Summary

This thesis consists of three parts; each part includes an introduction, literature review, and descriptive experiments, results and discussion for the all developed methods. It ends with references and a summary in Arabic.

Part I: DETERMINATION OF DANTROLENE SODIUM IN PRESENCE OF ITS MAJOR IMPURITY

In this part, synthesis of Dantrolene related compound C (DAN-C) was carried. Then, different analytical methods were developed and validated in compliance with ICH guidelines and was successfully applied for the determination of Dantrolene sodium (DAN) and DAN-C in their pure form, and laboratory prepared mixtures. Also, the methods were used for analysis of DAN in its dosage form. This part includes six sections as follow:

Section A: Introduction and Literature Review

In this section, the structures, physical properties, and review of the published methods developed for the analysis of the studied compounds; DAN and DAN-C.

Section B: Synthesis of 5-(4-Nitrophenyl)-2-Furaldehyde (Dantrolene Process Impurity)

Dantrolene related compound C is the synthetic precursor of DAN. It is considered to be the acidic and photolytic degradation product of DAN. Synthesis of DAN-C has been performed from p-nitroaniline with good yield and high purity. Structure elucidation has been confirmed by IR, MS and NMR analyses.

Section C: Determination of Dantrolene Sodium and Its Process Impurity by Dual Wavelength Spectrophotometric Method (DW)

Dual-wave length (DW) method, the selected wavelengths for determination of DAN were 315 nm and 380 nm in the concentration range of 2.00-25.00 µg/mL while 368 nm and 401 nm for DAN-C determination in the concentration range 0.20 -10.00 µg/mL. The method was found to be highly selective to determine DAN in presence of up to 30 % of DAN-C. The method was successfully applied for determination of DAN in pharmaceutical formulation. DW method has the advantages of being rapid, accurate, and simple which applied directly on the absorbance spectrum without any manipulation. The obtained results were statistically compared with results obtained from the official method showing no significant difference regarding both accuracy and precision.

Section D: Determination of Dantrolene Sodium and Its Process Impurity by Area Under The Curve Spectrophotometric Method (AUC)

In AUC method, the area under the curve in the wavelength region of 375-400nm and 340-365 nm were recorded for both DAN and DAN-C. Then, Cramer's rule was used for determination in the concentration range of 2.00-25.00 µg/mL and 0.30 -10.00 µg/mL for DAN and DAN-C, respectively. The proposed AUC method was successfully determined the studied components without interference from each other in their laboratory prepared mixtures in presence of 30 % of DAN-C. Satisfactory results were obtained by applying the AUC method to Dantrelex® capsules. When the developed method was compared with the official one using F-value and student's t-test, no significant difference was found between them.

Section E: Determination of Dantrolene Sodium and Its Process Impurity by First Derivative of Ratio Spectra Spectrophotometric Method (^{1}DD)

In ^{1}DD method, the absorption spectra of DAN solutions were divided by the absorption spectrum 5.00 µg/mL of DAN-C and ^{1}DD curves were obtained using $\Delta\lambda$ = 4 nm and scaling factor = 5. The peak amplitudes at 399.4 nm and 434.6 nm (peak to peak) were recorded in the concentration range of 1.00-25.00 µg/mL. On the other hand, the absorption spectra of DAN-C solutions were divided by the absorption spectrum 2.00 µg/mL of DAN and ^{1}DD curves were recorded with $\Delta\lambda$ = 8 nm and scaling factor = 10. The ^{1}DD peak amplitudes at 326.4 were measured in the concentration range of 0.30-10.00 µg/mL. It was noticed that this method was able to analyze DAN in presence of DAN-C in the range of 1-30 %. In order to confirm method specificity, it was applied to pharmaceutical formulation containing the studied drug and acceptable results were obtained. The results obtained by applying the proposed method for the analysis of DAN were statistically compared to those obtained by applying the official method and there was no significant difference regarding both accuracy and precision.

Section F: Determination of Dantrolene Sodium and Its Process Impurity by TLC-Densitometric Method

In this section, a highly selective and sensitive TLC-densitometric method was used for separation of the drug from its degradation product using chloroform: ethyl acetate: acetic acid (10:0.5:0.01, by volume) with UV scanning at 380 nm. It was successfully used for determination of DAN and DAN-C in the range of 0.10-1.50 µg/band and 0.10-2.00 µg/band, respectively. The method was applied to pharmaceutical formulation and was compared favorably with the official HPLC one. The proposed method is more

advantageous than other reported methods because it offers higher sensitivity, selectivity and can be used in laboratories lacking the facilities to conduct the HPLC methods.

Part II: STABILITY INDICATING METHODS FOR DETERMINATION OF GLIQUIDONE AND ITS MAJOR DEGRADATION PRODUCT

In this part, full stability study for Gliquidone (GQ) was developed to anticipate its degradation behavior. Different stability indicating methods were developed and validated according to ICH guidelines for simultaneous determination of the drug and its degradation product; Gliquidone sulfonamide (GQS). This part includes six sections:

Section A: Introduction and Literature Review

This section comprises a brief idea about the structures, properties and different reported methods for the analysis of GQ and GQS.

Section B: Preparation, Isolation and Structural Elucidation of Gliquidone Major Degradation Product

Full stability study of GQ has been carried out in different stress conditions; hydrolytic, oxidative, photolytic, and thermal conditions following ICH guidelines. The drug was found to be unstable under acidic, alkaline and oxidative conditions with the formation of one degradation product. The degradation product was identified to be Gliquidone sulfonamide (GQS) after its isolation and structural elucidation using IR, MS and NMR analyses. GQS is GQ pharmacopeial impurity A and also is considered as its synthetic intermediate.

Section C: Stability Indicating Ratio Difference Spectrophotometric Method (RD) for Determination of Gliquidone and Its Major Degradation Product

In RD method, the zero order spectra of different concentrations of GQ was divided by absorption spectrum of 8.00 µg/mL GQS while GQS was divided by absorption spectrum of 4 µg/mL GQ. The difference in amplitudes (ΔA) of the obtained ratio spectra was measured at 222 and 240 nm, ($\Delta A_{240\text{-}222\ nm}$) for GQ in concentration range of 2.00-25.00 µg/mL and ($\Delta A_{222\text{-}240\ nm}$) for GQS in the range of 1.00-20.00 µg/mL. The method succeeded for measuring of both GQ and GQS in laboratory prepared mixtures contain the ratio of 3-90 % of GQS, indicating high selectivity of the RD method. The proposed method was successfully applied to GQ in its pharmaceutical formulation. The obtained results were statistically compared with a reported HPLC method showing no significant difference regarding both accuracy and precision.

Section D: Stability Indicating First Derivative of Ratio Spectra Spectrophotometric Method (^{1}DD) for Determination of Gliquidone and Its Major Degradation Product

In ^{1}DD method, the absorption spectra of GQ solutions were divided by the absorption spectrum of standard 8.00 µg/mL of GQS and ^{1}DD curves were obtained using $\Delta\lambda = 8$ nm and scaling factor = 10. The peak amplitude at 233.6 and 245.5 nm (peak to peak) were measured in concentration range of 2.00-25.00 µg/mL. On the other hand, the absorption spectra of GQS solutions were divided by the absorption spectrum of standard 4.00 µg/mL of GQ and ^{1}DD curves were obtained with $\Delta\lambda = 8$ nm and scaling factor = 10. The ^{1}DD peak amplitude at 231.4 and 249.2 nm (peak to peak) were measured in the concentration range of 1.00-20.00 µg/mL. Specificity of the ^{1}DD method was confirmed by application for determination of the GQ and GQS in different laboratory prepared mixtures containing

GQS up to 90 %. The developed method was successfully applied for quantification of GQ in Glurenor® tablets with high selectivity. When the developed method was compared with the reported one using F-value and student's t-test, no significant difference was found.

Section E: Stability Indicating TLC-Densitometric Method for Determination of Gliquidone under Different Stress Conditions

In this section a specific stability indicating TLC-densitometric method was developed for estimation of both GQ and GQS. Moreover, this method was also used to study the degradation behavior of GQ following ICH recommendations. Chromatographic separation was carried out on TLC plates coated with silica gel 60 F_{254} using chloroform: glacial acetic acid: formic acid (10: 0.3: 0.1, by volume) as a developing system and scanned detected at 225 nm over a concentration range of 1.00–8.00 and 0.10-2.00 µg/band for GQ and GQS, respectively. The proposed method was successfully applied for quantification of GQ in the capsule. Statistical analysis using F-value and student's t-test proved no significant differences among the proposed method and the reported one. The proposed TLC-densitometric method is highly sensitive, accurate, time and cost effective.

Section F: Stability Indicating UPLC Method for Determination of Gliquidone under Different Stress Conditions

In this section, full stability study for GQ was developed and validated UPLC method. Chromatographic separation was carried out on Hypersil Gold RP8 column (50× 2.1 mm (i.d.), 1.9 µm particle size) using isocratic elution of methanol: water (70: 30, v/v), pH adjusted to 3 with orthophosphoric acid, and flow rate of 0.6 mL/min with UV scanning at

225 nm over a concentration range of 1.00-45.00 and 0.50-45.00 μg/mL of GQ and GQS, respectively. The utility of the suggested method was verified by application to Glurenor® tablets where no interference from additives was found. The proposed methods were also statistically compared to a reported HPLC method with no significant difference. The developed method needs short analysis time (1.5 min) and hence low mobile phase consumption that leads to lower environmental hazardous and lower analysis cost. Also it has good sensitivity and selectivity being a specific stability indicating method.

Part III: DETERMINATION OF WARFARIN SODIUM IN PRESENCE OF ITS PROCESS IMPURITIES

In this part, different analytical methods were developed and validated for the determination of Warfarin sodium (WF) and its process impurities; impurity B (Imp B) and impurity C (Imp C) in their pure form, in laboratory prepared mixtures and in WF pharmaceutical preparation. It includes four sections:

Section A: Introduction and Literature Review

This section describes the pharmacological actions, chemical structures, physical properties of WF and its process impurities. Review of the published methods was developed for WF determination either alone or in combination with other drugs, metabolites or impurities.

Section B: Multivariate Calibration Methods for Determination of Warfarin Sodium in Presence of Its Process Impurities

In this section, multivariate calibration models, namely; PCR and PLS were successfully used for determination of WF in presence of its process impurities. A five-level, three-factors calibration design was used for construction of calibration and

validation sets, the developed models were applied to predict the concentration of WF in presence of its process impurities in an external validation set in the concentration ranges of 10.00-30.00 (for WF) and 0.40-1.60 µg/mL for (Imp B and C). The multivariate calibration models were successfully applied to pharmaceutical formulation. Results obtained by the developed models were compared with the official method and no significant difference was found between them.

Section C: Simultaneous Determination of Warfarin Sodium and Its Process Impurities by TLC-Densitometric Method

In this section, simple and accurate TLC-densitometric have been developed for resolving WF and its two process impurities using chloroform: acetone: formic acid (10:0.5: 0.2, by volume) as a developing system. Densitometric scanning of the separated peaks was performed at 290 nm over a concentration range of 0.20-2.00, 0.05-1.40, 0.10-1.80 µg/band for WF, Imp B, and Imp C, respectively. The suggested TLC-Densitometric method was successfully applied for analysis of the studied drug in Marevan® tablets and the results showed good agreement with the labeled amount. Statistical analysis using F-value and student's t-test proved no significant differences among the proposed method and the official one. The proposed method offers higher sensitivity and selectivity over the reported methods. Also, it succeeded to resolve and quantify the parent drug along with its impurities in short analysis time and with low analysis cost.

Section D: Simultaneous Determination of Warfarin Sodium and Its Process Impurities by Eco-Friendly HPLC Method

In this section, the suggested HPLC method was used for simultaneous determination of WF and its process impurities without harming the environment. Chromatographic separation was carried out on ZOBRAX Eclipse Plus C18 (4.6 x 100 mm) using green mobile phase that consisted of ethanol: water (56:44, v/v), adjusting pH to 3 ± 0.05 by using acetic acid with UV detection at 220 nm and the analysis time was 3.5 min. In addition, the greenness profile of the developed HPLC method was compared to the official and the published HPLC methods. The proposed method was successfully applied for the determination of WF in its pharmaceutical dosage. When the developed method was compared with the official HPLC one using F-value and student's t-test, no significant difference was found between them. The main advantages of the proposed HPLC are that it is greener and more solvent-saving with shorter analysis time comparing to the reported ones. Moreover, the developed HPLC method provided the sensitivity required to meet BP requirements for detection and quantitation of drug impurities.

This thesis contains 138 references, 68 figures, 69 tables and ends with a summary in Arabic.