ANALYTICAL STUDY OF SOME NITROGEN CONTAINING PHARMACEUTICAL PREPARATIONS

Presented by

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Summary

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

Part I: Determination of Hydrochlorothiazide and Spironolactone in Their Binary Mixture and in Presence of Their Impurities and Degradation Products

This part includes five sections.

Section (A): Introduction and Literature Review

This section includes an introduction about Hydrochlorothiazide (HCT) and Spironolactone (SPR and summary of the published methods developed for their analysis in their single formulation and in their binary mixture.

Section (B): Simultaneous Determination of Hydrochlorothiazide and Spironolactone by Different Spectrophotometric Methods

In this section, zero order curve (^{0}D), isosbestic spectrophotometry (ISO) and the recently developed ratio subtraction (RS) methods have been applied for determination of HCT and SPR in their binary mixture using 0.1N HCl as a solvent. HCT concentrations were determined by measuring the absorbance at its λ_{max} (317.2 nm). The absorbance at the two isosbestic points (λ_{iso1} (232.4 nm) and λ_{iso2} (257.6 nm)) were used for calculating the total mixture concentration and by subtraction, SPR could be determined. Ratio subtraction was also used for determination of SPR by measuring its absorbance at its λ_{max} (243.8 nm).

The developed methods were applicable for determination of the studied drugs in different laboratory prepared mixtures. No significant difference was found between the proposed methods and the reported one.

Section (C): Stability Indicating Genetic Algorithm Based Wavelength Selection Method for Determination of Hydrochlorothiazide and Spironolactone

A wavelength selection method based on genetic algorithms (GAs) was demonstrated and compared with the conventional PLS method. In GA method several parameters were adjusted and the optimum parameters setting were determined using experimental design. The proposed chemometric methods were successfully used for quantitation of the cited drugs along with their degradation products and impurity. The investigated chemometric methods were successfully applied for quantitation of the chosen drugs in commercial tablets. No significant difference between the developed methods and the reported one.

Section (D): Stability Indicating TLC-Densitometric Method for Determination of Hydrochlorothiazide and Spironolactone

This section is concerned with the development of sensitive, economic and specific stability indicating TLC-Densitometric method for determination of HCT and SPR in their bulk powder and pharmaceutical formulations as well as in the presence of the impurity and the degradation products. The five proposed components were well separated using ethyl acetate – chloroform -formic acid - triethyl amine (7: 3: 0.1: 0.1, by volume) as a developing system and the separated bands were scanned at 235nm.The developed TLC-Densitometric method was applicable for determination of the two drugs in their tablets.

Section (E): Stability Indicating RP-HPLC Method for Determination of Hydrochlorothiazide and Spironolactone

In this section, a simple, accurate and selective RP-HPLC with gradient elution has been investigated and validated for quantitative analysis of HCT and SPR in presence of the impurities and the degradates.

The chromatographic separation was achieved by elution programming using water- acetonitrile. The separation was performed on C_{18} column maintaining the flow rate at 2 mL/min and scanning the eluted components at 230 nm. The suggested method has been applied for determination of the two proposed components in commercial tablets.

Part II: Determination of Furosemide and Spironolactone in Their Binary Mixture and in Presence of Spironolactone Degradate

This part consists of seven sections.

Section (A): Introduction and Literature Review

This section includes an introduction about the pharmacological action of Furosemide (FUR) and Spironolactone (SPR), their chemical structure, physical properties and summary of the published methods developed for their analysis.

Section (B): Simultaneous Determination of Furosemide and Spironolactone by Different Spectrophotometric Methods

In this section, different spectrophotometric methods have been investigated for determination of FUR and SPR in their binary mixture.

The first derivative (^{1}D) amplitudes at 242.2 nm were used for determination of FUR concentrations, while the absorbance at λ_{iso1} (241.8 nm) and λ_{iso2} (259 nm) were used for calculating the total mixture concentration and by subtraction, SPR concentration in the mixture could be obtained. By ratio subtraction method, using the absorbance at λ_{max} (244.2 nm), SPR concentrations could be obtained. The developed methods were successfully applied for quantitation of the studied drugs in Lasilactone® tablets.

Section (C): Determination of Furosemide and Spironolactone in Presence of Spironolactone Degradate by Derivative and Derivative Ratio Spectrophotometric Methods

In this section, ^{2}DD and ^{3}D methods were introduced; where the two drugs could be analyzed without interference from Spironolactone degradation product.

FUR concentrations could be obtained by using the third derivative amplitudes at 239 nm. By dividing the spectrum of the mixture by the spectrum of 10 µg/mL FUR, SPR could be calculated using the second derivative of ratio spectra amplitudes at 262.2 nm. This method was applicable for determination of the active drugs in presence of up to 50 % of SPR Deg.

Section (D): Determination of Furosemide and Spironolactone in Presence of Spironolactone Degradate by Mean Centering of Ratio Spectra Spectrophotometric Method

A recent and simple method was developed for the simultaneous determination of binary and ternary mixtures. In this method, the mean centered second ratio spectra

amplitudes at 276, 275 and 271 nm were used for quantitation of FUR, SPR and SPR Deg, respectively. It was used for determination of the parent compounds in their pharmaceutical preparations. Statistical comparison with the reported spectrophotometric method showed no significant difference.

Section (E) : Determination of Furosemide and Spironolactone in Presence of Spironolactone Degradate by Multivariate Calibration Methods

Multivariate calibration models, such as PCR and PLS has been successfully applied as selective stability indicating methods for determination of the ternary mixture of FUR, SPR and SPR Deg.

To validate the predictive ability of the developed models, they were applied to predict the concentrations of FUR, SPR and SPR Deg in an external validation set. Statistical analysis with the reported method showed no significant difference.

Section (F): Determination of Furosemide and Spironolactone in Presence of Spironolactone Degradate by TLC-Densitometric Method

In this section, a simple and accurate TLC-Densitometric method has been suggested for the analysis of the binary mixtures of FUR and SPR in presence of SPR Deg. Quantitative determination of the separated bands of both FUR and SPR was carried out at 235 nm upon using ethyl acetate – chloroform - formic acid - triethyl amine (5: 5: 0.1: 0.15, by volume) as a developing system. The suggested TLC-Densitometric method was successfully applied for analysis of the cited drugs in pharmaceutical formulations.

Section (G): Determination of Furosemide and Spironolactone in Presence of Spironolactone Degradate by RP-HPLC Method

A precise, specific, accurate and stability indicating RP-HPLC method was proposed for the determination of FUR and SPR in the presence of SPR Deg. In this method, an isocratic elution of the three components was performed at ambient temperature on C_{18} column with a mobile phase consisting of acetonitrile- deionized water (35: 65, v/v), using flow rate of 1mL/ min and UV-detection at 240 nm. Statistical comparison with the reported spectrophotometric one showed no significant difference.

Part III: Determination of Hydrochlorothiazide and Carvedilol in Their Binary Mixture and in Presence of Hydrochlorothiazide Impurities

This part comprises five sections.

Section (A): Introduction and Literature Review

This section includes an introduction about the pharmacological action of Hydrochlorothiazide (HCT) and Carvedilol (CV), their chemical structure, physical properties and a summary of the published methods developed for their analysis in their single formulation and in their binary mixture.

Section (B): Determination of Hydrochlorothiazide and Carvedilol in Their Binary Mixture by Mean Centering of Ratio Spectra Spectrophotometric Method

A simple spectrophotometric method has been investigated for simultaneous determination of both HCT and CV. In this method, HCT was determined by measuring the amplitudes of the mean centered ratio spectra at 270 nm using 10 µg/mL of CV as a divisor. While the amplitudes at 242 nm of the mean centered ratio spectra produced upon using the normalized spectrum of HCT as a divisor were used for assaying CV. The proposed method was used for quantitation of both HCT and CV in Codilatrol® tablets.

Section (C): Spectrofluorimetric Determination of Carvedilol in Presence of Hydrochlorothiazide and Its Impurities

This section deals with the development of simple, sensitive and economic spectrofluorimetric method for the selective determination of CV in the presence of HCT and its impurities, with successive application to spiked human plasma.

In this method, the native fluorescence of aqueous solutions of CV in the range of 0.3 - 1.6 µg/mL at λ_{em} = 680 nm upon excitation at λ_{ex} = 250 nm was measured and used for calculation of CV without interference from its combined drug or from its impurities. The high sensitivity of the proposed Spectrofluorimetric method allowed the determination of cited drug in spiked human plasma. The suggested method was used for determination of CV in commercial tablets.

Section (D): Determination of Hydrochlorothiazide and Carvedilol in Their Binary Mixture and in Presence of Hydrochlorothiazide Impurities by Genetic Algorithm Based Wavelength Selection Method

Wavelength selection by GA has been applied for the analysis of quaternary mixture of HCT, CV, Chlorothiazide (CT) and Salamide (DSA) in their laboratory prepared mixtures. This method was demonstrated and compared with the

conventional PLS method. The suggested models were used for determination of the parent drugs in their commercial tablets with satisfactory results. The results obtained by applying the developed models showed no significant difference with the results of the reported one.

Section (E): Determination of Hydrochlorothiazide and Carvedilol in Their Binary Mixture and in Presence of Hydrochlorothiazide Impurities by TLC-Densitometric Method

In this section, a sensitive and economic specific stability indicating TLC-Densitometric method has been investigated for determination of HCT and CV. The optimum separation was achieved by using ethyl acetate- methanol-ammonia solution (10: 1: 0.5, by volume) as a developing system. The suggested method is applicable for determination of both drugs in their commercial tablets. No significant difference was found between this method and the reported one.

All the suggested methods were validated according to the ICH guidelines. At the end of each part, statistical comparison of the proposed methods was performed using one way ANOVA test.

Part IV: Appendix

This part includes a brief idea about the instruments, solvents and chemicals used in other parts. In addition to the detailed preparation of the solutions used in each part throughout this work and also method of preparation of SPR Deg.

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