Beni-Suef University
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جامعة بني سويف

كلية الصيدلة

PhD Thesis Summary

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"Analytical Study Of Some Pharmaceutical Compounds Acting On The Respiratory System"

This thesis is concerned with analytical study of some pharmaceutical compounds acting on the respiratory system namely; Diphenhydramine HCl (DH), its mixture with Paracetamol (PC) and Oxeladin Citrate (OL),

The aim of this work is to develop new simple, rapid, sensitive and selective methods for the determination of the cited drugs in their mixtures and in presence of their different degradates and common interfering additives either in bulk forms or in pharmaceutical formulations.

Different analytical techniques are adopted in this thesis including; derivative spectrophotometry, second derivative of the ratio spectra spectrophotometry, isoabsorptive point spectrophotometry, ratio subtraction spectrophotometry,mean centering spectrophotometry, double divisor spectrophotometry, TLC-densitometry, high performance liquid chromatography and multivariate calibration of spectrophotometric data.

The thesis consists of four parts:

PART I :General introduction

This part includes a simple pharmacological introductionabout drugs acting on the respiratory system and their classification, followed by a general introduction about the chemistry of the studied drugs, their physical properties, modes of action and the literature review concerning their reported methods of analysis.

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PART II :Stability indicating spectrophotometric methods for determination of Diphenhydramine hydrochloride in presence of its N-oxide derivative.

This part includes the forced degradation and stability study of diphenhydramine hydrochloride, accompanied by the method of preparation and identification of its N-oxide derivative, followed by five different stability indicating techniques suggested forthe determination ofdiphenhydramine hydrochloride in presence of its N-oxide derivative.

This part consists of six sections:

Section A :Degradation and stability study of Diphenhydramine hydrochloride

This section discusses the stability study ofdiphenhydramine hydrochloride under stress degradation conditions including acid hydrolysis, alkaline hydrolysis, oxidation and photolysis using white cool lamp and UV lamp.

This section illustrates the oxidation pathway of diphenhydramine hydrochloride degradation, the method of preparation of the pure N-oxide derivative and its identification using IR and mass spectra.

Section B :Stability indicating third derivative spectrophotometric method for determination of Diphenhydramine hydrochloride in presence of its N-oxide derivative.

In this section diphenhydramine hydrochloride was determined using the third derivative spectrophotometric method in presence of its N-oxide derivative by measuring the peak amplitude at 281 nm, which is a zero crossing point (ZCP) of the derivative. The amplitude was linear with the concentration over a range of 3-35 μgmL^{-1}.

The selectivity of the method was checked by analyzing diphenhydramine hydrochloride concentration in different laboratory prepared mixtures containing different ratios of the drug and its N-oxide derivative.The method was successfully applied to Sultan® capsules with no interference from additives.The accuracy of the method was assured by applying the standard addition technique to the capsules.

The results of the method were statistically compared with the pharmacopoeial HPLC method, where the obtained t and F values were found to be less than the tabulated ones, indicating no significant difference with respect to accuracy and precision.

Section C :Stability indicating second derivative of the ratio spectra spectrophotometric method for determination of Diphenhydramine hydrochloride in presence of its N-oxide derivative.

In this section diphenhydramine hydrochloride was determined using the second derivative of the ratio spectra spectrophotometric method in presence of itsN-oxide derivative by measuring the peak amplitude at 274.5 nm, which is a zero crossing point (ZCP) of the N-oxide derivative. The spectrum of 20µgmL^{-1} solution of the N-oxide derivative was used as a divisor. The amplitude was linear with the concentration over a range of 10-35 µgmL^{-1}.

The selectivity of the method was checked by analyzing diphenhydramine hydrochloride concentration in different laboratory prepared mixtures containing different ratios of the drug and its N-oxide derivative.

The method was successfully applied to Sultan® capsules with no interference from additives.The accuracy of the method was assured by applying the standard addition technique to the capsules.

The results of the method were statistically compared with the pharmacopoeial HPLC method, where the obtained t and F values were found to be less than the tabulated ones, indicating no significant difference with respect to accuracy and precision.

Section D :Stability indicating isoabsorptive point spectrophotometric method for determination of Diphenhydramine hydrochloride in presence of its N-oxide derivative.

In this section diphenhydramine hydrochloride was determined using the isoabsorptive point spectrophotometric method in presence of itsN-oxide derivative by measuring the absorbance at 226 nm, which is the isoabsorptive point of the drug and its N-oxide derivative. At this point the total concentration of the mixture was considered as diphenhydramine hydrochloride. The concentration of the N-oxide derivative is calculated by measuring its absorbance at 271 nm , which is its λ_{max}, then the concentration of diphenhydramine hydrochloride is calculated by subtraction. The absorbance was linear with the concentration over a range of 2-50 μgmL^{-1}.

The selectivity of the method was checked by analyzing diphenhydramine hydrochloride concentration in different laboratory prepared mixtures containing different ratios of the drug and its N-oxide derivative.

The method was successfully applied to Sultan® capsules with no interference from additives.The accuracy of the method was assured by applying the standard addition technique to the capsules.

The results of the method were statistically compared with the pharmacopoeial HPLC method, where the obtained t and F values were found to be less than the tabulated ones, indicating no significant difference with respect to accuracy and precision.

Section E :Stability indicating ratio subtraction spectrophotometric method for determination of Diphenhydramine hydrochloride in presence of its N-oxide derivative.

In this section diphenhydramine hydrochloride was determined using the ratio subtraction spectrophotometric method in presence of itsN-oxide derivative. The interference of the degradate is removed by dividing the spectra of the mixtures by the spectrum of 20μgmL^{-1}

solution of the N-oxide derivative, then subtracting the resulting plateau and finally re-multiplication by the same divisor The amplitude of diphenhydramine hydrochloride at 207 nm was linear with the concentration over a range of 2-30 μgmL^{-1}.

The selectivity of the method was checked by analyzing diphenhydramine hydrochloride concentration in different laboratory prepared mixtures containing different ratios of the drug and its N-oxide derivative.

The method was successfully applied to Sultan® capsules with no interference from additives.The accuracy of the method was assured by applying the standard addition technique to the capsules.

The results of the method were statistically compared with the pharmacopoeial HPLC method, where the obtained t and F values were found to be less than the tabulated ones, indicating no significant difference with respect to accuracy and precision.

Section F :Stability indicating mean centering spectrophotometric method for determination of Diphenhydramine hydrochloride in presence of its N-oxide derivative.

In this sectiondiphenhydramine hydrochloride was determined using the mean centering spectrophotometric method in presence of its N-oxide derivative, where the interference of the N-oxide derivative is removed by dividing the spectra of the mixtures by the spectrum of 20μgmL^{-1} solution of the degradate, then resulting spectrum is mean centered. The concentration of diphenhydramine hydrochloride is calculated by measuring the amplitude at 280 nm, which was found to be linear with the concentration over a range of 2-45 μgmL^{-1}.

The selectivity of the method was checked by analyzing diphenhydramine hydrochloride concentration in different laboratory prepared mixtures containing different ratios of the drug and its N-oxide derivative.

The method was successfully applied to Sultan® capsules with no interference from additives.The accuracy of the method was assured by applying the standard addition technique to the capsules.

The results of the method were statistically compared with the pharmacopoeial HPLC method, where the obtained t and F values were found to be less than the tabulated ones, indicating no significant difference with respect to accuracy and precision.

The results obtained from the five proposed methods were compared together and to the official method using one way ANOVA test where no significant differences were found.

PART III :Simultaneous determination of Paracetamol and Diphenhydramine hydrochloride in presence of their degradates p-aminophenol and diphenhydramine N-oxide derivative

This part includes three different stability indicating techniques suggested forthe simultaneous determination ofparacetamol and diphenhydramine hydrochloride in presence of their degradates; p-aminophenol the hydrolytic degradate of paracetamol and one of its most common impurities and the N-oxide N-oxide derivative of diphenhydramine hydrochloride.

This part consists of three sections:

Section A :Double divisor spectrophotometric method for simultaneous determination of Paracetamol and Diphenhydramine hydrochloride in presence of their degradates

In this section the double divisor method has been used for the simultaneous determination of paracetamol and diphenhydramine hydrochloride in presence of their degradation products p-aminophenol and the N-oxide derivative of diphenhydramine hydrochloride.The interference caused by the two degradates was removed by dividing the spectra of the analyzed drugs or quaternary mixtures by the spectrum of a mixture

containing equal concentrations of the two degradates. The second derivative of the resulting spectra was obtained.

Paracetamol was determined by measuring theamplitude at 304 nm, where the contribution ofDiphenhydramine hydrochloride is zero. The amplitude was linear with the concentration over a range of 2-45 µgmL^{-1}.

Diphenhydramine hydrochloride was determined by measuring the amplitude at 256.4 nm, which is a ZCP for paracetamol. The amplitude was linear with the concentration over a range of 2-45 µgmL^{-1}.

The selectivity of the method was checked by analyzing paracetamol and diphenhydramine hydrochloride in different laboratory prepared mixtures containing different ratios of the 4 components.

The method was successfully applied to Panadol night® tablets with no interference from additives.The accuracy of the method was assured by applying the standard addition technique to the tablets.

The results of the method were statistically compared with the pharmacopoeial HPLC method, where the obtained t and F values were found to be less than the tabulated ones, indicating no significant difference with respect to accuracy and precision.

Section B :Multivariate calibration method for simultaneous determination of Paracetamol and Diphenhydramine hydrochloride in presence of their degradates

In this section two chemometric techniques; Principle component regression (PCR) and Partial least square (PLS) have been successfully applied for the simultaneous determination of paracetamol and diphenhydramine hydrochloridein their quaternary mixtures with their degradates either in bulk forms or in pharmaceutical formulation. The two models were constructed using 13 mixtures containing different concentrations of the four components.

The selectivity of the method was checked by analyzing paracetamol and diphenhydramine hydrochloride concentration in different laboratory prepared mixtures (a validation set consisting of 12 mixtures).

The method was successfully applied to Panadol night® tablets with no interference from additives.The accuracy of the method was assured by applying the standard addition technique to the tablets.

Section C :TLC-densitometric method for simultaneous determination of Paracetamol and Diphenhydramine hydrochloride in presence of their degradates

In this section the TLC-densitometric method has been used for the simultaneous determination of paracetamol and diphenhydramine hydrochloride in presence of their degradation products p-aminophenol and the N-oxide derivative of diphenhydramine hydrochloride. The 4 components were separated on silica gel plates using a developing system consisting of ethyl acetate - acetone -20%methanolic sodium lauryl sulphate - acetic acid (5:5:1:0.25 by volumes). Peaks were detected at 254 nm.

The four separated components had different R_f values; paracetamol (0.87), diphenhydramine hydrochloride (0.11), p-aminophenol (0.65) andN-oxide derivative of diphenhydramine hydrochloride (0.33).The method is applicable over concentration ranges of (1-60µg band^{-1}) for paracetamol and (1-30µg band^{-1}) for diphenhydramine hydrochloride

The method was successfully applied to Panadol night® tablets with no interference from additives.The accuracy of the method was assured by applying the standard addition technique to the tablets.

The results of the method were statistically compared with the pharmacopoeial HPLC method, where the obtained t and F values were found to be less than the tabulated ones, indicating no significant difference with respect to accuracy and precision. The results

obtained from the three proposed methods were compared together and to the official method using one way ANOVA test where no significant differences were found.

PART IV :Determination of Oxeladin citrate in presence of its hydrolytic degradate, its N-oxide derivative and the two preservatives methyl paraben and propyl paraben

This part includes the forced degradation and stability study of oxeladin citrate, accompanied by the method of preparation and identification of its alkaline induced hydrolytic degradate and its N-oxide derivative, followed by three different stability indicating techniques suggested forthe determination of oxeladin citrate in presence of its two different degradates and the two preservatives methyl paraben and propyl paraben.

This part consists of four sections:

Section A :Degradation and stability study of Oxeladin citrate

This section discusses the stability study of oxeladin citrate under stress degradation conditions including acid hydrolysis, alkaline hydrolysis, oxidation and photolysis using white cool lamp and UV lamp.

This section illustrates both the alkaline induced hydrolytic and the oxidation pathways of oxeladin citrate degradation and the method of preparation of pure degradation products and their identification using IR and mass spectra.

Section B :Multivariate calibration methods for determination of Oxeladin citrate in presence of its hydrolytic degradate, its N-oxide derivative and the two preservatives Methyl paraben and Propyl paraben.

In this section two chemometric techniques; Principle component regression (PCR) and Partial least square (PLS) have been successfully applied for the determination of oxeladin citrate in presence of its two degradates and the two preservatives methyl paraben and

propyl paraben either in bulk forms or in pharmaceutical formulations. The two models were constructed using 12 mixtures containing different concentrations of the five components.

The selectivity of the method was checked by analyzing oxeladin citrate concentration in different laboratory prepared mixtures (a validation set consisting of 13 mixtures).

The method was successfully applied to Oxeladine® and Paxeladine® syrups with no interference from other additives. The accuracy of the method was assured by applying the standard addition technique to the syrups.

Section C :High performance liquid chromatographic method for determination of Oxeladin citrate in presence of its hydrolytic, its N-oxide derivative and the two preservatives Methyl paraben and Propyl paraben.

In this section a RP-HPLC method has been used for the separation and determination of oxeladin citrate in presence of its 2 degradation products and the two preservatives methyl paraben and propyl paraben.

The 5 components were separated on a C_{18} column using a mobile phase consisting of methanol /water (1:1, maintained at pH=3 by trifluoroacetic acid)at a flow rate of 2 mL min^{-1} and detected at 220 nm.The five separated components had different t_R values; oxeladin citrate (2.6 min), the N-oxide derivative (6.2 min), the hydrolytic degradate (13.7 min) andthe two preservatives at the same t_R value(9.3 min).

The method is applicable over a concentration range of (5-200µg $band^{-1}$) of oxeladin citrate.The method was successfully applied to Oxeladine® and Paxeladine® syrups with no interference from other additives.The accuracy of the method was assured by applying the standard addition technique to the syrups.

The results of the method were statistically compared with the reported HPLC method, where the obtained t and F values were found to be less than the tabulated ones, indicating no significant difference with respect to accuracy and precision.

Section D :TLC-densitometric method for determination of Oxeladin citrate in presence of its hydrolytic degradate, its N-oxide derivative and the two preservatives Methyl paraben and Propyl paraben.

In this section the TLC-densitometric method has been used for the determination of oxeladin citrate in presence of its two degradation products and the two preservatives methyl paraben and propyl paraben.

The 5 components were separated on silica gel plates using a developing system consisting of acetone- ethyl acetate -7%methanolic sodium lauryl sulphate - acetic acid (6:5:3:0. 5 by volumes). Peaks were detected at 220 nm. The five separated components had different R_f values;oxeladin citrate (4.2), the N-oxide derivative (0.71), the hydrolytic degradate (0.19) andthe two preservatives had the same R_f value (0.87).

The method is applicable over a concentration range of (0.1-2µg $band^{-1}$) of oxeladin citrate

The method was successfully applied to Oxeladine® and Paxeladine® syrups with no interference from other additives.The accuracy of the method was assured by applying the standard addition technique to the syrups.

The results of the method were statistically compared with the reported HPLC method, where the obtained t and F values were found to be less than the tabulated ones, indicating no significant difference with respect to accuracy and precision.

The results obtained from the three proposed methods were compared together and to the official method using one way ANOVA test where no significant differences were found.

This thesis contains 164 references, 67 figures and 70 tables and ends with an Arabic summary.