RESEARCH ARTICLE

Novel Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) Method Development and Validation of Atorvastatin and Fenofibrate in Tablets

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ABSTRACT

A novel, simple, selective, precise, and accurate reverse-phase high-performance liquid chromatography (RP-HPLC) gradient method was developed for the simultaneous estimation of atorvastatin and fenofibrate in the combined formulation. The drugs atorvastatin calcium and fenofibrate were separated in the presence of their impurities atorvastatin related compound H, fenofibrate related compound A, and fenofibrate related compound B. The drugs and related compounds were separated on Kromasil C18 (250 x 4.6, 5µ) with reverse-phase gradient elution. Water adjusted pH 4.0 with phosphoric acid used as a buffer in pump A and acetonitrile used as a solvent in pump B as a mobile phase with gradient elution. The flow rate was 2.0 mL/min. 254 nm was the detection wavelength. The retention times were about 4.6 minutes for fenofibrate related compound A, 5.2 minutes for atorvastatin calcium, 5.7 minutes for fenofibrate related compound B, 8.7 minutes for atorvastatin related compound H, and 17.6 minutes for fenofibrate. The linearity ranges for atorvastatin calcium and fenofibrate were 5.00 to 15.00 and 80 to 240 mcg/mL, respectively, with correlation coefficient 0.999 for both. The proposed method validated statistically with respect to system suitability, specificity, linearity, precision, accuracy, range, robustness, and ruggedness. The method was accurate, linear, precise, specific, selective, and rapid suitable for the quantitative estimation of atorvastatin and fenofibrate in tablets.

Keywords: Atorvastatin, Fenofibrate, Method development, Related compound, RP-HPLC, Tablets.

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INTRODUCTION

Atorvastatin calcium chemically (3R,5R)-7-[3-(phenyl-carbamoyl)-5-(4-fluorophenyl)-2-iso-propyl-4-phenyl-1H-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid, calcium salt, monohydrochloride, fenofibrate chemically isopropyl 2-[p-(p-chlorobenzoyl)phenoxy]-2-methylpropanoate. Atorvastatin calcium and fenofibrate are official in Indian,[1] United States,[2] and European Pharmacopoeia.[3] But the combination of atorvastatin calcium and fenofibrate is not official in any one of the pharmacopeias. The tablet combination dosage form in the ratio of 10:160 mg atorvastatin calcium equivalent to atorvastatin and fenofibrate, respectively. The average weight of the tablet is about 312 mg. Many formulations are available with many trade names. Many methods have been reported for this combination by high performance liquid chromatography (HPLC),[4-10] and the combination with other drugs,[11-14] liquid chromatography (LC) method for the determination of atorvastatin, its related impurities, and its degradation products,[15] from literature reveals no related compounds were separated to estimate the combination of atorvastatin and fenofibrate. Moreover, as per USP, atorvastatin calcium tablets monograph atorvastatin calcium and atorvastatin related compound H used as a system suitability solution preparation and for fenofibrate tablets monograph fenofibrate related compound A and fenofibrate related compound B used as a system suitability preparation for their assay estimation.[2] So in this present proposed method, the atorvastatin and fenofibrate simultaneously estimate in tablet formulation by separating its related compounds atorvastatin related compound H, fenofibrate related compound A and B. The proposed method validated as per ICH guidelines.[16]

MATERIAL AND METHODS

Instrumentation

The separation was carried out on the Shimadzu HPLC system with quaternary gradient and UV and 20 MP PDA detectors,

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LC solutions software, and Kromasil C18 (250 x 4.6mm, 5 µm) column.

Chemicals and Reagents

The working standards of atorvastatin calcium and fenofibrate were provided as gift samples from Bio-Leo Analytical Labs, Hyderabad. The marketed formulation was purchased from the local market. Related compounds of atorvastatin and fenofibrate were provided as gift samples from JS Labs, Hyderabad. Ortho-phosphoric acid, water, and acetonitrile of HPLC grade from Rankem.

HPLC Conditions

The mobile phase consisting of a buffer, solution A, and acetonitrile, solution B was filtered through 0.45 µm PVDF membrane filter separately before use, degassed pumped into the column at a flow rate of 2.0 mL/min. The LC gradient program was set as (time in minutes/% B) = 0/55, 10/55, 13/80, 20/80, 25/55, and 30/55. The column maintained at 25°C temperature. The detection was monitored at 254 nm, and the run time was 30 minutes. The injection volume was 20 µL. The column was equilibrated for about 30 minutes prior to the injection of the drug solutions with the mobile phase initial % ratio.

Preparation of Buffer Solution A

Adjust the water to a pH 4.0 with dilute ortho-phosphoric acid.

Preparation of Solution B

The HPLC gradient grade acetonitrile.

Preparation of Diluent

The acetonitrile and water in the ratio 70:30.

Preparation of System Suitability Solution

Accurately transferred 10 mg each of atorvastatin calcium, fenofibrate related compound A and fenofibrate related compound B, and 20 mg each of fenofibrate and atorvastatin related compound H into 100 mL volumetric flask dissolve and diluted to volume with diluent. Further diluted to 10 to 100 mL with diluents.

Preparation of Standard Stock Solutions

Atorvastatin calcium: Weighed accurately 10 mg transferred into 100 mL of volumetric flask dissolve and diluted to volume with diluent and sonicate for 10 minutes.

Fenofibrate: Weighed accurately 160 mg transferred into 100 mL of volumetric flask dissolve and diluted to volume with diluent and sonicate for 10 minutes.

Preparation of Standard Solution

Accurately transferred 10 mL each of atorvastatin calcium and fenofibrate standard stock solutions into 100 mL volumetric flask dissolve and diluted to volume with diluent.

Preparation of Sample Solution

Accurately transferred sample powder not less than 10 tablets, equivalent to 10 mg of atorvastatin and 160 mg of fenofibrate into 100 mL of volumetric flask dissolve and dilute with diluent and sonicate for 10 minutes, passed through 0.45 µ PVDF membrane filter. Further diluted 10 to 100 mL with diluent.

EXPERIMENTAL

System Suitability Studies

System suitability testing is an integral part of the analytical procedure, the parameters like resolution, tailing factor, theoretical plates, and area % RSD determined for standard solution and system suitability solution. The system suitability solution chromatogram shown in Figure 1. The standard solution chromatogram is shown in Figure 2. The results tabulated in Table 1.

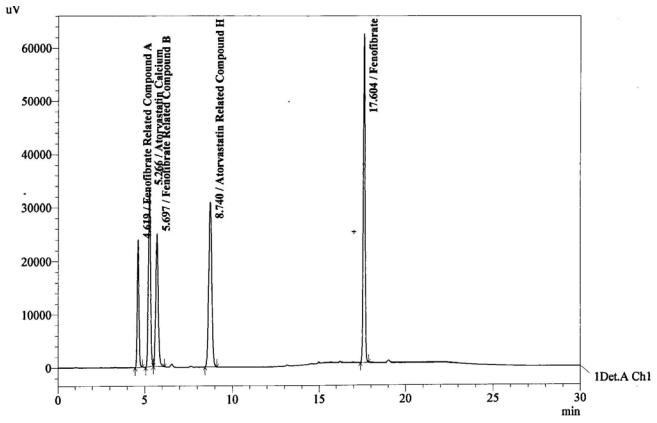

Figure 1: System suitability chromatogram

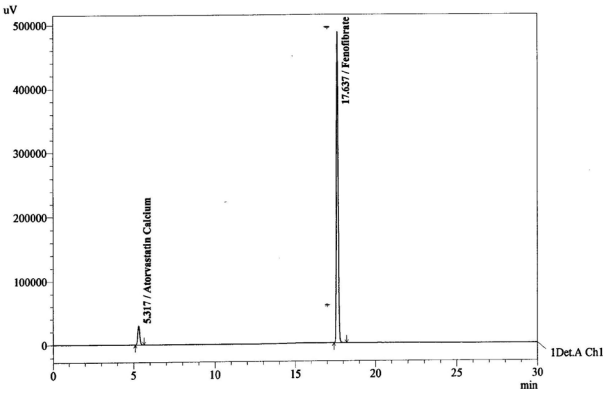

Figure 2: Standard chromatogram

Table 1: System suitability results

Parameters	*Fenofibrate related compound A*	*Atorvastatin calcium*	*Fenofibrate related compound B*	*Atorvastatin related compound H*	*Fenofibrate*	*Limit*
Retention time (min)	4.619	5.266	5.697	8.740	17.604	–
Resolution (R)	–	3.091	1.744	10.363	33.746	R > 1.5
Tailing factor (T)	1.17	1.12	1.17	1.09	1.10	T < 2
Theoretical plates (N)	10,053	8,104	7,644	11,451	125,691	N > 2000
Area % RSD	–	0.21	0.14	–	–	< 2.0 for n ≥ 5

Specificity

Specificity is the ability to assess the analyte unequivocally in the presence of components, which may be expected to be present and to provide an exact content or potency of the analyte in the sample. The test solution monitored on PDA is shown in Figure 3.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which, is accepted either as a conventional true value, or an accepted reference value, and the value found. The method accuracy was determined based on recovery experiments. The recovery studies were carried out at three concentration levels with triplicate preparations. The percentage recovery, mean recovery, and standard deviation of the percentage recovery were calculated and tabulated in Tables 2 and 3.

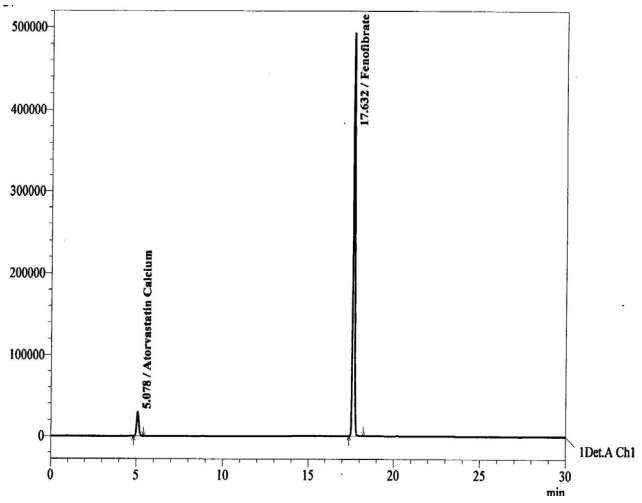

Figure 3: Test chromatogram

Table 2: Atorvastatin accuracy results

Spiked level	*Sample amount added (mcg/mL)*	*Sample amount recovered (mcg/mL)*	*% recovery*	*Mean % recovery*	*% RSD*
50%	4.9038	4.8855	99.63	99.16	0.37
	4.9455	4.8781	98.64		
	4.9423	4.8887	98.92		
	4.9295	4.8827	99.05		
	4.9167	4.8802	99.26		
100%	4.9135	4.8878	99.48	99.90	0.54
	9.9744	10.0103	100.36		
	9.9872	9.9912	100.04		
	10.0096	9.9395	99.30		
150%	14.9038	14.7236	98.79	98.98	0.15
	14.8782	14.7222	98.95		
	14.8590	14.7234	99.09		
	14.8237	14.7055	99.20		
	14.8205	14.6679	98.97		
	14.8045	14.6366	98.87		

Table 3: Fenofibrate accuracy results

Spiked level	*Sample amount added (mcg/mL)*	*Sample amount recovered (mcg/mL)*	*% recovery*	*Mean % recovery*	*% RSD*
50%	78.4615	78.5839	100.16	99.66	0.39
	79.1282	78.4429	99.13		
	79.0769	78.5806	99.37		
	78.8718	78.5225	99.56		
	78.6667	78.4765	99.76		
100%	78.6154	78.6240	100.01	98.66	0.41
	159.5897	157.8210	98.89		
	159.7949	158.0486	98.91		
	160.1538	157.2577	98.19		
150%	238.4615	234.1129	98.18	98.63	0.30
	238.0513	234.2281	98.39		
	237.7436	234.5193	98.64		
	237.1795	234.6043	98.91		
	237.1282	234.5060	98.89		
	236.8718	233.9187	98.75		

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The % assay calculated for six different preparations of the sample and tabulated in Table 4.

Linearity Range

The analytical procedure linearity is its ability (within a given range) to obtain test results. The results are directly proportional to the concentration (amount) of an analyte in the sample. The interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) has been demonstrated that the range of analytical procedure which has a suitable level of precision, accuracy, and linearity.

Linearity was determined triplicate at each level by plotting peak areas for atorvastatin calcium and fenofibrate against the concentration of solutions. The linearity of atorvastatin calcium and fenofibrate determined in the concentration ranges of 5.00 to 15.00 and 80 to 240 mcg/mL, respectively. The atorvastatin calcium regression equation is y = 25,531x – 5,677, with a coefficient of correlation (R2) of 0.999. The fenofibrate regression equation is y = 22,095x – 44,339, with a coefficient of correlation (R2) of 0.999 and shown in Figures 4 and 5. The range of the analytical method determined for atorvastatin calcium and fenofibrate tabulated in Table 5.

Robustness and Ruggedness

The analytical procedure robustness is small, but deliberate variations in method parameters measurement. The capacity of the method unaffected and provides as indication of its realibility, when compared to normal usage.

Robustness and ruggedness were determined by a change in column oven ± 5°C, buffer pH ± 0.1%, and different batch of the column with a different analyst.

RESULTS AND DISCUSSION

System suitability results were given by Table 1, and system suitability parameters are retention time, resolution, tailing, resolution, and plate count were within the acceptance limits. So the system is suitable for the analysis. The test chromatogram extracted on PDA detector the peak atorvastatin calcium and fenofibrate purities found 1, respectively, indicates no interference, so the method is specific. Atorvastatin recovery was found 98.64 to 100.36%, and % RSD was 0.15 to 0.37, fenofibrate recovery was found 98.18 to 100.16%, and % RSD was 0.30 to 0.41 indicates the method is accurate 50 to 150% of the target concentration. Six different % assay preparation values of the same homogeneous samples found 99.30 to

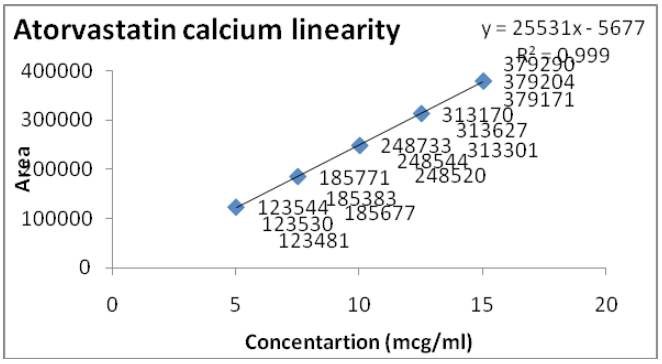

Figure 4: Atorvastatin calcium calibration curve

Table 4: Precision results

S. No.	*% assay (atorvastatin calcium equivalent to atorvastatin)*	*% assay (fenofibrate)*
Preparation-1	100.36	98.89
Preparation-2	100.04	98.91
Preparation-3	99.30	98.19
Preparation-4	99.86	98.51
Preparation-5	100.07	98.64
Preparation-6	100.23	98.72
Average assay	99.98	98.64
Std. dev	0.37	0.27
% RSD	0.37	0.27

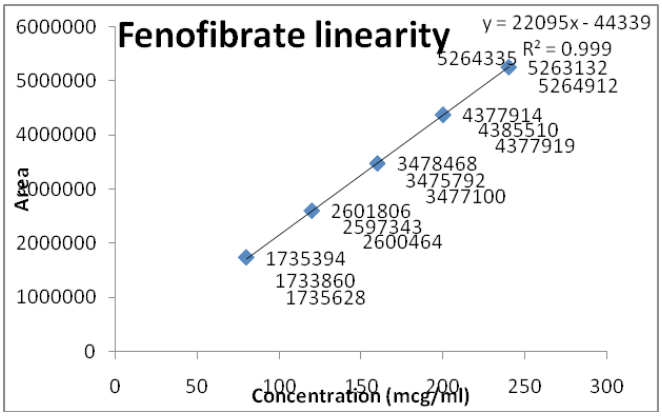

Figure 5: Fenofibrate calibration curve

Table 5: Range results

Parameter	*Unit*	*Atorvastatin calcium*	*Fenofibrate*
Range	mcg/mL	5.00–15.00	80.00–240.00
Linearity	Correlation coefficient (r^2)	0.999	0.999
Accuracy at 50%	% recovery	99.16	99.98
Accuracy at 150%	% recovery	98.98	98.63
Precision at 50%	% RSD	0.37	0.39
Precision at 150%	% RSD	0.15	0.30

100.36% for atorvastatin and 98.19 to 98.91% for fenofibrate indicates the method is precise. Atorvastatin calcium linear correlation was found to be y = 25,531x – 5,677, the correlation coefficient was 0.999, fenofibrate was y = 22,095x – 44,339, and the correlation coefficient was 0.999 indicates the method is linear across the target concentration. The method unaffected due to deliberate changes in flow rate, column oven temperature, and different batch column with different analyst proves the method is robust and rugged. The limit of detection (LoD) and limit of quantitation (LoQ) values were found to be 0.2 and 0.6 mcg/mL for atorvastatin calcium, and 0.3 and 0.9 mcg/mL for dutasteride, respectively. The proposed range 50 to 150% of the target concentration, i.e., 20 to 60 mcg/mL for atorvastatin calcium and 80 to 240 mcg/mL for fenofibrate found linear, accurate, and precise (Table 5).

CONCLUSION

A new, specific, selective, linear, accurate, and precise gradient RP-HPLC method was developed for the estimation of atorvastatin calcium and fenofibrate in their pharmaceutical tablet formulation. The proposed method was successfully separated atorvastatin calcium, fenofibrate, and their related compounds. The proposed method is specific, selective, and stability-indicating power. Hence, the developed method could be adapted to regular quality control analysis and stability analysis.

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