ANALYTICAL STUDIES OF SOME AMINOGLYCOSIDE AND MACROLIDE ANTIBIOTICS

A Thesis Presented by

Ehab Farouk Hassan El-Kady

B. Pharm. Sci. (1997)

M. Pharm. Sci. (2006)

Submitted for

The Degree of Doctor of Philosophy in Pharmaceutical Sciences

(Pharmaceutical Chemistry)

Supervised By

Prof. Dr. Sonia Talaat Hassib

Professor of Pharmaceutical Chemistry

Faculty of Pharmacy

Cairo University

Prof. Dr. Jos Hoogmartens

Professor of Pharmaceutical Chemistry

Faculty of Pharmaceutical Sciences

Katholieke University of Leuven-Belgium

Dr. Awatef El-Said Farag

Lecturer of Pharmaceutical Chemistry

Faculty of Pharmacy

Cairo University

Faculty of Pharmacy

Cairo University

2009

ACKNOWLEDGEMENT

*First and foremost, I am thankful to "**Allah**", the most merciful and the most generous.*

*I would like to express my deepest appreciation and sincere gratitude to **Prof. Dr. Sonia Talaat Hassib**, Professor of Pharmaceutical Chemistry , Faculty of Pharmacy, Cairo University, for suggesting the work, kind supervision, great effort, valuable advice and continuous support she kindly offered through all stages of the work.*

*I am deeply grateful to **Prof. Dr. Jos Hoogmartens**, Professor of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Katholieke University of Leuven-Belgium, for allowing me to work with his research group, and for his outstanding guidance that kept me on track. From the same laboratory, I sincerely thank **Prof. Dr. Erwin Adams**, for his constant support and guidance throughout my research. Parts I-B and I-C were carried out in this laboratory.*

*I wish to express my thanks to **Dr. Awatef El-Said Farag**, Lecturer of Pharmaceutical Chemistry, Faculty of Pharmacy, Cairo University, for her kind care, continuous support and cooperation.*

*I am grateful to **Dr. Mohammad Abdul-Azim**, Assistant Professor of Pharmaceutical Chemistry, Faculty of Pharmacy, Cairo University, for his contribution in part II-C of this thesis.*

Finally, I wish to extend my sincere thanks to my family who supported me to finish this work. I am also very grateful to all my professors and colleagues in the Pharmaceutical Chemistry Department, Faculty of Pharmacy, Cairo University, who supported me during this work.

Ehab F. El-Kady

2009

LIST OF ABBREVIATIONS

A	Absorbance
a	Intercept
AUP	Area Under the Peak
B	Slope
B.P.	British Pharmacopoeia
C	Concentration
C.I.	Confidence interval
CLS	Classical least-squares
cm	Centimeter
CT	Charge transfer
CZE	Capillary Zone Electrophoresis
DDQ	2,3-Dichloro 5,6-dicyano 1,4-benzoquinone
DMF	Dimethyl formamide
DMSO	Dimethyl sulfoxide
ELSD	Evaporative light scattering detection
Eq.	Equation
Eur.P.	European pharmacopoeia
F-C	Folin-Ciocalteau reagent
F-ratio	Variance ratio test
GC	Gas chromatography
HPLC	High performance liquid chromatography
HPTLC	High performance thin layer chromatography
K´	Capacity factor

l	Liter
LC	Liquid chromatography
M	Molar concentration
MBTH	3-Methylbenzothiazoline-2-one hydrazone
mg	Milligram
min.	Minute
Ml	Milliliter
mM	Millimolar concentration
MS	Mass spectroscopy
N	Number of theoretical plates
n	Number
ng	Nanogram (10^{-9} gram)
NP	Normal-phase
P=0.05	The probability of results in 95%
PCR	Principal component regression
PED	Pulsed electrochemical detection
PFPA	Pentafluoro propionic acid
PLS	Partial least squares regression
R	Resolution factor
r^2	regression coefficient
RP-HPLC	Reversed-phase high performance liquid chromatography
% R.S.D.	Relative standard deviation
S_a	Standard deviation of intercept
S_b	Standard deviation of slope

S.D.	Standard deviation
S.E.	Standard error
TCNQ	7,7,8,8-Tetracyanoquinodimethane
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
t_R	Retention time
t-test	Student "t" test
µg	Microgram
µl	Microliter
U.S.P.	United States Pharmacopoeia
UV	Ultraviolet
V	Volume
w	Weight
α	Separation factor
λ	Wavelength in nm

CONTENTS

Item	Page
Introduction	
Aminoglycoside and Macrolide Antibiotics ..…….……..	1
Classification and mechanism of action of antibiotics ...……..…….	1
Aminoglycosides……..…….……..	2
Macrolide……..…….…….	4
Methods of analysis of clarithromycin ……...	9
Methods of analysis of erythromycin …….	10
Methods of analysis of roxithromycin ..…….	15
Methods of analysis of spiramycin …….……	18
Methods of analysis of streptomycin sulphate ……..…….	20
Methods of analysis of tobramycin …….……...	24
The cited drugs in this thesis:	
Clarithromycin ……..……...	28
Erythromycin …….……..	29
Roxithromycin ..…….……..	30
Spiramycin …….……..	31
Streptomycin sulphate ……..……	32
Tobramycin …….....	33
Aim & basis of the present investigation……..	34
Experimental and discussion	
General considerations …….	36
Part I: Liquid Chromatographic Part	

Introduction to liquid chromatography…… 37

I-A- Liquid Chromatograpic Methods with UV-Detection

I-A-1- Simultaneous RP-HPLC method for the determination of erythromycin stearate and trimethoprim…… 39

Principle…….…….. 39

I-A-1-i- Materials and reagents …..……...……... 39

I-A-1-ii- Apparatus …..…….……... 39

I-A-1-iii- Preparation of stock solutions …….…… 40

I-A-1-iv-Choice of mobile phase…….…..... 40

I-A-1-v- Preparation of calibration curves …….…… 41

I-A-1-vi-Determination of laboratory prepared mixture ……..…….. 44

I-A-1-vii- HPLC determination of erythromycin and trimethoprim in erythroprim tablets ……..……. 46

I-A-2- RP-HPLC method for the determination of spiramycin and metronidazole in mixture…….…… 49

Principle …….……..…….…….. 49

I-A-2-i- Materials and reagents ……..……...……. 49

I-A-2-ii- Apparatus**..**……..……..… 49

I-A-2-iii-Choice of mobile phase.. …….. 50

I-A-1-iv- Preparation of stocksolutions…… 51

I-A-2-v-Construction of calibration curves…….. 51

I-A-2-vi-Determination of laboratory prepared mixture…….……. 54

I-A-2-vii- HPLC determination of spiramycin in spirazole tablets ……. 56

I-A-2-viii- HPLC determination of metronidazole in spirazole tablets …….. 58

Discussion of Part I-A-

I-A-1- Simultaneous RP-HPLC method for the determination of erythromycin stearate and trimethoprim…… 60

I-A-2- RP-HPLC method for the determination of spiramycin and metronidazole in mixture……..……..…….. 65

I-B- Liquid Chromatograpic methods with pulsed electrochemical detection……...……..…… 70

I-B-1-Theoretical background ……. 70

I-B-1-1-The need of "pulsed" electrochemical detection…...…….. 70

I-B-1-2-Triple step potential-time waveform..…….…… 71

I-B-1-3-Waveform optimization…….……. 72

I-B-1-4-Electrochemical cell 73

I-B-1-5-Maintenance of the electrodes of the detector (Decade II)…..…… 75

I-B-2- Analysis of tobramycin using liquid chromatography with pulsed electrochemical detection……..…… 76

Principle…… 76

I-B-2-i- Apparatus 76

……..……..……

I-B-2-ii- Materials and reagents ……..…….……...…. 77

I-B-2-iii-Preparation of tobramycin standard solute……..... 78

I-B-2-iv- Preparation of tobramycin test solution ……..….. 78

I-B-2-1-LC-PED method I.....…… 78

Principle..... 78

I-B-2-1-i- Method development……..…… 79

I-B-2-1-ii- Construction of the calibration curve for the determination of tobramycin using LC-PED method I 81

I-B-2-1-iii- Repeatability of the method……..... 82

I-B-2-1-iv- Application of the method to tobrex eye drops ….…….…….…….…….…….…….…….…….…….…….…….…….…….…. 82

I-B-2-2-LC-PED method II using volatile additives ……... 83

Principle 83

I-B-2-2-i- Method development…….…… 83

I-B-2-2-ii- Construction of the calibration curve for the determination of tobramycin using LC-PED method II .....……. 84

I-B-2-2-iii- Repeatability of the method……... 85

I-B-2-2-iv- Application of the method to tobrex eye drops…… 85

I-B-2-3- LC-PED method III using volatile additives..... 86

Principle 86

I-B-2-2-i- Method development…….…… 86

I-B-2-3-ii- Construction of the calibration curve for the determination of tobramycin using LC-PED method III 88

I-B-2-3-iii- Robustness study……... 89

I-B-2-3-iv- Repeatability of the method…… 94

I-B-2-3-v- Application of the method to tobrex eye drops ….…… 94

I-B-2-3-vi- Attempts for the simultaneous analysis of tobramycin and

dexamethasone by LC-PED……. 96

Discussion of Part I-B-…….……... 97

LC-PED method I ……..… 99

LC-PED method II using volatile additives..……..……..…. 100

3.1.2. LC-PED method III using volatile additive…….……..……. 101

I-C- Capillary Electrophoresis……..……..…… 103

I-C-1-Theoretical background……..…… 103

I-C-1-i-Instrumentation ……..…..…… 103

I-C-1- ii-Capillary……..…….. 104

I-C-1-iii-Sample injection……..……..……. 104

I-C-1-iv- Detection techniques……..…… 104

I-C-1-v- Electroosmotic flow (EOF) ……..…. 105

I-C-1-vi- The electropherogram……..…. 105

I-C-1-vii-Aspects of quantitative analysis in CE ……. 106

I-C-1-viii- Different types of CE …….. 106

I-C-2- An approach to the separation and simultaneous determination of spiramycin and metronidazole by capillary zone electrophoresis……..…… 107

Principle……..… 107

I-C-2-i-Apparatus……. 107

I-C-2-ii-Materials and reagents…… 107

I-C-2-iii- Spiramycin stock solution ……..... 107

I-C-2-iv- Metronidazole stock solution……..... 108

I-C-2-v- L-Histidine stock solution (internal standard)……. 108

I-C-2-vi- Electrophoretic Conditions…… 108

I-C-2-vii- Preparation of the capillary..…….. 109

I-C-2-viii- Preliminary capillary conditioning...……. 109

I-C-2-ix- Daily conditioning ……..……. 109

I-C-2-x- Capillary between-run rinsing procedure …….……...……..…… 109

I-C-2-xi- Choice of the background electrolyte …….…….. 110

I-C-2-xii- Choice of the internal standard ..…….…… 110

I-C-2-xiii- Preparation of the calibration curves …….…… 111

I-C-2-xiv- Repeatability of the method…….. 113

Discussion of part I-C…….…….. 114

II- Spectroscopic Part..... 116

Materials..... 116

Apparatus..... 117

II-A-Derivative ratio part..... 118

II-A-1- Spectrophotometric method for the determination of erythromycin stearate and trimethoprim in mixture..... 118

Principle..... 118

II-A-1-i- Reagents……...…….. 118

II-A-1-ii- Erythromycin stearate stock solutions…… 119

II-A-1-iii- Trimethoprim stock solution…… 119

II-A-1-iv- Optimization of the experimental conditions for the reaction of erythromycin stearate with perchloric acid…….……. 119

II-A-1-iv-1-Determination of the wavelength of maximum absorption……... 119

II-A-1-iv-2-Effect of molarity of perchloric acid……. 120

II-A-1-iv-3- Effect of the amount of perchloric acid……... 121

II-A-1-iv-4-Effect of the reaction time…… 122

II-A-1-iv-5-Stability of the colour in methanol……..... 122

II-A-1-v-Determination of wavelength of measurements for the mixture of erythromycin stearate and trimethoprim…….. 123

II-A-1-vi- Preparation of calibration curves……. 125

II-A-1-vii- Spectrophotometric determination of erythromycin stearate in bulk using perchloric acid ……..…. 127

II-A-1-viii-Determination of the laboratory prepared mixture……..… 128

II-A-1-ix- Spectrophotometric method for the determination of erythromycin stearate and trimethoprim in "erythroprim" tablets…….. 130

II-A-2-Spectrophotometric method (DR_3) for the determination of roxithromycin in the presence of erythromycin 133

Principle..... 133

II-A-2-i- Reagents……..... 133

II-A-2-ii- Roxithromycin stock solution..... 133

II-A-2-iii- Erythromycin stock solution..... 133

II-A-2-iv- Determination of wavelength of measurements…….. 134

II-A-2-v- Preparation of calibration curve…… 136

II-A-2-vi- Determination of roxithromycin in the presence of erythromycin in the laboratory prepared mixture…….. 137

II-A-2-vii -Determination of roxithromycin in "roxid" tablets…… 138

II-A-3- Spectrophotometric method for the determination of spiramycin and metronidazole in mixture……..…. 140

Principle..... 140

II-A-3-i- Reagents……..... 140

II-A-3-ii- Spiramycin stock solution…….. 140

II-A-3-iii- Metronidazole stock solution……. 140

II-A-3-iv- Determination of wavelength of measurements…….. 141

II-A-3-v- Preparation of calibration curves…….. 143

II-A-3-vi-Determination of the laboratory prepared mixture……... 145

II-A-3-vii- Spectrophotometric method for the determination of spiramycin and metronidazole in "spirazole" tablets……..…… 147

Discussion of Part II-A-

II-A-1- Spectrophotometric method for the determination of erythromycin stearate and trimethoprim in mixture..... 150

II-A-2-Spectrophotometric method (DR_3) for the determination of roxithromycin in the presence of erythromycin..... 156

II-A-3-Spectrophotometric method for the determination of spiramycin and metronidazole in mixture..... 160

II-B-Chemometric Part

II-B-1-Simultaneous determination of erythromycin stearate and trimethoprim by chemometric methods……... 164

II-B-1-i- Reagents……..….. 164

II-B-1-ii- Erythromycin stearate stock solution…… 164

II-B-1-iii- Trimethoprim stock solution…….. 165

II-B-1-iv-Procedures…… 165

II-B-1-iv-A-Calibration…… 165

1-Construction of the training set……. 165

2- Pre-processing the data……. 166

3- Constructing the models……... 166

4- Selection of the optimum number of factors to build the PCR and PLS models……. 167

II-B-1-iv-B- Validation…….. 169

Construction of the validation set……..... 169

II-B-1-iv-C-Application of the proposed methods for the analysis of erythromycin stearate and trimethoprim in erythroprim tablets…… 170

II-B-2- Simultaneous determination of spiramycin and metronidazole by chemometric methods…….…….. 173

II-B-2- i-Reagents…….…. 173

II-B-2-ii- Spiramycin stock solution……..… 173

II-B-2-iii- Metronidazole stock solution……..... 173

II-B-2-iv-Procedures……... 173

II-B-2-iv-A-Calibration…….. 173

1-Construction of the training set…… 173

2- Pre-processing the data……..... 175

3- Constructing the models…….. 175

4- Selection of the optimum number of factors to build the PCR and PLS models……. 175

II-B-2-v-B- Validation…… 177

Construction of the validation set……. 177

II-B-2-vi-C-Application of the proposed methods for the analysis of spiramycin and metronidazole in spirazole tablets…….. 178

Discussion of Part II-B……..…. 181

Theory of CLS method……..… 182

Theory of ILS method……... 182

Theory of Principle Component Regression (PCR)…….. 184

Theory of Partial Least Squares (PLS)…….…. 185

II-B-1-Simultaneous determination of erythromycin stearate and trimethoprim by chemometric methods…….…….. 185

II-B-2- Simultaneous determination of spiramycin and metronidazole by chemometric methods……..... 189

II-C-Spectrophotometric Part

II-C-1-Spectrophotometric determination of roxithromycin using DDQ reagent (2,3-dichloro 5,6-dicyano 1,4-benzoquinone)…….…… 192

Principle……. 192

II-C-1-i-Reagents…… 192

II-C-1-ii-Preparation of roxithromycin stock solution…….…… 193

II-C-1-iii-Determination of the wavelength of maximum absorption..…… 193

II-C-1-iv- Effect of different solvents…….…… 194

II-C-1-v- Effect of the amount of reagent……..…… 195

II-C-1-vi-Effect of the reaction time…… 196

II-C-1-vii- Stability of the colour in acetonitrile…… 196

II-C-1-viii- Determination of the reaction stoichiometry…….…… 197

II-C-1-ix- Preparation of calibration curve…….…….. 198

II-C-1-x-Spectrophotometric determination of roxithromycin in bulk using DDQ …….