (EC) 0,3,6 months. Liquid extract (LIQ) 0,3,6 months.]

53

Figure 3.2.9 Absorption spectrum of the hypericin standard solution B prepared in section 2.6.1.1

54

Figure 3.2.10 Hypericin standard curve	55
Figure 3.2.11 Degradation of hypericin in Hypericum herbal tablets at different storage conditions	57
Figure 3.2.12 Degradation of hypericin in Hypericum herbal capsules at different storage conditions	59
Figure 3.2.13 Degradation of hypericin in Hypericum extract capsules at different storage conditions	60
Figure 3.2.14 Degradation of hypericin in Hypericum herbal root powder at different radiation doses	61
Figure 3.2.15 Using linear regression analysis to calculate k for Hypericum herbal capsules at 25°C.	69
Figure 3.2.16 Arrhenius graph for data obtained for Hypericum herbal tablets.	70
Figure 3.3.1 TLC of Ginkgo leaf powder with mobile phase EMW (top), BEA (center) and CEF (bottom) and sprayed with <i>p</i> -anisaldehyde (left) and vanillin (right). Each plate shows extraction with Methanol (left), Acetone (center) and n-Hexane (right). R_f values for quercetin are indicated	80
Figure 3.3.2 Ginkgo identification according to the British Herbal Pharmacopoeia. (Ginkgo left and Rutin right)	81
Figure 3.3.3 Ginkgo samples stored at 25°C. [Sprayed with vanillin with mobile phase EMW (top), BEA (center) and CEF (bottom). From left: Herbal tablets (HT) 0,3,6 months. Herbal capsules (HC) 0,3,6 months. Extract capsules (EC) 0,3,6 months. Liquid extract (LIQ) 0,3,6 months.]	87

Figure 3.3.4 Ginkgo samples stored at 40°C. [Sprayed with vanillin with mobile phase EMW (top), BEA (center) and CEF (bottom). From left: Herbal tablets (HT) 0,3,6 months. Herbal capsules (HC) 0,3,6 months. Extract capsules (EC) 0,3,6 months. Liquid extract (LIQ) 0,3,6 months.]	88
Figure 3.3.5 Ginkgo samples stored at 60°C. [Sprayed with vanillin with mobile phase EMW (top), BEA (center) and CEF (bottom). From left: Herbal tablets (HT) 0,2,4,6 weeks. Herbal capsules (HC) 0,2,4,6 weeks. Extract capsules (EC) 0,2,4,6 weeks.]	89
Figure 3.3.6 Ginkgo samples stored at 80°C. [Sprayed with vanillin with mobile phase EMW (top), BEA (center) and CEF (bottom). From left: Herbal tablets (HT) 0,2,4,6 weeks. Herbal capsules (HC) 0,2,4,6 weeks. Extract capsules (EC) 0,2,4,6 weeks.]	90
Figure 3.3.7 Ginkgo samples at direct sunlight. [Sprayed with vanillin with mobile phase EMW (top), BEA (center) and CEF (bottom). From left: Herbal tablets (HT) 0,3,6 months. Herbal capsules (HC) 0,3,6 months. Extract capsules (EC) 0,3,6 months. Liquid extract (LIQ) 0,3,6 months.]	91
Figure 3.3.8 Ginkgo samples stored at direct sunlight and high humidity sprayed with vanillin with mobile phase EMW (top), BEA (center) and CEF (bottom). From left: Herbal tablets (HT) 0,3,6 months. Herbal capsules (HC) 0,3,6 months. Extract capsules (EC) 0,3,6 months. Liquid extract (LIQ) 0,3,6 months.	92
Figure 3.3.9 HPLC chromatogram of quercetin standard solution C (0.06mg/ml) prepared in section 2.7.1.2	93
Figure 3.3.10 Quercetin standard curve.	94
Figure 3.3.11 Degradation of quercetin in Ginkgo herbal tablets at different storage conditions	96
Figure 3.3.12 Degradation of quercetin in Ginkgo herbal capsules at different storage conditions	97

Figure 3.3.13 Degradation of quercetin in Ginkgo extract capsules at different storage conditions	98
Figure 3.3.14 Degradation of quercetin in Ginkgo dried leaf powder at different radiation doses	99
Figure 3.4.1 TLC of Kava Kava root powder with mobile phase EMW (top), BEA (center) and CEF (bottom) and sprayed with <i>p</i> -anisaldehyde (left) and vanillin (right). Each plate shows extraction with Methanol (left), Acetone (center) and n-Hexane (right)	112
Figure 3.4.2 Kava Kava identification according to the British Herbal Pharmacopoeia	113
Figure 3.4.3 Kava Kava samples stored at 25°C. [Sprayed with <i>p</i> -Anisaldehyde with mobile phase BEA (top) and CEF (bottom). From left: Herbal tablets (HT) 0,3,6 months. Herbal capsules (HC) 0,3,6 months. Extract capsules (EC) 0,3,6 months. Liquid extract (LIQ) 0,3,6 months.]	117
Figure 3.4.4 Kava Kava samples stored at 40°C. [Sprayed with <i>p</i> -Anisaldehyde with mobile phase BEA (top) and CEF (bottom). From left: Herbal tablets (HT) 0,3,6 months. Herbal capsules (HC) 0,3,6 months. Extract capsules (EC) 0,3,6 months. Liquid extract (LIQ) 0,3,6 months.]	118
Figure 3.4.5 Kava Kava samples stored at 60°C. [Sprayed with <i>p</i> -Anisaldehyde with mobile phase BEA (top) and CEF (bottom). From left: Herbal tablets (HT) 0,2,4,6 weeks. Herbal capsules (HC) 0,2,4,6 weeks. Extract capsules (EC) 0,2,4,6 weeks.]	119
Figure 3.4.6 Kava Kava samples stored at 80°C. [Sprayed with <i>p</i> -Anisaldehyde with mobile phase BEA (top) and CEF (bottom). From left: Herbal tablets (HT) 0,2,4,6 weeks. Herbal capsules (HC) 0,2,4,6 weeks. Extract capsules (EC) 0,2,4,6 weeks.]	120

Figure 3.4.7 Kava Kava samples stored at direct sunlight. [Sprayed with <i>p</i> -Anisaldehyde with mobile phase BEA (top) and CEF (bottom). From left: Herbal tablets (HT) 0,3,6 months. Herbal capsules (HC) 0,3,6 months. Extract capsules (EC) 0,3,6 months. Liquid extract (LIQ) 0,3,6 months.]	121
Figure 3.4.8 Kava Kava samples stored at direct sunlight and high humidity. [Sprayed with <i>p</i> -Anisaldehyde with mobile phase BEA (top) and CEF (bottom). From left: Herbal tablets (HT) 0,3,6 months. Herbal capsules (HC) 0,3,6 months. Extract capsules (EC) 0,3,6 months. Liquid extract (LIQ) 0,3,6 months.]	122
Figure 3.4.9 HPLC chromatogram of Kava Kava root powder prepared in section 2.7.2.3.	123
Figure 3.4.10 Degradation of kava-lactones in Kava Kava herbal tablets at different storage conditions	125
Figure 3.4.11 Degradation of kava-lactones in Kava Kava herbal capsules at different storage conditions	127
Figure 3.4.12 Degradation of kava-lactones in Kava Kava extract capsules at different storage conditions	128
Figure 3.4.13 Kava Kava dried root powder at different radiation doses	130