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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ATORVASTATIN AND FENOFIBRATE IN BULK AND FORMULATION

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ABSTRACT

The present work describes development and validation of a rapid and sensitive RP-HPLC method for simultaneous estimation of Atorvastatin Calcium and Fenofibrate in bulk and formulation. The separation was achieved using Methanol-Acetonitrile: Phosphate Buffer pH 5.0 (45:25:30 V/V) as mobile phase. Mean recovery was found to be 99.42% for Atorvastatin calcium and 99.86% for Fenofibrate. Limit of Detection and Limit of Quantitation was calculated from calibration curve. The LOD for AT and FB were found to be 0.20 µg/ml and 0.25 µg/ml, respectively, while LOQ were 0.50 µg/ml and 0.45 µg/ml, respectively. The method was validated as per ICH guidelines.

Keywords: Atorvastatin calcium, Validation, Fenofibrate

INTRODUCTION

Atorvastatin calcium **[1-4]** (AT) is (β R, δ R)-2-(4-fluorophenyl)-β,δ-dihydroxy-5-(1-methyl ethyl)-3-phenyl-4-((phenyl amino)carbonyl)-1H-pyrrole-1-hepatonoic acid, a HMG CoA reductase inhibitor. Fenofibrate **[3-7]** (FB) is 2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropanoic acid, 1-methylethyl ester, it is a lipid lowering agent. Literature survey reveals that, few HPLC and HPTLC methods have been reported for simultaneous estimation of ATR and FB as well as in combination with other drugs. N. Jain, *et al*. **[6]** has reported HPLC method for simultaneous estimation of ATR and FB using Methanol-Acetonitrile: Phosphate Buffer pH 5.0 (45:25:30 V/V) as eluting solvent. The methods reported by Jain and

Kadav **[7]** were excluding the internal standard, which was found to be the limitation of the method. Literature survey also revealed number of spectroscopic and RP-HPLC method development for both drugs in single component as well as in combination with other drugs **[5-22]**.

The present study illustrates development of a HPLC method for simultaneous estimation of Atorvastatin Calcium and Fenofibrate in bulk and Formulation.

MATERIAL AND METHOD

HPLC system consist of Cyberlab-chrom-HPLC V 4.0) with LCP-100 pump, variable wavelength programmable UV/Vis detector LC-UV 100 and operating software cyberstore version no 4-0512-039

C18 DDS5 column (4.6 mm LD x 250 mm i.d. particle size 5 µm) was used for method development.

HPLC grade acetonitrile for chromatography was procured from Merck Chemical Division Ltd., Mumbai. All other chemicals were of analytical reagent grade quality. Doubly distilled water was used to prepare mobile phase solutions.

Preparation of Stock Solutions

Standard stock solutions of Atorvastatin calcium and Fenofibrate were prepared separately by transferring accurately weighed quantity of 100 mg both drugs to 100ml volumetric flask, powder was dissolved in sufficient quantity of mobile phase and volume was made up to the mark to get the conc. of 1000µg/ml. The solution was further diluted with mobile phase to get the suitable concentration range.

Preparation of Sample Solution

To determine the content of the Atorvastatin calcium and Fenofibrate in pharmaceutical formulation, twenty tablets were weighed accurately, they were finely powdered and powder equivalent to 10 mg of Atorvastatin calcium and 10 mg of Fenofibrate was weighed accurately and transferred to a 100 ml volumetric flask containing 25 ml of mobile phase, the solutions were sonicated for 20 min. and diluted up to the mark with mobile phase. The resulting solution was filtered through Whatmann filter paper No.41. Filtrate obtained was used as sample stock solution. This solution was further diluted to desired concentration range with mobile phase.

Chromatographic conditions: Different mobile phases were tested in order to find the best conditions for determination of drugs. A reverse phase C18 column equilibrated with mobile phase of Methanol: Acetonitrile: Phosphate Buffer pH 5.0 (45:25:30 V/V) at a flow rate of 1ml per min. was selected as optimized parameters after several experimental runs. Quantitation was achieved with UV detection at 248 nm. The sample run time

was 10 min. All determinations were carried out at room temperature.

Calibration curve

Appropriate aliquots of stock solution were taken in different 10 ml volumetric flasks and diluted up to the mark with mobile phase to obtain final concentrations of 5-40mcg/ml of Atorvastatin calcium and Fenofibrate respectively and chromatograms were recorded. Calibration curve was constructed by plotting average peak area versus concentrations.

Method Validation

The method was validated as per the ICH and USP **[23-24]** guidelines for the parameters like accuracy, linearity, precision, detection limit, quantitation limit and robustness. The accuracy of the method was determined by calculating percentage recovery of AT and FB. For both the drugs, recovery studies were carried out by applying the method to preanalysed drug formulation to which known amount of AT and FB corresponding to 80, 100 and 120% had been spiked. At each level of the amount six determinations were performed.

RESULTS AND DISCUSSION

The optimized mobile phase consisting of Methanol: Acetonitrile: Phosphate Buffer pH 5.0 (45:25:30) adjusted with o-phosphoric acid, at 1ml/min flow rate which gave two well-resolved peaks with minimum tailing factor for AT and FB **(Figure 1)**. The retention times for AT and FB were 3.22 min and 8.02 min, respectively.

Linearity

The calibration curve for AT and FB was found to be linear over the range of 5-35 µg/ml and 5-40 µg/ ml, respectively. Calibration curve shows good linearity with the correlation coefficient of 0.9947 and 0.9967 for Atorvastatin Calcium and Fenofibrate respectively.

The proposed method was successfully applied to the determination of Atorvastatin and fenofibrate in their combined solid dosage form. The results for the combination were comparable with the corresponding labelled amounts **(Table 1)**.

Limit of Detection and Limit of Quantitation was calculated from calibration curve. The LOD for AT and FB were found to be 0.20 µg/ml and 0.25 µg/ml, respectively, while LOQ were 0.50 µg/ml and 0.45 µg/ml, respectively.

The results of system suitability test parameters are summarized in **Table 3**.

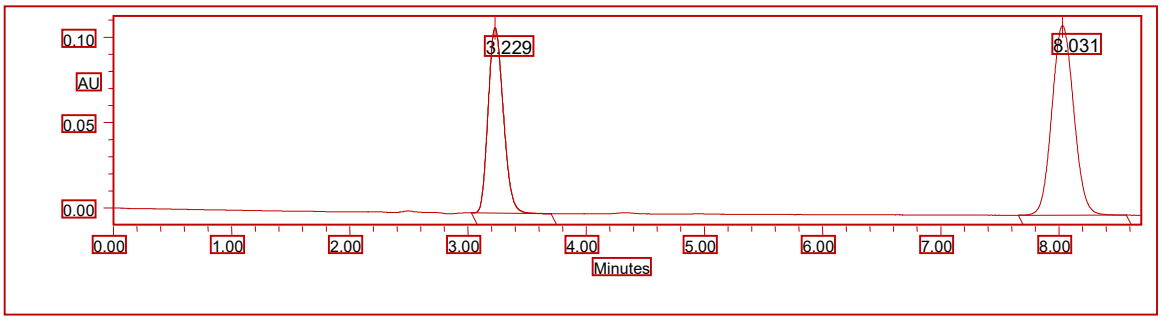

Figure 1: Chromatogram of standard AT and FB

Table 1: Results of formulation Analysis for Atorvastatin and Fenofibrate

Sr. No.	Amount present in (µg mL^{-1})		Total amount recovered (µg mL^{-1})		% Label claim	
	AT	FB	AT	FB	AT	FB
1	10	10	9.67	9.65	99.17	99.6
2	10	10	9.52	10.12	98.80	100.12
3	10	10	10.12	9.83	100.3	99.83
Mean			9.77	9.866	99.4233	99.8666
SD			0.3122	0.2371	0.7814	0.2371
% RSD			0.7851	0.2374	0.7859	0.2374

Accuracy:

Tablet sample -							
Amount of sample drug taken (µg mL^{-1})		Amount of standard drug added (µg mL^{-1})		Total amount recovered (µg mL^{-1})		% Recovery	
AT	FB	AT	FB	AT	FB	AT	FB
5	5	4.0	4.0	9.82	9.94	98.20	99.40
5	5	5.0	5.0	10.08	10.13	100.8	101.3
5	5	6.0	6.0	11.08	11.11	100.72	101.00
Mean						99.90	100.56
SD						0.1276	0.4483
% RSD						0.1288	0.4471

Table 2: Intra-day and Inter-days precision of Atorvastatin and Fenofibrate

Precision:

Theoretical concentration (µg mL^{-1})	Atorvastatin				Fenofibrate			
	Intra-day measured concentration		Inter -days measured concentration		Intra -day measured concentration		Inter -days measured concentration	
	Mean	RSD %	Mean	RSD %	Mean	RSD %	Mean	RSD %
10	9.91	1.57	9.78	0.95	10.05	0.389	10.15	0.389
10	9.94	0.39	9.89	0.05	10.08	1.12	10.75	1.57

Table 3: System suitability test parameters

Parameter	AT	FB
Retention Time (min) ± %RSD	3.22±0.10	8.031±0.20
Tailing Factor ± %RSD	1.19±0.18	1.07±0.14
Theoretical Plates ± %RSD	35911±0.88	40240±0.95
Resolution ± %RSD	1.89522	1.65±0.18

CONCLUSION

In this reported study, a RP- HPLC method was developed for the simultaneous determination of Atorvastatin calcium and Fenofibrate and validated as per ICH guidelines. Statistical analysis proved that the method developed was accurate, precise, and repeatable. The developed

method was found to be simple, sensitive and selective for analysis of AT and FB in combination without any interference from the excipients. The method was successfully used for determination of drugs in a pharmaceutical formulation.

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