**Summary**

This thesis consists of four parts, starts with introductory part and ends by description of the references included in the thesis. There are three parts which describe the experimental work and discussion of the results. The thesis includes a summary in Arabic.

**PART I: General introduction to anti-emetics**

This part introduces the anti-emetic drug metoclopramide hydrochloride (MCP-HCl) describing its physicochemical characters, stability and uses. In addition, the introduction summarizes different methods for the drug analysis single or mixture with some drugs and with paracetamol (PCM) and pyridoxine (VB6) in more detail.

**PART II: Spectrophotometric analysis of metoclopramide hydrochloride mixtures**

This part comprises analysis of MCP-HCl by spectrophotometry. The part consists of three sections. Section A represents analysis of MCP-HCl mixture with paracetamol while section B represents analysis of the drug mixture with pyridoxine. Section C covers analysis of the drug by a chemometric stability indicating method in presence of its H2O2 and acid induced degradation products.

All the suggested assays have the advantages that belongs the spectrophotometry which is accuracy, high precision, specificity, robustness, applicability and cost-effectiveness.

All validation procedures required by FDA and ICH guidelines are carried out and approved for all the proposed methods.

***Section [A]: Assay of Metoclopramide Hydrochloride/ Paracetamol Mixture***

This section is sub-divided into two sub-sections. The first sub-section describes a ratio subtraction spectrophotometric method whereas the second one describes a third derivative spectrophotometric assay.

***Sub-section [I]:*** *Assay of Metoclopramide Hydrochloride/ Paracetamol Binary Mixture by Ratio Subtraction Spectrophotometric Method*

In this sub-section MCP-HCl could be determined specifically by recording of its first and second orders spectra at 321 & 330 nm (R%: 99.83 ± 0.969 & 100.23± 0.768), respectively. While PCM could be determined by dividing the absorption spectrum of the drugs mixture by MCP-HCl spectrum (divisor) resulting in a new spectrum called ratio spectra, which include a constant values corresponding to division of MCP-HCl spectrum value in the mixture by that of the divisor. Subtraction of this value gives new spectrum which is multiplied by spectrum of the used divisor to give spectrum corresponding to concentration of PCM in the mixture so PCM concentration could be determined via measuring the amplitude at 294 nm (R%: 100.80 ± 0.509).

***Sub-section [II]:*** *Assay of Metoclopramide Hydrochloride/ Paracetamol Binary Mixture by Third Derivative Spectrophotometric Method*

This sub-section suggest third derivative spectrophotometric method for analysis of MCP-HCl/ PCM mixture which is characterize by simplicity and no need to long or complicated software handling. Simply both drugs could be determined specifically by recording of the third order spectrum of the drug mixture spectrum and measuring the peak amplitude at 334.5 nm for determination of MCP-HCl (R%: 99.60 ±1.471) and at 299 nm for determination of PCM (R%: 100.58 ± 0.867).

***Section [B]:Assay of Metoclopramide Hydrochloride/ Pyridoxine Mixture***

In this section, two assays are described for determination of MCP-HCl/ VB6 mixture. This section is sub-divided into two sub-sections as follows:

***Sub-section [I]:*** *Assay of Metoclopramide Hydrochloride/ Pyridoxine Binary Mixture by Derivative Ratio Spectrophotometric Method*

This sub-section shows determination of MCP-HCl/ VB6 mixture. The spectrum of MCP-HCl/ VB6 mixture is divided, one time, by spectrum of VB6 and recorded in first order to give derivative ratio spectrum corresponding to the concentration of MCP-HCl in the mixture, the peak amplitude could be measured at 318.5 nm (R%: 99.10 ± 0.644). Next, the spectrum of MCP-HCl/ VB6 mixture is divided by spectrum of MCP-HCl then recorded in first order to derivative ratio spectrum corresponding to the concentration of VB6 in the mixture the peak amplitude could be measured at 327.5 nm (R%: 100.40 ± 1.300).

***Sub-section [II]:*** *Assay of Metoclopramide Hydrochloride/ Pyridoxine Binary Mixture by Isosbestic Point Spectrophotometric Method*

In this sub-section, Isosbestic point method is described for the determination of MCP-HCl/ VB6 mixture. Total concentration of MCP-HCl/ VB6 mixture could be determined by measuring the peak amplitude at isosbestic point, the point at which the absorption spectra of MCP=HCl, VB6 and their mixture showed crossing, 299.5 nm. Since MCP-HCl can be determined separately by recording the first order of its spectrum and measuring the peak amplitude at λ= 321.5 nm (R%: 100.80 ± 0.509). Therefore VB6 concentration can be obtained by subtraction of MCP-HCl concentration from total concentration of the mixture (R%: 100.40 ± 1.157).

***Section [C]: Assay of Metoclopramide Hydrochloride in Presence of Acid Induced and Oxidative Degradation Products via the Principal Component and Partial Least Squares Methods***

Using mathematics, statistics and formal logic, to design or select optimal experimental procedures to provide maximum relevant information by analyzing chemical data, in methods like principal component regression (PCR) and partial least square (PLS) are described. Both methods are proposed for determination of MCP-HCl in presence of multi-mixture of its degradation products. The methods assume, like other analytical quantitative assays, a linear relationship between the absorbance values and the concentration of the drug in the mixture. Each method needs a calibration step, where the relationship between spectral data and concentration is deduced from a set of reference samples. Then a prediction step should be constructed frequently to predict the calibration.

PLS is related to PCR in that spectral decomposition is also performed but in different way. In PCR, the spectra are decomposed on the basis of the maximum variance between spectral data and without using information about concentration. PLS differs from PCR in that it uses both spectral and concentration data in modeling.

Few stability data have been reported for metoclopramide hydrochloride. In this work, MCP-HCl is exposed to stress acidic and oxidative degradation using 0.1 N HCl and 3% H2O2 solutions. For simultaneous analysis of a mixture of the drugs and the produced degradations, simplicity, accessibility and feasibility of spectrophotometry are combined with ability of chemomertry to analyze relatively complicated mixture using mathematical modeling and intelligent software to analyze MCP-HCl in presence of its acid induced degradation products (AC. Deg.) (R%: 100.00 ± 0.555) and peroxide induced degradation products (OX. Deg.) (R%: 100.36 ± 0.953).

**PART III: Densitometric analysis of metoclopramide hydrochloride mixtures**

This part involves analysis of MCP-HCl by densitometry. The suggested assays are highly specific, applicable, speed and cost-effective. All validation tests mentioned by FDA and ICHguidelines are carried out and approved for all the proposed methods.

This part consists of three sections as following:

***Section [A]: Assay of Metoclopramide Hydrochloride in Presence of Acid Induced and H2O2 Induced Degradation Products via HP-TLC***

This section includes a thin layer chromatographic (TLC) assay to determine MCP-HCl in presence (AC.Deg.) and (OX.Deg.), simultaneously, using the mobile phase; chloroform, methanol, toluene and conc. ammonium hydroxide solution (70: 30: 10: 0. 5; by volume) giving typical chromatogram of MCP-HCl and at Rf 0.65 ± 0.01, when the developed band is scanned via densitometer at 245 nm.

***Section [B]: Assay of Metoclopramide Hydrochloride/ Pyridoxine Binary Mixture via HP-TLC***

This section describes a densitometric assay to determine MCP-HCl/ VB6 mixture, using the mobile phase consisted of benzene, methanol, glacial acetic acid and acetone in ratio (10: 8: 0.5: 0.5; by volume) giving perfect chromatogram of both drugs with Rfs. 0.2 ± 0.02 & 0.51 ± 0.01, respectively. The detector is a densitometer and the developed bands of the drugs are scanned at 245 nm (R%: 99.82 ± 0.561& R%: 101.85 ± 0.571, respectively).

***Section [C]: Assay of Metoclopramide Hydrochloride/ Paracetamol Binary Mixture via HP-TLC***

This section includes a TLC assay for determination of MCP-HCl/ PCM mixture. The mobile phase: methanol, chloroform and conc. ammonia solution (10: 2: 0.15) gives typical chromatogram for MCP-HCl and PCM at Rfs 0.21 ± 0.02 & 0.59 ± 0.02 (R%: 99.83 ± 0.969 & R%: 100.80 ± 0.509), respectively, when the developed bands of the drugs are scanned via densitometer at 270 nm.

**PART IV: compatibility studies and high performance liquid chromatographic analysis of metoclopramide hydrochloride mixtures**

This part consists of three sections. Compatibility studies of MCP-HCl mixtures with VB6 and PCM and analysis of their mixture via RP-HPLC are covered through the first two sections. Whereas third section describes a stability indicating RP-HPLC method for analysis of MCP-HCl/ (AC.Deg.)/ (OX.Deg.) ternary mixture.

***Section [A]:***

This section covers a compatibility study of MCP-HCl/ VB6 mixture and its analysis via RP-HPLC through the next two sub-sections:

***Sub-section [I]:*** *Studying Compatibility of Metoclopramide Hydrochloride/ Pyridoxine Binary Mixture via IHCMC*

This compatibility study examines reaction of both drugs mixture in solid state or when dissolved in USP and bio-relevant GIT simulating fluids to determine their behaviors in GIT few hours after ingestion. As well, a comparison between effect of USP and bio-relevant GIT simulating fluids is done to show difference between both media in drug bio-studies. Detecting mixture compatibility and conducting stability study are carried out by ITMC. The best differentiating method to analyze, trace and detect reactions and resulting products which evolved in the calorimeter vials is RP-HPLC assay suggested in next subsection.

***Sub-section [II]:*** *Assay of Metoclopramide Hydrochloride/ Pyridoxine Binary Mixture via Isocratic RP-HPLC*

In this subsection, a sensitive accurate highly specific RP-HPLC

assay for estimation of the previously studied drugs mixture; MCP-HCl/ VB6, is developed and validated. Typical chromatogram of the drugs mixture is obtained by elusion of MCP-HCl/ VB6 mixture solution through C18 column using the mobile phase water, acetonitril and methanol in ratio 90: 10: 10 at flow rate 1 mL/min., at ambient temperature, and using spectrophotometer for peak detection at 212 nm (R%: 99.42 ± 0.860 & R%: 100.49 ± 0.587, repectively).

***Section [B]***

In this section, a compatibility study of MCP-HCl/ PCM mixture and its analysis via RP-HPLC are covered through the frequent sub- sections:

***Sub-section [I]:*** *Studying Compatibility of Metoclopramide Hydrochloride/ Paracetamol Binary Mixture via IHCMC*

The reactions of MCP-HCl in mixtures with PCM is examined in this sub-section, when it dissolved in USP and bio-relevant gastro-intestinal tract, GIT,-simulating fluids to determine their compatibility within a few hours after ingestion. Moreover, the stability of solid mixtures of MCP-HCl and PCM using stress conditions described by ICH and FDA guidelines are used to detect incompatibilities via IHCMC.

***Sub-section [II]:*** *Assay of Metoclopramide Hydrochloride/ Paracetamol Binary Mixture via Isocratic RP-HPLC*

The proposed RP-HPLC assay under this sub-section has the advantage of; it can analyze both drugs in presence of their degradation products. In this sub-division, hydrolysis degradation products has been identified by comparing with corresponding authentic products. The best chromatographic conditions are those which yield sharp peaks with good separation in presence of degradation products. The challenge of this method is to find a suitable chromatographic condition, which can separate the drug mixture and their degradation products, simultaneously. Typical chromatogram of the drugs mixture without interference from their decomposition products is obtained by using the mobile phase composed of phosphate buffer - acetonitrile - methanol (40:15:10 v/v/v) adjusted to pH 3 with phosphoric acid at flow rate 0.5 mL/min., ambient temperature and UV detection at 230nm .

***Section [C]: Multivariate Optimization of a RP.HPLC Assay for Determination of Metoclopramide Hydrochloride In Presence of Acid Induced and Oxidative Degradation Products***

This section of thesis treats the problem of identifying important variables in fractional factorial design by hypothesis testing techniques. Frequently, this work describes optimization of an analytical methodology for the determination of MCP-HCl in presence of its AC. Deg. and OX. Deg. products by HPLC using fractional factorial design. This kind of approach, multivariate, allows studying a lot of variances to make a decision which conditions give best chromatogram for drug analysis using fewer experiments. In the developed method, eluting water and acetonitrile in ratio (90: 10, v/v) at pH 6, adjusted with ortho-phosphoric acid through a C18 column at a flow rate of 0.5 ml/min. and ambient temperature with UV detection at 212 nm gives well-formed separated MCP-HCl peak at retention time 8.6 min. A complete validation process is performed to ensure specificity, robustness, repeatability and reliability of the established method.

**It includes 160 references and Arabic summary**