"Analytical and Stability Studies of Some Nitrogenous Compounds of Different Pharmacological Activities "

Presented by

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Summary

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

Part I: Quantitative Determination of Oxybutynin Hydrochloride in Presence of Its Degradation Product and Additives in Different Pharmaceutical Dosage Forms

This part includes five sections.

Section (A): Introduction and Literature Review

This section includes literature review about chemical structure, physical properties and pharmacology of Oxybutynin Hydrochloride (OX), it also includes review of methods of analysis developed for determination of OX either alone or in presence of its degradation product in pure form and in pharmaceutical formulations.

Section (B): Determination of Oxybutynin Hydrochloride in Presence of Its Degradation Product by First Derivative of Ratio Spectra Method (^{1}DD)

In this section the first derivative of ratio spectra spectrophotometric technique (^{1}DD) has been applied to improve selectivity for determination of OX in presence of its degradation product (OX Deg) using 0.1N HCl as

a solvent. OX was determined by dividing the absorption spectra of different concentrations of OX in the range of 6 - 28 µg.mL^{-1} by the absorption spectrum of 20µg.mL^{-1} of its degradation product. The obtained ratio spectra were differentiated with respect to wavelength and the ^{1}DD values at 216 nm were recorded. The proposed method was successfully applied for determination of OX in Uripan® and Detronin® tablets. The results obtained by applying the proposed method were statistically compared to those obtained by applying a reported HPLC method and no significant difference were found regarding both accuracy and precision.

Section (C): Determination of Oxybutynin Hydrochloride in Presence of its Degradation Product by Mean Centering of Ratio Spectra Method

A recent and simple method was developed for determination of OX in presence of its degradation product. In this method OX was determined by measuring the amplitudes of the mean centered ratio spectra at 217.8 nm using 20 µg.mL^{-1} of its degradation product as a divisor. The proposed method was used for determination of OX in tablets and the results of standard addition technique confirmed that tablet additives did not interfere.

Section (D): Determination of Oxybutynin Hydrochloride in Presence of its Degradation Product and Additives in Different Pharmaceutical Formulations by Multivariate Calibration Methods

Multivariate calibrations models, such as PCR and PLS have been successfully applied as selective stability indicating methods for determination of OX in presence of its degradation product. The two chemometric PCR and PLS methods were successfully applied for the determination of OX in Uripan® and Detronin ® tablets.

In order to apply the developed methods for determination of OX in Uripan ® syrup and Detronin ® syrup, model updating was performed.

To validate the predictive ability of the developed models, they were applied to predict the concentrations of OX and its degradation product in an external validation set. Statistical analysis of the results obtained by the developed models were compared with a reported HPLC one, indicating no significant difference regarding both accuracy and precision.

Section (E): Determination of Oxybutynin Hydrochloride in Presence of Its Degradation Product and Additives in Different Pharmaceutical Formulations by HPTLC-Densitometric Method

In this section HPTLC-Densitometric method was developed by separating OX from its degradation product, methylparaben and propylparaben successfully and efficiently using chloroform: methanol: ammonia solution: triethylamine (100: 3: 0.5: 0.2 by volume) as a mobile phase. The separated bands of OX were scanned at 220 nm in the range of 2–14 µg.band^{-1}. The proposed HPTLC-Densitometric method was applied successfully for determination of OX in different pharmaceutical formulations.

Part II: Stability Indicating Methods for Determination of Flavoxate Hydrochloride in Presence of Its Alkaline Induced Degradation Products

This part includes six sections.

Section (A): introduction and literature Review

This section includes literature review about chemical structure, physical properties and pharmacology of Flavoxate Hydrochloride (FL), it also includes review of methods of analysis developed for determination of FL either alone or in presence of its degradation product in pure form and in pharmaceutical formulations.

Section (B): Determination of Flavoxate Hydrochloride in Presence of Its Alkaline Induced Degradation Products by First Derivative Spectrophotometry (^{1}D)

In this section, a first derivative spectrophotometric method was developed for determination of FL in methanol at 275 and 331 nm. Good linearity was obtained in the range of 2- 16 µg.mL^{-1}. The proposed method retained its accuracy in presence of up to 80 % of FL alkaline induced degradation products (FL Degs). The proposed method has been applied for determination of FL in its pharmaceutical formulations.

Section (C): Determination of Flavoxate Hydrochloride in Presence of Its Alkaline Induced Degradation Products by First Derivative of Ratio Spectra method (^{1}DD)

In this section the first derivative of ratio spectra amplitudes at 268 nm and 291 nm were used for determination of FL in presence of its alkaline induced degradation products in the range of 2 - 16 µg.mL^{-1}. The suggested method was successfully applied for determination of FL in its pharmaceutical formulation.

Section (D): Determination of Flavoxate Hydrochloride in Presence of its Alkaline Induced Degradation Products by Multivariate Calibration Models.

In this section two chemometric models PCR and PLS were used for simultaneous determination of FL and its alkaline induced degradation products .Training set of 14 mixtures containing different ratios of FL and its alkaline induced degradation products was used for construction of the two models.. Satisfactory results were obtained on applying the proposed methods for the analysis of FL in its pharmaceutical formulation.

Section (E): Determination of Flavoxate Hydrochloride in Presence of its Alkaline Induced Degradation Products by TLC-Densitometry

In this section, TLC-Densitometric method was developed by separating FL from its alkaline induced degradation products using chloroform: methanol: glacial acetic acid (90: 6: 3 by volume) as a mobile phase. The proposed TLC-Densitometric method was applied successfully for determination of FL in its pharmaceutical formulation.

Section (F: Determination of Flavoxate Hydrochloride in Presence of Its Alkaline Induced Degradation Products by RP-HPLC Method

In this section, an accurate and selective RP-HPLC method has been investigated and validated for quantitative analysis of FL in presence of its alkaline induced degradation products. The chromatographic separation was achieved using methanol: acetonitrile: deionized water: triethylamine (100: 50: 36: 1, by volume; pH was adjusted to 6.5 with orthophosphoric acid) as a mobile phase, the flow rate was 2 $mL.min^{-1}$ with UV detection at 254 nm.

The proposed method was validated and applied for determination of FL in its pharmaceutical formulation. When results obtained by applying the proposed method for analysis of FL were compared to those obtained by applying a reported HPLC method, no significance difference was observed.

Part III: Determination of Methocarbamol and Ibuprofen in Their Binary Mixture and in Presence of Methocarbamol Degradation Product

This part comprises five sections

Section (A) : introduction and literature Review

This section includes literature review about chemical structure, physical properties and pharmacology of Methocarbamol (ME) and Ibuprofen (IB),

it also includes review of methods of analysis developed for determination in their single formulation and in their binary mixture.

Section (B): Simultaneous Determination of Methocarbamol and Ibuprofen by First (^{1}D) and Second (^{2}D) Derivative Spectrophotometry

This section includes two spectrophotometric methods for simultaneous determination of Methocarbamol and Ibuprofen in their binary mixture. The developed spectrophotometric methods include first and second derivative methods. The first derivative amplitudes at 283 nm were used for determination of ME concentrations, while second derivative amplitudes at 231.6 nm were used for determination of Ibuprofen.

Section (C): Simultaneous Determination of Methocarbamol and Ibuprofen by Mean Centering of Ratio Spectra Method.

A recent and simple method was developed for simultaneous determination of ME and IB in their binary mixture, without prior separation steps.

In this method, the mean centered ratio spectra amplitudes at 218.4 nm were used for determination of ME and IB. Moreover the suggested method has been applied for determination of the cited drugs in their commercial tablets.

Section (D): Simultaneous Determination of Methocarbamol and Ibuprofen by TLC- Densitometry in Presence of Methocarbamol Degradation Product.

In this section TLC-Densitometric method was developed by separating ME and IB in presence of Methocarbamol degradation product successfully and efficiently using ethylacetate: acetone: formic acid:

triethylamine (62: 35: 0.3: 6 by volume) as a developing system. The separated bands of ME and IB were scanned at 278 nm and 222 nm for ME and IB respectively

The suggested method is applicable for determination of both drugs in its pharmaceutical formulation.

Section (E): Simultaneous Determination of Methocarbamol and Ibuprofen by RP- HPLC method in Presence of Methocarbamol Degradation Product.

In this section, an accurate and selective RP-HPLC method has been investigated and validated for quantitative analysis of ME and IB in presence of Methocarbamol degradation product. 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 0.05 M KH_2PO4 (pH = 7): acetonitrile: methanol (90: 25: 15 by volume), using flow rate 1.2 $mL.min^{-1}$ and UV detection at 220 nm.

By applying the suggested RP-HPLC method, ME and IB could be quantified in the range of 6 – 20 and 6 – 28 $\mu g.mL^{-1}$ for ME and IB, respectively.

Statistical comparison of the results obtained by the proposed method and a reported HPLC one for analysis of pure ME and IB was carried out. The values of the calculated t and F are less than the tabulated ones which reveals that there is no significant difference between the two methods with respect to accuracy and precision.

Part IV: Determination of Methocarbamol and Diclofenac Potassium in Their Binary Mixture and in Presence of Methocarbamol Degradation Product

This part comprises six sections

Section (A) : introduction and literature Review

This section includes literature review about chemical structure, physical properties and pharmacology of Methocarbamol (ME) and Diclofenac Potassium (DI) it also includes review of methods of analysis developed for determination in their single formulation and in their binary mixture.

Section (B): Simultaneous Determination of Methocarbamol and Diclofenac potassium by First (^{1}D) and Second Derivative Spectrophotometry (^{2}D)

In this section, derivative spectrophotometric technique was used , for determination of Methocarbamol and Diclofenac Potassium in their binary mixture. Methocarbamol was determined by measuring peak amplitude at 225.4 nm for ^{2}D method while Diclofenac Potassium can be determined at 297 nm using ^{1}D method using 0.1 N HCl as blank. Satisfactory results were obtained on applying the proposed methods for the analysis of the two mentioned drugs in their commercial tablets, the results obtained were statistically compared with that obtained by the reported HPLC method indicating no significant difference between them.

Section (C): Simultaneous Determination of Methocarbamol and Diclofenac Potassium by Mean Centering of Ratio Spectra Method

A simple spectrophotometric method has been investigated for simultaneous determination of both Methocarbamol and Diclofenac Potassium without prior separation steps. in this method , ME was determined by measuring the amplitudes of the mean centered ratio spectra at 279.4 nm and 260.6 nm using 6 µg.mL^{-1} of DI as a divisor. While DI was determined by measuring the amplitudes of the mean centered ratio spectra at 260.8 nm. The proposed method was used for quantitation of both ME and DI in Dimra ® tablets where satisfactory results were obtained and its validity was further assessed by applying the standard addition technique.

Section (D): Simultaneous Determination of Methocarbamol and Diclofenac Potassium by Chemometric Method in Presence of Methocarbamol Degradation Product and Application of Model Updating for Determination Methocarbamol and Ibuprofen in Ibuflex® Tablets

In this section PLS model has been successfully applied as selective stability indicating method for determination of the ternary mixture of ME, DI and Methocarbamol degradation product.

To validate the predictive ability of the developed model it was applied to predict the concentrations of ME, DI and Methocarbamol degradation product in an external validation set.

In order to apply the developed method for determination of ME and IB in Ibuflex® tablets model updating was performed.

The investigated chemometric method was successfully applied for quantitation of the above mentioned drugs in Dimra® and Ibuflex® tablets with good percentage recoveries and good agreement with the labeled amounts. The results obtained by applying the developed model showed no significant difference when compared to those obtained by applying a reported HPLC.

Section (E): Simultaneous Determination of Methocarbamol and Diclofenac potassium by TLC- Densitometry in Presence of Methocarbamol Degradation Product

In this section TLC-Densitometric method was developed by separating ME and DI in presence of Methocarbamol degradation product successfully and efficiently using ethylacetate: acetone: formic acid:

triethylamine (62: 35: 0.3: 6 by volume) as a developing system. The separated bands of ME and DI were scanned at 278 nm

The suggested method is applicable for determination of both drugs in their pharmaceutical formulation.

Linear relationships were obtained between the integrated peak area $\times 10^{-4}$ and the corresponding concentrations in the concentration range of 2 - 12 µg.band^{-1} for ME and 0.2 - 2.2 µg.band^{-1} for DI.

Section (F): Simultaneous Determination of Methocarbamol and Diclofenac Potassium by RP- HPLC Method in Presence of Methocarbamol Degradation Product.

A precise, specific, accurate and stability indicating RP- HPLC method was proposed for the determination of ME and DI in presence of Methocarbamol degradation product. 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 0.05 M KH_2PO4 (pH = 7): acetonitrile (80: 30 v/v), using flow rate of 1.45 mL.min^{-1} and UV detection at 278 nm.

The suggested method has been applied for determination of the two proposed components in commercial tablets. When results obtained by applying the proposed method for analysis of pure ME and DI were compared to those obtained by applying a reported HPLC method, no significant difference was observed.

Part V: Appendix

This part includes a brief idea about the instruments, solvents and chemicals used in other parts. In addition, detailed preparation of the standard solutions used in each part through this work was described in addition to method of preparation of Oxybutynin and Flavoxate degradation products.

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