
Investigation of Different Analytical Techniques for the Quantitation of Some Selected UV- Blockers in Cosmetic Products

**Thesis presented by
Neven Nabil Abdo Ahmed Mikawy**

B.Sc. in Pharmaceutical Sciences
Faculty of pharmacy - Ain Shams University
2012

Submitted for the partial fulfillment of
**Master Degree in Pharmaceutical Sciences
(Pharmaceutical Analytical Chemistry)**

Under the Supervision of:

Prof. Dr. Maha Farouk Abd El-Ghany
Professor of Pharmaceutical Analytical Chemistry
Vice Dean for Graduate Studies and Research
Faculty of Pharmacy - Ain Shams University

Associate Prof. Dr. Omar Abd El-Aziz Ali Ghonim
Associate Professor and Head of Pharmaceutical Analytical
Chemistry Department
Faculty of Pharmacy - Ain Shams University

Dr. Miriam Farid Ayad
Lecturer of Pharmaceutical Analytical Chemistry
Faculty of Pharmacy - Ain Shams University

**Pharmaceutical Analytical Chemistry Department
Faculty of Pharmacy - Ain Shams University
2015**

Approval Sheet

Investigation of Different Analytical Techniques for the Quantitation of Some Selected UV-Blockers in Cosmetic Products

Name of the Candidate:

Neven Nabil Abdo Ahmed Mikawy

Submitted to:

Pharmaceutical Analytical Chemistry Department, Faculty
of Pharmacy, Ain Shams University, 2015

Committee in Charge:

1) Prof. Dr. Soher Abd El-Fatah El-Weshahy

Professor of Pharmaceutical Analytical chemistry
Faculty of Pharmacy, Modern University for Science and Technology

2) Prof. Dr. Mokhtar Mohamed Mabrouk

Professor of Pharmaceutical Analytical chemistry
Faculty of Pharmacy, Tanta University

3) Prof. Dr. Maha Farouk Abd El-Ghany

Professor of Pharmaceutical Analytical Chemistry
Vice Dean for Graduate Studies and Research
Faculty of Pharmacy, Ain Shams University

4) Associate Prof. Dr. Omar Abd El-Aziz Ali Ghonim

Associate Professor and Head of Pharmaceutical Analytical Chemistry
Department, Faculty of Pharmacy, Ain Shams University

24/5/2015

Acknowledgment

All thanks and gratitude is to my lord Allah, who has given me the strength and will to finish this work. Words and thanks will never suffice to praise Allah for his mercy, blessings and guidance that lead me throughout the tough times.

It's my great pleasure to express my sincere thanks and grateful appreciation to **Prof. Dr. Maha Farouk Abd El-Ghany**, Professor of Pharmaceutical Analytical Chemistry, Faculty of pharmacy Ain Shams University, for her valuable advice, expert supervision, constructive comments, helpful suggestions, generous assistance, and for the extensive effort, time, energy and guidance during the supervision of this work. I will always be indebted to her kindness and continuous encouragement that had made my continuation in the hard times attainable.

My profound thanks are to **Associate Prof. Dr. Omar Abd El-Aziz Ghonim**, Associate Professor of Pharmaceutical Analytical Chemistry, Faculty of pharmacy, Ain Shams University, who was always supporting me with constructive advice, persistent encouragement and valuable assistance throughout the whole work. He enriched me with spiritual power, capabilities and enthusiasm to achieve the goals I've been longing for.

I also like to express my sincere thanks to **Dr. Miriam Farid Ayad**, Lecturer of Pharmaceutical Analytical chemistry, Faculty of Pharmacy, Ain Shams University, for her keen interest, Kind guidance, constant advice and sincere help in all stages of the work.

I am very grateful to my parents, and sister for standing by my side in every step, their continuous support and encouragement all the way through and giving me all the strength and faith I've ever needed.

Finally my sincere appreciation and thanks go to all my colleagues and staff members in Pharmaceutical Analytical chemistry Department, for their friendly cooperation and constant support.

Neven Nabil

بسم الله الرحمن الرحيم

(قالوا سبحانك لا علم لنا الا ما علمتنا
انك انت العليم الحكيم)

صدق الله العظيم

سورة البقرة (32)

LIST OF ABBREVIATIONS

●ACN	Acetonitrile
●AUC	Area under curve
●AVO	Avobenzene
●BDS	Base Deactivated Silica
●BS	Benzyl Salicylate
●CD	Cyclodextrin
●K'	Capacity Factor
●DHHB	Diethyl amino hydroxyl benzoyl hexyl benzoate
●DHB	Dihydroxybenzophenone
●DAD	Diode Array Detector
●DDRD	Double divisor ratio spectra derivative
●°C	Degree Celsius
●DRZC	Derivative ratio spectra zero crossing
●HETP	Height Equivalent to Theoretical Plate
●HPLC	High performance liquid chromatography
●HS	Homo Salicylate
●ICH	International Conference on Harmonization
●LC-MS	Liquid Chromatography-Mass Spectroscopy
●LOD	Limit Of Detection
●LOQ	Limit Of Quantitation
●m/z	Mass to charge ratio
●MCR	Mean centering ratio spectra
●mp	Melting point
●MP	Methyl paraben
●MBC	4-methylbenzylidene camphor
●min	Minute
●ODS	Octadecylsilyl groups
●OMC	Octinoxate
●OCR	Octocrylene
●OS	Octyl salicylate
●OXY	Oxybenzone
●PABA	Para Amino Benzoic Acid
●PLS	Partial least square
● % R	Percentage Recovery
●PS	Phenyl Salicylate

●p53	Phosphoprotein 53
●PCR	Principle component analysis
●PP	Propyl paraben
●PDA	Photodiode array
● α	Selectivity Factor
● $\Delta \lambda$	Spectral bandwidth
●SD	Standard Deviation
●SPF	Sun Protection Factor
●T	Tailing Factor
●THF	Tetrahydrofuran
●N	Theoretical Plate
●TLC	Thin layer chromatography
●UV	Ultraviolet
●UV/VIS	Ultraviolet/Visible
●ROS	Reactive Oxygen Species
●RSD	Relative Standard Deviation
●R _s	Resolution
●RP-HPLC	Reversed Phase-High Performance Liquid Chromatography
●RP-TLC	Reversed Phase-Thin Layer Chromatography
●RMSEC	Root mean square error of calibration
●RMSECV	Root mean square error of cross validation
●RMSP	Root mean square error of prediction

CONTENTS
PART I: Introduction and Literature Review

Section A
General Introduction

	Page
I.A.1. UV-Light	1
I.A.2. Skin damage.....	2
I.A.3. UV filters.....	2
I.A.3.1. Classification.....	2
I.A.3.2. UV-filters stability.....	5

Section B
Literature Review

I.B.1. Chemical structure and physicochemical properties.....	6
I.B.1.1. Octinoxate.....	6
I.B.1.2. Oxybenzone.....	6
I.B.1.3. Octocrylene.....	7
I.B.1.4. Avobenzone.....	7
I.B.1.5. 4-methyl Benzylidene Camphor.....	8
I.B.2. Methods of Analysis.....	8
I.B.2.1. Spectroscopic Methods.....	8
I.B.2.2. Electrochemical Methods.....	9
I.B.2.3. Chromatographic Methods.....	9
I.B.2.3.1. Thin Layer Chromatography.....	10
I.B.2.3.2. High performance Chromatography.....	11
I.B.2.3.3. Gas Chromatography.....	17

PART II: High Performance Liquid Chromatographic Methods

Section A

HPLC Method for Simultaneous Determination of AVO and MBC in Sunscreen Formulations and Rat Skin Tissue

II.A.1. Experimental.....	21
II.A.1.1. Instrumentation.....	21
II.A.1.2. Samples.....	21
II.A.1.3. Chemicals and reagents.....	22
II.A.1.4. Standard solutions.....	22
II.A.1.5. Procedure.....	22
II.A.1.5.1. Chromatographic conditions.....	22
II.A.1.5.2. Method validation.....	22
II.A.1.5.2.1. Linearity.....	22
II.A.1.5.2.2. Accuracy.....	23
II.A.1.5.2.3. Precision.....	23
II.A.1.5.2.4. Specificity.....	23
II.A.1.5.2.5. Robustness.....	24
II.A.1.5.2.6. Limit of detection (LOD) and limit of quantitation (LOQ).....	24
II.A.1.5.3. Application.....	24
II.A.1.5.3.1. Application to the sunscreen formulation.....	24
II.A.1.5.3.2. Application to the skin tissue.....	25
II.A.2. Results and Discussion.....	25
II.A.2.1. Mobile phase composition.....	25
II.A.2.2. Method validation.....	25

Section B

Stability-Indicating HPLC Method for Determination of OMC, OXY and OCR in Presence of Their ‘Acid, Alkaline and Oxidative-Degradates’ and Their Structure Elucidation

II.B.1. Experimental.....	37
---------------------------	----

II.B.1.1. Instrumentation.....	37
II.B.1.2. Samples.....	37
II.B.1.3. Chemicals and reagents.....	38
II.B.1.4. Standard solutions.....	38
II.B.1.5. Preparation of the degraded solutions (20.00 $\mu\text{g mL}^{-1}$)....	38
II.B.1.6. Procedure.....	39
II.B.1.6.1. Chromatographic conditions.....	39
II.B.1.6.2. Conditions of Liquid chromatography-mass spectrometry for structural elucidation.....	39
II.B.1.6.3. Method validation.....	39
II.B.1.6.3.1. Linearity.....	39
II.B.1.6.3.2. Accuracy.....	40
II.B.1.6.3.3. Precision.....	40
II.B.1.6.3.4. Specificity.....	40
II.B.1.6.3.5. Robustness.....	41
II.B.1.6.3.6. Limit of detection (LOD) and limit of quantitation (LOQ).....	41
II.B.1.6.4. Application to the sunscreen formulation.....	41
II.B.2. Results and Discussion.....	42
II.B.2.1. Mobile phase composition.....	42
II.B.2.2. Method validation.....	42
II.B.2.3. Structural elucidation of Octinoxate and Octocrylene degradates using Liquid chromatography-mass spectrometric technique and their proposed degradation pathways	44

Section C

Kinetic Study of Acid and Alkaline Degradations of OMC and OCR

II.C.1. Introduction.....	67
II.C.2. Experimental study.....	67
II.C.2.1. Instrumentation	67
II.C.2.2. Pure samples	67
II.C.2.3. Reagents	67
II.C.2.4. Standard solutions	67

II.C.2.5. Procedure.....	68
II.C.2.5.1. Studying the kinetic Order of the reaction.....	68
II.C.2.5.2. Studying the effect of acid or base concentration on the reaction rate.....	69
II.C.2.5.3. Studying the temperature effect on the reaction rate.....	69
II.C.3. Results and Discussion.....	70

PART III: Thin Layer Chromatographic Methods

Section A

TLC Densitometric Method for Simultaneous Determination of OMC, OXY and OCR in Sunscreen Formulations

III.A.1. Experimental.....	88
III.A.1.1. Instrumentation.....	88
III.A.1.2. Samples.....	88
III.A.1.3. Chemicals and reagents.....	88
III.A.1.4. Standard solutions.....	89
III.A.1.5. Procedure.....	89
III.A.1.5.1. Chromatographic conditions.....	89
III.A.1.5.2. Method validation.....	89
III.A.1.5.2.1. Linearity.....	89
III.A.1.5.2.2. Accuracy.....	90
III.A.1.5.2.3. Precision.....	90
III.A.1.5.2.4. Specificity.....	90
III.A.1.5.2.5. Robustness.....	90
III.A.1.5.2.6. Limit of detection (LOD) and limit of quantitation (LOQ).....	91
III.A.1.5.3. Application.....	91
III.A.1.5.3.1. Application to the sunscreen formulation.....	91
III.A.2. Results and Discussion.....	91
III.A.2.1. Mobile phase composition.....	92
III.A.2.2. Method validation.....	92

Section B
TLC Densitometric Method for Simultaneous Determination of
AVO and MBC in Sunscreen Formulation

III.B.1. Experimental.....	102
III.B.1.1. Instrumentation.....	102
III.B.1.2. Samples.....	102
III.B.1.3. Chemicals and reagents.....	102
III.B.1.4. Standard solutions.....	102
III.B.1.5. Procedure.....	103
III.B.1.5.1. Chromatographic conditions.....	103
III.B.1.5.2. Method validation.....	103
III.B.1.5.2.1. Linearity.....	103
III.B.1.5.2.2. Accuracy.....	103
III.B.1.5.2.3. Precision.....	104
III.B.1.5.2.4. Specificity.....	104
III.B.1.5.2.5. Robustness.....	104
III.B.1.5.2.6. Limit of detection (LOD) and limit of quantitation (LOQ).....	104
III.B.1.5.3. Application.....	105
III.B.1.5.3.1. Application to the sunscreen formulation.....	105
III.B.2. Results and Discussion.....	105
III.B.2.1. Mobile phase composition.....	105
III.B.2.2. Method validation.....	106

Section C
Stability-Indicating TLC Spectrodensitometric Method for
Determination of OMC, OXY and OCR in Presence of Their
‘Acid, Alkaline and Oxidative-Degradates’

III.C.1. Experimental.....	115
III.C.1.1. Instrumentation.....	115
III.C.1.2. Samples.....	115
III.C.1.3. Chemicals and reagents.....	115
III.C.1.4. Standard solutions.....	116

III.C.1.5. Preparation of the degraded solutions ($120.00\mu\text{g mL}^{-1}$)	116
III.C.1.6. Procedure.....	116
III.C.1.6.1. Chromatographic conditions.....	116
III.C.1.6.2. Conditions of Liquid chromatography-mass spectrometry for structural elucidation.....	117
III.C.1.6.3. Method validation.....	117
III.C.1.6.3.1. Linearity.....	117
III.C.1.6.3.2. Accuracy.....	117
III.C.1.6.3.3. Precision.....	117
III.C.1.6.3.4. Specificity.....	118
III.C.1.6.3.5. Robustness.....	118
III.C.1.6.3.6. Limit of detection (LOD) and limit of quantitation (LOQ).....	118
III.C.1.6.4. Application to the sunscreen formulation.....	118
III.C.2. Results and Discussion.....	118
III.C.2.1. Mobile phase composition.....	119
III.C.2.2. Method validation.....	119

PART IV: Spectrophotometric Methods

Section A

Derivative Ratio Spectra Zero Crossing, Double Divisor Ratio Spectra Derivative and Mean Centering Ratio Spectra Methods for Simultaneous Determination of OMC, OXY and OCR in Sunscreen Formulation

IV.A.1. Experimental.....	140
IV.A.1.1. Instrumentation.....	140
IV.A.1.2. Samples.....	140
IV.A.1.3. Chemicals and reagents.....	140
IV.A.1.4. Standard solutions.....	141
IV.A.1.5. Procedure.....	141
IV.A.1.5.1. Spectral characteristics of OMC, OXY and OCR... 141	
IV.A.1.5.2. Method validation.....	142
IV.A.1.5.2.1. Linearity.....	142

IV.A.1.5.2.2. Accuracy.....	142
IV.A.1.5.2.3. Precision.....	142
IV.A.1.5.2.4. Specificity.....	143
IV.A.1.5.2.5. Limit of detection (LOD) and limit of quantitation (LOQ).....	143
IV.A.1.5.3. Application to the sunscreen formulation.....	143
IV.A.2. Results and Discussion.....	143

Section B

Mean Centering Ratio Spectra, Area Under the Curve, Dual Wavelength and Ratio Subtraction Methods for Simultaneous Determination of AVO and MBC in Sunscreen Formulation

IV.B.1. Experimental.....	167
IV.B.1.1. Instrumentation.....	167
IV.B.1.2. Samples.....	167
IV.B.1.3. Chemicals and reagents.....	167
IV.B.1.4. Standard solutions.....	167
IV.B.1.5. Procedure.....	168
IV.B.1.5.1. Spectral characteristics of OMC, OXY and OCR....	168
IV.B.1.5.2. Method validation.....	169
IV.B.1.5.2.1. Linearity.....	169
IV.B.1.5.2.2. Accuracy.....	170
IV.B.1.5.2.3. Precision.....	170
IV.B.1.5.2.4. Specificity.....	170
IV.B.1.5.2.5. Limit of detection (LOD) and limit of quantitation (LOQ).....	170
IV.B.1.5.3. Application to the sunscreen formulation.....	171
IV.B.2. Results and Discussion.....	171

PART V: Chemometric Methods

Section A

Simultaneous Determination of OMC, OXY and OCR Using Partial Least Square (PLS) and Principle Component Regression (PCR)

V.A.1. Experimental.....	195
V.A.1.1. Instrumentation.....	195
V.A.1.2. Samples.....	195
V.A.1.3. Chemicals and reagents.....	195
V.A.1.4. Standard solutions.....	195
V.A.1.5. Procedure.....	195
V.A.1.5.3. Application to the sunscreen formulation.....	196
V.A.2. Results and Discussion.....	196

Section B

Simultaneous Determination of AVO and MBC Using Partial Least Square (PLS) and Principle Component Regression (PCR)

V.B.1. Experimental.....	209
V.B.1.1. Instrumentation.....	209
V.B.1.2. Samples.....	209
V.B.1.3. Chemicals and reagents.....	209
V.B.1.4. Standard solutions.....	209
V.B.1.5. Procedure.....	209
V.B.1.5.3. Application to the sunscreen formulation.....	210
V.B.2. Results and Discussion.....	210

PART VI

General Discussion

VI. General Discussion.....	221
-----------------------------	-----

	References	
VII. References.....		228

Arabic Summary