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ANALYTICAL TECHNIQUES FOR THE ESTIMATION OF FENOFIBRATE IN BULK AND PHARMACEUTICAL DOSAGE FORMS: A REVIEW

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ABSTRACT

Fenofibrate which is chemically propan-2-yl 2-{4-[(4-chlorophenyl) carbonyl] phenoxy}-methyl propanoate. It is mainly used to reduce cholesterol levels in patients at risk of cardiovascular disease. Like other fibrates, it reduces low-density lipoprotein (LDL) and very low density lipoprotein (VLDL) levels, as well as increasing high density lipoprotein (HDL) levels and reducing triglycerides levels. It also appears to have a beneficial effect on the insulin resistance featured by the metabolic syndrome. . Literature survey reveals that Fenofibrate is estimated individually by UV spectrometry, RP-HPLC, LC/MS, LC/MS/MS and stability indicating HPLC.

Keywords: Fenofibrate, UV spectrometry, RP-HPLC, LC/MS, LC/MS/MS.

INTRODUCTION

Fenofibrate (Tricor) is a drug of the fibrate class. It is mainly used to reduce cholesterol levels in patients at risk of cardiovascular disease. Like other fibrates, it reduces both low-density lipoprotein (LDL) and very low density lipoprotein (VLDL) levels, as well as increasing high-densitylipoprotein (HDL) levels and reducing triglycerides level. Fenofibrate is mainly used for primary hypercholesterolemia or mixed dyslipidemia. Fenofibrate appears to decrease the risk of cardiovascular and possibly diabetic retinopathy in those with diabetes mellitus. It also appears to be helpful in decreasing amputations of the lower legs in this same group of people. It is used in addition to diet to reduce elevated low-density lipoprotein cholesterol (LDL), total cholesterol, triglycerides (TG), and apolipoprotein B (Apo B), and to increase high-density lipoprotein cholesterol (HDL) in adults with primary hypercholesterolemia or mixed dyslipidemia It is used in addition to diet for treatment of adults with severe hypertriglyceridemia. Improving glycemic control in diabetics showing fasting chylomicronemia will usually decrease the need for pharmacologic intervention. Three randomized, double-blind trials have shown that treatment with fenofibric acid plus a stain improved HDL and triglyceride levels better than a stain alone and improved LDL levels better than fenofibric acid monotherapy.Additionally, in Europe, Fenofibrate is indicated in mixed hyperlipidemia in those with high cardiovascular risk in addition to a stain when triglycerides and HDL are not adequately controlled.

Mass spectrometry (MS) has been described as the smallest scale in the world, not because of its size of what it weighs a molecule and a micro analytical technique that can be used selectively to detect and determine the amount of a given analyte [1-2]. MS is also used to determine the elemental composition and some aspect of the molecular structure of an analyte. Unique features of MS include its capacity for direct determination of the nominal mass of an analyte, and to produce an detect fragments of the molecule that correspond to discrete groups of atoms of different elements that reveal structure features [1]. The tools of MS are mass spectrometers, and

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data are called mass spectra that can be displayed in many different ways, which allow the desired information about the analyte to be easily extracted. A MS is an apparatus which produces a beam of gaseous ions from a sample, sorts out the resulting mixture of ions according to their mass-to-charge ratios, and provides output signals which are measures of relative abundance of each ionic species present. MS are usually classified on the basis of how the mass separation is accomplished, but they all can be described as ion optical devices which separate ions according to their mass-to-charge (*m/z*) ratios by utilizing electric and/or magnetic force fields (Figure. 1). The concept of MS is to form ions from a sample, to separate the ions based on their *m/z* ratio (this can be considered to be the same as the mass because the ion has only a single charge in most cases), and to measure the abundance of the ions. In modern MS instrumentation used in environmental analyses, all of the functions (ionization separation of the ions, rate of data acquisition, detection of the ions, and storage of the data) are under computer control. Gaseous molecules are ionized in the ion source to form molecular ions which some of that will fragment. By various processes, ions of differing *m/z* values pass through the mass analyzer one at a time to reach the detector. When the ions strike the detector, they are converted into an electrical signal which, in turn, is converted into a digital response that can be stored by the computer [3-4]. A mass spectrometer does not directly determine mass but, determines the mass of a molecule by measuring the *m/z* of its ion. The knowledge of the *m/z* of the ions enables one to determine what is present, while the measured ion intensities answer the question of how much is present. In addition, systematic interpretation of the mass spectra provides a detailed picture of the ionization process which, in turn, may be utilized in the elucidation of molecular structures. This definition of the term *m/z* is important to understanding of MS. It should be noted that the *m/z* value is a dimensionless number that is always used as an adjective, e.g. the ions with *m/z* 256, or the ion has an *m/z* value of 256. A recording of the number of ions (abundance) of a given *m/z* value as a function of the *m/z* value is a mass spectrum. The mass component that makes up the dimensionless *m/z* unit is based on an atomic scale rather than the physical scale normally considered as mass. Only ions are detected in mass spectrometer and any nonionic particles that have no charge are removed from the mass spectrometer by the continuous pumping that maintains the vacuum. The MS first must produce a collection of ions in the gas phase. These ions are separated according to their *m/z* values in a vacuum where the ions cannot collide with any other forms of matter during the separation process. Ions of individual *m/z* values are separated and detected in order to obtain the mass spectrum. Separation of ions in an evacuated environment is mandatory. If an ion collides with neutrals in an elastic collision during ion separation process, the ion's direction of travel could be altered and ion might not reach the detector. If an ion's collision with neutral is inelastic, sufficient energy transfer may cause it to decompose, meaning that the original ion will not be detected. Close encounters between ions of the same charge can be cause deflection in the path of each. Direct contact between ions of opposite charge sign will result in neutralization. Ions are positively or negatively charged atoms, groups of atoms, or molecules. The process whereby an electrically neutral atom or molecule becomes electrically charged, due to losing or gaining one or more of its extra nuclear electrons, is called ionization [5]. Although both positive and negative ions can be analyzed by MS, the majority of instruments are used to investigate positive ions because in most ion sources they are produced in larger number than negative ions. There is a minimum amount of energy, characterized by the "ionization potential," that must be provided in order for ion formation to occur. The first ionization potential of an atom or molecule is defined as the energy input required removing (to infinite distance) a valence electron from the highest occupied atomic or molecular orbital of the neutral particle to form the corresponding atomic or molecular ion, also in its ground state. When only one electron is removed the ion is called an atomic or molecular ion; often the term "parent ion" is used. The formation of parent ions may be considered as ionization without cleavage. The numerical magnitude of the ionization potential is influenced by such factors as the charge upon the nucleus, the atomic or molecular radius, the shielding effect of the inner electronic shells, and the extent to which the most loosely bound electrons penetrate the cloud of electric charge of the inner shells Because only ions can be detected in MS, any particles that are not ionic (molecules or radicals) are removed from the MS by the continuous pumping that maintains the vacuum. When only individual ions are present, they can be grouped according to their unique properties (mass and number of charges) and moved freely from one point to another. In order to have individual ions free from any other forms of matter, it is necessary to analyze them in a vacuum, which means that the ions must be in the gas phase. It is a fundamental requirement of MS that ions be in the gas phase before they can be separated according to their individual *m/z* values and detected. Due to ionization sources such as electro spray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI), MS has become an irreplaceable tool in the biological sciences. Over the past decade, MS has undergone tremendous technological improvements allowing for its application to proteins, peptides, carbohydrates, DNA, drugs, and many other biologically relevant molecules [6].

Instrumentation of mass spectrometric detection

Mass spectrometry is a particularly powerful scientific technique because it can be successfully applied even if you have only a tiny quantity available for

analysis—as little as 10-12 g, 10-15 moles for a compound of mass 1000 Daltons (Da). Compounds can be identified through mass spectrometry at very low concentrations (one part in 1012) in chemically complex mixtures. The basic mass spectrometry processes of instrumentation are consisted of (1) introduction of sample; a sample which can be a solid, liquid, or vapor is loaded onto a mass spectrometry device and is vaporized, (2) ionization; sample components are ionized by one of several available methods to create ions, (3) analyzer sorting; the ions are sorted in 445 an analyzer according to their *m/z* ratios through the use of electromagnetic fields, (4) detector; the ions then pass through a detector where the ion flux is converted into a proportional electrical current and (5) date conversion; the magnitude of the ion/electrical signals is converted into a mass spectrum. MS instruments consist of three modules: an ion source, which can convert gas phase sample molecules into ions (or, in the case of ESI, move ions that exist in solution into the gas phase); a mass analyzer*,* which sorts the ions by their masses by applying electromagnetic fields; and *a* detector, which measures the value of an indicator quantity and thus provides data for calculating the abundances of each ion present. The technique has both qualitative and quantitative uses. These include identifying unknown compounds, determining the isotopic composition of elements in a molecule, and determining the structure of a compound by observing its fragmentation. Other uses include quantifying the amount of a compound in a sample or studying the fundamentals of gas phase ion chemistry (the chemistry of ions and neutrals in a vacuum). MS is now in very common use in analytical laboratories that study physical, chemical, or biological properties of a great variety of compounds [7-10].

A reliable, fast, sensitive and selective Ultra Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC-MS/MS) method has been developed and validated for the determination of fenofibrate in marketed product (Lipanthyl) and human plasma. The chromatographic separation was performed on a reversed-phase Acquity®BEH C_{18} column (1.7 µm particle size, 50 mm x 2.1 mm ID) with an isocratic elution profile and mobile phase consisting of methanol and water (80:20, %, v/v). To achieve optimum chromatographic condition the influence of mobile phase composition and flow rate was investigated. The total chromatographic analysis time was as short as 2 min. Detection and quantification of the analyzed drug sample were carried out with a triple quadrupole mass spectrometer using Electro spray Ionization (ESI) operating in positive ionization mode. The data acquisition was performed in Multiple Reactions Monitoring (MRM) mode. The method was validated over a concentration range of 0.5-200ng/mL (r^2=0.993, n=6). The selectivity, matrix effect, recovery, accuracy, precision, and stabilities were validated for determination of fenofibrate in human plasma. Analytical recoveries of extracted fenofibrate from plasma were more than 92%. The validation results showed that the proposed method was sensitive, economical and less toxic and it could successfully be applied for evaluation of pharmacokinetics of fenofibrate in animals [11-14].

A simple and precise RP-HPLC method was developed and validated for the determination of Choline fenofibrate in pharmaceutical dosage forms. Chromatography was carried out on Agilent make Zorbax C18 column (4.6 mm x 15 cm), 5 µ particle size using a mobile phase of phosphate buffer (pH 6.8) : acetonitrile (70 : 30 % v/v) at a flow rate of 0.8 ml/min. The analyte was monitored using UV detector at 298 nm. The retention time was found to be 3.207 min for Choline fenofibrate. The proposed method was found to be having linearity in the concentration range of 5-35 µg/ml with correlation coefficient 0.999. The mean recoveries obtained for Choline fenofibrate were in the range of 99.4-100.65 %. The developed method has been statistically validated according to ICH guidelines and found to be simple, precise and accurate with the prescribed values. Thus the proposed method was successfully applied for the estimation of Choline fenofibrate in routine quality control analysis in bulk and its formulations.

Objective: Develop a simple isocratic reverse phase high performance liquid chromatography (RPHPLC) method and validate for the determination of Fenofibrate in bulk and Pharmaceutical dosage forms.

Methods: RPHPLC quantification was carried out using Zorbax C-18 column (5µm, 150cm 4.6mm, ID) with a mobile phase comprising phosphate buffer (pH 3.0) : Acetonitrile in the ratio of 30:70 (% v/v) at a flow rate of 1.0 ml/min. The detection was carried out using a diode array detector at 286 nm. The retention time was found to be 19.268 min and produced a linear response in the concentration range of 1-500 µg/mL (R2~0.999). The % RSD was found to be below 2%. The LOD and LOQ were found to be 0.229µg/ml and 0.765µg/ml respectively [15-16].

A stability-indicating LC assay method was developed for thequantitative determination of Fenofibrate (FFB) in pharmaceutical dosage form in the presence of its degradation products and kineticdeterminations were evaluated in acidic, alkaline and peroxidedegradation conditions.

Chromatographic separation was achieved by use of Zorbax C18 column (250 × 4.0 mm, 5 m). The mobile phasewas established by mixing phosphate buffer (pH adjusted 3 withphosphoric acid) and acetonitrile (30: 70, v/v). FFB degraded in acidic, alkaline and hydrogen peroxide conditions, while it was more stable inthermal and photolytic conditions. The described method was linearover a range of 1.0-500 g mL-1 for determination

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of FFB (r= 0.9999) [17-19]. The precision was demonstrated by relative standard deviation (RSD) of intra-day (RSD=0.56 – 0.91) and inter-day studies (RSD= 1.47). The mean recovery was found to be100.01%. The acid and alkaline degradations of FFB in 1M HCl and 1M NaOH solutionsshowed an apparent zero-order kinetics with rate constants 0.0736 and 0.0698 min−1respectively and the peroxide degradation with 5% H2O2 demonstrated an apparent first-orderkinetics with rate constant k = 0.0202 min−1. The t1/2, t90 values are also determined for allthe kinetic studies. The developed method was found to be simple, specific, robust, linear, precise, and accurate for the determination of FFB in pharmaceutical formulations [20-25].

The aim of present work is to develop and validate spectrophotometric methods for the determination of fenofibrate, an anti-hyperlipidemic, fibric acid derivative in pharmaceuticalformulation. Methanol was used as a solvent throughout the study. Quantitative determination offenofibrate in pharmaceutical formulation was carried out by UV-spectrophotometric method using the absorbtivity values at 287.5 nm and by comparison with standard (method 1a and 1b)and the first order derivative absorbance values at 249.2 nm were utilized for estimation of drug (method 2). The method showed high specificity in the presence of formulation excipients andgood linearity in the concentration range of 0-60 µg/mL. Percent recovery values at 287.5 nmwere 100.3% while it was 100.18% at in 1st order derivative spectrophotometry at 249.2 nm (n=3). SD values showed that both spectrophotometric methods were reproducible. The intra and interday precision data demonstrated that method is precise [26-29].

A simple, sensitive and reproducible UV visible spectrophotometric method has been developed for the quantitative determination of fenofibrate in bulk drug and pharmaceutical dosage forms using MBTH reagent. The method is based on the measurement of absorbance of fenofibrate in methanol (0.5% MBTH in 0.5% HCl and 1% FeCl3 in 0.5% HCl) at 596 nm. Beer's law is obeyed over the linear range 2-5µg /ml of fenofibrate for the method with apparent molar absorptivity value of 1909.5905 L mol-1cm-1. The method was validated in accordance with the current ICH guidelines. The precision results, expressed by reproducibility (RSD 1.7%) and repeatability (RSD 1.5%), were satisfactory. The accuracy is also satisfactory (RSD 0.200532%). The result demonstrated that the proposed method is accurate, precise and reproducible.

Figure 1. Sturacture of Finofibrate

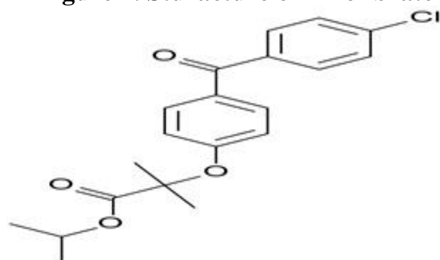


Figure 2. Structure of mass spectrum

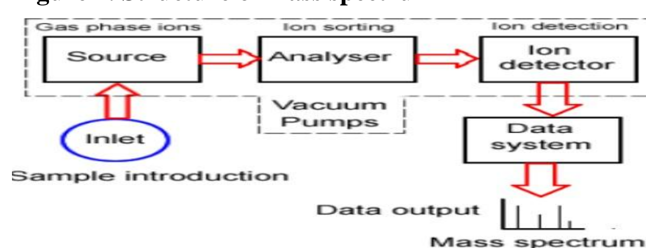
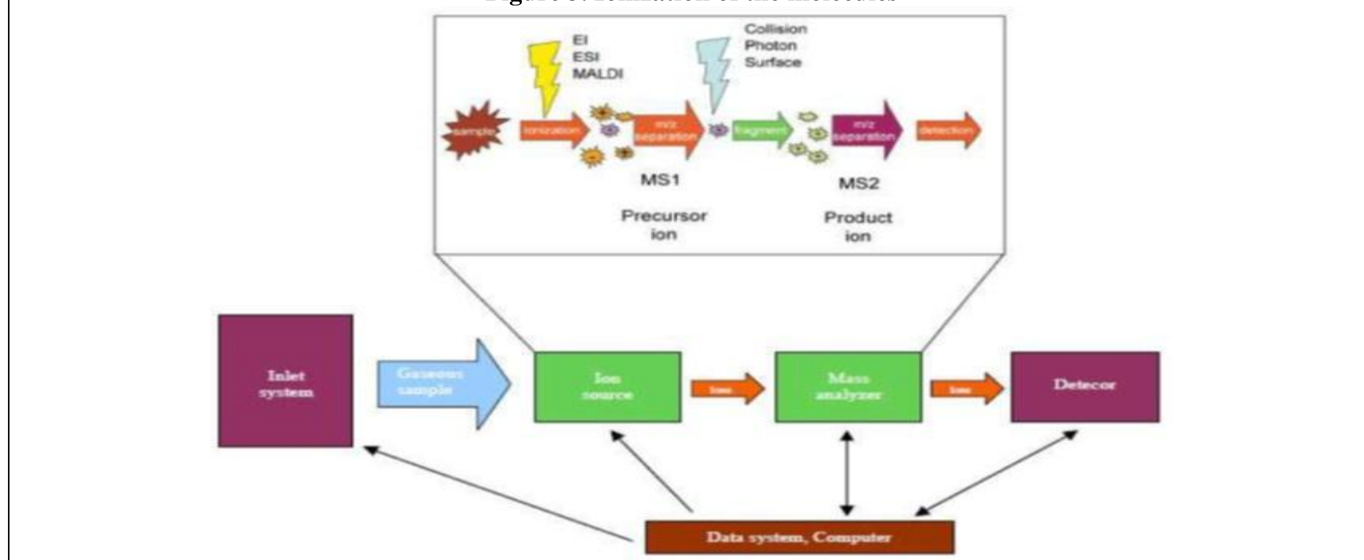


Figure 3. Ionization of the molecules



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