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 Quantitative Determination of Diacerein in Presence of Its degradate and Impurity in Different Pharmaceutical Formulations

This part includes seven sections.

Section (A): Introduction and Literature Review

This section includes an introduction about the pharmacological action of Diacerein (DIA), its chemical structure, physical properties and summary of the published methods developed for its analysis in binary mixture with its degradation product and active metabolite, Rhein (RH) and in ternary mixtures with RH and its impurity and related structure, Emodin (EMO).

Section (B): Determination of Diacerein and its Degradate and Active Metabolite, Rhein, by Q-Analysis Method

In this section, Q-Analysis method was developed. The method depends on the property that for the substance that obeys Beer's Lambert's law at all wavelengths, the ratio of absorptivity (or absorbance) values at any two wavelengths are constant, independent of the concentration or path length. This ratio is referred as Q-ratio. One of the two selected wavelengths is an isoabsorptive point and the other is the wavelength of maximum absorption of one of the two components.

Using the absorbance values at 365 nm (λ_{iso}) and 257 nm (λ_{max} for DIA) gave the best results regarding selectivity. The developed method has been applied for determination of the studied drugs in different laboratory prepared mixtures. The

results obtained by applying the investigated method for determination of DIA in Diacerein ® and Osteocerein ® capsules were statistically compared to those obtained by applying a reported HPLC one and no significant difference was found regarding both accuracy and precision.

Section (C): Determination of Diacerein by Ratio Subtraction and its Degradate and Active Metabolite, Rhein, by Extended Ratio Subtraction Methods

In this section, zero order curve (^{0}D), ratio subtraction (RS) and the recently developed extended ratio subtraction (ERS) methods have been applied for determination of DIA and RH, respectively in their binary mixture using methanol as a solvent. DIA concentrations were determined by measuring the absorbance at its λ_{max} (257 nm), while RH concentrations were determined by measuring the absorbance at its λ_{max} (230 nm).

The developed methods have been applied for determination of the studied drugs in different laboratory prepared mixtures. The results obtained by applying the investigated methods for determination of DIA in Diacerein ® and Osteocerein ® capsules were statistically compared to those obtained by applying a reported HPLC one and no significant difference was found regarding both accuracy and precision.

Section (D): Determination of Diacerein in Presence of its Degradate and Active Metabolite, Rhein, by Spectrofluorimetric Method

This section deals with the development of simple, sensitive, specific, stability indicating, and economic spectrofluorimetric method for the selective determination of DIA in the presence of its degradate, RH, with successive application to spiked human plasma.

In this method, the native fluorescence of DIA solutions in the range of 0.04-0.3 μg mL^{-1} at λ_{em} = 404 nm upon excitation at λ_{ex} = 255 nm was measured and used for calculation of DIA without interference from its degradate. Being RH is the

active metabolite of DIA on hand, and the proposed spectrofluorimetric method is highly sensitive on the other hand, in vivo application of the proposed method was of great importance. So, DIA was determined in spiked human plasma after drug extraction with methanol and good recoveries were obtained.

The suggested method was used for determination of DIA in Diacerein ® and Osteocerein ® capsules and the results were statistically compared to those obtained by applying a reported HPLC one and no significant difference was found regarding both accuracy and precision.

Section (E): Stability Determination of Diacerein, its Degradate and Active Metabolite, Rhein, and its Impurity, Emodin, by Different Spectrophotometric Methods

In this section, different spectrophotometric methods have been investigated for determination of DIA, RH, and EMO in their ternary mixture. The developed spectrophotometric methods include double divisor, Second derivative ratio (^{2}DD) and first derivative ratio (^{1}DD) methods.

Diacerein was determined at amplitudes at 355 nm using double divisor method, while peake amplitudes at 229 nm at ^{2}DD and ^{1}DD were used for determination of RH and EMO, respectively. The developed methods were successfully applied for quantitation of the studied drugs in Diacerein ® and Osteocerein ® capsules and the results were statistically compared to those obtained by applying a reported HPLC one and no significant difference was found regarding both accuracy and precision.

Section (F): Determination of Diacerein, its Degradate and Active Metabolite, Rhein, and Impurity, Emodin, by Chemometric Method

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

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

Section (G): Determination of Diacerein, its Degradate and Active Metabolite, Rhein, and its Impurity, Emodin, by TLC-Densitometric Method

In this section, a simple and accurate TLC-Densitometric method has been suggested for the analysis of the ternary mixtures of DIA, RH, and EMO in bulk powders and in capsules. The suitable mobile phase has been selected to achieve the best separation.

Quantitative determination of the separated bands of DIA, RH, and EMO was carried out at 230 nm upon using hexane: ethyl acetate: acetic acid (60: 40: 0.8, by volume) as mobile phase in the range of 0.5-10 μg band^{-1} for DIA and RH and 0.5 - 7 μg band^{-1} for EMO. The suggested TLC-Densitometric method was successfully applied for analysis of the cited drugs in pharmaceutical formulations and no significant difference was found comparing the reported HPLC method.

Part II: Determination Quantitative Determination of Metronidazole and Nystatin in Their Binary Mixtures

This part consists of seven sections.

Section (A): Introduction and Literature Review

This section includes an introduction about the pharmacological action of Metronidazole (MET) and Nystatin (NYS), their chemical structure, physical properties and summary of the published methods developed for their analysis in their single formulation and their binary mixture.

Section (B): Determination of Metronidazole and Nystatin by Dual Wavelength Method

In this section, dual wavelength method provides a simple method for simultaneous analysis of MET and NYS at zero order. The principle for dual wavelength method is that the absorbance difference at two points on the spectra is directly proportional to the concentration of the component of interest, independent of the interfering component.

Absorption values of NYS were the same at 266.5 and 328 nm, so that these wavelengths were selected for the determination of MET. The same as in 291 and 330 nm, the absorbance values of MET were the same and hence those two wavelengths were selected for estimation of NYS

The developed method was successfully applied for quantitation of the studied drugs in Amrizole N® vaginal suppositories and Nystazole® vaginal tablets and the standard addition technique has been applied to verify their validity.

Section (C): Determination of Metronidazole and Nystatin by Different Spectrophotometric Methods

In this section, different spectrophotometric methods have been investigated for determination of FUR and SPR in their binary mixture. The developed spectrophotometric methods include first derivative, isosbestic point and ratio subtraction methods.

The second derivative (^2D) amplitudes at 290 nm were used for determination of NYS concentrations, while isosbestic point (ISO) method was applied for determination of the total mixture concentration (MET and NYS) at the isosbestic point (λ_{iso} = 322 nm). Since the concentration of NYS in the mixture can be determined by using ^2D spectrophotometric method, therefore MET concentration can be obtained by subtraction.

The developed method was successfully applied for quantitation of the studied drugs in Amrizole N® vaginal suppositories and Nystazole® vaginal tablets

and the results were statistically compared to those obtained by applying a reported PLS regression chemometric method and no significant difference was found regarding both accuracy and precision.

Section (D): Determination of Metronidazole and Nystatin by TLC-Densitometric Method

This section is concerned with the development of sensitive, economic and specific stability indicating TLC-Densitometric method for determination of MET and NYS in their bulk powder and pharmaceutical formulations. The studied components were well separated using methanol: hexane: triethylamine (80: 20: 2, by volume) as a developing system and the separated bands were scanned at 305nm.

Linear relationships were obtained between the mean integrated peak area ($\times 10^{-4}$) and the corresponding concentrations of MET and NYS in the concentration range of 1 - 13 and 0.5 - 10 µg band^{-1}, respectively. The developed TLC-Densitometric method has been applied for determination of the two drugs in their pharmaceutical formulations.

Section (E): Determination of Metronidazole and Nystatin by HPLC Method

A precise, specific, accurate and stability indicating HPLC method was proposed for the determination of MET and NYS. In this method, an isocratic elution of the two components was performed at ambient temperature on ODS column using acetonitrile: 0.05 M phosphate buffer (30: 70, v/v) pH= 3.5 as a mobile phase with a flow rate of 1 mL min^{-1} and UV-detection at 305 nm.

By applying the suggested RP-HPLC method, MET and NYS could be quantified in the range of 3 - 50 and 15 - 50 µg mL^{-1}, respectively. Statistical comparison of the results obtained by the proposed method and a reported PLS regression chemometric method for analysis of MET and NYS in pharmaceutical formulations 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 III: Determination of Aspirin, Caffeine, and Paracetamol in Their Ternary Mixtures

This part comprises five sections.

Section (A): Introduction and Literature Review

This section includes an introduction about the pharmacological action of Aspirin (ASP), Caffeine (CAF), and Paracetamol (PAR), their chemical structure, physical properties and a summary of the published methods developed for their analysis in their single formulation and in their ternary mixture.

Section (B): Determination of Aspirin, Caffeine, and Paracetamol by Different Spectrophotometric Methods

The developed methods are successive ratio-derivative spectra and indirect spectrophotometric methods. Applying successive ratio-derivative spectra method, ASP and PAR could be selectively determined while, CAF could not be determined by this method, and so, it was determined indirectly using mathematical equation.

Aspirin was determined at the amplitudes at 241.2 nm, while PAR was determined at the amplitudes at 228.2 nm. CAF was indirectly determined using mathematical equations. The proposed methods were used for quantitation of the studied drugs in Aspicure-combi®, Excedrin®, and Markadel® tablets and results of standard addition technique confirmed that tablet additives did not interfere.

Section (C): Simultaneous Determination of Aspirin, Caffeine, and Paracetamol by Chemometric Methods

Multivariate calibration models, such as PCR and PLS has been successfully applied as selective methods for determination of the ternary mixture of ASP, CAF, and PAR.

To validate the predictive ability of the developed models, they were applied to predict the concentrations of ASP, CAF, and PAR in an external validation set. Statistical analysis of the results obtained by the developed models were compared with a reported RP-HPLC one and no significant difference was found regarding both accuracy and precision.

Section (D): Simultaneous Determination of Aspirin, Caffeine, and Paracetamol by TLC-Densitometric Method

In this section, a simple and accurate TLC-Densitometric method has been suggested for the analysis of the ternary mixtures of ASP, CAF, and PAR in bulk powders and in tablets. The suitable mobile phase has been selected to achieve the best separation.

Quantitative determination of the separated bands of ASP, CAF, and PAR was carried out at 230 nm upon using chloroform: methanol: glacial acetic acid: ammonia solution (95: 5: 0.5: 0.2, by volume) as a developing system in the range of 1 – 10 μg $band^{-1}$ for ASP and PAR and 0.5 - 7 μg $band^{-1}$ for CAF. The suggested TLC-Densitometric method was successfully applied for analysis of the cited drugs in pharmaceutical formulations.

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 DIA degradate, RH.