Analytical Study on Certain Fluorine-containing Drugs

Thesis

Presented for the partial fulfillment of the degree of
Master in Pharmaceutical Sciences (Pharmaceutical Analytical Chemistry)

By

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قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا
عَلَّمْتَنَا إِنَّكَ أَنْتَ الْعَلِيمُ
الْحَكِيمُ.

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β-CD	Beta-cyclodextrin
CTAB	Cetyl Trimethyl Ammonium Bromide
CE	Capillary Electrophoresis
r	Correlation Coefficient
CDs	Cyclodextrins
DOP	Di-octyl Phthalate
ESI	Electron Spray Ionization
EDTA	Ethylene Diamine Tetra Acetic acid
EE	Excitation Emission
EEM	Excitation Emission Matrix
^{1}D	First Derivative
FLU	Fluconazole
GC	Gas Chromatography
HPLC	High Performance Liquid Chromatography
ICH	International Conference for Harmonization
HP- β-CD	2-hydroxypropyl- β-cyclodextrin
IUPAC	International Union of Pure and Applied Chemistry
ISE	Ion Selective Electrode
LOD	Limit of Detection
LOQ	Limit of Quantitation
MET	Metformin
MPs	Micro Particles
NPs	Nano Particles
PDA	Photodiode array
PVC	Polyvinyl chloride
RSD	Relative Standard Deviation
RPC	Reverse Phase Chromatography
^{2}D	Second Derivative
SSM	Separate Solution Method

SLS	Sodium Lauryl Sulphate
STG	Sitagliptin
SD	Standard Deviation
SFS	Synchronous Fluorescence Scan or Spectroscopy
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TIN	Tinadazole
VIT B_{12}	Vitamin B_{12}

Preface

This thesis is concerned with certain fluorine-containing drugs, involving the development and validation of sensitive analytical methods for the determination of two fluorine-containing drugs; namely Sitagliptin and Fluconazole.

The aim is to determine these two drugs in bulk, mixtures, pharmaceutical formulaions, and biological fluids.

The thesis is directed to develop different analytical methods for determination of Sitagliptin and Fluconazole. These methods are potentiometric, spectrophotometric, chromatographic, and spectrofluorimetric techniques. These techniques were successfully applied for the determination of the studied fluorine-containing drugs in different matrices.

Five selective potentiometric sensors were developed for analysis of Sitagliptin and Fluconazole . The investigated electrodes were successfully applied for their determination in biological fluids. No sample pretreatment was required. Simplicity, sensitivity and lack of tedious separation were achieved.

Both isocratic and gradient high performance liquid chromatographic procedures using diode array detector were developed for separation and determination of Sitagliptin either in pure form, pharmaceutical formulation, or together with Metformin and Vitamin B_{12}. The suggested gradient procedure was further applied for separation and quantitation of Sitagliptin in spiked human urine. A simple pretreatment only was required.

Derivative spectroscopy was also used to separate and quantify Sitagliptin in mixtures with Metformin and/or Sitagliptin alkaline degradation product, as well

as, Fluconazole in mixtures with Tinidazole. Application to pharmaceutical formulations was, also, successful.

A synchronous spectrofluorimetric technique was suggestedfor the determination of Sitagliptin in its single and multi-ingredient pharmaceutical formulations as well as in spiked human plasma. A simple pretreatment only was required.

Summary

The thesis consisted of six parts:

Part I: General introduction

It explained the nature of fluorine-containing drugs, and their increased introduction into the pharmaceutical field.

Part II: Literature review

This part included a review of the physical and chemical properties of the studied fluorine-containing drugs, Sitagliptin and Fluconazole. Also, it included different analytical methods reported in the literature that aimed for determination of the chosen drugs in pure form, mixtures, pharmaceutical formulations, and biological fluids.

Part III: Spectrophotometric determination of fluorine-containing drugs

This part involved the development of two derivative methods for the analysis of the studied drugs, Sitagliptin and Fluconazole with further application to mixtures and pharmaceutical formulations. This part was divided into two sections:

Section A: Second derivative spectrophotometric determination of Sitagliptin in presence of Metformin and its alkaline degradation product

The zero-crossing method has been utilized to measure the second- derivative value of the derivative spectrum. Sitagliptin was measured at 278 nm from the second-derivative spectrum (zero-crossing of Metformin and Sitagliptin alkaline degradation product). Sitagliptin was determined in bulk powder, pharmaceutical formulation, and in presence of Metformin and Sitagliptin alkaline degradation product. The proposed method was easily applicable for routine analysis.

Section B: First derivative spectrophotometric determination of Fluconazole in presence of Tinidazole

The zero-crossing method has been utilized to measure the first- derivative value of the derivative spectrum. Fluconazole was measured at 218.4 nm from the first-derivative spectrum (zero-crossing of Tinidazole). The method was applied for the determination of Fluconazole in its pure form, in presence of Tinidazole, and in its pharmaceutical formulation with good accuracy and recovery.

Part IV: Potentiometric determination of fluorine-containing drugs

This part included the determination of Sitagliptin and Fluconazole using ion selective electrodes. It contained 3 sections.

Section A: Introduction to ion-selective electrodes

It described the different types of ion selective electrodes and their assembly.

Section B: Stability indicating ion selective electrodes for determination of Sitagliptin in presence of its alkaline degradation product

This section described the use of cyclodextrins and calix-8-arene as neutral ionophores for the development of novel sensors for the determination of Sitagliptin. Two sensors were investigated with dioctyl phthalate as a plasticizer in a polymeric matrix of polyvinyl chloride. Sensor 1 and 2 were constructed using β-cyclodextrin and calix-8-arene as ionophores, respectively. These sensors were used for determination of Sitagliptin in bulk powder, pharmaceutical formulations, and biological fluids (plasma and urine).

The performance of the proposed electrodes were assessed according to the IUPAC recommendations and showed that the electrodes had fast, stable and nernestian response.

Response time, effect of pH and temperature on the response, selectivity and stability of the electrodes were studied to determine the optimum conditions for the determination of Sitagliptin using the proposed electrodes.

Section C: Comparative study of normal, micro & nano-sized iron oxide particles for the potentiometric determination of Fluconazole

This section described the fabrication of three classical electrodes. It studied the effect of iron oxide particles on the response of the three sensors. The performance of the proposed electrodes were assessed according to the IUPAC recommendations and showed that they had fast, stable and nernestian response.

Response time, effect of pH and temperature on the response, selectivity and stability of the three sensors were studied to determine the optimum conditions for the determination of Fluconazole using the proposed electrodes.

The suggested electrodes were successfully applied for detection and quantification of Fluconazole in pure form, pharmaceutical formulation, and biological fluids (plasma, urine and milk).

Part V: Chromatographic Determination of Sitagliptin

High Performance Liquid Chromatographic technique was developed for the determination of Sitagliptin alone and in mixture with Metformin and Vitamin B_{12} .The proposed method was further applied to spiked human urine. This part involves two sections.

Section A: Isocratic chromatographic determination of Sitagliptin

This section was concerned with the application of HPLC method for the determination of Sitagliptin. The mobile phase of choice was acetonitrile: phosphoric acid at pH 3.0 (60:40, v/v) and Diode array detector was set at 210

nm. The proposed HPLC method was successfully applied for determination of the mentioned drug in pure form and pharmaceutical formulation.

Section B: Gradient chromatographic determination of Sitagliptin in presence of Metformin and Vitamin B_{12}

This section was concerned with the application of HPLC method for the simultaneous determination of Sitagliptin in the presence of Metformin and Vitamin B_{12}. The mobile phase of choice was acetonitrile: phosphoric acid at pH 3.0 (60:40, v/v) at a gradient program. Diode array detector was set at 210 nm. The proposed HPLC method was successfully applied for determination of the Sitagliptin in pure form, mixture, and spiked human urine.

Part VI: Synchronous spectrofluorimetric determination of Sitagliptin in presence of Metformin

This part was concerned with the determination of Sitagliptin down to a concentration approaching its C_{max} concentration in plasma. It was divided into two sections.

Section A: Introduction to Synchronous Spectrofluorimetry

This section discussed the theory of synchronous spectrofluorimetry and its advantages over ordinary spectrofluorimetry.

Section B: Synchronous spectrofluorimetric determination of Sitagliptin in presence of Metformin

Synchronous spectrofluorimetric technique has been utilized for the determination of Sitagliptin .Scanning of different wavelength differences was done in order to choose the most appropriate $\Delta\lambda$. Sitagliptin was determined at $\Delta\lambda$=40 nm. The technique was applied to the determination of Sitagliptin in pure form, pharmaceutical formulations, and spiked human plasma.

References and Arabic summary

This thesis contains 234 references, 54 tables and 40 figures and ends with an Arabic summary.

Part I: General Introduction

Part II: Literature review