ANALYTICAL STUDY OF SOME DRUGS CONTAINING CYCLIC NITROGEN

Introduction

Several drugs with different pharmacological actions share the cyclic nitrogen nucleus, including antihypertensive and antidiabetic drugs, which were the main focus in our study. During the past few years, most of medications introduced to the market as “polypill” tablets or capsules that combine multiple active pharmaceutical ingredients, especially those used for treatment of hypertension or diabetes mellitus. Under these circumstances, there are many advantages to patients and doctors in providing fixed combinations since it could help patients to adhere to recommended regimens, and overcome the risk of unexpected drug interaction. Various analytical techniques were used for the simultaneous analysis of such drug mixtures.

Abstract

The drug mixtures under study have been recently approved by the FDA to be marketed in their combined dosage form. The combinations of; (Amlodipine besylate - Olmesartan medoxomil - Hydrochlorothiazide), (Lercanidipine HCl - Enalapril maleate) and (Telmisartan - Amlodipine besylate) are prescribed as single dose polypill tablets for the clinical treatment of hypertension, while the combination (Saxagliptin HCl - Metformin HCl) is recommended for diabetes mellitus treatment. For their simultaneous determination, several methods were applied including spectrophotometric methods such as; derivative ratio spectra – zero crossing, double divisor derivative ratio spectra, mean centering and successive spectrophotometric resolution technique, dual wavelength, ratio subtraction, ratio difference and absorptivity factor methods, as well as chromatographic methods such as; High Performance Liquid Chromatographic Methods and Thin Layer Chromatographic methods.

Aim of this work

Our scientific motivation was to develop simple, reliable, fast and accurate analytical methods, which would be sensitive and specific for the determination of the studied drugs and to study the applicability of these methods in the routine analysis of
the studied drugs in pure form and in their pharmaceutical formulations in quality control laboratories.

**Summary**

This thesis consists of four parts in addition to references and an Arabic summary. Each part includes an introduction, literature review, descriptive experimental work for the studied drugs, results and discussion.

**Part I: Simultaneous Determination of Amlodipine besylate, Olmesartan medoxomil and Hydrochlorothiazide in Their Ternary Mixture** This part includes seven sections.

**Section A: Introduction and Literature Review**

This section includes an introduction about the pharmacological action of Amlodipine besylate (AM), Olmesartan medoxomil (OL) and Hydrochlorothiazide (HZ), their chemical structure, physical properties and summary of the published methods developed for their analysis in their ternary mixture.

**Section B: Simultaneous Determination of Amlodipine besylate, Olmesartan medoxomil and Hydrochlorothiazide by Direct Spectrophotometry Coupled with Derivative Ratio Spectra – Zero Crossing Method**

In this section, the simultaneous determination of three compounds in ternary mixtures is achieved by the measurements of the absorbance of the zero order spectra at max (for AM) and the peak amplitude at the zero - crossing points of the first and second derivative ratio spectra for HZ and OL, respectively. In the ternary mixture, the concentration of AM was proportional to the zero order absorbance at its max 364.6 nm, where no interference from either OL or HZ. The content of AM was calculated from the computed regression equation. The zero order absorption spectra of OL were divided by a standard spectrum of 20 µg/mL HZ and the second derivative of the ratio spectra was obtained. Similarly, the zero XXIII order absorption spectra of HZ were divided a standard spectrum of 20 µg/mL AM and the
first derivative of the ratio spectra was obtained. Two linear relationships were obtained using the peak amplitudes measured for OL at 254.4 nm (zerocrossing points for AM) and HZ at 308.6 nm (zero-crossing point for OL) versus their concentrations and the contents of OL and HZ were calculated from their corresponding regression equations. To assess the specificity of the developed method, different laboratory prepared mixtures of AM, OL and HZ were prepared and tested. The developed method was successfully applied for the quantification of the studied drugs in their pharmaceutical formulation and the standard addition technique has been applied to verify its validity. Statistical comparison with the official or reported methods showed no significant difference.

Section C: Simultaneous Determination of Amlodipine besylate, Olmesartan medoxomil and Hydrochlorothiazide by Direct Spectrophotometry Coupled with Double Divisor - Ratio Spectra Derivative Method

The determination of AM was carried out as previous section, using direct spectrophotometry. OL and HZ were determined by measuring the amplitude of the first derivative of the ratio spectra at 260.4 nm and 273.0 nm, respectively, which correspond to coinciding points. The sum of the spectra of 20 µg/mL HZ and 20 µg/mL AM as a ‘double divisor’ for determining OL and of 20 µg/mL OL and 20 µg/mL AM as a ‘double divisor’ for determining HZ in their ternary mixtures were found suitable. The developed method was successfully applied for the quantification of the studied drugs in their pharmaceutical formulation and the standard addition technique has been applied to verify its validity. Statistical comparison with the official or reported methods showed no significant difference.

Section D: Simultaneous Determination of Amlodipine besylate, Olmesartan medoxomil and Hydrochlorothiazide by Direct Spectrophotometry Coupled with Mean Centering Method

A recent and simple method was developed for the simultaneous determination of binary and ternary mixtures, without prior separation steps. In this method, the mean centered ratio spectra amplitudes at 252.0 nm and 256.0 nm were used for quantification of OL and HZ, respectively, while AM is determined as previously
mentioned in section B. It was used for determination of the parent compound in their pharmaceutical formulation. Statistical comparison with the official or reported methods showed no significant difference.

**Section E: Simultaneous Determination of Amlodipine besylate, Olmesartan medoxomil and Hydrochlorothiazide by Successive Spectrophotometric Resolution Technique**

This technique involved a series of steps in order to resolve the ternary mixture. Determination of AM was carried out as previously mentioned in section B, then the interference from AM is eliminated by ratio subtraction method. HZ determination was done by measuring the amplitude of the first derivative peak at 335.2 nm. The total concentration of OL and HZ was obtained at the isosbestic point at 260.0 nm, the concentration of OL was then obtained by subtraction. Statistical comparison with the official or reported methods showed no significant difference with respect to accuracy and precision.

**Section F: Simultaneous Determination of Amlodipine besylate, Olmesartan medoxomil and Hydrochlorothiazide by Thin Layer Chromatographic (TLC) - Densitometric Method**

In this section, a simple and accurate TLC – Densitometric method has been successfully applied for determination of the ternary mixture of AM, OL and HZ in bulk powder and in tablets. Quantitative determination of the separated bands of AM, OL and HZ was carried out at 254.0 nm using chloroform: methanol: acetone: formic acid (80: 15: 5: 0.3, by volume) as a developing system. The suggested TLC – Densitometric method was successfully applied for the quantification of the studied drugs in their pharmaceutical formulation and the standard addition technique has been applied to verify its validity. Statistical comparison with the official or reported methods showed no significant difference.

**Section G: Simultaneous Determination of Amlodipine besylate, Olmesartan medoxomil and Hydrochlorothiazide by High Performance Liquid Chromatographic (HPLC) Method**
A precise, specific, accurate HPLC method was proposed for the determination of AM, OL and HZ. In this method, an isocratic elution of the three components was performed at ambient temperature on C18 column with a mobile phase consisting of acetonitrile: phosphate buffer pH = 3.0 ± 0.1 (45: 55, v/v), at a flow rate of 1.0 mL/min and UV detection at 230.0 nm. Statistical comparison with the official or reported methods showed no significant difference with respect to accuracy and precision.

Part II: Simultaneous Determination of Lercanidipine HCl and Enalapril maleate in Their Binary Mixture

This part includes six sections.

Section A: Introduction and Literature Review

This section includes an introduction about the pharmacological action of Lercanidipine HCl (LER) and Enalapril maleate (ENA), their chemical structure, physical properties and summary of the published methods developed for their analysis in their single formulation and in their binary mixture.

Section B: Simultaneous Determination of Lercanidipine HCl and Enalapril maleate by Direct Spectrophotometry Coupled with Derivative Spectrophotometric Method

In this section, the simultaneous determination of two compounds in their binary mixture is achieved by the measurements of the absorbance of the zero order spectra at max (for LER) and the peak amplitude of the zero-crossing point of the second derivative spectra (for ENA). In the binary mixture, the concentration of LER was proportional to the zero order absorbance at its max 358.6 nm, where no interference from ENA, while concentration of ENA was proportional to the second derivative signals at 221.0 nm (zero crossing point for LER). The contents of LER and ENA were calculated from their corresponding regression equations. To assess the specificity of the developed method, different laboratory prepared mixtures of LER and ENA were prepared and tested. The developed method was successfully applied
for the quantification of the studied drugs in their pharmaceutical formulation and the standard addition technique has been applied to verify its validity. Statistical comparison with the official or reported methods showed no significant difference with respect to accuracy and precision.

**Section C: Simultaneous Determination of Lercanidipine HCl and Enalapril maleate by Direct Spectrophotometry Coupled with Dual Wavelength Method.**

In this section, LER was determined by direct spectrophotometry, as previously mentioned in section B. For the determination of ENA, two wavelengths (225.8 and 241.2 nm) were selected where the absorbance difference between the two wavelengths was directly proportional to the concentration of ENA, and the absorbance difference of LER at these wavelengths was zero. The content of ENA was calculated from the computed regression equation. The developed method was successfully applied for the quantification of the studied drugs in their pharmaceutical formulation and the standard addition technique has been applied to verify its validity. Statistical comparison with the official or reported methods showed no significant difference with respect to accuracy and precision.

**Section D: Simultaneous determination of Lercanidipine HCl and Enalapril maleate by Direct Spectrophotometry Coupled with Absorptivity Factor Method**

In this section, a recent and simple modification of isoabsorptive point method was applied for the simultaneous determination of the binary mixtures, without prior separation steps. LER was determined as previously mentioned in section B, while ENA was determined in this method by measuring the zero order absorption spectra of LER at 220.8 nm (absorptivity factor point) against its corresponding concentration. The total concentration (½ ENA + LER) was calculated by substituting in the computed regression equation. The concentration of ENA could be obtained after subtracting the concentration of LER and multiplying by 2 (F= ½). The developed method was successfully applied for the quantification of the studied drugs in their pharmaceutical formulation and the standard addition technique has been applied to verify its validity. Statistical comparison with the official or reported methods showed no significant difference with respect to accuracy and precision.
Section E: Simultaneous Determination of Lercanidipine HCl and Enalapril maleate by Mean Centering Method

In this section, mean centering method was successfully applied for the determination of LER and ENA, in their pure form and pharmaceutical formulations. For the determination of LER, the zero order absorption spectra were divided by standard spectrum of 10 µg/mL ENA and the obtained ratio spectra were then mean centered. The same procedure was applied for the determination of ENA using standard spectrum of 10 µg/mL LER as a divisor. Two linear relationships were obtained using the mean centered values measured at 292.0 nm and 210.0 nm for LER and ENA, respectively. The contents of LER and ENA were then calculated from the computed regression equations. The developed method was successfully applied for the quantification of the studied drugs in their pharmaceutical formulation and the standard addition technique has been applied to verify its validity. Statistical comparison with the official or reported methods showed no significant difference with respect to accuracy and precision.

Section F: Simultaneous Determination of Lercanidipine HCl and Enalapril maleate by High Performance Liquid Chromatographic (HPLC) Method

A precise, specific, accurate HPLC method was proposed for the determination of LER and ENA. In this method, an isocratic elution of the two components was performed at ambient temperature on C18 column with a mobile phase consisting of acetonitrile with mobile phase of methanol: phosphate buffer pH = 3.0 ± 0.1 (65: 35 v/v), using flow rate of 1.0 mL/min and UV detection at 209 nm. The developed method was successfully applied for the quantification of the studied drugs in their pharmaceutical formulation and the standard addition technique has been applied to verify its validity. Statistical comparison with the official or reported methods showed no significant difference with respect to accuracy and precision.

Part III: Simultaneous Determination of Telmisartan and Amlodipine besylate in Their Binary Mixture
This part includes seven sections.

**Section A: Introduction and Literature Review**

This section includes an introduction about the pharmacological action of Telmisartan (TEL) and Amlodipine besylate (AM), their chemical structure, physical properties and summary of the published methods developed for their analysis in their single formulation and in their binary mixture.

**Section B: Simultaneous Determination of Telmisartan and Amlodipine besylate by Direct Spectrophotometry Coupled with Dual Wavelength Method.**

AM was determined as previously mentioned in part I, section B. For the determination of TEL two wavelengths (266.0 and 335.0 nm) were selected where the absorbance difference between the two wavelengths was directly proportional to the concentration of TEL and the absorbance difference of AM at these wavelengths was zero. The content of TEL was calculated from the corresponding regression equation. The developed method was successfully applied for the quantification of the studied drugs in their pharmaceutical formulation and the standard addition technique has been applied to verify its validity. Statistical comparison with the official or reported methods showed no significant difference with respect to accuracy and precision.

**Section C: Simultaneous Determination of Telmisartan and Amlodipine besylate by Direct Spectrophotometry Coupled with Ratio Subtraction Method**

In this section, determination of AM was carried out as previously mentioned by direct spectrophotometry. The interference due to AM was then eliminated by applying the ratio subtraction method, by scanning the zero order absorption spectra of the laboratory prepared mixtures, dividing them by a carefully chosen concentration (25 µg/mL) of standard AM producing new ratio spectra. The absorbance in the plateau region (constant), where AM is extended, was then subtracted and followed by multiplication of the obtained spectra by the divisor. Finally, the original spectra of TEL could be obtained. TEL can be then directly determined at its absorption maxima (296.0 nm), and its concentration was calculated
from the corresponding regression equation. The developed method was successfully applied for the quantification of the studied drugs in their pharmaceutical formulation and the standard addition technique has been applied to verify its validity. Statistical comparison with the official or reported methods showed no significant difference with respect to accuracy and precision.

**Section D: Simultaneous Determination of Telmisartan and Amlodipine besylate by Ratio Difference Method**

In this section, the zero order absorption spectra of TEL and AM, in the region 200-345 nm, were divided by standard spectrum of 20 µg/mL AM and 10 µg/mL TEL, respectively. For the determination of TEL by this method, two selected wavelengths (245.0 and 294.8 nm) where the ratio spectra of the AM showed the same amplitudes (constant), whereas TEL ratio spectra showed significant difference at these two selected wavelengths. Similarly, another two wavelengths (244.2 and 266.8 nm) were selected for the determination of the AM. The calibration curves for each TEL and AM were constructed by plotting the amplitude ratio difference (ΔP_{245.0} – 294.8) and (ΔP_{244.2} – 266.8) for TEL and AM, respectively, versus the corresponding concentrations. The contents of TEL and AM were calculated from their computed regression equations. The developed method was successfully applied for the quantification of the studied drugs in their pharmaceutical formulation and the standard addition technique has been applied to verify its validity. Statistical comparison with the official or reported methods showed no significant difference with respect to accuracy and precision.

**Section E: Simultaneous Determination of Telmisartan and Amlodipine besylate by Mean Centering Method**

Mean centering method was successfully applied for the determination of TEL and AM without prior separation. The zero order absorption spectra of TEL and AM were divided by standard spectrum of 20 µg/mL AM and 10 µg/mL TEL, respectively, and the obtained ratio spectra were then mean centered. Two linear relationships were obtained using the mean centered values measured at 295.0 nm and 362.0 nm for TEL and AM, respectively, versus their corresponding concentrations. The contents of
TEL and AM were then calculated from the computed regression equations. The developed method was successfully applied for the quantification of the studied drugs in their pharmaceutical formulation and the standard addition technique has been applied to verify its validity. Statistical comparison with the official or reported methods showed no significant difference with respect to accuracy and precision.

Section F: Simultaneous Determination of Telmisartan and Amlodipine besylate by Thin Layer Chromatographic (TLC) - Densitometric Method

In this section, a simple and accurate TLC – densitometric method has been successfully applied for determination of TEL and AM in bulk powder and in tablets. Quantitative determination of the separated bands of TEL and AM was carried out at 240.0 nm using chloroform: methanol: acetone: formic acid (40: 35: 25: 0.3, by volume) as a developing system. The validity of the method has been further assessed by the application of the standard addition technique. Statistical comparison with the official or reported methods showed no significant difference with respect to accuracy and precision.

Section G: Simultaneous Determination of Telmisartan and Amlodipine besylate by High Performance Liquid Chromatographic (HPLC) Method

A precise, specific, accurate HPLC method was proposed for the determination of TEL and AM. In this method, an isocratic elution of the two components was performed at ambient temperature on C18 column with a mobile phase consisting of acetonitrile: phosphate buffer pH = 3.5 ± 0.1 (80: 20, v/v) and UV detection at 230.0 nm. The suggested HPLC method was successfully applied for the quantification of the studied drugs in their pharmaceutical formulation and the standard addition technique has been applied to verify its validity. Statistical comparison with the official or reported methods showed no significant difference with respect to accuracy and precision.

Part IV: Simultaneous Determination of Saxagliptin HCl and Metformin HCl in Their Binary Mixture
This part includes three sections.

**Section A: Introduction and Literature Review**

This section includes an introduction about the pharmacological action of Saxagliptin HCl (SAG) and Metformin (HCl), their chemical structure, physical properties and summary of the published methods developed for their analysis in their single formulation and in their binary mixture.

**Section B: Simultaneous Determination of Saxagliptin HCl and Metformin HCl by Thin Layer Chromatographic (TLC) - Densitometric Method**

In this section, a simple and accurate TLC – densitometric method has been successfully applied for determination of SAG and MET in bulk powder and in tablets. Quantitative determination of the separated bands of SAG and MET was carried out at 210.0 nm using chloroform: methanol: formic acid (80: 25: 0.3, by volume) as a developing system. The validity of the method has been further assessed by the application of the standard addition technique. Statistical comparison with the reported methods showed no significant difference with respect to accuracy and precision.

**Section C: Simultaneous Determination of Saxagliptin HCl and Metformin HCl by High Performance Liquid Chromatographic (HPLC) Method**

A precise, specific, accurate HPLC method was proposed for the determination of SAG and MET. In this method, an isocratic elution of the three components was performed at ambient temperature on C18 column with a mobile phase consisting of acetonitrile: phosphate buffer pH = 4.5 ± 0.1 (13: 87, v/v) and UV detection at 225.0 nm. The suggested HPLC method was successfully applied for the quantification of the studied drugs in their pharmaceutical formulation and the standard addition technique has been applied to verify its validity. Statistical comparison with the reported methods showed no significant difference with respect to accuracy and precision.

This thesis comprises 84 tables, 96 figures and 334 references.
**Conclusion**

This thesis discussed the analysis of four different mixtures; one ternary and three binary, in four separate parts. The problems raised upon the analysis of those mixtures were; the different chemical properties of the components, the different spectral profiles of the components which included partial or complete overlap and the different ratios of the components in the mixture.

The first part introduced the simultaneous analysis of Amlodipine besylate (AM), Olmesartan medoxomil (OL) and Hydrochlorothiazide (HZ) in Tribenzor® tablets in the ratio of 13.9: 40: 25. The spectrophotometric determination of the three components dealt with the determination of AM alone at its λmax due to the extension and partial overlap of its spectra with OL and HZ, while different manipulating techniques including; derivative ratio, double divisor, mean centering and successive spectrophotometric resolution were applied for OL and HZ. Two chromatographic techniques; TLC – densitometry and HPLC were applied successfully separation and quantification of the cited drugs.

The second part introduced the simultaneous analysis of Lercanidipine HCl and Enalapril maleate (ENA) in Zanipress® tablets in the ratio of 1: 1. The spectrophotometric determination of the two components dealt with the determination of LER alone at its λmax due to the extension and partial overlap of its spectra with ENA, while different manipulating techniques including; derivative, dual wavelength and absorptivity factor methods were applied for ENA. One spectrophotometric method, mean centering (MCN), was applied for both drugs in the mixture due to its simplicity and in addition to its high degree of sensitivity, accuracy and precision. One liquid chromatographic method was applied (HPLC) and the two drugs were successfully separated and quantified. Different developing systems were tried to separate LER and ENA on TLC plates, but complete separation of both drugs was not achieved as in HPLC.

The third part introduced the simultaneous analysis of Telmisartan (TEL) and AM in Twynsta® tablets in the ratio of 40: 13.9 and 80: 13.9. The same principle adopted in part I and II was applied in the spectrophotometric determination of both drugs. AM was determined directly at its λmax due to the extension and partial overlap of its spectra with TEL, while dual wavelength and ratio subtraction methods
were applied for the determination of TEL. In addition to another two spectrophotometric methods were applied to resolve both components in the binary mixture; ratio difference and mean centering methods, due to their simplicity and ease of their manipulation, in addition to their high degree of sensitivity, accuracy and precision. The previously mentioned spectrophotometric methods were successfully applied for the determination both drugs in their pure and combined dosage form in the ratio of 40: 13.9, while failed to determine them simultaneously in the other marketed dosage form in the ratio 80: 13.9. Chromatographic methods such as HPLC and TLC – densitometric methods were found to be convened for the determination of abnormal ratios of the drugs in their dosage form.

The fourth and the last part introduced the simultaneous determination of Saxagliptin HCl (SAG) and Metformin HCl (MET) in Kombiglyze® XR tablets in the ratio of 5.58: 1000. Spectrophotometric methods were found to be inconvenient for the determination of both drugs in their combined dosage form, due to huge difference between absorptivities of the two drugs and the abnormal ratio of the drugs in their dosage form, which made it difficult to determine SAG accurately. Two chromatographic techniques; HPLC and TLC – densitometric methods, were applied for the determination of both drugs in their pure and their combined dosage form.

Finally, all the proposed methods could be applied efficiently for the determination of the cited drugs in their pure and dosage form with satisfactory degree of accuracy and precision and could be easily applied in quality control laboratories.