Summary

This thesis consists of four 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: Stability Indicating Analytical Methods for Determination of Calcium Dobesilate and Its impurity and/or Degradation product (Hydroquinone)

This part includes eight sections.

Section (A): Introduction and literature Review

This section includes literature review about chemical structure, physical properties and pharmacology of calcium dobesilate (CD), it also includes review of methods of analysis developed for determination of CD either alone or in presence of its impurity and/ or degradation product, hydroquinone (HQ) in pure form and in pharmaceutical formulations.

Section (B): Application of first derivative spectrophotometric method for determination of calcium dobesilate and hydroquinone.

In this section, the first derivative spectrophotometric technique (D^1) has been applied to improve selectivity for determination of CD and HQ using methanol as a solvent. CD and HQ were determined in the range of 5.00- 70.00 and 5.00- 60.00 μg mL^{-1} using the peak amplitudes of the D^1 curves at 293.8 and 305 nm, respectively. The proposed method was successfully applied for the determination of CD in Doxium® and Dilasal® capsules. The results obtained by applying the proposed method were statistically compared to those obtained by applying the official

HPLC method and there was no significant difference regarding accuracy and precision.

Section (C): Application of dual wavelength spectrophotometric method for determination of calcium dobesilate and hydroquinone.

The difference between the amplitudes of the zero order spectra of CD and HQ at 285.4 and 301.8 nm (A285.4 – A301.8), and 299 and 311 nm (A299 – A311), respectively, was used to determine their concentration in the range of 5.00- 70.00 and 5.00- 60.00 μg mL^{-1}. Satisfactory results were obtained on applying the proposed methods for determination of CD in Doxium® and Dilasal® capsules.

Section (D): Application of extended ratio subtraction spectrophotometric method for determination of calcium dobesilate and hydroquinone.

In this method, 30.00 μg mL^{-1} CD spectrum, which is the more extended component, was used as a divisor for determination of HQ at 294 nm in the mixture after subtraction of the constant in the plateau region. For determination of CD, the obtained HQ spectra were divided by 30.00 μg mL^{-1} HQ as a divisor, the constant value at the plateau region was subtracted from its corresponding mixture, and CD was determined at 307 nm. The method was successfully applied for determination of CD in Doxium® and Dilasal® capsules and was compared to the official HPLC method with no significance difference between the two methods.

Section (E): Application of ratio difference spectrophotometric method for determination of calcium dobesilate and hydroquinone.

In this section a recently developed simple, accurate method was successfully applied for determination of CD and HQ, in their mixture, and determination of CD in its pharmaceutical formulations. 10.00 μg mL^{-1} HQ was used as a divisor for determination of CD, and the difference between the amplitudes of ratio spectra at 275.4 and 308 nm

(A 275.4- A 308) was used for determination of its concentration. The difference between the amplitudes of ratio spectra at 237.4 and 307 nm (A 237.4- A 307) after using 10.00 μg mL^{-1} CD as a divisor was used for determination of HQ concentration.

Section (F): Application of constant center spectrophotometric method for determination of calcium dobesilate and hydroquinone.

The method involves using the ratio spectra for calculation of a constant that can be used for obtaining the zero order spectra of either CD or HQ, from which their concentration could be determined at their λ_{max}. CD and HQ can be determined at 307 and 297 nm, respectively. The method was comparable to the official one regarding accuracy and precision.

Section (G): Application of mean centering of ratio spectra method for determination of calcium dobesilate and hydroquinone.

In this method, CD was determined by measuring the amplitudes of the mean centered ratio spectra at 270 nm using 30.00 μg mL^{-1} HQ as a divisor, while the amplitude at 226 nm was used for determination of HQ using 10.00 μg mL^{-1} CD as a divisor. The proposed method was used for quantitation of CD in Doxium® and Dilasal® capsules and the results of standard addition technique confirmed that tablets additives did not interfere.

Section (H): Application of TLC- densitometric method for determination of calcium dobesilate and hydroquinone.

In this section TLC-densitometric method was developed by separating CD from HQ successfully and efficiently using benzene: 1% sodium lauryl sulphate methanolic solution: ethyl acetate (7: 2.5: 2, v/v/v) as a mobile phase. The separated bands of CD and HQ were scanned at 225 nm in the range of 0.50-5.00 and 0.05- 3.00 μg $band^{-1}$, respectively. The proposed TLC-densitometric method was applied successfully for determination of CD in its pharmaceutical formulations.

Part II: Simultaneous Determination of Flumethsasone pivalate and Clioquinol in their Binary Mixture and their Ternary Mixture with Flumethasone.

This part includes six sections:

Section (A): Introduction and literature Review

This section includes literature review about chemical structure, physical properties and pharmacology of flumethasone pivalate (FP) and clioquinol (CL), it also includes review of methods of analysis developed for their determination in their single formulation, in binary mixture and in pharmaceutical formulations.

Section (B): Determination of Flumethasone pivalate and Clioquinol by ratio subtraction and zero order spectrophotometric methods.

This section includes two spectrophotometric methods for determination of FP and CL in their binary mixture. FP was determined at 234 nm by ratio subtraction method using 16.00 μg mL^{-1} CL as a divisor while CL was determined by direct absorbance measurement at 324.2 nm. The suggested methods have been applied for determination of the cited drugs in Viotic ® ear drops. The results obtained were statistically compared with that obtained by the official HPLC ones and no significant difference was found.

Section (C): Simultaneous determination of Flumethasone pivalate and Clioquinol by ratio difference spectrophotometric method.

In this section, the difference in the amplitudes of ratio spectra at 221 and 231nm using 16.00 μg mL^{-1} CL as a divisor was used for FP determination, while CL was determined using the difference between the amplitudes of ratio spectra at 234 and 262 nm after dividing CL spectra by 8.00 μg mL^{-1} FP as a divisor.

The investigated ratio difference method was successfully applied for the determination of FP and CL in Viotic ® ear drops, with good percentage recovery and good agreement with the labeled amounts. The results obtained by applying the developed method showed no significant difference when compared to those obtained by applying the official HPLC methods.

Section (D): Simultaneous determination of Flumethasone pivalate and Clioquinol by mean centering of ratio spectra method.

In this method, the mean centered ratio spectra amplitudes at 231 nm were used for determination of FP and CL. The suggested method has been applied for determination of FP and CL in their pharmaceutical formulation. Statistical comparison between the proposed method and the official HPLC ones showed no significant difference.

Section (E): Simultaneous determination of Flumethasone pivalate, Clioquinol and Flumethasone by TLC- densitometric method.

In this section TLC-densitometric method was developed for separation of the ternary mixture of FP, its potential impurity, flumethasone (FL) and CL successfully and efficiently using benzene: hexane: acetone: formic acid (5: 4: 2: 0.13, by volume) as a mobile phase. The separated bands were scanned at 235 nm in the range of 0.30–4.00, 0.30–3.00 and 1.50-5.00 µg $band^{-1}$ for FP, FL and CL, respectively. The proposed TLC-densitometric method was applied successfully for determination of both drugs in their pharmaceutical formulations.

Section (F): Simultaneous determination of Flumethasone pivalate, Clioquinol and Flumethasone by HPLC method.

In this section, an accurate and selective HPLC method has been investigated and validated for quantitative analysis of FP, FL and CL in their ternary mixture. 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: water (70: 30, v/v) delivered at a flow rate 1 mL min^{-1}. The injection volume was 20 µL with UV scanning at 235 nm at room temperature.

By applying the suggested HPLC method, FP, FL and CL could be quantified in the range of 5.00–50.00, 2.00–35.00 and 10.00–70.00 µg mL^{-1}, respectively.

Statistical comparison of the results obtained by the proposed method and the official HPLC ones, for the analysis of FP and CL was carried out. The values of calculated t and F are less than the tabulated ones, which reveals that there is no significant difference between the methods with respect to accuracy and precision.

Part III: Simultaneous Determination of Ciprofloxacin Hydrochloride and Tinidazole in Presence of their Potential Impurities.

This part includes five sections:

Section (A): Introduction and literature Review

This section includes literature review about chemical structure, physical properties and pharmacology of ciprofloxacin hydrochloride (CR) and tinidazole (TD). It also includes review of methods of analysis developed for determination of the cited drugs in their single formulation, in presence of their impurities and in mixture with other drugs.

Section (B): Simultaneous determination of Ciprofloxacin hydrochloride and Tinidazole in presence of their potential impurities by double divisor ratio spectra derivative method.

In this section, double divisor ratio spectra derivative method has been successfully applied for determination of CR and TD in presence of their potential impurities fluoroquinolonic acid (FQ) and menidazole (MD), respectively. DD^1 spectra were obtained using absorption spectrum of a

mixture containing 15.00 μg mL^{-1} of each of FQ and MD as a divisor. CR and TD were quantitatively determined at 315.6 and 330.6 nm in the range of 2.00 – 20.00, 5.00 – 45.00 μg mL^{-1}, respectively. The proposed method was successfully applied for determination of the suggested drugs in Tinifloxacin® tablets; satisfactory results were obtained with good agreement with the labeled amounts. The results obtained by the proposed method for the analysis of pure CR, TD were compared to the reported HPLC method; they showed no significant difference regarding accuracy and precision.

Section (C): Simultaneous determination of Ciprofloxacin hydrochloride and Tinidazole in presence of their potential impurities by chemometric methods.

Multivariate calibration models, such as PCR and PLS has been applied for determination of CR, TD, FQ and MD. Training set of 16 mixtures containing different ratios of the four proposed components was used for construction of the two models. Satisfactory results were obtained on applying the proposed methods for the analysis of CR and TD in Tinifloxacin® tablets.

Section (D): Simultaneous determination of Ciprofloxacin hydrochloride and Tinidazole in presence of their potential impurities by TLC- densitometric method.

In this section TLC-densitometric method was developed for separation of the quaternary mixture of CR, TD, FQ and MD using chloroform: methanol: triethylamine: glacial acetic acid (9.5:0.4:0.05:0.3, by volume) as a mobile phase. The separated bands were scanned at 300 nm in the range of 0.50-4.00, 0.50-3.00, 0.20-3.00 and 0.20-3.00 μg $band^{-1}$ for CR, TD, FQ and MD, respectively. The proposed TLC-densitometric method was applied successfully for determination of the two cited drugs in their commercial formulation. When results obtained by applying the proposed

method for the analysis of pure CR and TD were compared to those obtained by the reported HPLC method, no significant difference was observed.

Section (E): Simultaneous determination of Ciprofloxacin hydrochloride and Tinidazole in presence of their potential impurities by HPLC method.

A precise, accurate and specific RP- HPLC method was developed in this section. The separation was carried out in gradient mode on C8 column. The mobile phase contained a gradient mixture of solvents A and B. 0.05M phosphate buffer adjusted to (pH 3) using orthophosphoric acid was used as solvent A, while acetonitrile was used as solvent B. The gradient program (T (min)/ % B/ flow rate (mL min^{-1})) was set as 0/20/1, 6/20/1 and 6.5/60/1.5, respectively. The eluted compounds were monitored at 310 nm. The column temperature was maintained at 30°C
The method is comparable with the reported HPLC method.

Part IV: Appendix

This part includes a brief idea about the instruments, solvents and chemicals used in other parts, in addition to detailed preparation of the standard solutions used in each part through this work.

This thesis refers to 192 references, contains 84 tables, 100 figures and ends with an Arabic summary.