Summary

This thesis consists of three 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: DETERMINATION OF PARACETAMOL, ASCORBIC ACID AND PSEUDOEPHEDRINE HYDROCHLORIDE IN THEIR TERNARY MIXTURE AND IN PHARMACEUTICAL FORMULATION

This part includes four sections.

Section (A): Introduction and literature Review

This section includes an introduction about the pharmacological action of Paracetamol (PAR), Ascorbic acid (ASC) and Pseudoephedrine hydrochloride (PSH) their chemical structure, physical properties and summary of the methods reported for their analysis in their formulations and in their ternary mixture.

Section (B): Determination of Paracetamol, Ascorbic acid and Pseudoephedrine hydrochloride by Different Spectrophotometric Methods

In this section, derivative, ratio derivative and mean centering of ratio spectra spectrophotometric methods have been applied for determination of PAR, ASC and PSH in their ternary mixture using water as a solvent. The first method was derivative, ratio derivative method, where ^{1}DD spectra were obtained using the standard spectrum of 20 μg mL^{-1} of PAR as a divisor. The amplitudes at 266, 280 and 218 nm were measured for ASC and PSH, respectively. On the other hand, PAR in this mixture was determined by performing ^{1}D method, measuring the amplitudes at 257 nm. The second method was mean centering of ratio spectra method, which based on using the second mean centered of ratio spectra amplitudes at 286, 290 and 287 nm for quantitation of ASC, PSH and PAR, respectively. The developed methods have been applied for determination of the studied drugs in different laboratory prepared mixtures. The results obtained by applying the proposed methods for determination of

PAR, ASC and PSH in dosage form were statistically compared to those obtained by a reported RP-HPLC method and no significance difference was found regarding both accuracy and precision.

Section (C): Determination of Paracetamol, Ascorbic acid and Pseudoephedrine hydrochloride by Multivariate Calibration Models

Multivariate calibration models, such as PCR and PLS has been applied for determination of PAR, ASC and PSH, training set of 17 mixtures containing different ratios of PAR, ASC and PSH was used for construction of the two models. The selectivity of the proposed models was checked using laboratory prepared mixtures (external validation set of eight mixtures). Satisfactory results were obtained on applying the proposed methods for the analysis of PAR, ASC and PSH in Sudoflucet® tablets.

Section (D): Determination of Paracetamol, Ascorbic acid and Pseudoephedrine hydrochloride by Different Chromatographic Methods

In this section, TLC-Densitometric and RP-HPLC methods have been developed for simultaneous determination of PAR, ASC and PSH. The developed TLC-Densitometric method depended on chromatographic separation of PAR, ASC and PSH using chloroform: methanol: formic acid (8: 2: 0.2, by volume) as a mobile phase. The separated bands were scanned at 220 and 254 nm. While the proposed RP-HPLC method was also used for determination of PAR, ASC and PSH, RP-HPLC was achieved by using acetonitrile: water (10:90, v/v), the pH was adjusted to 4 with glacial acetic acid as a mobile phase, at flow rate 1 mL min^{-1} and DAD detection at 220 nm. The suitability of the proposed chromatographic methods was ascertained by the determination of system suitability testing parameters of the separated drugs. The suggested methods were successfully applied for the determination of PAR, ASC and PSH in their pharmaceutical formulation.

Part II: DETERMINATION OF FOILC ACID AND ITS TWO DEGRADATION PRODUCTS (IMPURITIES) IN THEIR TERNARY MIXTURE AND IN PHARMACEUTICAL FORMULATION

This part includes three sections.

Section (A): Introduction and literature Review

This section includes an introduction about the pharmacological action of Folic acid (FOL), its chemical structure, physical properties and summary of the methods developed for its analysis in its formulations and in the presence of other drugs.

Section B: Determination of Folic acid and its Two Degradation Products (Impurities) by Different Spectrophotometric Methods

In this section, different spectrophotometric methods have been applied to improve selectivity for determination of FOL in presence of PTR and PABA degradation products (possible impurities) using sodium hydroxide as a solvent. The first method is ratio difference spectrophotometric method, which depends on measuring the difference value in the ratio spectrum where the difference between 291 and 313 nm was used for determination of FOL, while the difference between 305 and 319 nm was selected for estimation of PABA, on the other hand PTR can be determined in this mixture using the first derivative of ratio spectra amplitudes at 262 nm. The second method is double divisor spectrophotometric methods, which based on using the ratio spectrum obtained by the division of the spectrum of ternary mixture by the spectrum of binary mixture containing two of the three mentioned components, in this method FOL, PABA and PTR were measured at 242, 313 and 258 nm, respectively. The third method is mean centering of ratio spectra spectrophotometric method, in this method FOL, PABA and PTR can be determined using the mean centered second ratio spectra amplitudes at 317-318 (peak to peak), 264-265 (peak to peak) and 232 nm, respectively. The results obtained by applying the proposed methods were statistically compared with those obtained by applying the official RP-HPLC methods and there was no significant difference regarding accuracy and precision.

Section C: Determination of Folic acid and its Two Degradation Products (Impurities) by Different Chromatographic Methods

This section is concerned with the development of sensitive, economic and specific TLC-Densitometric and RP-HPLC methods for determination of FOL, PABA and PTR in the bulk powder and pharmaceutical formulations. The three studied components were well separated using methanol: iso-propanol: water: acetic acid (9:0.5:0.5:0.2, by volume) as a developing system followed by Densitometric measurement of the separated bands at 280 nm. On the other hand, sensitive accurate and specific RP-HPLC method was developed for the separation of the mentioned components. The separation was carried out on C_{18} column using acetonitrile: water: triethylamine mixture (20:80:0.05, by volume) as the mobile phase at a flow rate 1 mL min^{-1} and UV scanning at 280 nm. The developed TLC-Densitometric and RP-HPLC methods have been applied for determination of the studied drug in its commercial tablets and capsules. Statistical comparison of the results obtained by the proposed methods and the official RP-HPLC method showed no significant difference.

Part III: FULL STABILITY STUDY OF DACLATASVIR AND DEVELOPMENT OF DIFFERENT STABILITY INDICATING METHODS OF ANALYSIS

This part includes five sections.

Section (A): Introduction and literature Review

This section includes an introduction about the pharmacological action of Daclatasvir (DAC), its chemical structure, physical properties and summary of the published methods developed for its analysis in pure form and in its tablets. **Section B:**

Section (B): Full Stability Study along with Kinetic Study and Characterization of Daclatasvir Hydrolytic Degradation Rate

In this section, Daclatasvir was subjected to full stability study. The drug was found to be sensitive only to acidic and alkaline hydrolysis, while it was stable to oxidative , photolytic and thermal degradation, also in this section, a kinetic study of Dacltasvir acidic and alkaline degradation as a function of drug concentration, acid and alkaline

concentration and temperature have been established utilizing dual wavelength spectrophotometric method as a resolving method in analytical chemistry. DAC concentrations were determined by measuring the absorbance difference at 215 nm and 275 nm allowed selective determination of DAC in presence of its degradation product.

The developed method has been applied for determination of the studied drug in different laboratory prepared mixtures also applied for determination of DAC in Daklanork® tablets. The results obtained by applying the proposed method for determination of DAC were statistically compared to those obtained by applying the reported RP-HPLC and no significant difference were found regarding both accuracy and precision. Moreover it was used for the kinetic study of the hydrolysis of DAC. The kinetic degradation of DAC obeyed Arrhenius equation and was found to follow pseudo-first order kinetics under the established experimental conditions and is pH and temperature dependent. Activation energy at different temperatures, kinetic rate constants and $t_{1/2}$ at different pH values were calculated. In addition, this section contains schematic diagram of the degradation pathway of DAC and structural illucidation of the prepared degradate.

Section C: Determination of Daclatasvir and its Hydrolytic Degradation Product by Different Sepectrophotometric Methods

In this section, four different spectrophotometric methods namely, Direct spectrophotometric combined with Ratio subtraction, Advanced absorbance subtraction, Area under curve and Dual wavelength spectrophotometric methods were developed for determination of DAC in the presence of its degradation product. In the first method, DAC was determined using zero order absorbance at 316 nm while the degradate was measured using the ratio subtraction method, where the spectra of mixtures were divided by the spectrum of 20 µg $mL^{-1}$of DAC as a divisor, the amplitude value in the plateau region at λ above 310 nm was subtracted from the spectra of the divided mixtures; the obtained spectra was then multiplied by the spectrum of the divisor. Finally, Degradate concentrations in the laboratory prepared

mixtures were measured from the last spectra obtained at its λ_{max}=250 nm. The second method was advanced absorbance subtraction, at which regression equations were constructed at λ_{iso} = 275 nm and used for calculating concentrations of DAC and DEG in the binary mixture after simple mathematical calculation.

The third method was Area under curve, where the simultaneous equations using AUC method, the absorptivity (Y) values of each of the two components were measured at the chosen wavelength ranges 215-230 nm (λ1- λ 2) and 235-250 nm (λ3- λ4). By applying Cramer rule, concentrations of Daclatasvir and its degradate can be obtained.

On the other hand, the forth method was Dual wavelength method, where the suggested method has been applied for determination of DAC and DEG in their binary mixture using methanol as a solvent. DAC concentrations were determined by measuring the absorbance difference at 215 nm and 275 nm. While Degradate concentrations were determined by measuring the absorbance difference at 240 nm and 270 nm.

The proposed methods were validated with different laboratory prepared mixtures and it was successfully applied for determination of DAC in Daklanork® tablets and the standard addition technique has been applied to verify its validity.

Section D: Determination of Daclatasvir in Presence of its Hydrolytic Degradation Product by spectrofluorimetric Method

In this section, sensitive, specific and economic spectrofluorimetric method was developed for determination of DAC in the presence of its hydrolytic degradation product, the emission intensity was measured at 382 nm using excitation wavelength of 317 nm for DAC, the proposed method was applied for the laboratory prepared mixtures and the marketed dosage form with no interference from the added excipients. Method validation was performed according to ICH guidelines. All the calculated parameters were within the accepted limits.

<u>Section E:</u> Stability Indicating TLC-Densitometrc Method for Determination of Daclatsvir in Presence of its Degradtion Product

Stability indicating TLC-Densitometric method has been optimized for determination of DAC in presence of its degradation product resulted from different sress conditions. In TLC-Densitometric method good separation of the binary mixture of DAC and DAC degradation product using chloroform: methanol (9.7:0.3, v/v) as a developing system. The separated bands were scanned at 225 nm. The proposed method was successfully applied for analysis of the cited drug in pharmaceutical formulation and the results showed good agreement with the label amount also, the obtained results were statistically compared to those obtained by the reported RP-HPLC one. The t and F values are less than the tabulated values indicating no significant difference between them.

This thesis refers to 268 references, contains 58 tables, 93 figures and ends with an Arabic summary.