European Journal of Applied Sciences 3 (2): 35-39, 2011
ISSN 2079-2077

Analytical Method Development and Validation of RP-HPLC for Estimation of Atorvastatin Calcium and Fenofibrate in Bulk Drug and Tablet Dosage Forms

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Abstract: A reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of Atorvastatin calcium and Fenofibrate in tablet formulation. The separation was achieved by Gemini C18, 250x4.6mm column and methanol: acetate buffer pH 3.7 (82:18 v/v) as mobile phase, at a flow rate of 1.0 ml/min. Detection was carried out at 248 nm. Retention time of Atorvastatin calcium and Fenofibrate was found to be 3.50 and 7.90 min, respectively. The method has been validated for linearity, accuracy and precision. Linearity for Atorvastatin calcium and Fenofibrate were in the range of 1-5 μg/ml and 16-80 μg/ml, respectively. The mean recoveries obtained for Atorvastatin calcium and Fenofibrate were found to be 101.76% and 100.06%, respectively. Developed method was found to be accurate, precise, selective and rapid for simultaneous estimation of Atorvastatin calcium and Fenofibrate in tablets.

Key words: Atorvastatin · Fenofibrate · Recovery · HPLC

INTRODUCTION

Atorvastatin calcium is chemically [R-(R, R*)]-2-(4-flurophenyl)-β, δ-dihydroxy-5(1-methylethyl)-3-phenyl-4-[phenyl amino) carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate is a synthetic HMG-CoA reductase inhibitor [1]. Fenofibrate is chemically propan-2-yl 2-{4-[(4-chlorophenyl)-carbonyl] phenoxy}-2-methylpropanoate. It has been demonstrated to be efficacious for the treatment of hypercholesterolemia and mixed dyslipidemia [2]. The typical dose of Atorvastatin calcium is 10-80 mg per day and introduces 40-60% LDL [3]. The present study was to develop a RP-HPLC method for estimation of Atorvastatin and Fenofibrate. Literature survey revealed that various analytical methods such as HPLC [4-6], LC [7] LC-MS [8], LC-MS-MS [9] and UV [10] have been reported for estimation of Atorvastatin calcium and Fenofibrate from its formulations and biological fluids. From the literature survey either in individual or combination several solvents system were tried to obtain good optimum resolution.

MATERIALS AND METHODS

A High pressure liquid chromatograph (Agilent 1200 Series) with quaternary pumps, variable wavelength programmable UV-Visible detector SPD-10 A VP, SCL-10A VP system controller and C-18 column was used. The HPLC system was equipped with the software Ezchrome (Agilent). Spectral runs were made on a Shimadzu UV-Visible spectrophotometer, model-1700 was employed with spectral bandwidth of 1 nm and wavelength accuracy of ± 0.3 nm with automatic wavelength corrections with a pair of 10 mm quartz cells.

Reagents and Solutions: All the reagents used in this assay were of analytical reagent grade and HPLC grade methanol was used. Distilled water filtered through 0.45 μm filter (Millipore) was used to prepare solutions. Mobile phase A consisted of methanol. Mobile phase B was buffer (Acetate buffer, pH 3.7, 4.1g of sodium acetate in 1 lit of water adjust the ph to 3.7 with glacial acetic acid and filter through 0.45 micron filter.) The mobile phase used was prepared by mixing mobile phase A and mobile phase

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B in the ratio, 82:18. Methanol was used as a diluent for the sample preparations. Atorvastatin and Fenofibrate pure drugs were obtained as a gift sample from msn labs, Hyd. Tablets of ATOREM F (Brand name) Atorvastatin (10mg) and Fenofibrate (160 mg) were purchased from local market for analysis.

Determination of λ Max: The standard solutions of 10µg/ml of Atorvastatin calcium and Fenofibrate were individually scanned in the range of 200-400nm and the λ max was determined. The overlain spectrum of both the drugs is also run.

Preparation of Calibration Curve: For each drug appropriate aliquots were pipetted out from standard solution into the series of 10 ml volumetric flask and the volume was made up to the mark with methanol to get concentrations of 8-24 µg/ml (n= 5) of Atorvastatin and 2-16 µg/ml (n=8) of Fenofibrate. Solutions of different concentrations for each drug were scanned at there respective wavelengths and absorbances are recorded. The calculation was done by simultaneous equation method.

Preparation of Stock Solution: Accurately 10 mg of Atorvastatin was weighed and transferred into 50 ml volumetric flask and 30 ml of methanol was added. It was sonicated for 10 minutes and volume made up to mark with methanol (Solution A). Accurately 40 mg of Fenofibrate was weighed and transferred into 25ml volumetric flask and 15 ml of methanol is added. It was sonicated for 10 minutes and volume made up to mark with methanol (Solution B). All solutions were freshly prepared prior to analysis. This stock solution is used for making dilutions for calibration curve.

Preparation of Standard Solution: By using pipette, 5ml of solution A and 10ml of solution B was taken and transferred into 20ml dry volumetric flask and volume was made up to mark with methanol (Mixed Standard).

Preparation of Sample Solution: Twenty Atorvastatin-Fenofibrate tablets (10mg Atorvastatin and 160 mg Fenofibrate-180mg total weight or per tablet were weighed and powdered. Weighed the tablet powder equivalent to average weight of the tablet and transferred into 200ml volumetric flask and 50ml methanol was added and sonicated for 20 min and diluted to volume with mobile phase. The solution was filtered through 0.45µm filter. First 5ml of the filtrate was discarded and the remaining solution was filtered.

Procedure: Separately injected 5 replicates of about 10µl of the standard preparation and two replicates of the sample solution, the chromatograms was recorded for 15min and the peak areas were measured and the % amount of Atorvastatin and Fenofibrate was calculated with respect to the individual average of standard area.

Calculation:

$$\% \text{ of amount present in tablet} = \frac{A \times C \times E \times P \times G}{B \times D \times L.A}$$

Where
A: sample area
B: standard area
C: dilution factor of standard
D: dilution factor of sample
E: weight of standard
P: potency of standard
G: a conv factor
L.A: labeled amount

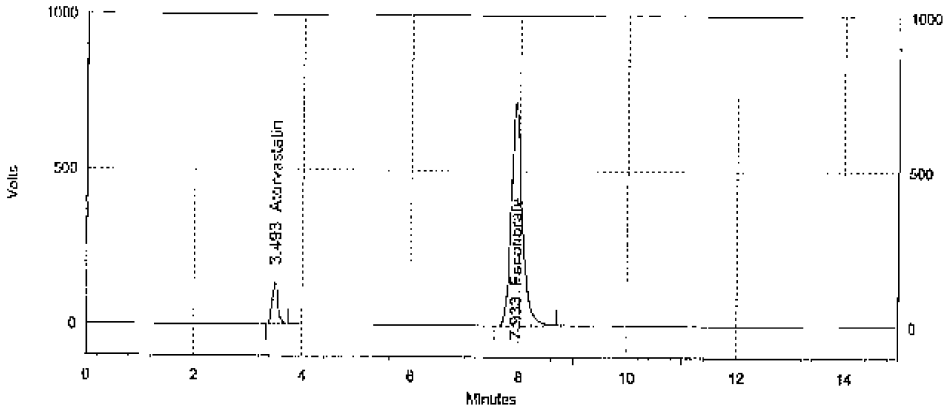

Fig. 1: Chromatogram of standard

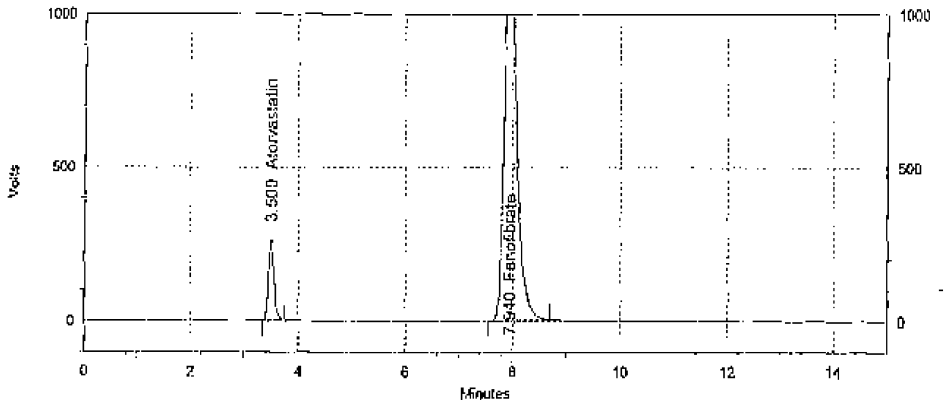

Fig. 1: Chromatogram of sample

RESULT AND DISCUSSION

Development of Optimum Mobile Phase: The present investigation was aimed to develop a simple, precise and accurate HPLC method to estimate Atorvastatin and Fenofibrate in tablets by using RP-HPLC C_{18} column (Gemini). Initially different mobile phase compositions were tried and finally the mobile phase was optimized with methanol and acetate buffer pH 3.7 in proportions of 82:18 v/v. With the above mobile phase a good resolution between Atorvastatin and Fenofibrate was achieved with a reasonably short runtime of 10 min. UV detection was carried out at 248 nm as Atorvastatin and Fenofibrate both showed good absorbance at this wavelength.

System Suitability Parameters: The retention time of Atorvastatin and Fenofibrate was found to be 3.50 and 7.90 min, respectively (Tab 1). The capacity factor (k`) of Atorvastatin and Fenofibrate was found to be 0.67 and 4.07respectively (Tab1). The peak shapes of both the drugs were symmetrical and the asymmetry factor was lesser than 2.0. The proposed method was validated as per the standard analytical procedures. Each of the samples was repeated 6 times and the same retention time was observed in all the cases. System suitability parameters of Atorvastatin and Fenofibrate are given in the Table 1.

Validation of the Method

Precision: Precision of proposed HPLC method was found to be 0.0345 (RSD) for Atorvastatin and 0.0079 (RSD) for Fenofibrate that indicates good precision of the samples analyzed. Linearity experiments were performed by giving six replicate for both the components and the response was found to be linear in the range of 1-5 mg/ml for Atorvastatin and 5-80 mg/ml for Fenofibrate. Linearity of Atorvastatin and Fenofibrate was plotted by a graph of response factor versus concentration. The correlation coefficient 'r' values (n=6) for both Atorvastatin and Fenofibrate were = 0.999.

Recovery Studies: Accuracy of the method was calculated by recovery studies (n=3) at three levels. Standard drug solutions containing drugs in the range 1-5 mg/ml for Atorvastatin and 16-80 mg/ml for Fenofibrate of concentration was added to previous analyzed test solution. Amount of drug recovered at each level (n=3) was determined. Percent recovery at each level was calculated. Table 2 shows data from the recovery study for Atorvastatin and Fenofibrate were 100.76 and 100.06 %, respectively. High percentage recovery showed that the method was free from interference of excipients used in formulations.

Table 1: System suitability parameters

PARAMETER	ATORVASTATIN	FENOFIBRATE
Tailing factor	1.23	1.08
No of theoretical plate	3161	7772
Capacity factor	0.67	4.07
Resolution factor	6.60	14.54
Capacity range	1-50µg/ml	16-80 µg/ml

Table 2: Recovery studies for Atorvastatin and Fenofibrate

Drug	Tablet amount	Amount added	Amount recovered	Recovery	Coefficient of variation
	1	1	1.04	103.8	
	1	2	2.01	100	
AC	1	3	3.1	102.3	0.017
	1	4	4.1	101.5	
	1	5	5.1	101.2	
	16	16	16.05	100.25	
FB	16	32	32.3	100.65	
	16	48	47.96	99.9	0.038
	16	64	63.82	99.7	
	16	80	79.9	99.8	

Chromatogram of standard: (Fig 1)

Chromatogram of sample: (Fig 2)

The method was simple and had short run time of 10 min, which make the method rapid. The results of the study indicate that the proposed HPLC method was simple, precise, highly accurate, specific and less time consuming. The developed method was validated based on ICH guidelines [11, 12].

CONCLUSION

The present work describes simple, economical and non interfering spectrophotometric method for estimation of Atorvastatin and Fenofibrate by using simultaneous equation method. The method was found to be economic, simple, precise, accurate and reproducible during analysis of drug formulations containing the two drugs.

ACKNOWLEDGEMENT

Authors thankful to Research lab, GIET School of Pharmacy, Rajahmundry, Andhra Pradesh, India, for their all kind of support to complete our research.

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