PREFACE

This thesis is concerned with analytical study of some pharmaceutical drugs acting on GIT containing different chemical classes namely Di-iodohydroxyquinoline (DIHQ), Metronidazole (MTN), Hyoscine N-Butyl Bromide (HBB), Paracetamol (PAR) and Dipyrone (DIP).

The aim of this work is to develop simple, rapid, sensitive and selective methods for simultaneous determination of the cited drugs in their mixtures in pure forms or in pharmaceutical preparations.

Different analytical techniques are adopted in this thesis including Spectrophotometric methods, namely [First derivative of ratio spectra, Formation of a highly stable orange-red ferroin chelate with o-phenanthroline (Colorimetric method) and chemometric manipulation of the spectrophotometric data], Spectrofluorimetry and chromatographic separation by TLC- densitometric methods, RP-HPLC and LC-MS techniques.

ABSTRACT

The thesis comprises five parts:

PART 1 *Introduction and literature review*

This part includes a general introduction about the chemistry and mode of action of all drugs mentioned in thesis, followed by the reported methods used for their quantitative analysis.

This part comprises five drugs:

(1) Diiodohydroxyquinoline (DIHQ) (2) Metronidazole (MTN)

(3) Hyoscine N-ButylBromide (HBB) (4) Paracetamol (PAR)

(5) Dipyrone (DIP).

PART II ***Determination of Mixture of Diiodohydroxyquinoline and metronidazole in Pure Form and in Pharmaceutical Preparations***

This part comprises four sections:

Section(A) Spectrophotometric determination of Diiodohydroxyquinoline in presence of metronidazole using iron(III)phenanthroline.

In this work a spectrophotometric method based on reduction of ferric-phenanthroline (ferriin) to ferrous-phenanthroline (ferroin) by DIHQ is used where the absorption of the obtained orange-red colored ferroin chelate was measured at λ_{max} 510 nm**.**

The reaction conditions including, volume of reagent, pH of buffer, volume of buffer, effect of temperature, time of heating and stability of color that can affect the extent of color development were optimized in order to maximize color intensity and drug sensitivity.

Under the optimum experimental conditions**,** a linear relationship was obtained in the range of 2.0 – 10.0 µg.mL^{-1}of DIHQ with mean percentage recovery 100.15 ± 0.538 at 510 nm**.**

The developed method was validated according to ICH guidelines.

The results of the proposed method were statistically compared with the reference method adopted by the manufacturer company. The student's t

and F values were found less than the tabulated ones indicating no significant difference with respect to accuracy and precision.

Section(B) ***Spectrofluorimetric determination of Diiodohydroxyquinoline in presence of metronidazole in pure form, pharmaceutical preparation and in spiked human plasma.***

This study is based upon measuring the native fluorescence intensity of DIHQ using λ_{ex}= 250 nm and at λ_{em} = 495 nm in water solvent at room temperature.

The effect of solvent, different excitation wavelengths and the stability of the fluorescence intensity by time were studied.

Under the optimum experimental conditions**,** a linear correlation was obtained between DIHQ emission intensity $x10^{-2}$ and the concentration in the range of 0.4- 0.9 $\mu g.mL^{-1}$ with mean percentage recovery of 100.21± 1.129. The method was applied successfully to spiked human plasma with mean percentage recovery of 100.53± 1.417.

The developed method was validated according to ICH guidelines.

The results of the proposed method were statistically compared with the reference method adopted by the manufacturer company. The student's t and F values were found to be less than the tabulated ones indicating no significant difference with respect to accuracy and precision.

Section(C) ***Simultaneous Determination of Mixture of Diiodohydroxyquinoline and Metronidazole by TLC-Spectrodesitometric Method.***

In this method, the two drugs were separated on a silica gel plate using chloroform: acetone: glacial acetic acid (7.5: 2.5: 0.1, by volume) as mobile phase and UV detection at 254 nm for each.

The two drugs were determined over a concentration range of 0.2-1.8 $\mu g.band^{-1}$ for DIHQ with mean percentage recovery of 100.21±1.537 and over a concentration range of 0.4-1.8 $\mu g.band^{-1}$ for MTN with mean percentage recovery of 100.14±1.807.

Applying this method to the analysis of laboratory prepared mixtures assessed the selectivity of the suggested method. The developed method was validated according to ICH guidelines. The results of the proposed method were statistically compared with the reference method adopted by the manufacturer company. The student's t and F values were found to be less than the tabulated ones indicating no significant difference with respect to accuracy and precision.

<u>Section (D)</u> Simultaneous determination of mixture of Diiodohydroxyquinoline and Metronidazole in pure form and in pharmaceutical preparations by RP-HPLC.

In this method, the two drugs were separated on a reversed-phase C18 column using water : methanol (60 :40, v/v, pH adjusted to 3.6 with H_3PO_4 acid) as mobile phase and HPLC analysis was performed by isocratic elution with a flow rate of 0.7 $mL.min^{-1}$ and UV detection at 220 nm for each drug .

The two drugs were determined over a concentration range of 5 – 50 $\mu g.mL^{-1}$ for DIHQ with mean percentage recovery of 99.88±0.774 and over

a concentration range of 5– 60 μg.mL^{-1} for MTN with mean percentage recovery of 99.89±0.909.

Applying this method to the analysis of laboratory prepared mixtures assessed the selectivity of the suggested method. The developed method was validated according to ICH guidelines. The results of the proposed method were statistically compared with the reference method adopted by the manufacturer company. The student's t and F values were found to be less than the tabulated ones indicating no significant difference with respect to accuracy and precision.

Part III LC-MS as a stability-indicating method for analysis of Hyoscine N-Butyl Bromide under stress degradation conditions and identification of degradation products.

This part comprises only one section.

Hyoscine N-Butyl Bromide (HBB) was subjected to different ICH recommended stress conditions of acid hydrolysis, base hydrolysis, oxidation and photolysis. It showed extensive decomposition under base hydrolytic conditions, while it was only moderately sensitive to stress acid hydrolytic conditions. It showed also moderate degradation in response to oxidation degradation conditions. The drug showed no changes under photolytic degradation conditions.

In total, a number of major degradation products were detected by LC/MS. For establishment of stability-indicating LC method, the reaction solutions in which different degradation products were formed were

prepared, and the separation was optimized by varying the LC conditions. The percent of degradation was calculated in each run by measuring the intensity of the peak area of the intact drug at 6.2 min. Complete degradation only occurred in case of 5 N NaOH indicating that the drug is liable to alkaline hydrolysis.

The LC-MS study was carried out to identify the major degradation products. The *m*/*z* values of the main peaks were used to identify and characterize the chemical structure of degradates.

Part IV Determination of Mixture of Hyoscine N-Butyl Bromide (HBB) and Paracetamol (PAR) in Pure Form and in Pharmaceutical Preparations.

This part comprises four sections:

Section A Determination of Mixture of Hyoscine N-Butyl Bromide (HBB) and Paracetamol (PAR) by zero order spectra and ^{1}DD First Derivative Ratio Spectra.

In this method, PAR could be determined in presence of HBB by use of zero order spectra with measurements at 248.0 nm over a concentration range of (2-12µg.mL^{-1}) with mean percentage recovery 99.74±0.731.

HBB could be determined in presence of PAR by using first derivative of the ratio spectra (^{1}DD) method with measurements at 220.5 nm using the spectrum of PAR 100 µg.mL^{-1} as a divisor over a concentration range of 2 – 45 µg.mL^{-1}with mean percentage recovery 99.52±1.327.

The selectivity of the proposed method was checked using laboratory prepared mixtures and it was successfully applied to the analysis of the pharmaceutical preparations containing the above drugs with no interference from other dosage form additives.

The developed method was validated according to ICH guidelines.

The results of the proposed method were statistically compared with the reference method adopted by the manufacturer company. The student's t and F values were found to be less than the tabulated ones indicating no significant difference with respect to accuracy and precision.

Section B Multivariate Spectrophotometric Technique for Determination of Hyoscine N-Butyl Bromide(HBB), Paracetamol (PAR)and para-aminophenol(PAP) Mixture in pure form and in pharmaceutical preparations .

Two chemometric techniques, namely; Principle component regression (PCR) and Partial least squares (PLS) have been successfully applied for simultaneous determination of Hyoscine N-Butyl Bromide(HBB), Paracetamol (PAR) and para-aminophenol (PAP) (main degradate of paracetamol) mixture in pure forms and in pharmaceutical preparations. Training set of 15 mixture containing different ratios of the two drugs and degradate is used for construction of the two models.

The selectivity of the proposed method was checked using laboratory prepared mixtures (validation set consisting of 10 mixtures). Also it was successfully applied to the analysis of the cited drugs in different pharmaceutical preparations.

Section(C) *Simultaneous Determination of Mixture of Hyoscine N-Butyl Bromide (HBB) and Paracetamol (PAR) in pure form and in pharmaceutical preparations by RPTLC-Spectrodesitometric Method.*

In this method, the two drugs were separated on a reversed phase C18 silica gel F_{254} plate using methanol: citrate buffer (pH=1.5): triflouroacetic acid (70:30:0.1, by volume) as mobile phase and UV detection at 210 nm for each.

The two drugs were determined over a concentration range of 2.0-12.0 $\mu g.band^{-1}$ for HBB with mean percentage recovery of 100.22±0.733 and over a concentration range of 2.0-14.0 $\mu g.band^{-1}$ for PAR with mean percentage recovery of 99.84±1.035.

Applying this method to the analysis of laboratory prepared mixtures assessed the selectivity of the suggested method. The method was successfully applied to the analysis of the pharmaceutical preparations containing the above drugs with no interference from other dosage form additives. The developed method was validated according to ICH guidelines. The results of the proposed method were statistically compared with the reference method adopted by the manufacturer company. The student's t and F values were found to be less than the tabulated ones indicating no significant difference with respect to accuracy and precision.

Section (D) *Simultaneous determination of mixture of Hyoscine N-Butyl Bromide(HBB) and Paracetamol (PAR)*

in pure form and in pharmaceutical preparations by RP-HPLC.

In this method, the two drugs were separated on a reversed-phase C18 column using water: methanol (50:50, v/v, pH adjusted to 3.9 with triflouroacetic acid) as mobile phase and HPLC analysis was performed by isocratic elution with a flow rate of 1.0 $mL.min^{-1}$ and UV detection at 210 nm for each drug .

The two drugs were determined over a concentration range of 2.0-50.0 $\mu g.mL^{-1}$ for HBB with mean percentage recovery of 100.10±0.475 and over a concentration range of 5.0-200.0 $\mu g.mL^{-1}$ for PAR with mean percentage recovery of 99.86±0.953.

Applying this method to the analysis of laboratory prepared mixtures assessed the selectivity of the suggested method. The method was successfully applied to the analysis of the pharmaceutical preparations containing the above drugs with no interference from other dosage form additives. The developed method was validated according to ICH guidelines. The results of the proposed method were statistically compared with the reference method adopted by the manufacturer company. The student's t and F values were found to be less than the tabulated ones indicating no significant difference with respect to accuracy and precision.

<u>Part V</u> Simultaneous Determination of Mixture of Hyoscine N-Butyl Bromide (HBB) and Dipyrone (DIP) in Pure Form and in Pharmaceutical Preparations

This part comprises two sections:

Section(A) Simultaneous Determination of Hyoscine N-Butyl Bromide(HBB) and Dipyrone (DIP)Mixture in pure form and in pharmaceutical preparations by RPTLC-Spectrodesitometric Method.

In this method, the two drugs were separated on a reversed phase C18 silica gel F_{254} plate using methanol: citrate buffer (pH=1.5): triflouroacetic acid (70:30:0.1, by volume) followed by addition of 0.05 gram of sodium lauryl sulphate as mobile phase and UV detection at 210 nm for each.

The two drugs were determined over a concentration range of 2.0-10.0 $\mu g.band^{-1}$ for HBB with mean percentage recovery of 100.05±0.763 and over a concentration range of 1.0-12.0 $\mu g.band^{-1}$ for DIP with mean percentage recovery of 100.29±0.614 .

Applying this method to the analysis of laboratory prepared mixtures assessed the selectivity of the suggested method. The developed method was validated according to ICH guidelines. The results of the proposed method were statistically compared with the reference method adopted by the manufacturer company. The student's t and F values were found to be less than the tabulated ones indicating no significant difference with respect to accuracy and precision.

Section (B) Simultaneous determination of mixture of Hyoscine N-Butyl Bromide(HBB) and Dipyrone (DIP) in pure form and in pharmaceutical preparations by RP-HPLC.

In this method, the two drugs were separated on a reversed-phase C18 column using water: methanol (50: 50 v/v, pH adjusted to 7.0 with triflouroacetic acid and triethylamine) as mobile phase and HPLC analysis was performed by isocratic elution with a flow rate of 1.0 $mL.min^{-1}$ and UV detection at 210 nm for each drug .

The two drugs were determined over a concentration range of 2.0-50.0 $\mu g.mL^{-1}$ for HBB with mean percentage recovery of 100.28±0.571 and over a concentration range of 5.0-120.0 $\mu g.mL^{-1}$ for DIP with mean percentage recovery of 99.92±0.424 .

Applying this method to the analysis of laboratory prepared mixtures assessed the selectivity of the suggested method. The developed method was validated according to ICH guidelines. The results of the proposed method were statistically compared with the reference method adopted by the manufacturer company. The student's t and F values were found to be less than the tabulated ones indicating no significant difference with respect to accuracy and precision.

This thesis refers to **274** references, contains **62** figures, **59** tables and ends with Arabic summary.