

**Analytical study on certain antihypertensive drugs containing amino
group**

Thesis

Presented for the partial fulfillment of

Master Degree in Pharmaceutical sciences

(Pharmaceutical Analytical Chemistry)

By

Michael Abdelmessih Nassif Ibrahim

B.SC. Pharmaceutical sciences 2009

Department of Pharmaceutical Analytical Chemistry

Faculty of Pharmacy

Ain Shams University

Under the supervision of

Prof. Dr. Lobna Abd El-Aziz Hussein

Professor and Head of Pharmaceutical Analytical Chemistry Department

Faculty of Pharmacy

Ain Shams University

The late Associate Prof. Dr. Omar Abd El-Aziz Ali Ghoneim

Associate Professor of Pharmaceutical Analytical chemistry

Faculty of Pharmacy

Ain Shams University

Associate Prof. Dr. Nancy Magdy Hanna

Associate Professor of Pharmaceutical Analytical chemistry

Faculty of Pharmacy

Ain Shams University

Pharmaceutical Analytical Chemistry Department

Faculty of Pharmacy – Ain Shams University

2019

Approval sheet

Title of the master Degree Thesis in Pharmaceutical Sciences

(Pharmaceutical Analytical Chemistry)

**Analytical study on certain antihypertensive drugs containing amino
group**

Name of the Candidate:

Michael Abdelmessih Nassif Ibrahim

Submitted to:

Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy,
Ain Shams University, 2019

Committee in Charge:

1. Prof. Dr. Sawsan Mohamed Amer.....

Professor of pharmaceutical Analytical chemistry, Faculty of pharmacy,
Cairo University.

2. Prof. Dr. Lobna Abdel-Aziz Hussein.....

Professor and head of pharmaceutical Analytical chemistry, Faculty of
pharmacy, Ain Shams University.

3. Associate Prof. Dr. Miriam Farid Ayad.....

Associate Professor of pharmaceutical Analytical chemistry, Faculty of
pharmacy, Ain Shams University.

4. Associate Prof. Dr. Nancy Magdy Hanna.....

Associate Professor of pharmaceutical Analytical chemistry, Faculty of
pharmacy, Ain Shams University.

Acknowledgement

All thanks and gratitude is to my lord who has given me the strength, patience and will to finish this work. Words and thanks will never suffice to praise my lord for his blessings and guidance.

I would like to express my deepest gratitude and sincere appreciation to **Prof. Dr. Lobna Abdel-Aziz**, Professor and head of Pharmaceutical Analytical Chemistry department, Faculty of pharmacy, Ain Shams University, for her kind and expert supervision, patience, supporting my desired topic help during the whole time of my work.

I am deeply indebted to **Dr. Nancy Magdy Hanna**, Associate professor of Pharmaceutical Analytical Chemistry department, Faculty of pharmacy, Ain Shams University, for her outstanding effort, valuable suggestions and unfailing help.

I would like also to express my appreciation to the late professor **Dr. Omar Abdel-Aziz Ali Ghoneim** for his support and help, may god rest his soul.

My sincere appreciation and thanks to **Eva Pharma Company** for the ultimate support and providing me with materials, reagents equipment funding and all what I needed to complete this work.

Last but not least, no appreciation nor gratitude could ever pay to **my family** for their long lasting help, love and support throughout my entire life specially my **father** and **mother**, and special thanks to my dear **wife Dr. Mary Atta** for her endless help, support and her belief in me.

Thank You

Michael Abdelmessih Nassif Ibrahim

Contents

Part I: General introduction

I.1. Hypertension	1
I.2. Antihypertensive drugs	2
I.2.1. Classification of antihypertensive drugs	2
I.2.1.1. Diuretics	2
I.2.1.2. Adrenergic System Inhibitors	2
I.2.1.3. Calcium Channel Antagonists	2
I.2.1.4. Converting Enzyme Inhibitors	3
I.2.1.5. Direct Vasodilators	3

Part II: Literature review

II.1. Azilsartan Medoxomil	4
II.1.1. Structure	4
II.1.2. Properties	4
II.1.3. Pharmacology	4
II.1.4. Methods of analysis.....	4
II.1.4.1. Spectroscopic techniques.....	5
II.1.4.1.1. Spectrophotometric methods	5
II.1.4.1.2. Spectrofluorimetric methods	5
II.1.4.2. Separation techniques.....	5
II.1.4.2.1. TLC methods	5
II.1.4.2.2. HPLC methods	6
II.2. Chlorthalidone.....	8
II.2.1. Structure	8
II.2.2. Properties	8
II.2.3. Pharmacology	8
II.2.4. Methods of analysis.....	8
II.2.4.1. Spectroscopic techniques.....	9
II.2.4.1.1. Spectrophotometric methods	9
II.2.4.1.2. Spectrofluorimetric methods	11

II.2.4.2. Separation techniques.....	11
II.2.4.2.1. TLC methods	11
II.2.4.2.2. HPLC methods	12
II.2.4.2.3. Capillary electrophoresis methods.....	14
II.3. Perindopril Arginine	15
II.3.1. Structure	15
II.3.2. Properties	15
II.3.3. Pharmacology	15
II.3.4. Methods of analysis.....	15
II.3.4.1. Spectroscopic techniques.....	16
II.3.4.1.1. Spectrophotometric methods	16
II.3.4.1.2. Spectrofluorimetric methods	17
II.3.4.2. Separation techniques.....	17
II.3.4.2.1. TLC methods	17
II.3.4.2.2. HPLC methods	18
II.4. Amlodipine Besylate.....	20
II.4.1. Structure	20
II.4.2. Properties	20
II.4.3. Pharmacology	20
II.4.4. Methods of analysis.....	21
II.4.4.1. Spectroscopic techniques.....	21
II.4.4.1.1. Spectrophotometric methods	21
II.4.4.1.2. Spectrofluorimetric methods	23
II.4.4.2. Electrochemical techniques.....	24
II.4.4.3. Separation techniques.....	25
II.4.4.3.1. TLC methods	25
II.4.4.3.2. HPLC methods	27
II.4.4.3.3. Capillary electrophoresis methods.....	30
Part III: UPLC methods for determination of some antihypertensive drugs coupled with LC-MS technique	
III.1. Introduction	32

Section A: Stability-indicating RP-UPLC method for simultaneous estimation of Azilsartan Medoxomil and Chlorthalidone in dosage forms and in presence of degradation products

III.A.1. Introduction.....	34
III.A.2. Experimental	34
III.A.2.1. Instrument.....	34
III.A.2.2. Materials	35
III.A.2.2.1. Pure standard	35
III.A.2.2.2. Pharmaceutical dosage form	35
III.A.2.2.3. Chemicals and reagents	35
III.A.2.2.4. Standard solutions	36
III.A.2.2.4.1. Stock solution preparation.....	36
III.A.2.2.4.2. Working solution preparation.....	36
III.A.2.3. Procedures	36
III.A.2.3.1. Chromatographic conditions.....	36
III.A.2.3.2. Method validation.....	37
III.A.2.3.2.1. Linearity and construction of calibration graphs.....	37
III.A.2.3.2.2. Accuracy	37
III.A.2.3.2.3. Precision	38
III.A.2.3.2.3.1. Repeatability	38
III.A.2.3.2.3.2. Intermediate precision.....	39
III.A.2.3.2.4. Limit of detection and limit of quantification	39
III.A.2.3.2.5. Specificity	40
III.A.2.3.2.5.1. Forced degradation	40
III.A.2.3.2.5.1.1. For Azilsartan Medoxomil	40
III.A.2.3.2.5.1.2. For Chlorthalidone	40
III.A.2.3.2.5.2. Placebo	41
III.A.2.3.3. Application to pharmaceutical formulation.....	42
III.A.3. Results and discussion.....	42
III.A.3.1. Validation of the method	44
III.A.3.1.1. Linearity and range.....	44
III.A.3.1.2. Limit of detection and limit of quantification	45

III.A.3.1.3. Accuracy	45
III.A.3.1.4. Precision	45
III.A.3.1.5. System suitability test	45
III.A.3.1.6. Robustness	46
III.A.3.1.7. Specificity	46
III.A.3.1.7.1. Forced degradation	46
III.A.3.1.7.1.1. For Azilsartan Medoxomil	46
III.A.3.1.7.1.2. For Chlorthalidone	48
III.A.3.1.7.2. Placebo	49
III.A.3.2. Structure elucidation for the degradation products by LC-MS.....	50
III.A.3.2.1. Azilsartan Medoxomil	50
III.A.3.2.2. Chlorthalidone.....	50
III.A.3.3. Application to pharmaceutical formulation.....	50
III.A.3.4. Statistical analysis of results.....	51
III.A.4. Conclusion	51

**Section B: Stability-indicating RP-UPLC method for the simultaneous estimation of
Perindopril Arginine and Amlodipine Besylate in pharmaceutical formulation in the
presence of their degradation products**

III.B.1. Introduction.....	77
III.B.2. Experimental	77
III.B.2.1. Instrument.....	77
III.B.2.2.4. Standard solutions.....	79
III.B.2.2.4.1. Stock solution preparation.....	79
III.B.2.2.4.2. Working solution preparation.....	79
III.B.2.3. Procedures.....	79
III.B.2.3.1. Chromatographic conditions.....	79
III.B.2.3.2. Method validation	80
III.B.2.3.2.1. Linearity and construction of calibration graphs	80
III.B.2.3.2.2. Accuracy	80
III.B.2.3.2.3. Precision	81
III.B.2.3.2.3.1. Repeatability	81

III.B.2.3.2.3.2. Intermediate precision	82
III.B.2.3.2.4. Limit of detection and limit of quantification	82
III.B.2.3.2.5. Specificity	83
III.B.2.3.2.5.1. Forced degradation	83
III.B.2.3.2.5.1.1. For Perindopril Arginine	83
III.B.2.3.2. 5.1.2. For Amlodipine Besylate	83
III.B.2.3.3. Application to pharmaceutical formulation	85
III.B.3. Results and discussion	85
III.B.3.1. Validation of the methods	87
III.B.3.1.1. Linearity and range	87
III.B.3.1.2. Limit of detection and limit of quantification	88
III.B.3.1.3. Accuracy	88
III.B.3.1.4. Precision	88
III.B.3.1.5. System suitability test	89
III.B.3.1.6. Robustness	89
III.B.3.1.7. Specificity	89
III.B.3.1.7.1. Forced degradation	89
III.B.3.1.7.1.1. For Perindopril Arginine	89
III.B.3.1.7.1.2. For Amlodipine Besylate	91
III.B.3.1.7.2. Placebo	92
III.B.3.2. Structure elucidation for the degradation products by LC-MS	92
III.B.3.2.1. Perindopril Arginine	92
III.B.3.2.2. Amlodipine Besylate	93
III.B.3.3. Application to pharmaceutical formulation	93
III.A.3.4. Statistical analysis of results	93
III.B.4. Conclusion	93

**Part IV: TLC-Densitometric method for the simultaneous estimation of Azilsartan
Medoxomil and Chlorthalidone in solid dosage forms**

IV.1. Introduction	129
IV.2. Experimental	130
IV.2.1. Instrument	130

IV.2.2. Materials.....	130
IV.2.2.1 Pure standard	130
IV.2.2.2 Pharmaceutical dosage form	130
IV.2.2.3 Chemicals and reagents	130
IV.2.2.4. Standard solutions.....	130
IV.2.2.4.1. Stock solution preparation.....	130
IV.2.2.4.2. Working solution preparation.....	131
IV.2.3.2.2. Accuracy	132
IV.2.3.2.3. Precision	133
IV.2.3.2.3.1. Repeatability	133
IV.2.3.2.3.2. Intermediate precision.....	133
IV.2.3.2.4. Limit of detection and limit of quantification	134
IV.2.3.2.5. Specificity	134
IV.2.3.2.5.1. Placebo	134
IV.2.3.3. Application to pharmaceutical formulation.....	135
IV.3. Results and Discussion	135
IV.3.1. Validation of the method	136
IV.3.1.1. Linearity and range.....	136
IV.3.1.2. Limit of detection and limit of quantification	136
IV.3.1.3. Accuracy	137
IV.3.1.4. Precision	137
IV.3.1.5. Specificity	137
IV.3.1.5.1. Placebo	137
III.A.3.2. Application to pharmaceutical formulation.....	137
III.A.3.3. Statistical analysis of results.....	137
IV.4. Conclusion	138

Part V: Spectroscopic determination of some antihypertensive drugs

Section A: Spectrophotometric Absorptivity factor method, Q absorbance ratio method and absorbance subtraction method for the simultaneous estimation of Azilsartan Medoxomil and Chlorthalidone in solid dosage forms

V.A.1. Introduction	148
---------------------------	-----

V.A.2. Theory	148
V.A.2.1. Theoretical background for Absorptivity factor method.....	148
V.A.2.2. Theoretical background for Q absorbance method	150
V.A.2.3. Theoretical background for Absorbance subtraction method	151
V.A.3. Experimental	152
V.A.3.1. Instrument	152
V.A.3.2. Materials	152
V.A.3.2.1. Pure standard.....	152
V.A.3.2.2. Pharmaceutical dosage form	152
V.A.3.2.3. Chemicals and reagents	153
V.A.3.2.4. Standard solutions	153
V.A.3.2.4.1. Stock solution preparation	153
V.A.3.3. Procedures	153
V.A.3.3.1. Spectral characteristics of AZL and CLT	153
V.A.3.3.2. Method validation.....	154
V.A.3.3.2.1. Linearity and construction of calibration graphs.....	154
V.A.3.3.2.2. Accuracy	154
V.A.3.3.2.3. Precision.....	155
V.A.3.3.2.3.1. Repeatability	155
V.A.3.3.2.3.2. Intermediate precision	155
V.A.3.3.2.4. Limit of detection and limit of quantification.....	156
V.A.3.3.2.5. Specificity	156
V.A.3.3.2.5.1. Placebo.....	156
V.A.3.3.2.6. Selectivity	157
V.A.3.3.3. Application to pharmaceutical formulation	158
V.A.3. Results and Discussion	158
V.A.3.1. Validation of the methods	159
V.A.3.1.1. Linearity and range	159
V.A.3.1.2. Limit of detection and limit of quantification.....	160
V.A.3.1.3. Accuracy	160
V.A.3.1.4. Precision.....	160

V.A.3.1.5. Selectivity	161
V.A.3.1.6. Specificity	161
V.A.3.1.6.1. Placebo	161
V.A.3.2. Application to pharmaceutical formulation	161
V.A.3.3. Statistical analysis of results	161
V.A.4. Conclusion	162
Section B: Spectrophotometric Absorptivity factor method, Q absorbance ratio method and absorbance subtraction method for the simultaneous estimation of Perindopril Arginine and Amlodipine Besylate in solid dosage forms	
V.B.1. Introduction	178
V.B.2. Theory	178
V.B.2.1. Theoretical background for Absorptivity factor method	178
V.B.2.2. Theoretical background for Q absorbance method	178
V.B.2.3. Theoretical background for Absorbance subtraction method	178
V.B.3. Experimental	178
V.B.3.1. Instrument	178
V.B.3.2. Materials	178
V.B.3.2.1. Pure standard	178
V.B.3.2.2. Pharmaceutical dosage form	179
V.B.3.2.3. Chemicals and reagents	179
V.B.3.2.4. Standard solutions	179
V.B.3.2.4.1. Stock solution preparation	179
V.B.3.3. Procedures	179
V.B.3.3.1. Spectral characteristics of PER and AML	179
V.B.3.3.2. Method validation	180
V.B.3.3.2.1. Linearity and construction of calibration graphs	180
V.B.3.3.2.2. Accuracy	180
V.B.3.3.2.3. Precision	181
V.B.3.3.2.3.1. Repeatability	181
V.B.3.3.2.3.2. Intermediate precision	181
V.B.3.3.2.4. Limit of detection and limit of quantification	182

V.B.3.3.2.5. Specificity	182
V.B.3.3.2.5.1. Placebo	182
V.B.3.3.2.6. Selectivity	183
V.B.3.3.3. Application to pharmaceutical formulation.....	184
V.B.3. Results and Discussion	184
V.B.3.1. Validation of the methods	185
V.B.3.1.1. Linearity and range	185
V.B.3.1.2. Limit of detection and limit of quantification	185
V.B.3.1.3. Accuracy	186
V.B.3.1.4. Precision	186
V.B.3.1.5. Selectivity	186
V.B.3.1.6. Specificity	187
V.B.3.1.6.1. Placebo	187
V.B.3.2. Application to pharmaceutical formulation.....	187
V.B.3.3. Statistical analysis of results	187
V.B.4. Conclusion	188
Section C: First derivative synchronous spectrofluorimetric method for the simultaneous estimation of Perindopril Arginine and Amlodipine Besylate in solid dosage forms	
V.C.1. Introduction	204
V.C.2. Experimental	205
V.C.2.1. Instrument.....	205
V.C.2.2. Materials	205
V.C.2.2.1. Pure standard	205
V.C.2.2.2. Pharmaceutical dosage form	205
V.C.2.2.3. Chemicals and reagents	205
V.C.2.2.4. Standard solutions	206
V.C.2.2.4.1. Stock solution preparation.....	206
V.C.2.3. Procedures	206
V.C.2.3.1 Spectral characterization	206
V.C.2.3.2. Method validation.....	206
V.C.2.3.2.1. Linearity and construction of calibration graphs.....	207

V.C.2.3.2.2. Accuracy	207
V.C.2.3.2.3. Precision	208
V.C.2.3.2.3.1. Repeatability	208
V.C.2.3.2.3.2. Intermediate precision.....	208
V.C.2.3.2.4. Limit of detection and limit of quantification	209
V.C.2.3.2.5. Specificity	209
V.C.2.3.2.5.1. Placebo	209
V.C.2.3.3. Application to pharmaceutical formulation.....	210
V.C.3. Results and Discussion	210
V.C.3.1. Validation of the methods	211
V.C.3.1.1. Linearity and range	211
V.C.3.1.2. Limit of detection and limit of quantification	211
V.C.3.1.3. Accuracy	211
V.C.3.1.4. Precision	212
V.C.3.1.5. Specificity	212
V.C.3.1.5.1. Placebo	212
V.C.3.2. Application to pharmaceutical formulation.....	212
V.C.3.3. Statistical analysis of results	212
VI.4. Conclusion	212
 Part VI: General discussion	
References.....	230
Summary in Arabic	250

List of Figures

Figure 1: UPLC chromatogram for AZL (199.24 µg/mL) and CLT (100 µg/mL) at 254nm	52
Figure 2: Calibration curve between the peak areas and the corresponding concentrations of AZL (79.69 - 318.78 µg/mL)	52
Figure 3: Calibration curve between the peak areas and the corresponding concentrations of CLT (40 - 160 µg/mL)	53
Figure 4: Peak purity for AZL (199.24 µg/ml) and CLT (100 µg/ml) using the UPLC proposed method	53
Figure 5: UPLC chromatogram of 199.24 µg/mL AZL in 1N HCl for 20 minutes at 50°C	54
Figure 6: UPLC chromatogram of 199.24 µg/mL AZL in 0.5N NaOH initially without heat	54
Figure 7: UPLC chromatogram of 199.24 µg/mL AZL in 3% H ₂ O ₂ for 20 minutes at 50°C	55
Figure 8: Degradation profile of AZL in 1N HCl, 0.5N NaOH and 3% H ₂ O ₂	55
Figure 9: UPLC chromatogram of 100 µg/mL CLT in 1N HCl for 90 minutes at 70°C.....	56
Figure 10: UPLC chromatogram of 100 µg/mL CLT in 0.5N NaOH for 90 minutes at 70°C	56
Figure 11: UPLC chromatogram of 100 µg/mL CLT in 3% H ₂ O ₂ for 90 minutes at 70°C.....	57
Figure 12: Degradation profile of CLT in 1N HCl, 0.5N NaOH and 3% H ₂ O ₂	57
Figure 13: UPLC chromatogram of AZL placebo	58
Figure 14: UPLC chromatogram of CLT placebo.....	58
Figure 15: LC-MS chromatograms for acid induced degradation products of AZL.....	59
Figure 16: LC-MS chromatograms for alkaline induced degradation products of AZL.....	60
Figure 17: LC-MS chromatograms for oxidation induced degradation products of AZL	61
Figure 18: LC-MS chromatograms for acid induced degradation products of CLT	62
Figure 19: LC-MS chromatograms for alkaline induced degradation products of CLT	62
Figure 20: LC-MS chromatograms for oxidation induced degradation products of CLT	63
Figure 21: UPLC chromatogram for PER (40 µg/mL) and AML (55.40 µg/mL) at 210nm	94
Figure 22: Calibration curve between the peak areas and the corresponding concentrations of PER (20 – 64 µg/mL)	94
Figure 23: Calibration curve between the peak areas and the corresponding concentrations of AML (27.70 – 88.64 µg/mL).....	95
Figure 24: Peak purity for PER (40 µg/ml) and AML (55.40 µg/ml) using the UPLC proposed method	95
Figure 25: UPLC chromatogram of 40 µg/mL PER in 1N HCl for 120 minutes at 70°C	96

Figure 26: UPLC chromatogram of 40 µg/mL PER in 1N NaOH for 20 minutes at 70°C	96
Figure 27: UPLC chromatogram of 40 µg/mL PER in 3% H ₂ O ₂ for 60 minutes at 70°C	97
Figure 28: Degradation profile of PER in 1N HCl, 1N NaOH and 3% H ₂ O ₂	97
Figure 29: UPLC chromatogram of 55.40 µg/mL AML in 1N HCl for 40 minutes at 70°C	98
Figure 30: UPLC chromatogram of 55.40 µg/mL AML in 1N NaOH for 40 minutes at 70°C	98
Figure 31: UPLC chromatogram of 55.40 µg/mL AML in 3% H ₂ O ₂ for 120 minutes at 70°C ...	99
Figure 32: Degradation profile of AML in 1N HCl, 1N NaOH and 3% H ₂ O ₂	99
Figure 33: UPLC chromatogram of PER placebo	100
Figure 34: UPLC chromatogram of AML in placebo	100
Figure 35: LC-MS chromatograms for acid induced degradation products of PER	101
Figure 36: LC-MS chromatograms for the Alkaline-induced degradation products of PER ...	102
Figure 37: LC-MS chromatograms for the Oxidation-induced degradation products of PER.	103
Figure 38: LC-MS chromatograms for the Acid-induced degradation products of AML	104
Figure 39: LC-MS chromatograms for the Alkaline-induced degradation products of AML ..	105
Figure 40: LC-MS chromatograms for the Oxidation-induced degradation products of AML	106
Figure 41: TLC densitometric chromatogram for 249.05 µg/Spot of AZL and 125 µg/Spot of CLT	139
Figure 42: AZL and CLT derivatization reaction with Phosphomolybdic acid	140
Figure 43: Time optimization for the derivatization reaction of 249.05 µg/Spot AZL and Phosphomolybdic acid	141
Figure 44: Time optimization for the derivatization reaction of 125 µg/Spot CLT and Phosphomolybdic acid	141
Figure 45: Stability of the developed compounds from derivatization reaction of AZL and Phosphomolybdic acid	142
Figure 46: Stability of the developed compounds from derivatization reaction of CLT and Phosphomolybdic acid	142
Figure 47: Calibration curve between the peak areas and the corresponding concentrations of AZL (99.62 – 348.67 µg/spot)	143
Figure 48: Calibration curve between the peak areas and the corresponding concentrations of CLT (50 – 175 µg/spot)	143
Figure 49: Zero order absorption spectrum for the iso absorptive point of 12.45 µg/mL AZL and 10.0 µg/mL CLT at 213 nm	163