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

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ABSTRACT

Pitavastatin Calcium is a member of the medication class of statins. The IUPAC name \((3R, 5S, 6E)-7-[2\text{-cyclopropyl}-4-(4\text{-fluorophenyl})\text{quinolin-3-yl}]-3,5\text{-dihydroxyhept-6-enoic acid with molecular formula C25H24FNO4 (421.461)}\). Pitavastatin completely inhibits HMG CoA reductase, the rate-determining enzyme in hepatic cholesterol synthesis. Consequently, LDL-C receptors in the liver are increased, thereby increasing the removal of LDL-C from the blood. The primary route of metabolism of pitavastatin was hepatic glucuronidation with minimal metabolism by cytochrome P450 2C9 (CYP 2C9) and CYP 2C8. It is being marketed under license in South Korea and in India. It is likely that pitavastatin will be approved for use in hypercholesterolaemia. Literature survey reveals that meloxicam is estiamted individually by RP-HPLC, determination of impurity and assay by RP-HPLC, Stability indicating UPLC and HPLC, HPTLC and and stability indicating HPTLC, LC-MS/MS by using solid phase extraction Technique.

KEYWORDS: Pitavastatin, RP-HPLC, UPLC, HPTLC, LC-MS/MS.

INTRODUCTION

Pitavastatin (usually as a calcium salt) is a member of the blood cholesterol lowering medication class of statins, marketed in the United States under the trade name Livalo. Like other statins, it is an inhibitor of HMG-CoA reductase, the enzyme that catalyses the first step of cholesterol synthesis. It has been available in Japan since 2003, and
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is being marketed in South Korea and in India. It is expected that pitavastatin will be approved outside Southeast Asia as well. In the US, it received FDA approval in 2009. Kowa Pharmaceuticals is the owner of the American patent to pitavastatin. USES: Like the other statins, pitavastatin is indicated for hypercholesterolaemia (elevated cholesterol) and for the prevention of cardiovascular disease. A 2009 study of the 104 week LIVES trial found pitavastatin increased HDL cholesterol, especially in patients with HDL lower than 40 mg/dl, who had a 24.6% rise, in addition to greatly reducing LDL cholesterol 31.3%. HDL improved in patients who switched from other statins and rose over time. In the 70-month CIRCLE observational study, pitavastatin increased HDL more than atorvastin. It has neutral or possibly beneficial effects on glucose control. As a consequence, pitavastatin is likely to be appropriate for patients with metabolic syndrome plus high LDL, low HDL and diabetes mellitus.[1]

![Pitavastatin](image.png)

**Fig No 1: Pitavastatin**

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. 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. The tools of MS are mass spectrometers, and 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. 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. 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. 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.\[1\]
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 10^12) 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 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.

**Fig No 3: LC-MS/MS**

**ANALYTICAL METHODOLOGIES**

Mrinalini C. Damle et al., Pitavastatin Ca is a HMG-CoA reductase inhibitor, blood cholesterol lowering agent. An approach for the stress degradation was successfully applied for the development of stability indicating HPTLC method for the determination of Pitavastatin Ca in the presence of its degradation product on the plates precoated with silica gel 60 F254. The mobile phase used was Chloroform: Methanol in the ratio of 8:2 v/v. The drug showed considerable absorbance at 244nm. Stress testing of Pitavastatin Ca was carried out according to the international conference of harmonization (ICH) guideline Q1A (R2). The drug was subjected to acid, base, neutral hydrolysis, oxidation, thermal degradation and photolysis. There was no interference between the drug peak and peak of product of degradation; therefore the method was specific for the determination of Pitavastatin Ca in the presence of the degradation product. This system showed a peak for Pitavastatin Ca at Rf value of 0.44 ± 0.02. The data of linear regression analysis indicated a good linear relationship over the range of 200–1000 ng/band concentrations. The method was validated for robustness, precision and recovery. The LOD and LOQ were 16.23 and 49.20 ng/band, respectively. Under various stressed conditions, Pitavastatin Ca showed degradation product only under acidic hydrolysis at Rf value of 0.70 ± 0.02. \[2\]
E. SASIKIRAN GOUD et al., Objective: The main objective of current study was to develop and validate RP-HPLC, simple, precise, accurate and specific chromatographic method for the determination of related impurities of pitavastatin in pharmaceutical formulations. Methods: A high performance liquid chromatography instrument and Phenomenex, Kinetex C18, 75 X 4.6 mm, 2.6 μ 100A were used for determination of pitavastatin and its related impurities (Desfluoro impurity, anti isomer, Z-isomer, methyl Ester impurity, lactone impurity and tertiary butyl ester impurity). Buffer was prepared by using 0.82 g of sodium acetate in 1000 mL of water and adjusts its pH to 3.8 with acetic acid. Filter this solution through 0.22 μm nylon filter and sonicate to degas. The mobile phase-A was prepared by mixing of buffer and acetonitrile in the ratio of 0 90:10(v/v).The mobile phase-B was prepared by mixing acetonitrile and water in the ratio of 90:10(v/v).The flow rate of 1.0 mL/min was set with gradient program, the temperature of column compartment maintained at 25°C and Ultra violet detection done at 250nm wavelength. The pitavastatin and its related impurities peaks eluted at 9.13, 7.41, 9.71, 10.78, 14.86, 15.54 and 21.82 minutes and then run time was set as about 30 minutes. Results: The correlation coefficient (≥ 0.998) shows the linearity of response against concentration over the range of LOQ to 200%. The observed result shows that the method was rapid, precise, accurate and simple. The method was validated as per ICH guidelines. Conclusion: The developed and validated High performance liquid chromatographic method was suitable for determination of pitavastatin and its related impurities in pharmaceutical formulations which is more useful with respect to regular Laboratory analysis.[3]

Antony Raj Gomas et al., Degradation pathway for pitavastatin calcium is established as per ICH recommendations by validated and stability indicating reverse phase liquid chromatographic method. Pitavastatin is subjected to stress conditions of acid, base, oxidation, thermal and photolysis. Significant degradation is observed in acid and base stress conditions. Four impurities are studied among which impurity-4 is found prominent degradant. The stress samples are assayed against a qualified reference standard and the mass balance is found close to 99.5%. Efficient chromatographic separation is achieved on a BEH C18 stationary phase with simple mobile phase combination delivered in gradient mode and quantification is carried at 245 nm at a flow rate of 0.3 mL min-1. In the developed UPLC method the resolution between pitavastatin calcium and four potential impurities is found to be greater than 4.0. Regression analysis shows an r value (correlation coefficient) of greater than 0.998 for pitavastatin calcium and four potential impurities. This method is capable to
detect the impurities of pitavastatin calcium at a level of 0.006% with respect to test concentration of 0.10 mg/mL for a 2-μL injection volume. The developed UPLC method is validated with respect to specificity, linearity & range, accuracy, precision and robustness for impurities determination and assay determination.[4]

Nanjappan Satheesh Kumar et al., A simple, sensitive, reliable and rapid reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been developed and validated for the determination of pitavastatin calcium using paracetamol as internal standard. The chromatographic system consisted of Shimadzu LC-10ATVP Pump, SPD-M10 AVP with PDA detector. Separation was achieved on the phenomenex C18 (250 x 4.60), 5 μ particle size column in isocratic mode at room temperature. The sample was introduced through an injector valve with a 20 μl, sample loop. 0.5% Acetic acid: Acetonitrile 35:65 (%, v/v), was used as mobile phase with flow rate of 1 ml/min. UV detection was performed at 245 nm. A calibration graph was plotted which showed a linearity range between 1-5 μg/ml with the correlation coefficient of 0.9986. The LOD was 5 ng/ ml, while the LOQ was 20 ng/ml. Validation studies revealed the method is specific, rapid, reliable, and reproducible. To study the validity of the method, recovery studies and repeatability studies were carried out using the same optimum conditions. The system suitability studies were also calculated which includes column efficiency, resolution, capacity factor and peak asymmetrical factor. Therefore the proposed method is reliable, rapid, precise and selective so may be used for the quantitative analysis of pitavastatin calcium.[5]

B. Neelima et al., A new simple, selective, accurate Stability- Indicating RP-HPLC method has been developed and validated for quantitative determination of pitavastatin in bulk and pharmaceutical dosage form. The chromatographic separation was achieved with Agilent Eclipse XDB, C18, (150 x 4.6 mm, 5μ) column. The optimized mobile phase consisting phosphate buffer: Acetonitrile (65:35% v/v pH 3.5 adjusted with o-phosphoric acid. The flow rate was 0.9 mL/min and eluent was detected at 244nm using PDA detector. The retention time of pitavastatin was found to be 3.05. The percentage recoveries were found to be in the range of 99.00-100%. The calibration curve was constructed between peak area vs concentration and demonstrated good linear in the range of 25 - 150μg/ml. Degradation studies were studied for pitavastatin under various stress conditions such as acid hydrolysis, base hydrolysis, oxidation, thermal, photochemical and UV. All the degradation peaks were resolved effectively using developed method with different retention times. The developed
method was validated according to ICH guidelines. As the method could effectively separates the degradation products from active ingredient, it can be used for routine analysis of drug both in bulk and pharmaceutical dosage form.\[7\]

**Boligarla Gopi Kalyan Kumar1 et al.,** A rapid and sensitive liquid chromatography–tandem mass spectrometric (LC–MS/MS) assay method has been developed and fully validated for the quantitative determination of pitavastatin in human plasma. A pitavastatin stable labeled isotope (pitavastatin d4) was used as an internal standard. Analyte and the internal standard were extracted from human plasma via solid phase extraction technique. The chromatographic separation was achieved on a C18 column by using a mixture of acetonitrile–0.1% formic acid (90:10, v/v) as the mobile phase at a flow rate of 0.85 mL/min. The calibration curve obtained was linear (r2 0.99) over the concentration range of 0.05–160 ng/mL. Method validation was performed as per FDA guidelines and the results met the acceptance criteria. A run time of 1.5 min for each sample made it possible to analyze more than 450 plasma samples per day. The proposed method was found to be applicable to clinical studies.

**K.Venkat et al.,** A simple, sensitive, reliable, and rapid HPTLC method has been developed for the determination of pitavastatin calcium in tablet dosage form. Identification and determination were performed on aluminum backed silica gel 60F\textsubscript{254} washed with methanol. The mobile phase of ethyl acetate-methanol-ammonia-1 drop formic acid (7:2:0.8) calibration plots were established showing the dependence of response (peak area) on the amount chromatographed. The spot were scanned at 245 nm. The method has a linear range of 50–250 ng/spot. The method was validated for selectivity, repeatability, and accuracy. The method was used for determination of the compound in commercial pharmaceutical dosage forms. It is a more effective option than other chromatographic techniques in routine quality control.

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