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A New rp-hplc Method for Simultaneous Estimation of Atorvastatin and Fenofibrate in its Bulk and Tablet Dosage form

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Commentary

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ABSTRACT

Objective: A New method was established for simultaneous estimation of Atorvastatin And Fenofibrate by RP-HPLC method.

Methods: Chromatographic separations were carried using Phenomenex C18 column (4.6 × 250 mm, 5 µm) column with a mobile phase composition of 0.1 M Ammonium acetate: acetonitrile (60:40) have been delivered at a flow rate of 1ml/min and the detection was carried out using waters HPLC auto sampler, separation module 2695 with PDA detector at wavelength 236 nm.

Results: The retention time for Atorvastatin And Fenofibrate were 2.991 and 5.931 minute respectively. The correlation coefficient values in linearity were found to be 0.999 and concentration range 10-30 µg/ml for Atorvastatin and 160-480 µg/ml for Fenofibrate respectively. For accuracy The total recovery was found to be 99.08% and 100% for Atorvastatin And Fenofibrate.

Conclusion: The results of study showed that the proposed RP-HPLC method is a simple, accurate, precise, rugged, robust, fast and reproducible, which may be useful for the routine estimation of Atorvastatin And Fenofibrate in pharmaceutical dosage form.

INTRODUCTION

Atorvastatin is used primarily for lowering blood cholesterol and for prevention of events associated with cardio vascular disease. It may be used in combination with bile acid sequestarants to increase the reduction in cholesterol levels. It is a competitive inhibitor of HMG-COA reductase. HMG-COA reductase catalyses the reduction of 3-hydroxy-3-methyl-glutaryl-coenzymeA to mevalonate which is rate limiting step in hepatic cholesterol biosynthesis1. Iupac name is (3R,5R)-7-[2-(4-fluorophenyl) -3-phenyl-4-(phenyl carbomyl)-5-propan-2-ylpyrrol-1-yl]-3,-5-dihydroxypeptanoic acid. It is soluble in Water [1].

Fenofibrate is an antilipidemic agent which reduces both cholesterol and triglycerides in blood. It exerts therapeutic effects through activation of peroxisome proliferators activated receptor a(PPARa). This increases lypolysis and elimination of triglyceride-rich particles from plasma by activating lipo protein lipase and reducing production of apoprotein. The compound be longs to class of organic compound known as benzophenones. These are organic compound containing a ketone attached to two phenyl groups 2. Iupac name is propan-2-yl-2-[4-((4-chlorophenyl) carbonyl) phenoxy-4-(phenoxy)]-2-methyl propanoate. It is soluble in Water [2].

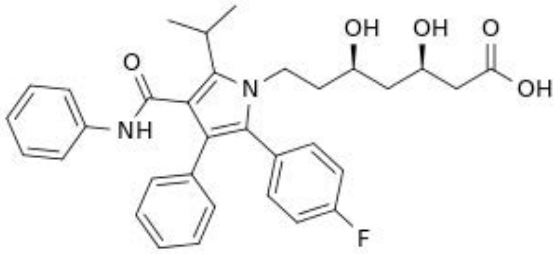

Figure 1. Structure of Atorvastatin.

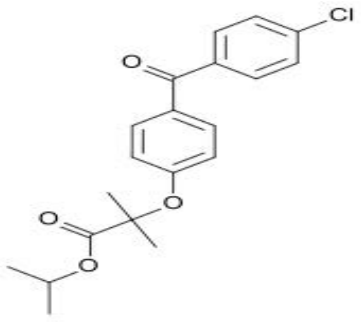

Figure 2. Structure of Fenofibrate.

Literature survey shows that a number of methods have been reported for estimation of Atorvastatin And Fenofibrate individually or in combination with other drugs Those are HPLC Methods [3-8], Ultraviolet spectrophotometry. However, there is only few HPLC method is reported for the simultaneous estimation of these drugs in combined dosage forms.

The aim of the present study was A New Rp-Hplc Method For Simultaneous Estimation Of Atorvastatin And Fenofibrate In Its Bulk And Tablet Dosage Form.

MATERIALS AND METHODS

Chemicals and Reagents

Atorvastatin And Fenofibrate were obtained as a gift sample from Ranbaxy laboratories limited, Hyderabad. KH2PO4 was analytical grade supplied by Finer chemical LTD, Mumbai, Orthophosphoric acid (Standard reagents Hyd), Acetonitrile (Molychem, HPLC grade) and Water for HPLC (LICHROSOLV (MERCK).

Equipment and Chromatographic Conditions

The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, PDA detector and Empower 2 software. Analysis was carried out at 236 nm with an symmetry shield C18 column (4.6× 250mm, 5µm), dimensions at ambient temperature. The optimized mobile phase consists of 0.1 M Ammonium acetate:acetonitrile (60:40). Flow rate was maintained at 1 ml/min and run time for 30 min.

Preparation of solutions

Preparation of buffer

Accurately measured 1 ml of OPA (orthophosphoric acid) was transferred to 1000 ml volumetric flask and 250 ml of HPLC Grade water added initially, after sonicating for 30 min volume is makeup with HPLC Grade water, to give in 0.1% solution

Preparation of mobile phase

A mixture of above prepared buffer 200 ml, and 800 ml of HPLC grade methanol were mixed and degassed in ultrasonic water bath for 5 minutes. The mobile phase was filtered through 0.45 µ filter under vacuum.

The diluents

The Mobile phase was used as the diluent.

Preparation of standard stock solution

Accurately weighed and transferred 10 mg of Atorvastatin and 160 mg fenofibrate into a 100 ml clean dry volumetric flask and added about 40 ml of diluents (mobile phase). It was sonicated to dissolve completely and made volume up to mark with the same mobile phase. From this, 3ml of the solution was pipetted out into another 25 ml of volumetric flask and diluted up to mark with mobile phase.

Preparation of Sample stock solution

Accurately weighed 20 tablets and calculated the average weight of 20 tablets. The tablets were triturated and transferred the tablet powder equivalent to 10mg of Atorvastatin and 160 mg of Fenofibrate into a 100 ml clean dry volumetric flask and added about 40 ml of mobile phase. It was sonicated to dissolve completely and made volume up to the mark with the same mobile phase. From this, 3 ml of the solution was pipetted out into another 25 ml of volumetric flask and diluted up to mark with mobile phase.

Procedure

10 ml of the standard and sample solutions were injected into chromatographic system and areas for the Atorvastatin and Fenofibrate peaks were measured.

METHOD

The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min for 10 minutes to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 10 µL of standard into Phenomenex C18 column (4.6x250 mm,5 µm), the mobile phase of composition 0.1% OPA buffer and acetonitrile in the (50:50) was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1

Table 1. System suitability parameters.

Parameters	Atorvastatin	Fenofibrate
Retention time	2.991	5.931
USP Plate count	16111	15478
USP Tailing	1.20	1.09

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Atorvastatin and Fenofibrate in their tablet dosage form. The result obtained for Atorvastatin and Fenofibrate was comparable with the corresponding labeled amounts and they were shown in Table-2.

Table 2. Assay results for Atorvastatin and Fenofibrate.

Chemicals	**Label Claim (mg)**	**% Assay**
Atorvastatin	10	99
Fenofibrate	160	99.7

Validation of Analytical method

Linearity and Range

Stock solution was prepared by dissolving the appropriate amount of Atorvastatin and Fenofibrate in 10 ml of diluent and further diluted to the required concentrations with diluent. The solution was prepared at five concentration levels ranging from 10 µg/ml to 30 µg/ml of Atorvastatin and 160 µg/ml to 480 µg/ml of Fenofibrate. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The resulte are shown in table 3.

Table 3. Linearity results for Atorvastatin and Fenofibrate.

Atorvastatin		**Fenofibrate**	
Concentration(µg/ml)	**Sample Absorbance**	**Concentration(µg/ml)**	**Sample Absorbance**
10	0.2018	160	0.3614
15	0.3162	240	0.5429
20	0.4186	320	0.7264
25	0.5284	400	0.9125
30	0.6253	480	1.0834
Correlation coefficient	0.999	Correlation coefficient	0.999

Accuracy studies

The accuracy was determined by help of recovery study. The recovery method carried out at three level 50%, 100%, 150%. Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Atorvastatin and Fenofibrate and calculate the individual recovery and mean recovery values. The resulte are shown in table 4,5.

Table 4. Accuracy results for Atorvastain.

Atorvastain						
Spiked level	**Sample weight**	**Sample Absorb**	**µg/ml added**	**µg/ml founded**	**%Recovery**	**%Mean**
50%	205.50	0.2085	5.940	5.92	99.08	99
100%	411.00	0.4139	11.880	11.82	98.98	
150%	616.50	0.6232	17.280	17.82	98.97	

Precision Studies

Precision was calculated from Coefficient of variance for six replicate injections of the standard. The standard solution was injected for six times and measured the area for all six Injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 6.

Table 5. Accuracy results for Fenofibrate.

Fenofibrate						
Spiked level	**Sample weight**	**Sample Absorb**	**µg/ml added**	**µg/ml founded**	**%Recovery**	**%Mean**
50%	205.5	0.3612	96.00	95.84	100	100
100%	411.00	0.7245	192.1	192.85	100	
150%	616.50	1.0845	287	287.30	100	

Table 6. Precision results for Atorvastatin and Fenofibrate.

Injection	Atorvastatin Area	Fenofibrate Area
Injection-1	2976098	5375506
Injection-2	2978524	5376291
Injection-3	2972780	5372816
Injection-4	2977965	5370378
Injection-5	2975011	5379820
Average	2976195.33	5374752.33
%RSD	0.34	0.23

Ruggedness

To evaluate the intermediate precision of the method, Precision was performed on different day. The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found. The resulte are shown in table 7.

Table 7. Intermediate precision resultes for Atorvastain and Fenofibrate.

S. NO	Sample Weight	Sample Absorbance-1	Sample Absorbance-2	% Assay	% Assay
1	411	0.4252	0.7325	99	100
2	411	0.4254	0.7355	99	100
3	411	0.4352	0.7412	100	100
4	411	0.4384	0.7452	99	100
5	411	0.4980	0.7521	99	100
6	411	0.4998	0.7541	99	100
Average assay	--	--	--	99	100
STD	--	--	--	0.20	0.22
%RSD	--	--	--	0.19	0.21

Robustness

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The flow rate was varied at 0.8 ml/min to 1.2 ml/min. The Organic composition in the Mobile phase was varied from 50% to 50%.

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines?

LOD = 3.3σ/S and

LOQ = 10 σ/S, where

σ= Standard deviation of y intercept of regression line,

S = Slope of the calibration curve

CONCLUSION

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Atorvastatin and Fenofibrate in pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Atorvastatin and Fenofibrate in pure and its pharmaceutical dosage forms

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