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# New Developed and Validated, Rapid HPLC Method for the Determination of Rosuvastatin Calcium and Fenofibrate Simultaneously in Combined Tablet Dosage Form.

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## ABSTRACT

A new developed and validated simple ,precise, accurate, and rapid HPLC method for the determination of rosuvastatin calcium and fenofibrate simultaneously, in combined tablet dosage form the mobile phase used was a mixture of 1% TEA : Acetonitrile (60:40%v/v), PH: 5.8. the detection of rosuvastatin calcium and fenofibrate was carried out at 287 nm with a flow rate of 1.0ml/min. The retention time (min) for rosuvastatin calcium and fenofibrate were 2.7, 4.28 respectively. Results of the analysis were validated statistically, and by recovery studies. The proposed method can be successfully used to determine the drug contents of marketed formulation.

**Keywords:** HPCL, Fenofibrate, Rosuvastatin Calcium, Validation

**INTRODUCTION:** Pharmaceutical analysis simply means analysis of pharmaceuticals. Webster' dictionary defines pharmaceutical as a medical drug. It is generally known that a pharmaceutical is a therapeutic interest. A more appropriate term for a pharmaceutical is active pharmaceutical ingredient (API) or active ingredient to distinguish it from a formulated product or drug product is prepared by formulating a drug substance with inert ingredient (excipients) to prepare a drug product that is suitable for administration to patients. It is well known in the pharmaceutical industry that pharmaceutical analysts in research and development (R&D) play a very comprehensive role in new drug development and follow up activities to ensure that a new drug product meets the established standards is stable and continue to approved by regulatory authorities ,assuring that all batches of drug product are made to the specific standards utilization of approved ingredients and production method becomes the responsibility of pharmaceutical analysts in the quality control (QC) or quality assurance department. The methods are generally developed in an analytical R&D department and transferred to QC or other departments as needed. At times they are transferred to other divisions. By now it should be quite apparent that pharmaceutical analysts play a major role in assuring the identity, safety, efficacy, and quality of drug product safety and efficacy studies required that drug substance and drug product meet two critical requirements. 1. Established identity and purity. 2. Established bio availability/ dissolution.

### *High Performance Liquid Chromatography (HPLC)*

High performance liquid chromatography (HPLC)[3-7] is a process, which separates mixture containing two or more components under high pressure. in this the stationary phase is packed in a column one end of which is attached to a source of pressurized liquid mobile phase. High performance liquid chromatography is the fastest growing analytical technique for the analysis of drugs. Its simplicity, high specificity and wide range of sensitivity makes it ideal for the analysis of many drugs in both dosage forms and biological fluids. It is presently used in pharmaceutical research and developments in the following ways: To purify synthetic or natural products, To characterize metabolites, To assay active ingredients, impurities, degradation products and in dissolution assays In Pharmacodynamic and pharmacokinetic studies.

***Isocratic and Gradient Elution:*** A separation in which the mobile phase composition remains constant throughout the procedure is termed isocratic (meaning *constant composition*). The word was coined by Csaba Horvath who was one of the Chromatographic Parameters: Retention time (Rt): Adjusted retention time: Retention volume (VR): Adjusted retention volume (VR): Relative retention volume: Height equivalent to a theoretical plate (HETP), Resolution: Column Efficiency (N): Two related terms are widely used as quantitative measures of the efficiency of the chromatographic columns.

## METHOD

For achieving of stable base line Equilibration of the column with the prescribed mobile phase and flow rate and room temperature or at the temperature specified in the monograph and preparation of sample solution and to be examined and the reference solution is require d the solutions must be free from solid particles.

***Optimization of the method:*** During optimization of the method the initial set of conditions have evolved from the first stages of development are improved or maximized in terms of resolution and peak shape plate counts asymmetry capacity elution time detection limits limit of quantification and overall ability to quantify the specific analyte of interest.

The various parameters is that include to be optimized during method development

- Modes of separation, Selection of stationary phase, Selection of mobile phase
- Selection of the detector

***Selection modes of separation:*** Reverse phase mode the mobile phase is comparatively more polar than the stationary phase for the separation of polar or moderately polar compounds the most preferred mode is reverse phase The nature of the analyte is the primary factor in the selection of modes of separation. The second factor is based on nature of the matrix.

### *HPLC METHOD DEVELOPMENT*

Systematic approach to HPLC method development should be based on the knowledge of the chromatographic process. In most cases, a considerable amount of experimentation may be needed. A good method development strategy should require only as many experimental runs as are necessary to achieve desired final result.

***HPLC method validation:*** Method validation[8-10] is defined as a process of providing that an analytical method is acceptable for its intended use. Method validation provides the method development extremely specific, linear, precise, accurate and sensitive.

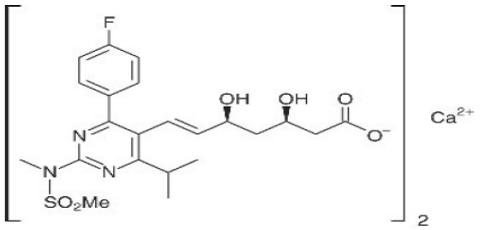
**Table 1: System Suitability Parameters and Recommendations**

| Parameter | Recommendation |
|---|---|
| Capacity Factor (k') | The peak should be well-resolved from other peaks and the void |
| Repeatability | RSD $\leq$ 1% for N $\geq$ 5 is desirable. |
| Relative retention | Not essential as long as the resolution is stated. |
| Resolution ($R_S$) | $R_S$ of > 2 between the peak of interest and the closest eluting |
| TailingFactor(T) | T of $\leq$ 2 |
| Theoretical Plates (N) | N > 2000 |

### *The Specific Aim of the Research*

To develop and validate RP-HPLC method for the estimation of Rosuvastatin and finofibratein bulk and dosage form. The developed method can successfully be applied to estimate the amount of Rosuvastatin and finofibratein bulk and dosage form. The proposed method was validated as per ICH guidelines. Based on the objective the plan of the work is as follows: Development of new analytical method, Selecting the HPLC separation mode, Selecting/ optimizing the mobile phase, Selecting column for analysis, Selecting the appropriate detector system, Selecting appropriate gradient/ isocratic medium, Selecting appropriate flow rate, temperature and pH, Validation parameters,Linearity, Assay of Rosuvastatin and finofibrate tablet, System suitability, Accuracy, Precision, Robustness, LOD, LOQ.

**DRUG PROFILE: Name**: **Rosuvastatin**[26-33]
**Structure**:




**Functional Category:** Anti cholesteremic Agents
**MATERIALS AND INSTRUMENTS:** The following materials used were either AR/LR grade or the best possible Pharma grade

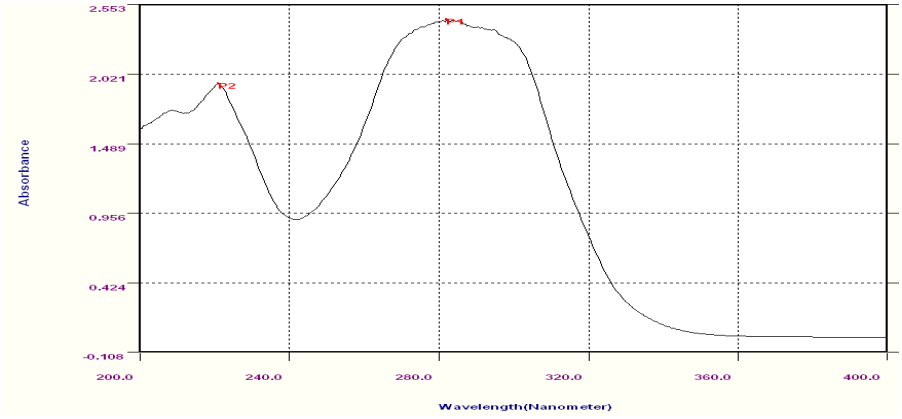
**Drug Sample:** Rosuvastatin - Comprime Labs Pvt. Ltd., Hyderabad. 2. Fenofibrate - Comprime Labs Pvt. Ltd., Hyderabad.

**Chemicals and Solvents Used:** 1. Water –HPLC grade, 2. Methanol – HPLC grade, 3. Acetonitrile - HPLC grade, 4. Tri Ethyl Amine -HPLC grade, 5. Potassium Dihydrogen Phosphate , 6. DiPotassium Hydrogen Phosphate above chemicals and solvents were supplied by S.D. Fine Chemicals Ltd., India; Qualigens Fine Chemicals Ltd., Mumbai, India and Ranbaxy Chemicals Ltd., New Delhi, India.

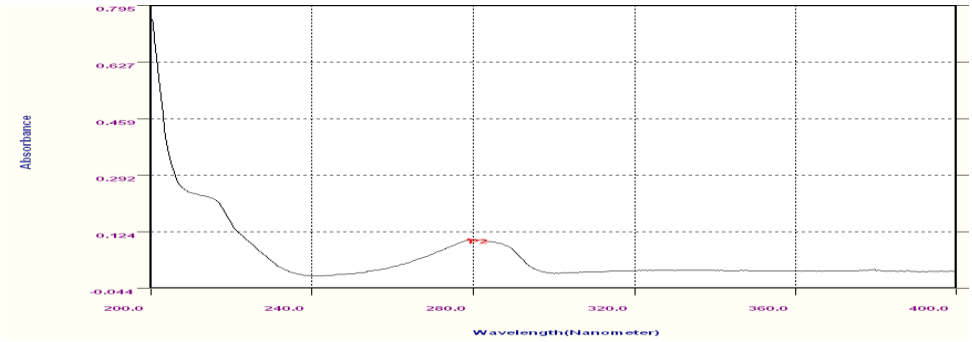
**Instruments Used:** 1. Shimadzu ATY224 Digital Electronic Balance, 2. Wensar Pvt. Limited, India, pH meter, 3. Vacuum Pump Filter (PCI), 4. Life Care Sonicator, 5. Elico SL 210 Double beam UV/Vis Spectrophotometer, 6. Hitachi Elite Lachome HP LC model, Containing Pump: L2130,Detector: UV, L2400, Software: D2000 Elite.

## *METHOD DEVELOPMENT*

***Selection of wavelength:*** Selectivity of HPLC method that uses UV detector depends on proper selection of Wavelength. A wavelength which gives good response for the drug to be detected should be Selected. From the UV spectra 287 nm was selected as the wavelength for rosuvastatin & 288 for fenofibrate The λ max selected for work was 287nm.




**Uv-Spectrum for Rosuvastatin**




**Uv-Spectrum for fenofibrate**

***Selection of Mobile phase:*** Solvent selectivity (solvent type), solvent strength (percentage of organic solvent in the mobile phase), strength and pH of buffer, flow rate etc were varied to determine the chromatographic conditions, that gave the best separation. The standard solutions of Fenofibrate and Rosuvastatin were run and combination of solvents have been tried to get a symmetric and stable peak. Each mobile phase was filtered through 0.45 µ membrane filter. From the various mobile phase tried, mobile phase containing 1% tri ethyl amine: acetonitrile (60:40) %v/v was selected, since it gave sharp peak with symmetry within limits and significant retention time.

***Chromatographic experiment:*** Different chromatographic conditions were tried to optimize the method:

**Trial 8: Optimized Method:** Mobile phase: - 1% TEA : Acetonitrile (60:40%v/v), Ph: 5.8

Column: C18 Hypersil, 150*4.6, particle size 3µ, Flow rate: 1 ml/min, Run time: 10 min

Detection Wavelength: 287 nm, Retention time: 2.73 min for Rosuvastatin, 4.64 for Fenofibrate.

Result: optimized method.

| S.NO. | DRUG | RT | PEAK AREA | TAILING FACTOR | THEORETICAL PLATES |
|---|---|---|---|---|---|
| 1 | FENOFIBRATE | 2.7 | 943810 | 1.58 | 4578 |
| 2 | ROSUVASTATIN | 4.28 | 4765505 | 0.98 | 6389 |

## EXPERIMENTAL METHOD

| | |
|---|---|
| Stationary phase | C18 Hypersil, 150*4.6, particle size 3µ |
| Injector | Rheodyne |
| Column | C18 Hypersil, 150*4.6, particle size 3µ |
| Mobile phase | 1% TEA : Acetonitrile (60:40%v/v) Ph: 5.8 |
| Flow rate | 1ml/min |
| Injection volume | 20µl |
| Wavelength | 287nm |
| Temperature | 250C |
| Run time | 10 min |

## VALIDATION PARAMETER

***System Suitability Studies:*** System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (Rt), number of theoretical plates (N) and tailing factor (T) were evaluated for six replicate injections of the drug at a concentration of 50 µg/ml. The results which are given in Table 5 were within acceptable limits. System suitability test is a pharmacopoeia requirement.

**For fenofibrate**

| S.NO. | Concentration | Retention time | Peak area | Theoretical plates | Tailing factor |
|---|---|---|---|---|---|
| 1 | 50 | 2.69 | 946633 | 4557 | 1.6 |
| 2 | 50 | 2.7 | 943810 | 4578 | 1.58 |
| 3 | 50 | 2.7 | 941462 | 4573 | 1.54 |
| 4 | 50 | 2.69 | 932398 | 4576 | 1.56 |
| 5 | 50 | 2.7 | 949637 | 4556 | 1.55 |
| 6 | 50 | 2.7 | 943945 | 4570 | 1.58 |
| Avg | | 2.69666 | 5891.744 | 4568.333 | 1.568 |
| SD | | 0.00516 | 942980.8 | 9.56382 | 0.022 |
| %RSD | | 0.191495 | 0.6248 | 0.20935 | 1.421 |

**For Rosuvastatin**

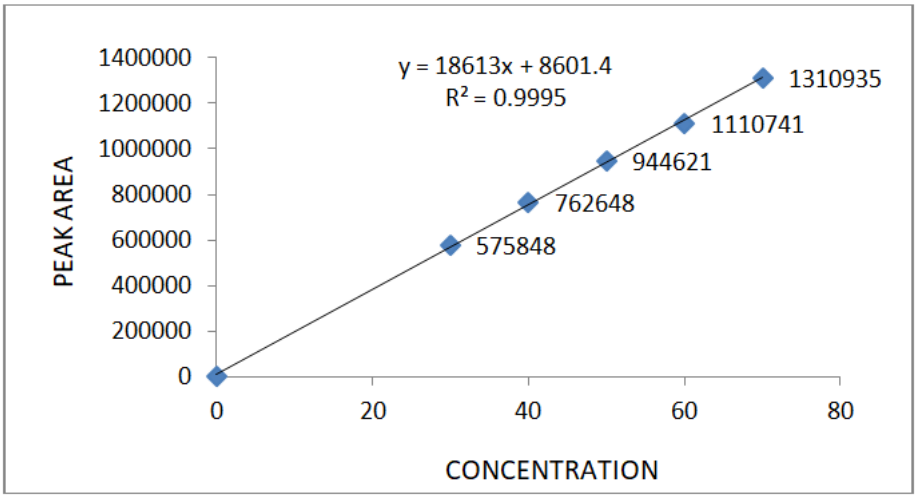
| S.NO. | Concentration | Retention time | Peak area | Theoretical plates | Tailing factor |
|---|---|---|---|---|---|
| 1 | 50 | 4.27 | 4853049 | 6320 | 1.01 |
| 2 | 50 | 4.28 | 4765505 | 6389 | 0.98 |
| 3 | 50 | 4.28 | 4711296 | 6366 | 0.99 |
| 4 | 50 | 4.27 | 4870347 | 6312 | 0.99 |
| 5 | 50 | 4.28 | 4892550 | 6353 | 0.96 |
| 6 | 50 | 4.28 | 4812980 | 6308 | 0.97 |
| Avg | | 4.27333 | 68836.2568 | 6341.333 | 0.98333 |
| SD | | 0.005164 | 4817621.17 | 32.99495 | 0.017512 |
| %RSD | | 0.120842 | 1.42884329 | 0.520316 | 1.780871 |

## Acceptance Criteria and Result

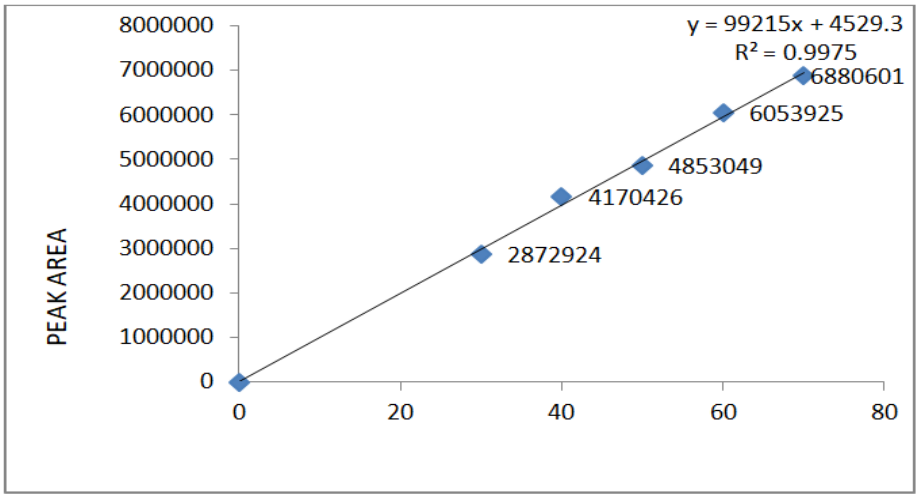
| S.No. | Parameter | Limit | Result |
|---|---|---|---|
| 1 | Resolution | Rs > 2 | 3.15 |
| 2 | Tailing factor | T ≤ 2 | Rosuvastatin =0.98<br>Fenofibrate =1.568 |
| 3 | Theoretical plate | N > 2000 | Rosuvastatin =6341<br>Fenofibrate= 4568 |

***Linearity:*** To evaluate the linearity, serial dilution of analyte were prepared from the stock solution was diluted with mobile phase to get a series of concentration ranging from 30, 40, 50, 60 and 70µg/ml. The prepared solutions were filtered through whatmann filter paper (No.41). From these solutions, 20µl injections of each concentration were injected into the HPLC system and chromatographed under the optimized conditions. Calibration curve was constructed by plotting the mean peak area (Y-axis) against the concentration (X-axis). The results which are given in Table 6 were within acceptable limits. Correlation coefficient should not be less than 0.99.

### *Calibration Curve Of Fenofibrate*




### *Calibration Curve Of Rosuvastatin*




***Accuracy: Recovery study:*** Fenofibrate and Rosuvastatin: To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of Rosuvastatin & fenofibrate were taken and injected in three replicates. From that percentage recovery values were calculated from standard graph.

***Repeatability:*** The precision of each method was ascertained separately from the peak areas obtained by actual determination of five replicates of a fixed concentration of Fenofibrateand Rosuvastatin. The Percent Relative Standard Deviations Were Calculated For Fenofibrate and Rosuvastatin.

***Sensitivity:*** The Sensitivity of measurement of Rosuvastatin and Fenofibrate by use of the proposed method was estimated in terms of the limit of detection (LOD) and the Limit of Quantitation (LOQ). The LOD and LOQ were calculated by the use of the equations LOD = 3.3 × σ / S and LOQ = 10 × σ / S where σ is the standard deviation of intercept of Calibration plot and S is the average of the slope of the corresponding Calibration plot.

### *Ruggedness*

Readings by Analyst 1:

| s.no | Injection number | Fenofibrate | | Rosuvastatin | |
|---|---|---|---|---|---|
| | | area | Retention time | Area | Retention time |
| 1 | Rosu+ Feno 50+50 | 2.7 | 944621 | 4.28 | 4953049 |
| 2 | Rosu+ Feno 50+50 | 2.7 | 943810 | 4.28 | 4895505 |
| | Avg | 2.7 | 944215.5 | 4.28 | 40689.75 |

| | | | | | |
|---|---|---|---|---|---|
| | S.D. | 0 | 573.4636 | 0 | 4924277 |
| | %RSD | 0 | 0.060734 | 0 | 0.826309 |

Readings by Analyst 2:

| s.no | Injection number | Fenofibrate | | Rosuvastatin | |
|---|---|---|---|---|---|
| | | area | Retention time | Area | Retention time |
| 1 | Rosu+ Feno 50+50 | 2.69 | 926633 | 4.27 | 4711296 |
| 2 | Rosu+ Feno 50+50 | 2.7 | 914621 | 4.28 | 4765505 |
| | Avg | 2.695 | 920627 | 0.007071 | 38331.55 |
| | S.D. | 0.007071 | 8493.767 | 4.275 | 4738401 |
| | %RSD | 0.262377 | 0.922607 | 0.165405 | 0.808956 |

***Robustness:*** Robustness is a measure of capacity of a method to remain unaffected by small, but deliberate variations in the method conditions, and is indications of the reliability of the method. A method is robust, if it is unaffected by small changes in operating conditions. To determine the robustness of this method, the experimental conditions were deliberately altered at three different levels and retention time and chromatographic response were evaluated. One factor at a time was changed to study the effect. Variation of wavelength (235 and 239 nm) and mobile phase flow rate by 0.1 ml/min (0.9 and 1.1ml/min) had no significant effect on the retention time and chromatographic response of the 50 µg/ml solution, indicating that the method was robust.

**RESULT AND DISCUSSION:** To develop a precise, linear, specific RP-HPLC method for analysis of Fenofibrate and Rosuvastatin different chromatographic conditions were applied & the results observed are presented in the thesis. Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution. In case of RP-HPLC various columns are available, but here develo Sil, C-18, V size (150mm*4.6mmØ) column was preferred because using this column peak shape, resolution and absorbance were good. Mobile phase & diluent for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (methanol, acetonitrile, dichloromethane, water, 0.1N NaOH, 0.1NHCl). Fenofibrate was found to be insoluble in water and soluble in acetonitrile & methanol. Rosuvastatin was found to be soluble in water and soluble in methanol & acetontrile. Detection wavelength was selected after scanning the standard solution of drug over 200 to 800nm. From the U.V spectrum of Rosuvastatin& Fenofibrate it is evident that most of the HPLC work can be accomplished in the wavelength range of 215-290 nm conveniently. Further, a flow rate of 1.0 ml/min & an injection volume of 20 µl were found to be the best analysis. The result shows the developed method is yet another suitable method for assay which can help in the analysis of Rosuvastatin& Fenofibrate in different formulations.

**CONCLUSION:** A sensitive & selective stability indicting RP-HPLC method has been developed & validated for the analysis of Fenofibrate & RosuvastatinAPI. Based on peak purity results, obtained from the analysis of samples using described method, it can be concluded that the absence of co-eluting peak along with the main peak of Fenofibrate & Rosuvastatin indicated that the developed method is specific for the estimation of Fenofibrate & Rosuvastatin. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility.

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