Abstract

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

Part I: STABILITY INDICATING METHODS FOR DETRMINATION OF NICLOSAMIDE IN PRESENCE OF ITS DEGRADATION PRODUCTS

This part includes:

Introduction and Literature Review

This introduction describes the pharmacological action of Niclosamide, its chemical structure, physical properties and review of the published methods developed for its analysis.

Section (A): Stability Indicating Double Divisor-Ratio Spectra Spectrophotometric Method for Determination of Niclosamide With Kinetic Study of its Alkaline Degradation

In this section, double divisor ratio spectra spectrophotometric method was applied to determine intact Niclosamide in presence of its degradation products without prior separation. The absorption spectra of the solutions prepared of different concentrations of pure Niclosamide and the ternary mixture were recorded and divided by the absorption spectrum of the mixed solution of CNA and CSA with equal concentration as a double divisor. First derivative of these ratio spectra were obtained and then the peak amplitudes values at 243 nm were plotted against the corresponding concentrations of Niclosamide to obtain the calibration curve with the corresponding regression equation. The double divisor ratio spectra spectrophotometric method was used to determine the order of the alkaline degradation rate of Niclosamide by following the decrease in the concentration of the drug within 6 hours at one hour time interval. The kinetic of Niclosamide alkaline degradation was found to follow pseudo-first order reaction.

<u>Section (B):</u> Stability Indicating Modified Ratio Difference Spectrophotometric Method for Determination of Niclosamide

In this section modified ratio difference spectrophotometric method was proposed for determination of Niclosamide in presence of its degradation products, Different concentrations of Niclosamide have been divided by standard spectrum of 20 µg/mL CNA (as a divisor) to obtain division spectra, then the difference in the amplitude values at 244 and 283 nm were measured (at which CNA was constant and CSA has equal amplitudes). The suggested method was applied for determination of Niclosamide in its pharmaceutical formulations and the validity of the method was Furfuralther assessed by applying the standard addition technique.

<u>Section (C):</u> Stability Indicating Mean Centering of Ratio Spectra Spectrophotometric Method for Determination of Niclosamide

In this method the mean centered ratio spectra amplitudes at 234 nm was used for quantitation of Niclosamide in presence of its degradation products. The suggested method was applied for determination of Niclosamide in its pure form and pharmaceutical formulations. The validity of the method was Furfuralther assessed by applying the standard addition technique.

<u>Section (D):</u> Stability Indicating Multivariate Calibration Method for Determination of Niclosamide and Its Degradation Products (Related Substances)

Two chemometric techniques; principal component regression (PCR) and partial least squares (PLS) were used for the determination of Niclosamide in presence of its degradation products. Training set consisted of 25 mixtures containing different ratios of Niclosamide and its degradation products was used for construction of the models. The selectivity of the proposed method was checked using laboratory prepared mixtures (validation set consisted of eight mixtures). Satisfactory results were obtained upon applying the proposed methods for the analysis of Niclosamide in its pharmaceutical formulations.

Section (E): Stability Indicating TLC-Densitometric Method for Determination of Niclosamide and Its Degradation Products (Related Substances)

In this section TLC-densitometric method was used for separation of the drug from its degradation products. The proposed method was applied by using benzene: ethyl acetate: methanol: triethylamine (9:1:1:0.1, by volume) as a developing system. The bands measured quantitatively at 230 nm. The suitability of the proposed chromatographic method was Ascorbic Acidertained by the determination of system suitability parameters of the separated components. The suggested methods was successfully applied for the determination of Niclosamide in its pharmaceutical formulations.

Section (F): Stability Indicating and RP-HPLC-DAD Method for Determination of Niclosamide and Its Degradation Products (Related Substances)

In this section RP-HPLC-DAD method was used for determination of Niclosamide and its degradation products. RP-HPLC was achieved by using water (adjusted to pH 3.5 using glacial acetic acid) : acetonitrile (30:70, v/v) as a mobile phase, at a flow rate 1 mL/min, and DAD detection at 230 nm. The suitability of the proposed chromatographic method was Ascorbic Acidertained by the determination of system suitability parameters of the separated components. The suggested method was successfully applied for the determination of Niclosamide in its pharmaceutical formulations.

Part II: DETERMINATION OF HYDROXYZINE HYDROCHLORIDE, EPHEDRINE HYDROCHLORIDE AND THEOPHYLLINE

This part includes:

Introduction and Literature Review

This introduction comprises a brief idea about the structure, properties and different methods for the analysis of Theophylline either alone or in its ternary mixture with Hydroxyzine Hydrochloride and Ephedrine Hydrochloride.

Section (A): Zero Order Combined with Manipulating Ratio Spectrophotometric Methods for Simultaneous Determination of Hydroxyzine Hydrochloride, Ephedrine Hydrochloride and Theophylline

In this work a newly developed spectrophotometric method has been established utilizing ratio-subtraction and ^{3}D derivative spectrophotometry to resolve the overlap between the studied components. Theophylline can be determined directly at its λ_{max} 272 nm, (without any interference from Ephedrine Hydrochloride and Hydroxyzine Hydrochloride). For determination of Hydroxyzine Hydrochloride, different concentration of Hydroxyzine Hydrochloride were divided by the standard spectrum of 22 µg/mL Theophylline, then the peak amplitude of the obtained spectra were measured at 234.2 nm, while for determination of Ephedrine Hydrochoride, different concentrations of Ephedrine Hydrochloride were divided by the standard spectrum of 22 µg/mL Theophylline, then derivatized using $\Delta\lambda$=4 and scaling factor 100 to obtain ^{3}D spectra and measuring the peak amplitudes at 222 nm for ^{3}D spectra of Ephedrine Hydrochloride. The proposed method was successfully applied for determination of Hydroxyzine Hydrochloride, Ephedrine Hydrochloride and Theophylline in their pharmaceutical formulation (Bronchaline® tablets).

Section (B): Multivariate Calibration Method for Simultaneous Determination of Hydroxyzine Hydrochloride, Ephedrine Hydrochloride and Theophylline

Two chemometric techniques; principal component regression (PCR) and partial least squares (PLS) were used for simultaneous determination of Theophylline, Hydroxyzine Hydrochloride and Ephedrine Hydrochloride. Training set consisted of 25 mixtures of Theophylline, Hydroxyzine Hydrochloride and Ephedrine Hydrochloride, containing different ratios was used for construction of the models in the spectral region 210-230 nm. Seven mixtures were used as validation set; the proposed method was successfully applied for determination of the ternary mixture in their pharmaceutical formulation. Results obtained by the proposed method were statistically compared with that obtained by the reported method indicating no significant difference between them.

Section (C): TLC-Densitometric Method for Simultaneous Determination of Hydroxyzine Hydrochloride, Ephedrine Hydrochloride and Theophylline

In this section, a simple and accurate TLC-densitometric method was suggested for simultaneous determination of Theophylline, Hydroxyzine Hydrochloride and Ephedrine Hydrochloride in bulk powder and in pharmaceutical formulation. The method is based on the difference in R_f values of the cited drugs. Satisfactory separation was obtained by using chloroform: ammonium acetate buffer (9.5: 0.5, v/v) adjusting to pH 6.5 using ammonia solution as a developing system. The bands were scanned at 220 nm giving maximum sensitivity for the drugs. The proposed method was successfully applied for determination of the ternary mixture in their pharmaceutical formulation and validity was assessed by applying standard addition technique.

Part III: DETERMINATION OF SALICYLIC ACIDICYLAMIDE AND ASCORBIC ACIDORBIC ACID IN THEIR BINARY MIXTURE AND IN PRESENCE OF THEIR IMPURITIES

This part includes:

Introduction and Literature Review

This introduction describes the pharmacological action of Salicylic Acidicylamide, its chemical structure, physical properties and review of the published methods developed for it either alone or in presence of its binary mixture with Ascorbic Acidorbic acid.

Section (A): Area Under Curve and Dual Wavelength Spectrophotometric Methods for Simultaneous Determination of Salicylic Acidicylamide and Ascorbic Acidorbic acid in Their Binary Mixture

In this section, the Area under curve and dual wavelength spectrophotometric methods were developed for determination of Salicylic Acidicylamide and Asocrbic Acid in their binary mixture. In the simultaneous equations using AUC method, the absorptivity (Y) values of each of the two drugs were determined at the selected wavelength ranges, 225-

245 nm (λ_1-λ_2) and 265-285 nm (λ_3-λ_4). By applying Cramer's rule, concentrations of Salicylic Acidicylamide and Ascorbic Acidorbic Acid can be obtained. While The dual wavelength spectrophotometric method, the absorbance values of Ascorbic Acidorbic Acid are the same at 240.4 and 286.4 nm therefore these two wavelengths were selected for determination of Salicylic Acidicylamide. The same for the two wavelengths 249.8 and 285.8 nm, the absorbance values of Salicylic Acidicylamide are the same, hence those two wavelengths were selected for determination of Ascorbic Acidorbic Acid. The suggested methods were used for determination of Salicylic Acidicylamide and Ascorbic Acidorbic acid in their pharmaceutical formulation, where satisfactory results were obtained.

<u>Section (B):</u> Derivative Spectrophotometric Method Combined With Isoabsorptive Point or Ratio Subtraction Spectrophotometric Methods for Determination of Salicylamide and Ascorbic Acid in Their Binary mixture

In this section Salicylamide can be measured by ^{1}D spectra at which (Ascorbic Acid showed zero-crossing) at 315.4 nm, using $\Delta\lambda$= 4 nm and scaling factor=10. While determination of Asocrbic Acid could be achieved either by isoabsorptive point or by ratio-subtraction spectrophotometric method. The isoabsorptive point spectrophotometric method was applied by measuring the absorbance value at the chosen isoabsorptive point, the total concentration of the mixture could be calculated. By applying the suggested procedure the absorbance at 246.4 nm ($Aiso_1$) and 287 nm ($Aiso_2$) for Ascorbic Acid was obtained over different concentrations. Then the concentration of Ascorbic Acid could be calculated by subtraction of Salicylamide concentration from total mixture concentration. The ratio subtraction spectrophotometric method, the spectra of mixtures containing different concentration of Salicylamide and Ascorbic Acid were divided by the spectrum of 10 µg/mL of Salicylamide as a divisor. The amplitude value in the plateau region at λ above 304 nm was subtracted from the spectra of the divided mixtures; the obtained spectra were then multiplied by the spectrum of the divisor. Finally, Ascorbic Acid concentrations in laboratory prepared mixtures were measured from the last spectra obtained at its λ_{max}= 265.4 nm. The suggested methods were successfully applied for

determination of Salicylamide and Ascorbic Acid in their pure forms and in pharmaceutical formulation and the results obtained were statistically compared with the reported one.

Section (C): Double Divisor-Ratio Spectra Spectrophotometric Method for Determination of Salicylamide and Ascorbic Acid in presence of Their Impurities

In this section, double divisor ratio spectra spectrophotometric method has been successfully applied for determination of Salicylamide and Ascorbic Acid in presence of their potential impurities Salicylic Acid and Furfural, respectively. ^{1}D spectra were obtained using absorption spectrum of a mixture containing 5 µg/mL of each of Salicylic Acid and Furfural as a divisor. Salicylamide and Ascorbic Acid were quantitatively determined at 248 and 242.8 nm, respectively. The proposed method was successfully applied for determination of the suggested drugs in their pharmaceutical formulation; satisfactory results were obtained. The results obtained by the proposed method were compared to the reported HPLC method; they showed no significant difference regarding accuracy and precision.

Section (D): Multivariate Calibration Method for Determination of Salicylamide and Ascorbic Acid in presence of Their Impurities

Multivariate calibration models, such as PCR and PLS have been applied for determination of Salicylamide, Ascorbic Acid, Salicylic Acid and Furfural. Training set of 25 mixtures containing different ratios of the four proposed components was used for construction of the two models, Six mixtures were used as validation set. Satisfactory results were obtained on applying the proposed methods for the analysis of Salicylamide and Ascorbic Acid in their pharmaceutical formulation.

Section (E): RP-HPLC-DAD Method for Determination of Salicylamide and Ascorbic Acid in Presence of Their Impurities

A precise, accurate and specific RP- HPLC-DAD method was developed in this section. The separation was carried out on C_{18} column using 0.1% sodium lauryl sulfate aqueous

solution (adjusted to pH 4 using orthophosphoric acid): methanol (45:55, v/v), at a flow rate 1 mL/min. The eluted compounds were monitored at 240 nm. The method is comparable with the reported HPLC method.

This thesis refers 197 references, contains 71 figures and 91 tables and ends with a summary in Arabic.