SIMULTANEOUS ESTIMATION OF ROSUVASTATIN AND FENOFIBRATE BY RP-HPLC METHOD

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1. ABSTRACT
An accurate, sensitive and precise RP-HPLC method has been developed and validated for the simultaneous estimation of Rosuvastatin Calcium (ROS) and Fenofibrate (FEN) from bulk and Pharmaceutical Dosage form. The separation was achieved by Zorbax Eclipse plus C18 column (100mm X 4.6mm, 3.5μm) in isocratic mode, with mobile phase comprises of Acetonitrile: Water in proportion of 90:10v/v, pH 3 of the water was adjusted with Ortho Phosphoric Acid. The flow rate of mobile phase was 0.6 ml/min and the detection wavelength was 243nm. The retention time of Rosuvastatin calcium and Fenofibrate were 1.93min and 4.35min respectively. The calibration curve range was 2-16μg/ml for ROS and 14-112μg/ml for FEN. The regression data shows good linear relationship with 0.9974 for ROS and 0.9954 for FEN. The method was validated in accordance with the requirements of ICH guidelines. Moreover, the proposed analytical method was applied to monitor the formulation commercially available.

KEYWORDS: Rosuvastatin Calcium (ROS) and Fenofibrate (FEN), Validation, RP-HPLC.

ABBREVIATIONS

<table>
<thead>
<tr>
<th>Symbols</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>μg</td>
<td>Microgram</td>
</tr>
<tr>
<td>μL</td>
<td>Microlitre</td>
</tr>
<tr>
<td>AB</td>
<td>AmlodipineBesilate</td>
</tr>
<tr>
<td>ACE</td>
<td>Angiotensinconvertingenzyme</td>
</tr>
<tr>
<td>AR</td>
<td>Analytical reagent</td>
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</table>

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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>BDS</td>
<td>Base deactivated silane</td>
</tr>
<tr>
<td>Conc.</td>
<td>Concentration fico gram</td>
</tr>
<tr>
<td>Fig.</td>
<td>Figure</td>
</tr>
<tr>
<td>GC</td>
<td>Gas chromatography gmGram</td>
</tr>
<tr>
<td>GPC</td>
<td>Gel permeation chromatography</td>
</tr>
<tr>
<td>HETP</td>
<td>Height equivalent to Theoretical Plates</td>
</tr>
<tr>
<td>HIC</td>
<td>Hydrophobic interaction chromatography HILIC</td>
</tr>
<tr>
<td>Hydrophilic interaction chromatography HPLC</td>
<td>High-performance liquid chromatography</td>
</tr>
<tr>
<td>I.D</td>
<td>Internal diameter</td>
</tr>
<tr>
<td>ICH</td>
<td>International Conference on Harmonization</td>
</tr>
<tr>
<td>IEC</td>
<td>Ion exchange chromatography</td>
</tr>
<tr>
<td>IP</td>
<td>Ion-pair</td>
</tr>
<tr>
<td>LC</td>
<td>Liquid chromatography</td>
</tr>
<tr>
<td>LC-MS</td>
<td>Liquid chromatography-mass spectroscopy</td>
</tr>
<tr>
<td>LOD</td>
<td>Limit of detection</td>
</tr>
<tr>
<td>LOQ</td>
<td>Limit of quantification</td>
</tr>
<tr>
<td>LP</td>
<td>Losartan Potassium</td>
</tr>
<tr>
<td>Min</td>
<td>Minutes mL Millilitre</td>
</tr>
<tr>
<td>mm</td>
<td>millimetre mM Millimolar</td>
</tr>
<tr>
<td>MS</td>
<td>Mass spectroscopy</td>
</tr>
<tr>
<td>NARP</td>
<td>Non-aqueous reverse phase ng Nano gram</td>
</tr>
<tr>
<td>NPC</td>
<td>Normal phase chromatography Picogram</td>
</tr>
<tr>
<td>RPC</td>
<td>Reversed phase chromatography</td>
</tr>
<tr>
<td>Rs</td>
<td>Resolution</td>
</tr>
<tr>
<td>RSD</td>
<td>Relative standard deviation</td>
</tr>
<tr>
<td>RT</td>
<td>Retention time</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SEC</td>
<td>Size-exclusion chromatography</td>
</tr>
<tr>
<td>TF</td>
<td>Tailing factor</td>
</tr>
<tr>
<td>TP</td>
<td>Theoretical plates</td>
</tr>
</tbody>
</table>
INTRODUCTION

1.1 ANALYTICAL CHEMISTRY
Chemistry is a science that deals with the composition, structure and properties of substance and with the transformation that they undergo.

Analytical chemistry Is a branch of chemistry involved with the analysis of chemical composition of natural and artificial materials. It is the measurement of science consisting of a set of powerful ideas and method that are useful in all fields of science and medicine.

1.2 General introduction to Pharmaceutical chemistry
Is a specialized branch of analytical chemistry which is involved in separating, identifying and determining the relative amounts of components in a sample of matter. Pharmaceutical analysis plays a very important role in quality assurance and quality control of bulk drugs and their formulations.

Method of analysis is routinely developed improved validated collaboratively studied and applied. The discipline of analytical chemistry consists of qualitative and quantitative analysis.

QUALITATIVE ANALYSIS: Refers to analyses in which substances are identified or classified on the basis of their chemical or physical properties, such as chemical reactivity, solubility, molecular weight, melting point, radiative properties (emission, absorption), mass spectra, nuclear half-life, etc.

QUANTITATIVE ANALYSIS: Refers to analyses in which the amount or concentration of an analyte may be determined (estimated) and expressed as a numerical value in appropriate units.

1.3 Instrumental method of chemical analysis
Instrumental method of chemical analysis interacts with all the areas of chemistry and other areas of pure and applied science.

Analytical techniques play an important role in
- Production and evaluation of new drugs in bulk and formulation and also estimation from biological fluids.
- Detection and quantification of impurities and metabolites.
Accelerated stability studies
Invitro dissolution studies
Pharmacokinetic studies and drug metabolism studies
Determination of bioavailability of two or more formulation

Method of estimation of drugs are divided into

- Physical methods
- Chemical methods.
- Physicochemical methods.

A. Physical method: Physical method of analysis involves the studying of physical properties of a substance. They include determination of the solubility transparency or degree of turbidity, density or specific gravity moisture content, melting, freezing and boiling points.

B. Chemical methods: The chemical methods include the gravimetric and volumetric procedures which are based on complex formation acid base precipitation and redox reactions.

TYPES OF CHEMICAL ANALYSIS
1. Proximate analysis: The amount of each element in a sample is determined with no concern as to the actual compounds present.
2. Partial analysis: Deals with the determination of selected constituent in the sample.
3. Trace constituent analysis: Concerned with the determination of specified compounds present in minute quantity.
4. Complete analysis: Proportion of each component of sample is determined.

C. Physicochemical methods: Physicochemical methods include.

A. SPECTROPHOTOMETRIC TECHNIQUES
- UV-visible spectrophotometric techniques
- Fluorescence and Phosphorescence techniques
- Atomic spectrophotometric
- Infrared spectrophotometry
- X-ray spectrophotometry
- Nuclear magnetic resonance spectroscopy
B. ELECTROCHEMICAL TECHNIQUES
- Potentiometer
- Voltametry
- Electrogravimetry
- Conductometry
- Amperometry

C. CHROMATOGRAPHIC TECHNIQUES
- High performance liquid chromatography
- Gas chromatography
- High performance thin layer chromatography
- Thin layer chromatography
- GC-MS
- LC-MS

D. MISCELLANEOUS TECHNIQUES
- Thermal analysis
- Kinetic technique
- Electrophoresis.

1.4 INTRODUCTION TO HIGH PERFORMANCE LIQUID CHROMATOGRAPHY
Chromatography may be defined as a non destructive procedure for separating mixture of components into individual components through equilibrium distribution between two phases. The technique of chromatography is based on the differences in the rate at which the components of a mixture move through a porous medium under the influence of solvent or gas.

1.4.1: CLASSIFICATION OF CHROMATOGRAPHIC PROCESS
ADSORPTION CHROMATOGRAPHY
- Gas-solid chromatography
- Liquid-column chromatography
- High performance liquid chromatography
• Thin-layer chromatography

PARTITION CHROMATOGRAPHY
• Gas liquid chromatography
• Super critical fluid chromatography
• Liquid liquid chromatography
• Paper chromatography
• High performance liquid chromatography

ION EXCHANGE CHROMATOGRAPHY
PERMEATION CHROMATOGRAPHY
• Size exclusion chromatography

AFFINITY CHROMATOGRAPHY
• DNA affinity chromatography

ELECTROPHOROSIS
Capillary electrophorosis

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)
It has also been referred to as High Pressure LC)
HPLC is a separation technique that involves:
• The injection of a small volume of liquid sample into a tube packed with tiny particles (3 to 5 micron (µm) in diameter called the stationary phase where individual components of the sample are moved down the packed tube (column) with a liquid (mobile phase) forced through the column by high pressure delivered by a pump.

• These components are separated from one another by the column packing that involves various chemical and/or physical interactions between their molecules and the packing particles. These separated components are detected at the exit of this tube (column) by a flow-through device (detector) that measures their amount. An output from this detector is called a “liquid chromatogram”.

TYPES OF HPLC TECHNIQUES
A. BASED ON PRINCIPLE OF SEPERATION
• Adsorption
Ion exchange  
Size exclusion  
Affinity  
Chiral phase  

B. BASED ON MODES OF CHROMATOGRAPHY  
Normal phase chromatography  
Reverse phase chromatography  

C. BASED ON ELUTION TECHNIQUE  
Isocratic elution  
Gradient elution  

D. BASED ON SCALE OF OPERATION  
Analytical HPLC  
Preparative HPLC  

REVERSE PHASE HPLC  
Reversed phase HPLC (RP-HPLC or RPC) has a non-polar stationary phase and an aqueous, moderately polar mobile phase. One common stationary phase is a silica which has been treated with RMe₂SiCl, where R is a straight chain alkyl group such as C₁₈H₃₇ or C₈H₁₇. With these stationary phases, retention time is longer for molecules which are less polar, while polar molecules elute more readily.

Table 1.1: Difference between normal phase and reverse phase in HPLC.  

<table>
<thead>
<tr>
<th>Properties</th>
<th>Normal phase</th>
<th>Reversed phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polarity of Stationary Phase</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Polarity of Mobile Phase</td>
<td>Low to medium</td>
<td>Low to high</td>
</tr>
<tr>
<td>Sample elution order</td>
<td>Lower polar first</td>
<td>Most polar first</td>
</tr>
<tr>
<td>Retention will increase by</td>
<td>Increasing surface of stationary phase</td>
<td>Increasing surface of stationary phase.</td>
</tr>
<tr>
<td></td>
<td>Increasing of alkyl chain length of stationary phase.</td>
<td>Increasing polarity of mobile phase</td>
</tr>
<tr>
<td></td>
<td>Decreasing polarity of mobile phase</td>
<td>Decreasing polarity of sample molecules</td>
</tr>
<tr>
<td></td>
<td>Increasing polarity of sample molecules</td>
<td>Decreasing polarity of sample molecules</td>
</tr>
</tbody>
</table>
Principle
The principle involved in HPLC is adsorption. The separation mechanism in reversed phase chromatography depends on the hydrophobic binding interaction between the solute molecule in the mobile phase and the immobilised hydrophobic ligand, i.e. the stationary phase. The solute molecules partition (i.e. an equilibrium is established) between the mobile phase and the stationary phase. The distribution of the solute between the two phases depends on the binding properties of the medium, the hydrophobicity of the solute and the composition of the mobile phase.

Chemically bounded octadecylsilane (ODS) and alkane with 18 carbon atom is the most popular stationary phase used in pharmaceutical industry. Organic solvents water and buffers are used as mobile phase.

HPLC is a method of choice in the field of analytical chemistry since the method is specific, robust, linear, precise and accurate; LOD is low and also offers,
1. Speed (many analysis may be accomplished in 20min)
2. Greater sensitivity (various detectors can be employed)
3. Improved resolution
4. Reusable column
5. Ideal for substance of low viscosity
6. Easy sample recovery
7. Precise and reproducible

HPLC INSTRUMENTATION

![Schematic representation of HPLC system.](image-url)
Pump
- The role of the pump is to force a liquid (called the mobile phase) through the liquid chromatograph at a specific flow rate, expressed in milliliters per min (ml/min).
- Normal flow rates in HPLC are in the 1- to 2-mL/min range.
- Typical pumps can reach pressures in the range of 6000-9000 psi (400- to 600-bar).
- During the chromatographic experiment, a pump can deliver a constant mobile phase composition (isocratic) or an increasing mobile phase composition (gradient).

Injector
- The injector serves to introduce the liquid sample into the flow stream of the mobile phase.
- Typical sample volumes are 5- to 20-microliters (µL).

Manual Injector
User manually loads sample into the injector using a syringe and then turns the handle to inject sample into the flowing mobile phase which transports the sample into the beginning (head) of the column, which is at high pressure.

Auto sampler
User loads vials filled with sample solution into the autosampler tray (100 samples) and the autosampler automatically measures the appropriate sample volume, injects the sample, then flushes the injector to be ready for the next sample, etc., until all sample vials are processed…An autosampler is the automatic version for when the user has many samples to analyze or when manual injection is not practical.
Column

Considered the “heart of the chromatograph” the column’s stationary phase separates the sample components of interest using various physical and chemical parameters. The small particles inside the column are what cause the high backpressure at normal flow rates. The pump must push hard to move the mobile phase through the column and this resistance causes a high pressure within the chromatograph.

Types of columns in HPLC

- Analytical [internal diameter (i.d.) 1.0 - 4.6-mm; lengths 15 – 250 mm]
- Preparative (i.d.> 4.6 mm; lengths 50 – 250 mm)
- Capillary (i.d. 0.1 - 1.0 mm; various lengths)
- Nano (i.d.< 0.1 mm, or sometimes stated as < 100 μm)

Materials of construction for the tubing

- Stainless steel (the most popular; gives high pressure capabilities)
- Glass (mostly for biomolecules)
- PEEK polymer (biocompatible and chemically inert to most solvents)

Columns are packed with small diameter porous particles. The most popular sizes are: 5-μm, 3.5- μm and 1.8-μm.

Columns are packed using high-pressure to ensure that they are stable during use most users purchase pre-packed columns to use in their liquid chromatographs These porous particles in the column usually have a chemically bonded phase on their surface which interacts with the sample components to separate them from one another.

For example, C18 is a popular bonded phase

- The process of retention of the sample components (often called analytes) is determined by the choice of column packing and the selection of the mobile phase to push the analytes through the packed column.

4. Detector

- The detector can see (detect) the individual molecules that come out (elute) from the column. A detector serves to measure the amount of those molecules so that the chemist can quantitatively analyze the sample components.
The detector provides an output to a recorder or computer that results in the liquid chromatogram (i.e., the graph of the detector response).

A. Ultraviolet (UV) Absorption
- An ultraviolet light beam is directed through a flow cell and a sensor measures the light passing through the cell.
- If a compound elutes from the column that absorbs this light energy, it will change the amount of light energy falling on the sensor.
- The resulting change in this electrical signal is amplified and directed to a recorder or data system.
- A UV spectrum is sometimes also obtained which may aid in the identification of a compound or series of compounds.

B. Mass Spectroscopy (MS)
- An MS detector senses a compound eluting from the HPLC column first by ionizing it then by measuring its mass and/or fragmenting the molecule into smaller pieces that are unique to the compound.
- The MS detector can sometimes identify the compound directly since its mass spectrum is like a fingerprint and is quite unique to that compound.

C. Refractive Index (RI) Detection
- The ability of a compound or solvent to deflect light provides a way to detect it.
- The RI is a measure of molecule’s ability to deflect light in a flowing mobile phase in a flow cell relative to a static mobile phase contained in a reference flow cell.
- The amount of deflection is proportional to concentration.
- The RI detector is considered to be a universal detector but it is not very sensitive

E. Fluorescence Detection
Compared to UV-Vis detectors fluorescence detectors offer a higher sensitivity and selectivity that allows quantifying and identifying compounds and impurities in complex matrices at extremely low concentration levels (trace level analysis).
- Fluorescence detectors sense only those substances that fluoresce.
5. Computer

- Frequently called the data system, the computer not only controls all the modules of the HPLC instrument but it takes the signal from the detector and uses it to determine the time of elution (retention time) of the sample components (qualitative analysis) and the amount of sample (quantitative analysis).

**IMPORTANCE OF HPLC**

A. **Quantitative Analysis** - The measurement of the amount of a compound in a sample (concentration); There are two main ways to interpret a chromatogram (i.e. perform quantification):

1. Determination of the peak height of a chromatographic peak as measured from the baseline.
2. Determination of the peak area (see figure below).

In order to make a quantitative assessment of the compound, a sample with a known amount of the compound of interest is injected and its peak height or peak area is measured. In many cases, there is a linear relationship between the height or area and the amount of sample.

B. **Preparation of Pure Compound(s)**

- By collecting the chromatographic peaks at the exit of the detector, and concentrating the compound (analyte) by removing/evaporating the solvent, a pure substance can be prepared for later use (e.g. organic synthesis, clinical studies, toxicology studies, etc.). This methodology is called preparative chromatography.

C. **Trace analysis**: A trace compound is a compound that is of interest to the analyst but it’s concentration is very low, usually less than 1% by weight, often parts per million (ppm).

- The determination of trace compounds is very important in pharmaceutical, biological, toxicology and environmental studies since even a trace substance can be harmful or poisonous; in a chromatogram trace substances can be difficult to separate or detect.
- High resolution separations and very sensitive detectors are required.

1.5 **GUID LINE TO METHOD DEVELOPMENT**

Prior to the initiation of method development, all the information about the analyte such as its structure, physical and chemical properties, toxicity, purity, hygroscopicity, solubility and stability should be determined. The goals and requirements of the hplc method that needs to
be developed should be known as well as the analytical figures of merit, which include the required detection limits selectivity, range, accuracy and precision.

**METHOD DEVELOPMENT CONSIDERATIONS**

**A. SAMPLE PROPERTIES**

Analyte structure and pka: If the target substance is ionizable, the pka of the analyte should be determined. The optimal pH, to commence method development, is at a pH that is at least 1-2 units from the analyte pka in the particular hydro-organic mixture that is employed.

Solubility of components: solubility of components is also very important. The analyte must be in the diluent and must not react with any of the diluent components. The diluent should match to the starting eluent composition of the essay to ensure that no peak distortion will occur, especially for early eluting components.

**B. DETECTOR CONSIDERATION**

Choice of the proper detection scheme is dependent on the properties of the analyte. Different types of detectors are available such as ultraviolet (UV), fluorescence, electrochemical, light scattering, refractive index (RI), flame ionization (FID), evaporating light scattering detection (ELSD), corona aerosol detection (CAD), mass spectrophotometric (MS), NMR and others. However, the majority of reversed phase and normal phase HPLC method development in the pharmaceutical industry is carried out with UV detection. A wavelength for UV detection must be chosen so that an accurate mass balance may be determined. Therefore if area % normalization is to be used then all the impurities and the active pharmaceutical ingredient must have similar relative response factors (area response/weight). The UV spectra of target analyte and impurities must be taken and overlaid with each other and the spectra should be normalized due to different amounts present in the mixture.

A wavelength must be chosen such that adequate response is obtained for the active and that at least a 0.05 v/v% solution of the active at target concentration should be quantified (S/N greater than 10). The wavelength chosen should not be distinct slope of the spectrum and the relative difference in the absorbance at a certain wavelength is not significantly different from the impurities/degradation products present.
C. MOBILE PHASE PARAMETERS

i. ORGANIC SOLVENT STRENGTH AND SELECTIVITY
Solvent strength or % organic solvent content in the mobile phase controls the retention time of analyte and that different organic solvents (MeOH, ACN, THF) can have a dramatic effect on selectivity. In the development of reverse phase separation methods the organic part of the eluent is considered the strong solvent. Organic solvent increases the solvent strength and allows for elution of the species in the mixture, resulting in smaller analyte retention factors or retention volumes. Increasing the concentration of organic modifier generally leads to an exponential increase in the analyte retention volume. The general rule of thumb is that for every 10v/v% increase in organic modifier there is two to three fold decrease in the analyte retention factors or analytes with molecular weights of less than 1000Da. Increasing the fraction of mobile phase strength depends not only on the concentration of the organic modifier, but also on the type of organic modifier used. The solvent strength of the most common organic eluents used at the same volume percentage (v/v%) in the reversed-phase chromatography would be methanol < Acetonitrlyl < Tetrahydrofuran.

ii. BUFFERS
Many drugs have either acidic or basic functional groups and can exist in solutions in ionized or unionized forms. The ionic state and the degree of ionization greatly affect their chromatographic retention in RPLC. Typically, the ionic form does not partition well into the hydrophobic stationary phase and therefore has significantly lower k’ than the neutral, unionized form. Buffers are commonly used to control the pH of the mobile phase for the separation of acidic or basic analytes. Volatile acids and their ammonium salts are used for the development of mass spectrometer (MS) compatible HPLC methods. Since a buffer is only effective with in 1-2 ph unit from its pka judicious selection of the proper buffer with in its buffering range is paramount.

iii. ACIDIC MOBILE PHASE
A mobile phase at acidic pH of 2.5-3 is a good starting point for most pharmaceutical applications because the low pH suppresses the ionization of most acidic analytes resulting in their higher retention. Common acids used for mobile phase preparations are phosphoric acid, formic acid and acidic acid. Low pH also minimizes the interaction of basic analytes with surface silanols on the silica packing (because silanol do not ionize at acidic pH) also, the
lifetime of the silica based columns is excellent in the pH range of 2.5. However the basic analytes are ionized at low pH and might not be retained unless ion pairing reagents are used.

IV. ION PAIRING REAGENTS

Ion pairing reagents are detergent like molecules added to the mobile phase to provide additional retention or selectivity for the analytes with opposite charge. Long chain alkyl sulphonates are commonly used for water soluble basic analytes. Amine modifiers such as tetra ethyl amine(TEA) are often used in the mobile phase to reduce peak tailing caused by the strong interaction of basic analytes with acidic surface silanols. For acidic analytes, ion pairing reagents such as tetra alkyl ammonium salts are often used.

V. HIGH pH MOBILE PHASE

The advent of silica based columns stable under high pH conditions offers an important alternate approach for the separation of basic analytes. the application of this approach is increasing for assay and impurity testing of many water soluble basic drugs. The advantage of high pH separation as opposed to ion-pairing is mass spectrometry compatibility, better sensitivity and unique selectivity.

D: ISOCRATIC Vs GRADIENT ANALYSIS

Traditionally, most pharmaceutical assays are isocratic analysis employing the same mobile phase throughout the elution of the sample. Isocratic analysis are particularly common in quality control application since they use simpler HPLC equipment and premixed mobile phases. Notable disadvantage of isocratic analysis are limited peak capacity(the maximum number of peaks that are obtained in the chromatogram) and problems with samples containing analytes of diverse polarities. also, late eluters (such as dimmers) are particularly difficult to quantitative in isocratic analysis due to excessive band broadening with long retention times.

In contrast the gradient analysis in which the strength of mobile phase is increased with time during sample elution, is suited for complex samples and those containing analytes of wide polarities. Gradient chromatography is amenable have high throughput screening applications and for impurity testing. The disadvantage of gradient analysis is the requirements for more complex instrumentation and greater skills in method development and difficulties in method transfer.
E: STATIONARY PHASES FOR REVERSE PHASE HPLC

Silica based packing materials dominate in application for RP separations in pharmaceutical industries. Hydrophobic surface of these packings typically are made by covalent bonding of organosilanes on the silica surface. This modification involves the reaction on monofunctional alkyl dimethylchlorosilanes with the surface silanol groups. Octadecysilane was the first commercially available silica based bonded phase and is still the most commonly utilized. Also alkyl type ligands of different number of carbon atoms (C1, C4, C8, C12) are often used as well as phases with phenyl functionality. Polar embedded phases provide an additional avenue for potential modification of the chromatographic selectivity, and some of these phases offer an enhancement of retention of polar analytes. These phases can be used with high aqueous mobile phases, even 100% aqueous, without loss of analyte retention that sometimes could be observed for more hydrophobic forces. Most silica-based reversed-phase packing materials have a relatively narrow applicable pH range.

1.2.1 Method goals

Analytical method goals are often defined as method acceptance criteria for peak resolution, precision, specificity and sensitivity. For instance, pharmaceutical methods for potency assays of an active pharmaceutical ingredient (API) typically require the following: resolution > 1.5 from the close steluting components; precision of retention time and peak area, <1–2% RSD; and linearity in the range of 50–150% of the label claim. Other desirable characteristics include:

- Analysis time ~ 5–30 min (~60 min for complex samples)
- Minimal sample work-up (extract and inject if possible)
- Robust method that doesn’t require extensive execution.
- Low cost per analysis.

Table 1.2: Method goals.

<table>
<thead>
<tr>
<th>Goal</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resolution</td>
<td>Precision and rugged quantitative analysis require that Rs be greater than 1.5.</td>
</tr>
<tr>
<td>Separation time</td>
<td>&lt;5-10 min is desirable for routine procedures.</td>
</tr>
<tr>
<td>Quantitation</td>
<td>≤2% for assays; ≤5% for less-demanding analyses; ≤15% for trace analyses.</td>
</tr>
<tr>
<td>Pressure</td>
<td>&lt;150 bar is desirable, &lt;200 bar is usually essential (new column assumed).</td>
</tr>
<tr>
<td>Peak height</td>
<td>Narrow peaks are desirable for large signal/noise ratios.</td>
</tr>
<tr>
<td>Solvent consumption</td>
<td>Minimum mobile-phase use per run is desirable.</td>
</tr>
</tbody>
</table>
1.5.2 Sample and analyte information

This information is useful for the selection of appropriate sample preparation procedures as well as the initial detection and chromatographic modes. If critical data are not available (e.g., pKa, solubility), separate studies should be initiated as soon as possible. The chemical structure of the analyte furnishes data on molecular weight and the nature of the functional groups. Particular attention should be directed to acidic, basic, aromatic, or reactive functional groups from which estimates of pKa, solubility, chromophoric, or stability data can be inferred. The sample-related Information is summarized.

Table 1.3: Sample and analyte information.

<table>
<thead>
<tr>
<th>Sample/Analyte</th>
<th>Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>Number of components, Concentration range of analytes</td>
</tr>
<tr>
<td>Analyte(s)</td>
<td>Chemical structure and molecular weight, pKa, Solubility, Chromophore</td>
</tr>
<tr>
<td></td>
<td>wavelength (λmax) Chiral centres, isomers, Stability and toxicity</td>
</tr>
</tbody>
</table>

1.6 METHOD VALIDATION

Method validation is the process to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Analytical method validation is the process of demonstrating that an analytical method is reliable and adequate for its intended purpose. Any method that is utilized to determine results during drug substance and formulation development will have to be validated.

Validation of HPLC method focuses on the following:

- Identification tests
- Quantitative measurements of the content of related substance
- Quantitative and limit tests for the control of related substance
- Quantitative tests for the assay of major components (e.g., drug substance and preservatives) in sample of drug substance or drug product (assay, content uniformity, dissolution rate, etc).

Analytical method validation is established through documented evidence demonstrating the accuracy, precision, linearity, selectivity, ruggedness and robustness of that particular test method which will be utilized to generate test results for a drug substance or drug product.
PARAMETERS FOR THE METHOD VALIDATION

1. SPECIFICITY/SELECTIVITY
The term specific generally refers to a method that produces a response for a single analyte only while the term selectivity refers to a method, which provides responses for a number of chemical entities that may or may not be distinguished from each other. If the response is distinguished from each other. If the response is distinguished from all other responses the method is said to be selective.

2. PRECISION AND REPRODUCIBILITY
Precision of a method is the extent to which individual test results of multiple injections of a series of standards agree the measured standard deviation can be subdivided into three categories.

Repeatability: Repeatability expresses the precision under the same operating conditions over a short interval of time. it is also termed as intra-day precision.

Intermediate precision: intermediate precision expresses within laboratories variations: different day, different analyst, different equipment etc.

Reproducibility: Reproducibility expresses the precision between laboratories collaborative studies, usually supplied to standardization of methodology.

3. RUGGEDNESS
The precision obtained when the assay is performed by multiple analysis, using multiple instruments, on multiple days, in one laboratory, different sources of reagents and multiple lots of columns should also be included in this study.

4. ACCURACY
The test for accuracy is intended to demonstrate the closeness of agreement between the value found and the value that is accepted either as conventional true value or as an accepted reference value. Thus the accuracy of the method is the closeness of the measured value to the true value for the sample. The accuracy can also be determined by recovery of the impurity spiked to a drug substance or into placebo with drug substance. The percentage recovery with the certain acceptance criteria at each defined level is reported. Accuracy should be assessed using a minimum of nine determination at a minimum of three concentration levels covering the specified range.
5. **LINEARITY**

The purpose of the test for linearity is to demonstrate that the entire analytical system (including detector and data acquisition) exhibits a linear response and is directly proportional over the relevant concentration range for the target concentration of the analyte. It is recommended to perform the linearity of the API and related substances independently and once linearity has demonstrated, linearity could be performed containing both API and specific related substance if necessary. At least five concentration within the range specified above for linearity test should be used.

6. **LIMIT OF DETECTION**

LOD is defined as lowest concentration of the analyte that can be detected, but not necessarily quantified, by the analytical method.

**Based on visual evaluation:** The detection limit is determined by the analysis of samples with known concentrations of the analyte and by establishing the minimum level at which the analyte can be reliably detected.

**Based on signal to noise ratio:** A signal to noise ratio between 3 or 2:1 is generally considered acceptable for estimating the detection limit.

**Based on standard deviation of the response and slope**
The limit of detection (LOD) may be expressed as,

\[
\text{LOD} = 3.3 \frac{\sigma}{S}
\]

Where \( \sigma \) = the standard deviation of the response

\( S \) = slope of calibration curve of analyte

7. **LIMIT OF QUANTIFICATION**

LOQ is defined as the lowest concentration of the analyte that can be determined with acceptable accuracy and precision by the analytical method.

**Based on visual evaluation:** The quantization limit is determined by the analysis of the samples with known concentration of analyte.

**Based on signal to noise ratio:** Signal to noise ratio between 10:1 is generally considered.
Based on standard deviation of the response and slope
The limit of detection (LOD) may be expressed as

\[ \text{LOD} = \frac{10 \sigma}{S} \]

Where \( \sigma \) = the standard deviation of the response
\( S = \) slope of calibration curve of analyte

8. ROBUSTNESS
Robustness tests examine the effect operational parameters have on the analysis results.

9. RANGE
The range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample.

10. SYSTEM SUITABILITY PARAMETERS
The purpose of the system suitability test is to ensure that the complete testing system (including instrument, reagents, columns, and analysts) is suitable for intended application.

After the method has been validated an overall system suitability test should be routinely run to determine if the operating system is performing properly.

Commonly used system suitability parameters are as follows:-

i. RETENTION TIME (Rt)
Retention time is the time of elution of peak of maximum after injection of compound.

ii. THEORITICAL PLATES (N)
It is also called as column efficiency. A column can be considered as being made of large number of theoretical plates where distribution of sample between liquid-liquid or solid-solid phase occurs. The number of theoretical plates in the column is given by the relationship,

\[ N = 16\left(\frac{t}{w}\right)^2 \]

Where \( t \) is the retention time and \( w \) is the width at the base of the peak.

\[ \text{HETP} = \frac{L}{N} \]

Where \( L \) is length of the column

Theoretical plates should be more than 2000.
iii. **RESOLUTION (R)**

It is the function of column efficiency and is specific to ensure that closely eluting compounds are resolved from each other to establish the general resolving power of the system.

For the separation of two components in the mixture the resolution is determined by equation.

\[
R = \frac{2 (t_2 - t_1)}{W_1 + W_2}
\]

Where \( t_1 \) and \( t_2 \) are the retention time of second and first compounds respectively, where as \( W_1 \) and \( W_2 \) are the corresponding widths at the bases of peak obtained by extrapolating straight sides of the peaks to baselines.

\( R \) should be more than 2 between peak of interest and the closest eluted potential interferences (impurities, excipients, degradation products or internal standard).

iv. **TAILING FACTOR (T)**

It is the measure of peak symmetry, is unity for perfectly symmetrical peaks and its value increases as tailing becomes more pronounced.

\[
R = \frac{W_{0.05}}{2F}
\]

Where \( W_{0.05} \) is the width of peak at 5% height and \( F \) is the distance from the peak maximum to the leading edge of the peak height from the baseline. Tailing factor should be less than 2.

v. **CAPACITY FACTOR (K')**

It is calculated by the formula,

\[
K' = \frac{t}{t_a} - 1
\]

Where \( t \) is the retention time of the drug, \( t_a \) is the retention time of non retarded component, air with thermal conductivity detection.

vi. **SELECTIVITY**

Also known as separation factor, it is measure of peak spacing and expressed as,

\[
\text{Selectivity} = \frac{k''_2}{k'_1}
\]
System suitability parameters and their standard values.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capacity factor</td>
<td>k'&gt;2</td>
</tr>
<tr>
<td>Injection precision</td>
<td>RSD &lt; 1% for n≥5</td>
</tr>
<tr>
<td>Resolution</td>
<td>Rs&gt; 2</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>T ≤ 2</td>
</tr>
<tr>
<td>Theoretical plate</td>
<td>N&gt;2000</td>
</tr>
</tbody>
</table>

REVIEW OF LITERATURE

- Gajjar Anuradha K et.al has reported simultaneous UV spectrophotometric estimation of rosuvastatin and ezetimibe in their combined dosage form(3).
- Vishal V et.al has reported characterisation and method development for estimation and validation of rosuvastatin calcium by UV – Visible spectrophotometry(4).
- Alka Gupta et.al has reported simple UV spectrophotometric determination of rosuvastatin calcium in pure form and in pharmaceutical formulations(5).
- Gajjar Anuradha K et.al has reported simultaneous estimation of rosuvastatin and ezetimibe by ratio spectra derivative spectrometry method in their fixed dosage forms(6).
- Naman Doshi et.al has reported a validated RP-HPLC method for simultaneous estimation of rosuvastatin calcium and telmisartan in pharmaceutical dosage form(7).
- Devika G S et.al has reported a new improved RP-HPLC method for simultaneous estimation of rosuvastatin calcium and fenofibrate in tablets(8).
- Chirag B et.al has developed and validated RP-HPLC method for determination of rosuvastatin calcium in bulk and pharmaceutical dosage form.(9)
- Uma devi S et.al has developed and validated HPTLC method for estimation of rosuvastatin calcium in bulk and pharmaceutical dosage forms(10).

M. Saravana kumar et al

A simple, sensitive and rapid ionpair high performance liquid chromatographic methodwas developed for the estimation ceftriaxone sodium (CS) and tazobactum sodium (TS) in pharmaceutical dosage forms. LichrocartR100-RP18e5)m-C18 column was used with a mobile phase containing mixture of 0.012M tetra butyl ammonium hydroxide in 0.01M potassium dihydrogen phosphate : acetonitrile in the ratio of 70:30% v/v. The flow rate was 0.8ml/min and effluents were monitored at 220nm and eluted at 4.5 and 6.7 min for tazobactum sodium (TS) and ceftriaxone sodium (CS) respectively. Calibration curve was plotted with a range from 2 to 12)g/ml (CS) and 0.26 to 1.56 (TS) The assay was validated for the parameters like accuracy, precision, robustness and system suitability parameters.
The proposed method can be useful in the routine analysis for the determination of ceftriaxone sodium and tazobactum sodium in pharmaceutical dosage forms.

**Abdel-Hamid ME et al**[2]**., Have developed accurate and precise Spectrophotometric full spectrum quantitation (FSQ) and High-Performance Liquid Chromatography (HPLC) procedures for the quantitation of some selected Cephalosporins namely, Cefotaxime, Ceftazidime and Ceftriaxone in the presence of their alkali-induced degradation products and in commercial injections. The Spectrophotometric method was based on the use of FSQ software of multicomponent analysis for Simultaneous determination of the examined antibiotics in the presence of their alkali-induced degradation products.

**P el SA etal**[3]**., Have studied on Spectrophotometric method for the estimation of Cefotaxime and Ceftriaxone using Folin-Ciocalteu reagent in presence of 20% sodium carbonate solution. The blue colourchromogen formed is measured at wavelength of maximum absorption 752nm and 750nm for Cefotaxime and Ceftriaxone respectively against reagent blank. The chromogen obeyed linearity over 5.0 to 60μg/mL for Cefotaxime and 2.0 to 36μg/mL for Ceftriaxone.

**Lakshmi KS et al**[4]**., Have been developed Three Spectrophotometric methods (A, B and C) in the UV region for the determination of Ceftriaxone sodium in vials. Method A was based on the measurement of intensity of UV radiation at 258.8nm using 0.1M NaOH, Method B was based on the measurement of intensity of UV radiation at 266.4nm using 0.1M Hydrochloric acid and Method C was based on the reaction with Imidazole - mercury (II) reagent in slightly acidic condition and heating at 83°C for 20 min, which showed absorbance at 236nm. Beer's Law was obeyed in the concentration range of 1-5 µg/ml for method A, 10-60µg/ml for method B and 0.4 - 2.0µg/mL for method C.

**Rind FMA et al**[5]**., Have been developed Spectrophotometric method for the determination of the potent Antibiotic Ceftriaxone (CF) by derivatization with 4-dimethyl amino benzaldehyde (DAB). The derivative indicated molar absorptivity of 5.3x 103l mol⁻¹ cm⁻¹ at lambdamax 397nm and obeyed the Beer’s law within 100μg/mL.

**Natarajan Sunder et al**[6]**., Have developed the RP-HPLC method and validated for the Simultaneous estimation of Ceftriaxone sodium and Tazobactam sodium in injectable preparations. The separation was obtained using a mobile phase of mixture of 600:400 ratio
of 0.02M phosphate buffer and 0.4% w/v solution of tetra heptyl ammonium bromide solution in acetonitrile, with final pH of 6.6-6.8 on an octa-decyl-silane (ODS) column (4.6mm x 250 mm, 5μ) with UV detection at 230nm at 1.5mL per minute flow rate. For stress studies, a diode array detector was used.

Sanjay MS et al\cite{7}, Have been developed an Isocratic Liquid Chromatographic method with UV detection at 220nm is described for Simultaneous determination of Ceftriaxone sodium and Sulbactam sodium in Sulbactomax. Chromatographic separation of two drugs was achieved on a Hypersil ODS C\textsubscript{18} column using a mobile phase consisting of a binary mixture of acetonitrile and tetra butyl ammonium hydroxide adjusted to pH7.0 with orthophosphoric acid in ratio 70:30.

George GG et al\cite{8}, Have developed a High-Performance Liquid Chromatographic assay to measure Ceftriaxone in serum, urine and cerebrospinal fluid. The mobile phase was a combination of acetonitrile and water (46:54), adjusted to pH 9.0 with 10mM K\textsubscript{2}HPO\textsubscript{4}, which contained 10mM hexadecyl tri methyl ammonium bromide as the ion- pairing agent. A silica- packed precolumn (3cm long) was used to prevent rapid deterioration of the analytical column (30 by 0.4cm) by the alkaline pH of the mobile phase.

De diegoglaria Met al\cite{9}, Have studied the Stability of Ceftriaxone in water and cerebrospinal fluid in the presence or the absence of buffer at different storage temperatures was performed by Liquid Chromatography on c\textsubscript{18} column (125 x4mm, 5μm) using a mobile phase consisting of acetonitrile (300ml), 0.1M phosphate buffer (Ph 7.4; 50 mL), tetra butyl ammonium bromide (3.2g) made up to a volume of 1L with water.

Deyi Z et al\cite{10}, Have developed a novel determination method for Ceftriaxone sodium using a flow-injection technique based on the Chemiluminescence (CL) emission generated from the oxidation of Ceftriaxone sodium alkali hydrolysis by potassium permanganate in polyphosphoric acid (PPA). The proposed method has been successfully utilized for the determination of Ceftriaxone sodium in Pharmaceutical Formulations, while the CL reaction mechanisms were investigated.
DRUGS INTRODUCTION

INTRODUCTION
Rosuvastatin Calcium is official in Indian Pharmacopoeia. It is chemically Bis [(E)-7-[4- (4-fluorophenyl)-6-isopropyl-2-[methyl(methylsulfonyl) amino] pyrimidi-5-yl] (3R, 5S)-3, 5-di hydroxyhept-6-enoic acid] calcium. It is used in the treatment of Hyperlipidemia. Rosuvastatin Calcium is a selective and competitive inhibitor of HMG CoA reductase, the rate-limiting enzyme that converts 3- hydroxyl-3-methylglutaryl coenzyme A to mevalonate, a precursor of cholesterol. Simultaneous Estimation Of Rosuvastatin Calcium And Aspirin In Pharmaceutical Dosage Form By UV Spectrophotometric Method (S. Patel et al., 2012), methods have been reported for the simultaneous estimation of Rosuvastatin Calcium in combination with other drugs. Fenofibrate is official in Indian Pharmacopoeia. It is chemically Propane-2-yl-[4-(4- chlorobenzoyl) phenoxy]-2-methyl propanate. It is the lipid regulating drug. Fenofibrate increases lipolysis and elimination of triglyceride-rich particles from plasma by activating lipoprotein lipase and reducing production of apoprotein C-III (an inhibitor of lipoprotein lipase activity).

ROSUVASTATIN
Rosuvastatin: It is a synthetic lipid lowering agent for oral administration.

Drug profile
IUPACNAME: (E)-(3R,5S)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl(methylsulphonyl amino)]pyrimidin-5-yl]-3,5-di hydroxyhepten-6-oic acid calcium.
Empirical formula: C_{22}H_{27}FN_{3}O_{6}S.Ca
Molecular weight: 1001.1
Solubility: Sparingly soluble in water and methanol, and slightly soluble in ethanol.
**Pharmacologic class:** HMG-CoA reductase inhibitor.

**Therapeutic class:** Antilipemic.

**Pregnancy risk category X.**

**Action:** Selectively and competitively inhibits HMG-CoA reductase, which catalyzes its conversion to the cholesterol precursor mevalonate and thus limits cholesterol synthesis. This action increases high-density lipoprotein level and decreases low-density lipoprotein (LDL) level.

**Availability**
Tablets: 5 mg, 10 mg, 20 mg, 40 mg.

**Indications and dosages**

- **Primary heterozygous hypercholesterolemia; mixed dyslipidemia (Fredrickson types IIa and IIb).**

**Adults:** Initially, 10 mg/day P.O. Patients who need less aggressive cholesterol reduction or have predisposing factors for myopathy may start at 5 mg/day. Patients with marked hypercholesterolemia (LDL above 190 mg/dl) and more aggressive LDL goals may start at 20 mg/day. For maintenance, 5 to 40 mg/day P.O.

- **Homozygous familial hypercholesterolemia.**

**Adults:** 20 mg/day P.O. Maximum recommended dosage is 40 mg/day.

- **Hypertriglyceridemia (Fredrickson type IV).**

**Adults:** Initially, 10 mg/day P.O. For maintenance, 5 to 40 mg/day P.O.

**Contraindications**

Hypersensitivity to drug or its components active hepatic disease or persistent, unexplained hepatic enzyme elevations pregnancy or breastfeeding.
Precautions

Use cautiously in

• Women of childbearing age (except those who are highly unlikely to conceive and have been informed of potential hazards).
• Children (safety and efficacy not established).

Administration

Check liver function tests before therapy starts. Give with or without food.

• Measure lipid levels within 2 to 4 weeks after therapy starts and after titration.
• Know that drug should be used as adjunct to other lipid-lowering treatments, such as diet.

<table>
<thead>
<tr>
<th>Route</th>
<th>Onset</th>
<th>Peak</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>P.O.</td>
<td>Unknown</td>
<td>3-5 hr</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

Adverse reactions

CNS: Headache, dizziness, anxiety, depression, insomnia, hypertonia, paresthesia, asthenia, tremor, vertigo, neuralgia.

CV: Palpitations, tachycardia, chest pain, angina pectoris, hypertension, vasodilation, peripheral edema.

EENT: Rhinitis, sinusitis, pharyngitis.

GI: Nausea, vomiting, diarrhea, constipation, abdominal pain, dyspepsia, flatulence, gastritis, gastroenteritis.

GU: Urinary tract infection, acute renal failure.

Hematologic: Anemia.

Metabolic: Hypokalemia, hyperglycemia, hypoglycemia.

Musculoskeletal: Myalgia; myopathy; arthritis; pathologic fractures; back, pelvic, neck, or joint pain; rhabdomyolysis.

Respiratory: Respiratory tract infection, bronchitis, increased cough, dyspnea, pneumonia, asthma.
Skin: Rash, pruritus, bruising.

Other: Periodontal abscess, flulike symptoms, infection.

Interactions

Drug-drug. Antacids: decreased rosuvastatin blood level
Cyclosporine, gemfibrozil: increased rosuvastatin bioavailability
Hormonal contraceptives: increased contraceptive blood level
Warfarin: increased International Normalized Ratio.

Drug-diagnostic tests. Alanine aminotransferase (ALT), alkaline phosphatase, aspartate aminotransferase (AST), bilirubin, creatine kinase (CK), glucose: increased levels
Potassium: decreased level
Thyroid function tests: altered results
Urine protein: present beyond trace.

Drug-food. Caffeine-containing foods and beverages: increased stimulant effect
Oat bran, pectin: impaired drug absorption
Urine-acidifying foods: increased drug blood level.

Drug-herbs. Caffeine-containing herbs (such as cola nut, yerba maté), ephedra (ma huang): increased stimulant effect.

Patient monitoring
☞ Monitor CK, creatinine and urine protein levels closely. Also watch for signs and symptoms of rhabdomyolysis with acute renal failure: CK level above 10 times normal limits, muscle ache or weakness, creatinine elevation and urine protein level beyond trace, accompanied by hematuria. If these findings occur, withhold drug and notify prescriber immediately.

☞ Monitor liver function tests 12 weeks after therapy begins, after dosage increases, and at least semiannually thereafter. Reduce dosage or withdraw drug if ALT or AST persists at Three times normal levels.
Temporarily withhold drug in patients with acute, serious conditions predisposing to renal failure caused by rhabdomyolysis (such as sepsis, hypotension, major surgery, trauma, uncontrolled seizures, or severe metabolic, endocrine and electrolyte disorders).

- Monitor blood glucose, electrolyte levels and lipid panel.
- Assess vital signs and cardiovascular status, especially for tachycardia and palpitations.
- Monitor for signs and symptoms of respiratory tract infection.
- Stay alert for tremor and asthenia.

**Patient teaching**

- Tell patient he may take with or without food. If he's using antacids, instruct him to take these 2 hours after rosuvastatin.
- Instruct patient to maintain a standard cholesterol-lowering diet.

Tell patient to immediately report unexplained muscle pain, tenderness, or weakness (particularly if accompanied by malaise or fever). Caution female patient of childbearing age not to take drug if she is pregnant, plans to become pregnant, or is breastfeeding.

- Teach patient how to check blood or urine glucose level and recognize signs and symptoms of hyperglycemia and hypoglycemia.
- Tell patient that foods, beverages and preparations containing caffeine or ephedra may increase drug's stimulant effect.
- Advise patient against heavy alcohol use, which increases risk of liver disease.
- As appropriate, review all other significant and life-threatening adverse reactions and interactions, especially those related to the drugs, tests, foods and herbs mentioned above.

**FENOFIBRATE**

**NAME:** Fenofibrate

**IUPAC NAME:** Propane-2-yl 2-\{4-[(4-chlorophenyl) phenoxy]-2-methylpranoate

Structure:
Dosage
The pharmaceutical form and the strength may change from one country to another and from one brand to another. In the United States, Tricor was reformulated in 2005 and is available in tablets of 48 and 145 mg.

Mechanism of action
Fenofibrate is a fibric acid derivative. It lowers lipid levels by activating Peroxisome proliferators-activated receptor alpha (PPARα). PPARα activates lipoprotein lipase and reduces apoprotein CIII, which increases lipolysis and elimination of triglyceride-rich particles from plasma.

PPARα also increases apoproteins AI and AII, reduces very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) containing apoprotein B and increases high-density lipoprotein (HDL) containing apoprotein AI and AII.

In addition, by reducing the synthesis and increasing the catabolism of VLDL, fenofibrate increases LDL clearance and reduces small and dense LDL, which are associated with coronary heart disease.

Indications
Fenofibrate is primary therapy for hypercholesterolaemia and hypertriglyceridaemia alone or combined (types IIa, IIb, III, IV and V dyslipidaemias) and/or in situations in which first line therapy is insufficient or has unacceptable side-effects.

Additionally, in Europe, fenofibrate is indicated in mixed hyperlipidemia in patients with high cardiovascular risk in addition to a statin when triglycerides and HDL are not adequately controlled. Fenofibrate is contraindicated in children, during pregnancy or lactation, in patients with liver insufficiency, presence of gallstones, renal insufficiency, in patients hypersensitive to fenofibrate and/or its excipients, known photoallergy or phototoxic reaction during treatment with fibrates or ketoprofen.

Efficacy
Three randomized, double-blind, multicenter, phase III trials have shown that treatment with fenofibric acid plus a statin (Atorvastatin, rosuvastatin or simvastatin) improved HDL and triglyceride levels significantly better than statin monotherapy and improved LDL levels better than fenofibric acid monotherapy.
The Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study (2005), the largest, with 9795 patients with type 2 diabetes mellitus did not show a lower risk for the primary end point (non-fatal myocardial infarction and coronary heart disease death). The secondary end-point (total cardiovascular disease events) showed a relative risk reduction of 11% for total CVD events. A large proportion of placebo patients took statins during the trial, which weakened the effect. After an adjustment for statin drop in, the relative risk reductions were 19% for Non-Fatal MI and CHD Death, and 15% for total CVD events.\cite{7}

**Side effects**
Gastrointestinal: Digestive, gastric or intestinal disorders (abdominal pain, nausea, vomiting, diarrhea and flatulence). Skin Reactions: Rashes, Pruritus, urticaria or photosensitivity reactions.

**Scientific**
Fenofibrate exhibits anticonvulsant properties comparable to the ketogenic diet in adult rats, using pentylenetetrazol and lithium-pilocarpine models.\cite{16}

**Brand names**
Fenofibrate is sold under the brand name Tricor and Trilipix by Abbott Labs, Lipofen by Kowa Pharmaceuticals America Inc, Lofibra by Teva, Lipanthyl, Lipidil and Supralip by Abbott Laboratories, Fenocor-67 by Ordain Health Care, Fenogal by SMB Laboratories, Antara by Oscient Pharmaceuticals, Tricheck by Zydus (CND), Atorva TG by Zydus Medica and Golip by Golgi USA.

**AIM AND SCOPE OF WORK**
The scope of developing and validating a method is to ensure a suitable strategy for a particular analyte which is more stable, cheap specific accurate precise and less time consuming. here the main focus is drawn to achieve improvement in conditions and standard operating procedure to be followed.

The existing available literature reveals that very few analytical methods are available for the drug, which is very costly and less stable.

Hence it was felt that there is a need to develop new analytical method for the estimation of valsartan in a single step, simple stable, less time consumable, cheap and more economical process using RP-HPLC methods by optimizing the chromatographic conditions and to
validate the developed method by establishing the parameters like accuracy, precision, linearity range LOD, LOQ, ruggedness etc.

METHOD AND METHADOLOGY
Present study was conducted to obtain a new, affordable, cost-effective and convenient method for HPLC determination of ceftriaxone and tazobactam inj dosage form. The experiment was carried out according to the official specifications of USP–30, ICH- 1996 and Global Quality Guidelines-2013. The method was validated for the parameters like system suitability, selectivity, linearity, accuracy, precision, LOD, LOQ and robustness.

SYSTEM SUITABILITY
System suitability study of the method was carried out by six replicate analysis of solution containing 100% target concentration of ceftriaxone and tazobactam. Various chromatographic parameters such as retention time, peak area tailing factor, theoretical plates (Tangent) of the column and resolution between the peaks were determined and the method was evaluated by analyzing these parameters.

SELECTIVITY
Selectivity test determines the effect of excipients on the assay result. To determine the selectivity of the method, standard sample of ceftriaxone and tazobactam were injected first. Then commercial product, blank and excipients solution were run in the instrument one after another.

MATERIAL

MATERIALS

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV-Visible Spectrophotometer</td>
<td>Analytical Technologies Ltd</td>
</tr>
<tr>
<td>HPLC</td>
<td>Auto sampler Egilient</td>
</tr>
<tr>
<td>Ultra sonicator</td>
<td>Citizen, Digital Ultrasonic Cleaner</td>
</tr>
<tr>
<td>pH meter</td>
<td>Elico</td>
</tr>
<tr>
<td>Electronic balance</td>
<td>Shimadzu</td>
</tr>
<tr>
<td>Syringe</td>
<td>Hamilton</td>
</tr>
<tr>
<td>HPLC Column</td>
<td>Waters symmetry C18 (150x4.6 ID) 3.5µm</td>
</tr>
</tbody>
</table>
Chemicals and reagents used for study

Table 3.1.2 reagents used.

<table>
<thead>
<tr>
<th>REAGENT</th>
<th>SPECIFICATIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium dihydrogen orthophosphate</td>
<td>Mol. Formula – KH₂PO₄</td>
</tr>
<tr>
<td>(Merck – HPLC grade)</td>
<td>Mol. Weight – 136.09</td>
</tr>
<tr>
<td>Ortho phosphoric acid</td>
<td>Mol. Formula – H₃PO₄</td>
</tr>
<tr>
<td>(Merck – HPLC grade)</td>
<td>Mol. Weight – 98</td>
</tr>
<tr>
<td>Ammonium acetate</td>
<td>Mol. Formula-CH₃COONH₄</td>
</tr>
<tr>
<td>( Merck-GR)</td>
<td>Mol. Weight-77.0825</td>
</tr>
<tr>
<td>Methanol (Merck HPLC grade)</td>
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</tr>
<tr>
<td></td>
<td>Mol. Weight – 32.04</td>
</tr>
<tr>
<td>Acetonitrile (Merck – HPLC grade)</td>
<td>Mol. Formula – CH₃CN</td>
</tr>
<tr>
<td></td>
<td>Mol. Weight – 41.05</td>
</tr>
<tr>
<td>WATER</td>
<td>Milli Q grade</td>
</tr>
</tbody>
</table>

Table 4.22: Optimised condition.

<table>
<thead>
<tr>
<th>Mobile phase</th>
<th>Acetonitrile: Buffer pH6(45:55) v/v</th>
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<tbody>
<tr>
<td>Column</td>
<td>Inertsil ODS C18(150x4.6 ID) 3.5 µm</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1.0mL/min</td>
</tr>
<tr>
<td>Column temperature</td>
<td>Room temperature(20-25°C)</td>
</tr>
<tr>
<td>Sample temperature</td>
<td>Room temperature(20-25°C)</td>
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<td>Wavelength</td>
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<tr>
<td>Injection volume</td>
<td>20µL</td>
</tr>
<tr>
<td>Runtime</td>
<td>10 min</td>
</tr>
<tr>
<td>Retention time</td>
<td>4.730 min for Ceftriaxone and 2.453min for Tazobactam</td>
</tr>
</tbody>
</table>

METHOD VALIDATION

Preparation of samples for Assay

Preparation of Standard stock solution

About 100 mg of ceftriaxone and 425 mg of tazobactam were weighed into a 10 mL volumetric flask, to this 10mL of mobile phase was added.

Preparation of Standard solution

1ml of each solution pipette out make in to 10ml by using mobile phase. 2.3ml ceftriaxone Add 1 ml of tazobactam added as mixture.
Preparation of Sample solution

Sample name: ALZON-T inj

100mg of ceftriaxone and 425mg of tazobactamin 50mL volumetric flask and makeup mark with mobile phase. From above solution Pipette 2.3mL of ceftriaxone clear solution in to 10mL volumetric flask and make up volume with mobile phase. Pipette 1ml of tazobactam to 10 ml volumetric flask. Pipette 1ml of tazobactam and 2.3 ml ceftriaxone as mixture. used to record the chromatogram.

PREPARATION AND SELECTION OF MOBILE PHASE

The preliminary isocratic studies on a reverse phase C18 column with different mobile phase combination of pot. phosphate buffer 5.6 and Methanol and ACN were studied for simultaneous separation of both the drugs. The optimal composition of mobile phase determined to be Buffer:ACN (55:45 v/v) and filtered through 0.45µ membrane filter.

CHROMATOGRAPHIC CONDITIONS

The mobile phase, a mixture of PHOSPHATE buffer and ACN (55:45v/v) pumped at a flow rate of 1.0 ml/min through the column (C18; 5µ, 4.6 X 250 mm, ODS) at 25C. The mobile phase was degassed prior to use under vacuum by filtration through a 0.45µ membrane filter. Both drugs showed good absorbance at 211 nm, which was selected as wavelength for further analysis.

DEVELOPMENT AND VALIDATION OF HPLC METHOD

Present study was conducted to obtain a new, affordable, cost-effective and convenient method for HPLC determination of ceftriaxone and tazobactam in injectable dosage form. The experiment was carried out according to the official specifications of USP–30, ICH-1996 and Global Quality Guidelines-2013. The method was validated for the parameters like system suitability, selectivity, linearity, accuracy, precision, LOD, LOQ and robustness.

SYSTEM SUITABILITY

System suitability study of the method was carried out by six replicate analysis of solution containing 100% target concentration of CEFTRIAXONE and TAZOBACTAM various chromatographic parameters such as retention time, peak area tailing factor, theoretical plates (Tangent) of the column and resolution between the peaks were determined and the method was evaluated by analyzing these parameters.
SELECTIVITY
Selectivity test determines the effect of excipients on the assay result. To determine the selectivity of the method, standard sample of ceftriaxone and tazobactam were injected first. Then commercial product, blank and excipients solution were run in the instrument one after another.

SPECIFICITY
The specificity studies were carried out by attempting deliberate degradation of the sample with exposure to stress conditions like acidic (0.1N HCl), alkaline (0.1N NaOH), 105°C Heat, Oxidizing agents (H₂O₂), Water.

Preparation of standard solution for CEFTRIAXONE & TAZOBACTAM
About 100 mg of ceftriaxone and 425 mg of tazobactam were weighed into a 10 mL volumetric flask, to this 10 mL of mobile phase was added, sonicated for 10 min and the 1 ml of each solution pipette out make in to 10 ml by using mobile phase. 2.3 ml ceftriaxone Add 1 ml of tazobactam added as mixture.

A) With 0.1M HCl
ALZONE-T inj About 100 mg of ceftriaxone and 425 mg of tazobactam were weighed into a 10 mL volumetric flask, to this 10 mL of mobile phase was added, sonicated for 10 min and the 1 ml of each solution pipette out make in to 10 ml by using mobile phase and filter the solution. Take 10 ml of filtrate and add 10 ml of 0.1N HCl solution and reflexed for 30 min and Dilute to 100 ml with mobile phase. Further filter the solution through 0.45µ filter paper.

B) With 0.1M NaOH
ALZONE-T inj About 100 mg of ceftriaxone and 425 mg of tazobactam were weighed into a 10 mL volumetric flask, to this 10 mL of mobile phase was added, sonicated for 10 min and the 1 ml of each solution pipette out make in to 10 ml by using mobile phase and filter the solution. Take 10 ml of filtrate and add 10 ml 0.1N NaOH solution and reflexed for 30 min and Dilute to 100 ml with mobile phase. Further filter the solution through 0.45µ filter paper.
C. With 1% H$_2$O$_2$
ALZONE-T inj About100 mg of ceftriaxone and 425mg of tazobactam were weighed into a 10 mL volumetric flask, to this10mL of mobile phase was added, sonicated for 10 min and the 1ml of each solution pipette out make in to 10ml by using mobile phase and filter the solution. Take 10 ml of filtrate and add10ml of 1% H$_2$O$_2$ solution and reflexed for 30min and Dilute to 100 ml with mobile phase. Further filter the solution through 0.45µ filter paper.

C. With H$_2$O
ALZONE-T inj About100 mg of ceftriaxone and 425mg of tazobactam were weighed into a 10 mL volumetric flask, to this10mL of mobile phase was added, sonicated for 10 min and the 1ml of each solution pipette out make in to 10ml by using mobile phase and filter the solution. Take 10 ml of filtrate and add10ml water solution and reflexed for 6 hours and Dilute to 100 ml with mobile phase. Further filter the solution through 0.45µ filter paper.

C) With Heat
ALZONE-T inj About100 mg of ceftriaxone and 425mg of tazobactam were weighed into a 10 mL volumetric flask, to this10mL of mobile phase was added, sonicated for 10 min and the 1ml of each solution pipette out make in to 10ml by using mobile phase and filter.

The solution. Take 10 ml of filtrate and Dilute to 100 ml with mobile phase. Further filter the solution through 0.45µ filter paper.

2 PRECISION

Preparation of Standard stock solution
About100 mg of ceftriaxone and 425mg of tazobactam were weighed into a 10 mL volumetric flask, to this 10mL of mobile phase was added.

Preparation of Standard solution
1ml of each solution pipette out make in to 10ml by using mobile phase.2.3ml ceftriaxone Add 1 ml of tazobactam added as mixture.

Preparation of Sample solution
Sample name: ALZON-T inj 100mg of ceftriaxone and 425mg of tazobactamin 50mL volumetric flask and makeup mark with mobile phase. From above solution Pipette
2.3mL of ceftriaxone clear solution in to 10mL volumetric flask and make up volume with mobile phase. pipette 1ml of tazobactam to 10 ml volumetric flask. Pipette 1ml of tazobactam and 2.3 ml ceftriaxone as mixture. used to record the chromatogram.

**System precision**

ALZONE-T inj About100 mg of ceftriaxone and 425mg of tazobactam were weighed into a 10 mL volumetric flask, to this 10mL of mobile phase was added, sonicated for 10 min and the 1ml of each solution pipette out make in to 10ml by using mobile phase and filter the solution. Dilute 10 ml of filtrate to 100 ml with mobile phase. The peak areas were noted down and %RSD were calculated.

**Method precision**

ALZONE-T inj About100 mg of ceftriaxone and 425mg of tazobactam were weighed into a 10 mL volumetric flask, to this 10mL of mobile phase was added, sonicated for 10 min and the 1ml of each solution pipette out make in to 10ml by using mobile phase. Further filter the solution through 0.45µ filter paper. Dilute 10 ml of filtrate to 100 ml with mobile phase. Peak areas were noted down. Average, Standard deviation, %RSD were calculated.

**ACCURACY**

Accuracy is a measure of the closeness of test results obtained by a method to the true value. Accuracy indicates the deviation between the mean value found and the true value. It is determined by applying the method to samples to which known amounts of analyte have been added. These should be analysed against standard and blank solutions to ensure that no interference exists. The accuracy is then calculated from the test results as a percentage of the analyte recovered by the assay.

**Preparation of Standard stock solution**

About100 mg of ceftriaxone and 425mg of tazobactam were weighed into a 10 mL volumetric flask, to this 10mL of mobile phase was added.

**Preparation of Standard solution**

1ml of each solution pipette out make in to 10ml by using mobile phase.2.3ml ceftriaxone Add 1 ml of tazobactam added as mixture.
**Preparation of Test Solutions**

1. **Preparation of 50% Solution**
   Take ALNAZOE-T inj power Transfer Sample quantitatively equivalent to 50 mg of ceftriaxone and 212.5 mg of tazobactam 10 mL volumetric flask, to this 10 mL of mobile phase was added, sonicate to dissolve for 10 minutes and dilute to volume with diluent. Further filter the solution through 0.45µ filter paper. Dilute 10 ml of filtrate to 100 ml with mobile phase.

2. **Preparation of 100% Solution**
   Take ALNAZOE-T inj power Transfer Sample quantitatively equivalent to 100 mg of ceftriaxone and 425 mg of tazobactam 10 mL volumetric flask, to this 10 mL of mobile phase was added, sonicate to dissolve for 10 minutes and dilute to volume with diluent.

   Further filter the solution through 0.45µ filter paper. Dilute 10 ml of filtrate to 100 ml with mobile phase.

3. **Preparation of 150% Solution**
   Take ALNAZOE-T inj power Transfer Sample quantitatively equivalent to 150 mg of ceftriaxone and 637.5 mg of tazobactam 10 mL volumetric flask, to this 10 mL of mobile phase was added, sonicate to dissolve for 10 minutes and dilute to volume with diluent.

   Further filter the solution through 0.45µ filter paper. Dilute 10 ml of filtrate to 100 ml with mobile phase.

**Acceptance**
The mean % recovery at each level should not be less than 97% and should not be more than 103%.

**CONCLUSION**
The above results reveal that the method is accurate.

4. **LINEARITY**
   **Standard stock solution preparation**
   About 100 mg of ceftriaxone and 425 mg of tazobactam were weighed into a 10 mL volumetric flask, to this 10 mL of mobile phase was added.
Table: 3.1.4 Linearity studies.

<table>
<thead>
<tr>
<th>Linear solutions (%)</th>
<th>Stock solution taken in (mL)</th>
<th>Diluted to volume (mL) with diluents</th>
</tr>
</thead>
<tbody>
<tr>
<td>25%</td>
<td>2.5</td>
<td>100</td>
</tr>
<tr>
<td>50%</td>
<td>5.0</td>
<td>100</td>
</tr>
<tr>
<td>75%</td>
<td>7.5</td>
<td>100</td>
</tr>
<tr>
<td>100%</td>
<td>10.0</td>
<td>100</td>
</tr>
<tr>
<td>125%</td>
<td>12.5</td>
<td>100</td>
</tr>
<tr>
<td>150%</td>
<td>15.0</td>
<td>100</td>
</tr>
</tbody>
</table>

Procedure
Inject each concentration into the chromatographic system and measure the peak area. Plot the graph of peak area on y axis versus concentration on x axis. Calculate the correlation coefficient.

5. ROBUSTNESS

Preparation of Standard stock solution
Preparation of Standard solution
1 ml of each solution pipette out make in to 10 ml by using mobile phase. 2.3 ml ceftriaxone. Add 1 ml of tazobactam added as mixture.

Temperature was varied to 25°C and 35°C (± 5°C)
About 100 mg of ceftriaxone and 425 mg of tazobactam were weighed into a 10 mL volumetric flask, to this 10 mL of mobile phase was added. Transfer 10 mL of standard stock solution into 100 mL volumetric flask and dilute to volume with diluent.

Flow rate was varied to 0.8 ml/min and 1.2 ml/min (± 0.2 ml/min)
Weigh and transfer 100 mg of OLM working standard and 425 mg of ceftriaxone working standard into 10 mL volumetric flask, add 10 mL of diluent and sonicate to dissolve and dilute to volume with diluent. Transfer 10 mL of standard stock solution into 100 mL volumetric flask and dilute to volume with diluent.

RUGGEDNESS
A wide range of the method was studied by carrying out the experiments by changing the conditions such as,

a. Different operators in the same laboratory (using waters HPLC system & spectra physics HPLC system).
b. Different instruments in the same lab (using waters HPLC system & spectra physics HPLC system).

**Preparation of standard solution of CEFTRIAXONE & TAZOBACTAM**

Weigh and transfer 100 mg of ceftriaxone working standard and 425 mg of tazobactam working standard into 10 mL volumetric flask, add 10 mL of diluent and sonicate to dissolve and dilute to volume with diluent. Transfer 10 mL of standard stock solution into 100 mL volumetric flask and dilute to volume with diluent.

The solution was injected once into the present chromatographic system by Analyst 1. Again the solution was injected once into the same chromatographic system by analyst 2. The peak areas were noted down and %RSD were calculated. To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions.

**SYSTEM SUITABILITY**

A Standard solution of ceftriaxone and tazobactam working standards was prepared as per procedure and was injected six times into the HPLC system. The system suitability parameters were evaluated from standard Chromatograms obtained by calculating the % RSD of retention times, tailing factor, theoretical plates and peak areas from six replicate injections.

1. The % RSD for the retention times of principal peak from 6 replicate injections of each Standard solution should be not more than 2.0%.
2. The number of theoretical plates (N) for the Sum and Nap peaks should be NLT 2000.
3. The Tailing factor (T) for the ceftriaxone and tazobactam peaks should be NMT 2.0.

**ASSAY OF CEFTRIAXONE & TAZOBACTAM**

**Preparation of Standard stock solution**

About 100 mg of ceftriaxone and 425 mg of tazobactam were weighed into a 10 mL volumetric flask, to this 10 mL of mobile phase was added.

**Preparation of Standard solution**

1 ml of each solution pipette out make in to 10ml by using mobile phase. 2.3 ml ceftriaxone Add 1 ml of tazobactam added as mixture.
Preparation of Sample solution

Sample name: ALZON-T inj 100mg of ceftriaxone and 425mg of tazobactamin 50mL volumetric flask and makeup mark with mobile phase. From above solution Pipette 2.3mL of ceftriaxone clear solution in to 10mL volume tric flask and make up volume with mobile phase. pipette 1ml of tazobactam to 10 ml volumetric flask. Pipette 1ml of tazobactam and 2.3 ml ceftriaxone as mixture. used to record the chromatogram.

Assay% = \[(AT/AS)*(WS/DT)*(WT/DS)*(P/100)*(AVG WT/label claim)\]*100

Where: AT = Peak Area of obtained with test preparation.
AS = Peak Area of obtained with standard preparation.
WS = Weight of working standard taken in mg
WT = Weight of sample taken in mg
DS = Dilution of Standard solution
DT = Dilution of sample solution
P = Percentage purity of working standard

RESULT AND DISCUSSION

SPECIFICITY

SAMPLE PREPARATION

Five samples are prepared
1) To 1ml stoke solution add 0.1ml NaOH
2) To 1ml stoke solution add 0.1ml HCL
3) To 1ml stoke solution add 2drops of H2O2
4) 1ml stoke solution kept under uv light for 10min (Heat)
5) 1ml stoke solution kept under heat for 10min

Specificity

Specificity of the methods was determined by the complete separation of Rosuvastatin calcium and Fenofibrate with parameters like retention time, asymmetry and capacity factor. The condition of the method was exactly effective and efficient.
2.

3.
4.

5.
LINEARITY

From the standard stock solution five samples are prepared mobile phase is used as diluents.

1) 0.6ml standard stalk solution+10ml mobile phase----------60%
2) 0.8ml standard stalk solution+10ml mobile phase ---------80%
3) 1ml standard stalk solution+10ml mobile phase ----------100%
4) 1.2ml standard stalk solution+10ml mobile phase -------120%
5) 1.4ml standard stalk solution+10ml mobile phase -------140%
Concentration Vs Area graph is plotted

1. LINEARITY 60%

2. LINEARITY 80%
3. **LINEARITY 100%**

![Graph](image1)

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<th>Sample ID</th>
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<th>Amount (mg)</th>
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</tr>
<tr>
<td></td>
<td>Linearly</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Linearly</td>
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<table>
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<th>Result Table (Linear )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearly03_Rosuvastatin(10mg)+Fenofibrate(160mg)</td>
</tr>
<tr>
<td>Time (min)</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
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<table>
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<th>Column Performance Table (From 30% - Linearly03_Rosuvastatin(10mg)+Fenofibrate(160mg))</th>
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</thead>
<tbody>
<tr>
<td>Retention Time</td>
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<tr>
<td>----------------</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
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</table>

4. **LINEARITY 120%**

![Graph](image2)

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<tr>
<td></td>
<td>Linearly</td>
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<tr>
<td></td>
<td>Linearly</td>
<td>0.02</td>
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<tr>
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<td>Linearly</td>
<td>0</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Result Table (Linear )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearly04_Rosuvastatin(12mg)+Fenofibrate(192mg)</td>
</tr>
<tr>
<td>Time (min)</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>Total</td>
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</table>

<table>
<thead>
<tr>
<th>Column Performance Table (From 30% - Linearly04_Rosuvastatin(12mg)+Fenofibrate(192mg))</th>
</tr>
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<tbody>
<tr>
<td>Retention Time</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>1</td>
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<td>2</td>
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5. LINEARITY 140%

Linearity of Rosuvastatin

<table>
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<tr>
<th>Rosuvastatin mcg</th>
<th>Area</th>
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<tbody>
<tr>
<td>6</td>
<td>476.798</td>
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<tr>
<td>8</td>
<td>640.983</td>
</tr>
<tr>
<td>10</td>
<td>795.678</td>
</tr>
<tr>
<td>12</td>
<td>955.234</td>
</tr>
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<td>14</td>
<td>1101.635</td>
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Linearity of Fenofibrate

<table>
<thead>
<tr>
<th>Fenofibrate Mcg</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>96</td>
<td>2227.433</td>
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<tr>
<td>128</td>
<td>2932.702</td>
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<tr>
<td>160</td>
<td>3718.406</td>
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<tr>
<td>192</td>
<td>4448.597</td>
</tr>
<tr>
<td>224</td>
<td>5122.222</td>
</tr>
</tbody>
</table>
RESULTS

$R^2$ value for Rosuvastatin is 0.9998 and $R^2$ value for Fenofibrate is 0.998 with in limit as the concentration increases are also increases.

The correlation coefficient values were found to be within the acceptance limits for both the drugs i.e. correlation coefficient not less than 0.999.

PRECISION

Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. Percentage relative standard deviation ($\%{\text{RSD}}$) was found to be less than 2% for within a day and day to day variations, which proves that method is precise.
ACCEPTANCE CRITERIA: The results obtained indicate that the % RSD was found to be less than 2% which was in the acceptance limits.
ROBUSTNESS

To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in the optimized method parameters were done. The effect of change in flow rate and mobile phase ratio on the retention time and tailing factor were studied. The method was found to be unaffected by small changes like ± 0.2 change in flow rate and ± 2 change in mobile phase.

FLOW RATE 1

![Flow Rate 1 Diagram]

FLOW RATE-2

![Flow Rate 2 Diagram]
Accuracy (Recovery studies)
To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 80%, 100% and 120%. Known amounts of standard RST and FB were added to pre-analyzed samples and were subjected to the proposed HPLC method. Results of recovery studies. From the stalk solution of standard and sample prepare 100%, 120%, 140% by adding 1ml, 1.2ml, 1.4ml of solution.
Bheemudu et al. World Journal of Pharmaceutical Research

Sample Info:
Sample ID: Recovery
Sample: Rosuvastatin(10mcg)+Fenofibrate(160mcg)
Amount: 0
Volume [ml]: 0.02
Solvent subtracted: (None)

Result Table (Linear):

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Sample</th>
<th>Amount</th>
<th>Volume [ml]</th>
<th>Solvent subtracted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery 01</td>
<td>Recovery</td>
<td>0</td>
<td>0.02</td>
<td>(None)</td>
</tr>
</tbody>
</table>

Column Performance Table (From 50% - Recovery03_Rosuvastatin(12mcg)+Fenofibrate(192mcg))

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Sample</th>
<th>Amount</th>
<th>Volume [ml]</th>
<th>Solvent subtracted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery 01</td>
<td>Recovery</td>
<td>0</td>
<td>0.02</td>
<td>(None)</td>
</tr>
</tbody>
</table>
Rosuvastatin

<table>
<thead>
<tr>
<th>Standard Area</th>
<th>8</th>
<th>640.983</th>
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<tr>
<td></td>
<td>10</td>
<td>795.678</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>955.234</td>
</tr>
</tbody>
</table>

8+2 mcg

10 mcg

816.624

815.647

755.977

Avrg 796.083

result 9.94 mcg

% rec 99.36%

10+2 mcg

12 mcg

979.409

972.428

961.508

Avrg 971.115

result 12.20 mcg

% rec 101.71%

12+2 mcg

14 mcg

1124.603

1127.485

1105.652

Avrg 1119.247

result 14.06 mcg

% rec 100.43%
FORCE DEGRADATION

Forced Degradation Studies

Forced degradation studies were carried out in presence of acid, alkali, H₂O₂ and heat. To the sample bearing concentration 2μg/ml and 26.8μg/ml of Rosuvastatin calcium and Fenofibrate respectively, added 0.1ml of 0.1N Hcl, 0.1ml of 0.1N NaOH and 0.1ml of 5%H₂O₂ and at temperature of 50°C for 6 hours individually.

Forced degradation in presence of 0.1N Hcl

Two different degraded products of Rosuvastatin were found at a RT of 2.740 and 3.220, but the peaks of RST and FB was found to be undisturbed and intact when subjected to forced degradation in 0.1N HCl.
FORCED DEGRADATION IN PRESENCE OF 0.1N NAOH

Two different degraded products of Rosuvastatin were found at a RT of 1.953 and 2.953, but the peaks of RST and FB was found to be undisturbed and intact when subjected to forced degradation in 0.1N NaoH.
Forced degradation in presence of $5\% \text{H}_2\text{O}_2$

No change was observed when subjected to forced degradation in the presence of $5\% \text{H}_2\text{O}_2$. 

---

Sample info:
- Sample ID: Forced degradation
- Amount: 0
- Sample: Rosuvastatin(10mcg)+Fenofibrate(160mcg)
- ISTD Amount: 0
- Inj. Volume [μl]: 0.02
- Dilution: 1
- Solvent subtracted: (None)

Result Table (Uncal. - Forced degradation (NaOH) Rosuvastatin(12mcg)+Fenofibrate(192mcg))

<table>
<thead>
<tr>
<th></th>
<th></th>
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</tr>
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<tbody>
<tr>
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<td>2</td>
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<td>5970.712</td>
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<td>Total</td>
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</table>

Column Performance Table (For 56% - Forced degradation (NaOH) Rosuvastatin(12mcg)+Fenofibrate(192mcg))

<table>
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---

Sample info:
- Sample ID: Forced degradation
- Amount: 0
- Sample: Rosuvastatin(10mcg)+Fenofibrate(160mcg)
- ISTD Amount: 0
- Inj. Volume [μl]: 0.02
- Dilution: 1
- Solvent subtracted: (None)

Result Table (Uncal. - Forced degradation (H2O2) Rosuvastatin(12mcg)+Fenofibrate(192mcg))

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</tbody>
</table>

Column Performance Table (For 56% - Forced degradation (H2O2) Rosuvastatin(12mcg)+Fenofibrate(192mcg))

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.677</td>
<td>0.153</td>
<td>1.630</td>
<td>3717</td>
<td>37172</td>
</tr>
<tr>
<td>2</td>
<td>4.355</td>
<td>0.153</td>
<td>2.050</td>
<td>4425</td>
<td>44247</td>
</tr>
</tbody>
</table>
FORCED DEGRADATION IN PRESENCE OF 50°C TEMPERATURE

No change was observed when the forced degradation was carried out at 50°C temperature.
RUGGEDNESS

Ruggedness of the proposed methods was determined by analysis of aliquots from homogeneous slot in different laboratories, by different analysts, using similar operational and environmental conditions. The % R.S.D. values were found to be less than 2%.  

![Graphical representation of the ruggedness analysis](image)
ASSAY

Sample Info:
- Sample ID: Assay
- Amount: 0
- Solvent added: None

Result Table (Local -
Assay(Sp011)_Rosuvastatin(10mcg)+Fenofibrate(160mcg))

<table>
<thead>
<tr>
<th>Retention Time [min]</th>
<th>Area [mm²]</th>
<th>Height [mm]</th>
<th>Area [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.633</td>
<td>783.442</td>
<td>120.425</td>
</tr>
<tr>
<td>2</td>
<td>4.280</td>
<td>2236.872</td>
<td>333.221</td>
</tr>
<tr>
<td>Total</td>
<td>4510.315</td>
<td>4182.745</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Column Performance Table (From 50% -
Assay(Sp011)_Rosuvastatin(10mcg)+Fenofibrate(160mcg))

<table>
<thead>
<tr>
<th></th>
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<th></th>
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<tbody>
<tr>
<td>1</td>
<td>0.032</td>
<td>1.782</td>
<td>0.949</td>
<td>3916</td>
<td>7.580</td>
</tr>
<tr>
<td>2</td>
<td>0.163</td>
<td>2.000</td>
<td>0.948</td>
<td>4316</td>
<td>7.580</td>
</tr>
<tr>
<td>Total</td>
<td>0.265</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sample Info:
- Sample ID: Assay
- Amount: 0
- Volume [ml]: 0.02
- Dilution: 1

Result Table (Local -
Assay(Sp021)_Rosuvastatin(10mcg)+Fenofibrate(160mcg))

<table>
<thead>
<tr>
<th>Retention Time [min]</th>
<th>Area [mm²]</th>
<th>Height [mm]</th>
<th>Area [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.633</td>
<td>783.442</td>
<td>120.425</td>
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<tr>
<td>2</td>
<td>4.470</td>
<td>3667.331</td>
<td>372.221</td>
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<tr>
<td>Total</td>
<td>4460.316</td>
<td>4182.745</td>
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Column Performance Table (From 50% -
Assay(Sp021)_Rosuvastatin(10mcg)+Fenofibrate(160mcg))

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.163</td>
<td>1.682</td>
<td>4126</td>
<td>4126</td>
<td>7.580</td>
</tr>
<tr>
<td>2</td>
<td>0.163</td>
<td>2.000</td>
<td>4108</td>
<td>4700</td>
<td>7.580</td>
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</tbody>
</table>
### Rosuvastatin vs Fenofibrate

<table>
<thead>
<tr>
<th></th>
<th>Rosuvastatin</th>
<th>Fenofibrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard Area</td>
<td>1 776.162</td>
<td>3667.253</td>
</tr>
<tr>
<td></td>
<td>2 793.295</td>
<td>3714.023</td>
</tr>
<tr>
<td></td>
<td>3 777.156</td>
<td>3661.750</td>
</tr>
<tr>
<td></td>
<td>4 795.514</td>
<td>3679.772</td>
</tr>
<tr>
<td></td>
<td>5 792.922</td>
<td>3649.541</td>
</tr>
<tr>
<td>Average</td>
<td><strong>782.204</strong></td>
<td><strong>3674.468</strong></td>
</tr>
</tbody>
</table>

| Sample area | 1 783.949    | 3656.973    |
|             | 2 790.984    | 3667.331    |
|             | 3 783.954    | 3689.410    |
|             | 4 796.362    | 3719.872    |
|             | 5 797.71     | 3726.651    |
| Average     | **790.592**  | **3692.047**|

| Tablet average weight | 220.1mg | 220.1 mg |
| Standard weight       | 10 mg   | 160 mg   |
| Sample weight         | 220.1mg | 220.1 mg |
| Label amount          | 10 mg   | 160 mg   |
| Std. purity           | 99.6%   | 99.9%    |
| **Cal.:**             | **10.07mg** | **160.60mg** |
| % Assay               | **100.67%** | **100.38%** |

Assay = Avg. Sample Area x Standard Wt. x Standard dilution x Purity of Standard
Avg. Standard area x Sample wt. x Sample dilution

% Assay = Assay in mg x Avg. wt.
Label Claim

### LOD – LOQ

<table>
<thead>
<tr>
<th></th>
<th>Rosuvastatin</th>
<th>Fenofibrate</th>
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<tbody>
<tr>
<td>mcg</td>
<td>mcg</td>
<td>mcg</td>
</tr>
<tr>
<td>6</td>
<td>476.798</td>
<td>2227.433</td>
</tr>
<tr>
<td>8</td>
<td>640.983</td>
<td>2932.702</td>
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<tr>
<td>10</td>
<td>795.678</td>
<td>3718.406</td>
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<td>12</td>
<td>955.234</td>
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<td>14</td>
<td>1101.635</td>
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<td>SD</td>
<td>3.2</td>
<td>247</td>
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<tr>
<td>Slope</td>
<td>78.2</td>
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<tr>
<td>mcg</td>
<td>mcg</td>
<td>mcg</td>
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<td>LOD</td>
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<tr>
<td>LOQ</td>
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| Slope            | 78.2         | 22.83       |

Slope
LOD
LOQ
INDEX

<table>
<thead>
<tr>
<th>Chapter No</th>
<th>Name</th>
<th>Page No</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Abbreviations</td>
<td>1-2</td>
</tr>
<tr>
<td>2</td>
<td>Abstract</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>Introduction to Hplc</td>
<td>4-14</td>
</tr>
<tr>
<td>4</td>
<td>Method Development Considerations (Trials)</td>
<td>14-24</td>
</tr>
<tr>
<td>5</td>
<td>Method Validation</td>
<td>24-29</td>
</tr>
<tr>
<td>6</td>
<td>Review of Literature</td>
<td>30-32</td>
</tr>
<tr>
<td>7</td>
<td>Drug Introduction (profile)</td>
<td>33-40</td>
</tr>
<tr>
<td>8</td>
<td>Aim &amp; Scope of Work</td>
<td>41</td>
</tr>
<tr>
<td>9</td>
<td>Method and Methodology (Validation)</td>
<td>42-86</td>
</tr>
<tr>
<td>10</td>
<td>References</td>
<td>87</td>
</tr>
</tbody>
</table>

LIST OF TABLES

<table>
<thead>
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<th>S.No</th>
<th>Name of the table</th>
<th>Page No</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Difference between normal phase &amp; reverse phase in HPLC</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>Method goals (sample and analyte information)</td>
<td>19</td>
</tr>
<tr>
<td>3</td>
<td>Materials</td>
<td>42</td>
</tr>
<tr>
<td>3.1</td>
<td>Instruments used</td>
<td>42</td>
</tr>
<tr>
<td>3.1.2</td>
<td>Chemicals &amp; Reagents Used</td>
<td>43</td>
</tr>
<tr>
<td>3.1.3</td>
<td>Optimised Condition</td>
<td>44</td>
</tr>
<tr>
<td>3.1.4</td>
<td>Linearity Study</td>
<td>49</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of the Figure</th>
<th>Page No</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>High Performance Liquid Chromatography (HPLC)</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>HPLC Chromatogram</td>
<td>20-23</td>
</tr>
<tr>
<td>3</td>
<td>Specificity</td>
<td>54-56</td>
</tr>
<tr>
<td>4.1</td>
<td>Linearity 60%</td>
<td>57</td>
</tr>
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<td>4.2</td>
<td>Linearity 80% &amp; 100%</td>
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<td>4.3</td>
<td>Linearity 120% &amp; 140%</td>
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<td>5</td>
<td>Linearity of Rosuvastatin &amp; Fenofibrate</td>
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<tr>
<td>6</td>
<td>Precession</td>
<td>61-65</td>
</tr>
<tr>
<td>7</td>
<td>Robustness</td>
<td>65-66</td>
</tr>
<tr>
<td>8</td>
<td>Accuracy (Recovery Studies)</td>
<td>67-72</td>
</tr>
<tr>
<td>9</td>
<td>Forced Degradation</td>
<td>73-76</td>
</tr>
<tr>
<td>10</td>
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<td>Assay</td>
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<tr>
<td>12</td>
<td>LOD – LOQ</td>
<td>86</td>
</tr>
</tbody>
</table>

CONCLUSION

A simple, specific, linear, precise and accurate RP-HPLC method has been developed and validated for quantitative determination of Rosuvastatin Calcium and Fenofibrate in new tablet formulation. The results of stress testing of the drug, undertaken accordingly reveal that the degradation products were formed in hydrolytic (acid and base) conditions and the method being able to separate the main drug from its degraded product. The method is very simple, specific and rapid with the total run time of 5 min which makes it especially suitable for routine quality control analysis.

The proposed RP-HPLC method was validated as per International Conference on Harmonization (ICH) Guidelines and found to be applicable for routine quality control analysis for the simultaneous estimation of ROS and FEN using isocratic mode of elution. The results of linearity, precision, accuracy and specificity, proved to be within the limits. The method provides selective quantification of ROS and FEN without any interference. The proposed method is highly sensitive, reproducible, reliable, rapid and specific.

ACKNOWLEDGMENT

I take the immense pleasure in expressing gratitude to our guide Mr. SHIVADEV, M. pharm. Dept. of Analysis, vathsalya college of pharmacy, Anantharam, Bhongir, Nalgonda. I express my profound gratitude for her encouragement, observation and suggestion throughout the dissertation work.
We are thankful to **Dr. Hemanth P. Joshi, M.pharm., Ph.D.**, Principal, vathsalya college of pharmacy, Anantharam, Bhongir, Nalgonda for his constant encouragement throughout our course work and also for the facilities provided for our project work.

I express my sincere thanks to entire faculty members of vathsalya college of pharmacy, Anantharam, Bhongir, Nalgonda for their support and their help during the course of our study and to carry out this project work.

I take this opportunity to express my deep sense of gratitude and sincere thanks to **Mr. Ganesh**, lab Technician, Dept. of Pharmaceutics, Vathsalya College of Pharmacy, for his help during the experimental work.

I owe my special thanks to non-teaching faculty who helped me to complete my project work.

I am thankful to **Chief Librarian, Mr. Seetharamaraju** and **Asst. Librarian, Mrs. R. Supraja** for providing the valuable books journals and reference during the project work.

I am indebted to the management “Vathsalya College of Pharmacy” for providing the necessary infrastructure facilities to carry out the project.

I am very grateful to **my parents, friends** who have been given a constant inspiration which enabled me to reach this exuberant moment.

**D. BHEEEMUDU**

**REFERENCES**