



Ain Shams University

Faculty of Pharmacy

Department of Pharmaceutical
Analytical Chemistry

ANALYTICAL STUDY ON CERTAIN NATURAL ANTIDIABETICS

THESIS

Presented for the Partial Fulfillment of Master's Degree in Pharmaceutical
Sciences
(Pharmaceutical Analytical Chemistry)

By

Hend Zaki Hassan Saleh

B.S.C Pharmaceutical Sciences, 2009
Faculty of Pharmacy
Ain Shams University

Under Supervision of

Prof. Dr. Maha Farouk Abdel-Ghany

Professor of Pharmaceutical Analytical Chemistry
Faculty of Pharmacy
Ain Shams University

Assoc. Prof. Dr. Lobna Abdel-Aziz Hussein

Associate Professor of Pharmaceutical Analytical Chemistry
Faculty of Pharmacy
Ain Shams University

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

2014

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

(وَمَا أُوتِيْتُمْ مِّنَ الْعِلْمِ إِلَّا قَلِيلًا)

صَدَقَ اللَّهُ الْعَظِيمُ

Approval Sheet

This Thesis was approved on 22/6/ 2014 by the committee in charge:

Prof. Dr. Fatth Allah Fatth Allah Belal

Professor of Pharmaceutical Analytical Chemistry

Faculty of Pharmacy

Mansoura University

Prof. Dr. Khadiga Mohammed Omar Kelani

Professor of Pharmaceutical Analytical Chemistry

Faculty of Pharmacy

Cairo University

Vice Dean & Head of Analytical Chemistry Department

Faculty of Pharmacy

MTI University

Prof. Dr. Maha Farouk Abdel-Ghany

Professor of Pharmaceutical Analytical Chemistry

Faculty of Pharmacy

Ain Shams University

Acknowledgements

First, I feel always grateful and intended to "**Allah**", the most kind and the most merciful.

I would like to express my deep thanks and gratitude to **Prof. Dr. Maha Farouk Abdel-Ghany**, Professor of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Ain Shams University, for her valuable supervision, continuous encouragement and effective support during this work.

My deep appreciation to **Assoc. Prof. Dr. Lobna Abdel-Aziz Hussein**, Associate Professor of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Ain Shams University, for her sincere help, guidance and continuous support during my work.

I am deeply grateful to **Prof. Dr. Sayed Badawy**, Professor of Chemistry, Chemistry Department, Faculty of Science, Cairo University, for his beneficial discussion, patience and helpful support concerning ion selective electrodes.

Special Thanks to **Dr. Nehad Amin**, PhD in Analytical Chemistry, Experimental and Advanced Pharmaceutical Research Unit (EAPRU), Faculty of Pharmacy, Ain Shams University, for her valuable support.

My deep thanks to all staff members and my colleagues in Pharmaceutical Analytical Chemistry Department, Ain Shams University, especially **Dr. Nancy Magdy**, **Dr. Mona Hamdy** and **Sherif Okil** for their friendly cooperation, helpful advice and sincere support.

I wish to express my special thanks to my husband, **Mohammed Hussien**, for his patience, kindness and continuous support to me. Special

thanks to my father in law, **Hussein Ahmed** and my sister **Marwa Zaki** for their continuous encouragement and help. Also, my lovely thanks to my sweet daughter, **Reem**, the source of happiness and optimism in my life.

Last but not least, **my parents**, the most valuable gift in my life. Words really could not express how I am grateful to them not only for continues support and encouragement to me in this work, but for everything they are doing to build a brighter tomorrow for me. Their endless care was the main cause standing behind every success in my life. Thanks for them being my parents.

Hend Zaki

Note

Besides the practical work carried on in this thesis, the candidate has attended and successfully passed the pre-master courses examination with the grades mentioned below:

Subject	Grade
Instrumental Analysis	Excellent
Physical Chemistry	Excellent
Computer Science	Excellent
Statistics	Excellent
Chromatography	Excellent
Selected Topics in Specialization	Excellent
Analytical Chemistry	Excellent
Spectrophotometry	Excellent
Electrochemistry	Excellent
General Grade	Excellent

Head of Pharmaceutical Analytical Chemistry Department

Prof. Dr. Amira Mabrouk El-Kosasy

Professor of Pharmaceutical Analytical Chemistry

Faculty of Pharmacy

Ain Shams University

List of Contents

List of Contents.....	I
List of Tables	XIII
List of Figures	XVII
List of Abbreviations	XXII
Preface	XXIII
Summary.....	XXIV

Part (1) General Introduction

I.1.	Introduction to Diabetes	2
I.1.1.	Definition of Diabetes.....	2
I.1.2.	Types of Diabetes	2
I.1.2.1.	Type 1 Diabetes	2
I.2.2.2.	Type 2 Diabetes	3
I.1.3.	Signs and Symptoms of Diabetes	3
I.1.4.	Complications of Diabetes	4
I.2.	Introduction to Herbal Drugs	4
I.2.1.	Importance of Herbal Drugs	4
I.2.2.	Factors Leading to Increased Growth of Herbal Drugs.....	5
I.2.3.	Quality Control of Herbal Drugs	5
I.2.4.	Natural Anti-Diabetics	7

Part (II) Literature Review

Section (A) Literature Review of Trigonelline

II.A.1.	Structure and Physical Properties	10
II.A.2.	Natural Sources	11
II.A.3.	Pharmacological Effects	12
II.A.4.	Mechanism of Action	12
II.A.5.	Thermal Stability	13
II.A.6.	Methods of Analysis	13
II.A.6.1.	Titrimetric Methods	13
II.A.6.2.	Spectroscopic Techniques	14
II.A.6.2.1.	Spectrophotometric Methods	14

II.A.6.2.2.	Proton and Carbon Nuclear Magnetic Resonance Spectroscopic Methods	14
II.A.6.3.	Flow Injection Analysis.....	17
II.A.6.4.	Voltammetric Methods	17
II.A.6.5.	Chromatographic Techniques.....	18
II.A.6.5.1.	High Performance Liquid Chromatographic Methods	18
II.A.6.5.2.	Gas Chromatographic Methods	23
II.A.6.5.3.	High Performance Thin Layer Chromatographic Methods	24
II.A.6.5.4.	Overpressure Layer Chromatographic Methods	25
II.A.6.6.	Capillary Electrophoretic Technique.....	26

Section (B) Literature Review of Gallic Acid

II.B.1.	Structure and Physical Properties	28
II.B.2.	Natural Sources	29
II.B.3.	Pharmacological Effects	30
II.B.4.	Mechanism of Action	30
II.B.5.	Thermal Stability.....	30
II.B.6.	Methods of Analysis	31
II.B.6.1.	Spectroscopic Techniques	31
II.B.6.1.1.	Colorimetric Methods	31
II.B.6.1.2.	Spectrofluorimetric Methods	31
II.B.6.2.	Flow Injection Analysis.....	32
II.B.6.3.	Electrochemical Techniques	33
II.B.6.3.1.	Polarographic and Voltammetric Methods	33
II.B.6.3.2.	Analyte Pulse Perturbation Methods	35
II.B.6.4.	Chromatographic Techniques	36
II.B.6.4.1.	High Performance Liquid Chromatographic Methods	36
II.B.6.4.2.	Gas Chromatographic Methods	40
II.B.6.4.3.	Thin Layer Chromatographic Methods	42
II.B.6.5.	Capillary Electrophoretic Technique.....	45

Part (III) Analytical Study on Trigonelline

Section (A) HPTLC Determination of Trigonelline

III.A.1.	Introduction	54
III.A.2.	Experimental.....	54
III.A.2.1.	Apparatus	54
III.A.2.2.	Samples	55
III.A.2.2.1.	Pure Sample	55
III.A.2.2.2.	Market Sample	55
III.A.2.3.	Chemicals and Reagents	55
III.A.2.4.	Standard Solution	56
III.A.2.4.1.	Trigonelline Standard Stock Solution	56
III.A.2.4.2.	Trigonelline Standard Working Solution	56
III.A.2.5.	Procedures	56
III.A.2.5.1.	Extraction Techniques	56
III.A.2.5.2.	Chromatographic Conditions	57
III.A.2.5.3.	Method Validation	57
III.A.2.5.3.1.	Linearity	57
III.A.2.5.3.2.	Limit of Detection and Limit of Quantification ..	58
III.A.2.5.3.3.	Accuracy	58
III.A.2.5.3.4.	Precision	58
III.A.2.5.3.4.1.	Repeatability	58
III.A.2.5.3.4.2.	Intermediate Precision	59
III.A.2.5.3.5.	Specificity	59
III.A.2.5.3.6.	Robustness	60
III.A.2.5.3.7.	System Suitability Testing	60
III.A.2.5.4	Application to <i>Trigonella foenum-graecum</i> Seeds Extract Analysis	60
III.A.3.	Results and Discussion.....	60
III.A.3.1.	Development of the Optimum Mobile Phase	60
III.A.3.2.	Method Validation	61
III.A.3.2.1.	Linearity	61
III.A.3.2.2.	Limit of Detection and Limit of Quantification ..	62
III.A.3.2.3.	Accuracy	62
III.A.3.2.4.	Precision	62
III.A.3.2.5.	Specificity	63
III.A.3.2.6.	Robustness	64
III.A.3.2.7.	System Suitability Testing	63
III.A.3.3.	Application to <i>Trigonella foenum-graecum</i> Seeds Extract Analysis	64

III.A.3.4.	Statistical Comparison between the Results of Analysis of Trigonelline by the Proposed HPTLC Method and the Reported HPTLC Method	64
III.A.4.	Conclusion.....	64

Section (B) Optimization of Microwave Assisted Extraction of Trigonelline from *Trigonella foenum-graecum* Seeds and Comparison with Other Conventional Techniques

III.B.1.	Introduction	77
III.B.2.	Advantages and Applications of Microwave Assisted Extraction.....	78
III.B.3.	Microwave Theory	78
III.B.4.	Microwave Assisted Extractor	80
III.B.2.	Experimental	81
III.B.2.1.	Apparatus.....	81
III.B.2.2.	Samples	81
III.B.2.2.1.	Pure Sample	81
III.B.2.2.2.	Market Sample	81
III.B.2.3.	Chemicals and Reagents	82
III.B.2.4.	Standard Solutions	82
III.B.2.4.1.	Trigonelline Hydrochloride Standard Stock Solution	82
III.B.2.4.2.	Trigonelline Hydrochloride Standard Working Solutions	82
III.B.2.5.	Procedures	82
III.B.2.5.1.	Extraction Techniques	82
III.B.2.5.1.1.	Microwave Assisted Extraction	83
III.B.2.5.1.2.	Heat Reflux Extraction	83
III.B.2.5.1.3.	Maceration Extraction	83
III.B.2.5.2.	Thermal Stability of Trigonelline	84
III.B.2.5.3.	Repeatability.....	84
III.B.2.5.4.	HPTLC Analysis.....	84
III.B.3.	Results and Discussion	84
III.B.3.1.	Optimization of Microwave Assisted Extraction Parameters	84

III.B.3.1.1.	Effect of Methanol Concentration	85
III.B.3.1.2.	Effect of Solid/Liquid Ratio	86
III.B.3.1.3.	Effect of Irradiation Time	87
III.B.3.1.4.	Effect of Irradiation Power	87
III.B.3.1.5.	Effect of Extraction Cycles	88
III.B.3.2.	Trigonelline Thermal Stability.....	88
III.B.3.3.	Comparison of Different Extraction Techniques..	89
III.B.3.4.	Repeatability	90
III.B.4.	Conclusion	90

Section (C) HPLC Determination of Trigonelline

III.C.1.	Introduction	99
III.C.2.	Experimental	100
III.C.2.1.	Apparatus	100
III.C.2.2.	Samples	101
III.C.2.2.1.	Pure Sample	101
III.C.2.2.2.	Market Sample	101
III.C.2.3.	Chemicals and Reagents	101
III.C.2.4.	Standard Solutions	101
III.C.2.4.1.	Trigonelline Hydrochloride Standard Stock Solution	101
III.C.2.4.2.	Trigonelline Hydrochloride Standard Working Solutions	102
III.C.2.5.	Procedures	102
III.C.2.5.1.	Extraction Techniques	102
III.C.2.5.2.	Chromatographic Conditions	102
III.C.2.5.3.	Method Validation	103
III.C.2.5.3.1.	Linearity	103
III.C.2.5.3.2.	Limit of Detection and Limit of Quantification ..	103
III.C.2.5.3.3.	Accuracy	104
III.C.2.5.3.4.	Precision	104
III.C.2.5.3.4.1.	Repeatability	104
III.C.2.5.3.4.2.	Intermediate Precision	105
III.C.2.5.3.5.	Specificity	105
III.C.2.5.3.6.	System Suitability Testing	105
III.C.2.5.3.7.	Robustness	105

III.C.2.5.4.	Application to <i>Trigonella foenum-graecum</i> Seeds Extract Analysis	106
III.C.3.	Results and Discussion	106
III.C.3.1.	Optimization of Chromatographic Conditions ...	106
III.C.3.2.	Method Validation	107
III.C.3.2.1.	Linearity	107
III.C.3.2.2.	Limit of Detection and Limit of Quantification .	108
III.C.3.2.3.	Accuracy.....	108
III.C.3.2.4.	Precision	108
III.C.3.2.5.	Specificity	108
III.C.3.2.6.	System Suitability Testing	109
III.C.3.2.7.	Robustness	109
III.C.3.3.	Application to <i>Trigonella foenum-graecum</i> Seeds Extract Analysis	109
III.C.3.4.	Statistical Comparison between the Results of Analysis of Trigonelline by the Proposed HPLC Method and the Reported HPTLC Method	110
III.C.4.	Conclusion	110

Section (D) Comparative Study of Calix-6-arene, 2-Hydroxy Propyl β -Cyclodextrin and 18-Crown-6 as Ionophores in Potentiometric Ion-Selective Electrodes for Determination of Trigonelline

III.D.1.	Introduction	119
III.D.2.	Experimental	121
III.D.2.1.	Apparatus	121
III.D.2.2.	Samples	121
III.D.2.2.1.	Pure Sample	121
III.D.2.2.2.	Market Sample	122
III.D.2.3.	Chemicals and Reagents	121
III.D.2.4.	Standard Solutions	122
III.D.2.4.1.	Trigonelline Hydrochloride Standard Stock Solution	123
III.D.2.4.2.	Trigonelline Hydrochloride Standard Working Solutions	123
III.D.2.5.	Procedures	123
III.D.2.5.1.	Electrodes Fabrication.....	123

III.D.2.5.2.	Electrodes Calibration	124
III.D.2.5.3.	Study of Experimental Conditions	124
III.D.2.5.3.1.	Identification of Slope, Response Time and Operative Life of the Studied Sensor	124
III.D.2.5.3.2.	Effect of pH	125
III.D.2.5.3.3.	Effect of Temperature	125
III.D.2.5.3.4.	Electrodes Selectivity	125
III.D.2.5.4.	Direct Potentiometric Determination of Trigonelline Hydrochloride in <i>Trigonella</i> <i>foenum-graecum</i> Seeds Extract	126
III.D.3.	Results & Discussion	127
III.D.3.1.	Electrodes Fabrication	127
III.D.3.2.	Electrodes Performance Characteristics	128
III.D.3.3.	Effect of pH & Temperature	130
III.D.3.4.	Sensor Selectivity	130
III.D.3.5.	Direct Potentiometric Determination of Trigonelline in <i>Trigonella foenum-graecum</i> Seeds Extract	130
III.D.3.6.	Figures of Merit	131

Part (IV) Analytical Study on Gallic Acid

Section (A) HPLC Determination of Gallic Acid

IV.A.1.	Introduction.....	142
IV.A.2.	Experimental.....	142
IV.A.2.1.	Apparatus	142
IV.A.2.2.	Samples	142
IV.A.2.2.1.	Pure Sample	142
IV.A.2.2.2.	Market Sample	143
IV.A.2.3.	Chemicals and Reagents	143
IV.A.2.4.	Standard Solutions	143
IV.A.2.4.1.	Gallic Acid Standard Stock Solution	143
IV.A.2.4.2.	Gallic Acid Standard Working Solutions	144
IV.A.2.5.	Procedures	144
IV.A.2.5.1.	Extraction Technique	144
IV.A.2.5.2.	Chromatographic Conditions	144
IV.A.2.5.3.	Method Validation	145
IV.A.2.5.3.1.	Linearity	145

IV.A.2.5.3.2.	Limit of Detection and Limit of Quantification...	146
IV.A.2.5.3.3.	Accuracy	146
IV.A.2.5.3.4.	Precision	146
IV.A.2.5.3.4.1.	Repeatability	146
IV.A.2.5.3.4.2.	Intermediate Precision	147
IV.A.2.5.3.5.	Specificity	147
IV.A.2.5.3.6.	System Suitability Testing	147
IV.A.2.5.3.7.	Robustness	147
IV.A.2.5.4.	Application to <i>Acacia arabica</i> Bark Extract Analysis	148
IV.A.3.	Results and Discussion	148
IV.A.3.1.	Development of the Optimum Mobile Phase	148
IV.A.3.2.	Method Validation	149
IV.A.3.2.1.	Linearity	149
IV.A.3.2.2.	Limit of Detection and Limit of Quantification ...	149
IV.A.3.2.3.	Accuracy	149
IV.A.3.2.4.	Precision	149
IV.A.3.2.5.	Specificity	150
IV.A.3.2.6.	System Suitability Testing	150
IV.A.3.2.7.	Robustness	150
IV.A.3.3.	Application to <i>Acacia arabica</i> Bark Extract Analysis	150
IV.A.3.4.	Statistical Comparison between the Results of Analysis of Gallic Acid by the Proposed HPLC Method and the Reported HPTLC Method	151
IV.A.4.	Conclusion	151

Section (B) Optimization of Microwave Assisted Extraction of Gallic Acid from *Acacia arabica* Bark and Comparison with Other Conventional Techniques

IV.B.1.	Introduction.....	160
IV.B.2.	Experimental.....	161
IV.B.2.1.	Apparatus	161
IV.B.2.2.	Samples	161
IV.B.2.2.1.	Pure Sample	161
IV.B.2.2.2.	Market Sample	161
IV.B.2.3.	Chemicals and Reagents	161

IV.B.2.4.	Standard Solution	161
IV.B.2.4.1.	Gallic Acid Standard Stock Solution	161
IV.B.2.4.2	Gallic Acid Standard Working Solutions.....	161
IV.B.2.5.	Procedures	162
IV.B.2.5.1.	Extraction Techniques	162
IV.B.2.5.1.1.	Microwave Assisted Extraction	162
IV.B.2.5.1.2.	Heat Reflux Extraction	162
IV.B.2.5.1.3.	Maceration Extraction	163
IV.B.2.5.2.	Thermal Stability of Gallic Acid	163
IV.B.2.5.3.	Repeatability.....	163
IV.B.2.5.4.	High performance Liquid Chromatographic Analysis.....	163
IV.B.3.	Results and Discussion	163
IV.B.3.1.	Optimization of Microwave Assisted Extraction Parameters	163
IV.B.3.1.1.	Effect of Methanol Concentration	164
IV.B.3.1.2.	Effect of Solid/Liquid Ratio	164
IV.B.3.1.3.	Effect of Irradiation Time	165
IV.B.3.1.4.	Effect of Irradiation Power	165
IV.B.3.1.5.	Effect of Extraction Cycles	166
III.B.3.2.	Gallic Acid Thermal Stability.....	166
IV.B.3.3.	Comparison of Different Extraction Techniques ...	167
IV.B.3.4.	Repeatability.....	167
IV.B.4.	Conclusion.....	167

Section (C): Determination of Gallic Acid by Synchronous Spectrofluorimetry

IV.C.1.	Introduction.....	175
IV.C.2.	Experimental	176
IV.C.2.1.	Apparatus	176
IV.C.2.2.	Samples	176
IV.C.2.2.1.	Pure Sample	176
IV.C.2.2.2.	Market Sample	177
IV.C.2.3.	Chemicals and Reagents	177
IV.C.2.4.	Standard Solution	177
IV.C.2.4.1.	Gallic Acid Standard Stock Solution	177
IV.C.2.4.2.	Gallic Acid Standard Working Solution	177