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METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF IN ATORVASTATIN AND FENOFIBRATE BULK AND PHARMACEUTICAL DOSAGE FORM BY USING RP-HPLC METHOD

Sanjay Kumar[1*], Darsh Gautam[2] and Poonam Talwan[2]

[1]Gautam College of Pharmacy Hamirpur,
Himachal Pradesh, India.
[2]Himachal Institute of Pharmaceutical Education and
Research, Hamirpur, Himachal Pradesh, India.

ABSTRACT

A simple, accurate, economical and reproducible reverse phase high performance liquid chromatographic (RP-HPLC) method was developed and validated for the determination of Atorvastatin and Fenofibrate in bulk and pharmaceutical formulations. The separation was achieved on a Thermo Scientific BDS C18 column (250 × 4.6 mm i.d 5m) using a mixture of 25mMSodium acetate (pH adjusted to 5.0 With 1.0 M Glacial acetic acid): Acetonitraile (10::90 % v/v)as mobile phase in an isocratic elution mode, at a flow rate of 1 ml/min. The detection was monitored at 254 nm. The retention time of Atorvastatin and Fenofibrate was found to be around 2.672±0.05 min (Atorvastatin) 4.971±0.07 min (Fenofibrate) respectively. Excellent linearity range was found between 1-5 µg/ml for Atorvastatin and 1-5 µg/ml for Fenofibrate. The method was validated with respect to linearity, robustness, precision and accuracy and was successfully applied for the simultaneous determination of Atorvastatin and Fenofibrate from the combined dosage formulation.

1. INTRODUCTION

Atorvastatin (ATOR) is chemically7[2(4fluorophenyl)3phenyl4(phenylcarbamoyl)-5-(propan-2-yl)-1H-pyrrol-1-yl]-3,5-dihydroxyheptanoate. Atorvastatin is a HMG-COA reductase inhibiter actas anti- hyper lipidemic drug clinically effective drug in the treatment of Hyper cholestrimia . It issoluble in methanol, ethanol, and acetonitrile. Practically insoluble in water. Fenofibrate (FENO),propan2-yl2-{4-[(4-chlorophennyl)carbonyl] phenoxy}-2methyl ropionate is a widely used asAnti-cholesrtimic agent as ppar receptor inhibiter. Atorvastatin and Fenofibrate is available incombined dosage forms as film coated tablets (LIPIKIND). Each tablet contains 10mg of Atorvastatin and 160 mg of Fenofibrate. It is used for the treatment of Hyper Cholestrimia. For this combination derivative spectroscopic methods and reverse phase liquid chromatographic methods are reported. However, there is no work reported on combination of these drugs by standard addition simultaneous equation method. Hence fast, simple, and accurate and validated spectrophotometric method was developed by standard addition of both drugs by applying simultaneous equation method, the developed method was simple, accurate, precise, specific, sensitive and reproducible which can be efficiently and easily applied to pharmaceutical dosage forms.

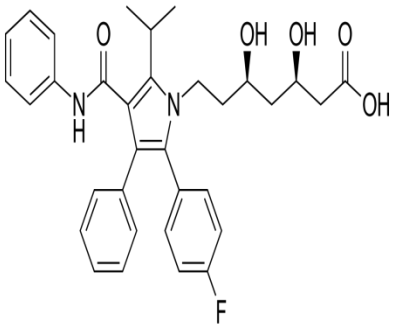

Fig. 1: Structure of Atorvastatin

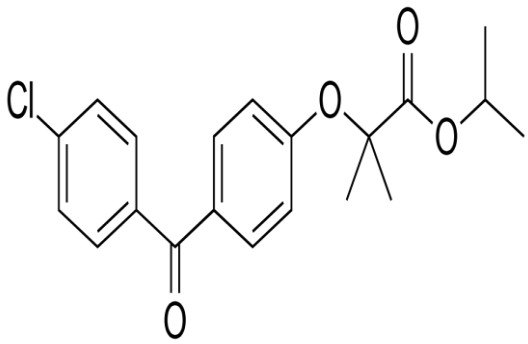

Fig. 2: Structure of Fenofibrate

2. Experimental

2.1. Apparatus

A SHIMADZU (Japan) HPLC instrument (LC-20AD) equipped with a UV-Visible detector, rheodyne injector with 20 µL loop Thermo Scientific BDS C18 column (250 × 4.6 mm i.d 5m) and LC-Solution software were used. Other instruments included are SHIMADZU electronic balance, BL-220H (SHIMADZU corp., Japan), fast clean ultrasonic cleaner and value 1 stage vaccum pump (model: VE115).

CHEMICALS AND REAGENTS

All chemicals obtained from local market, Pure Atrovastatin and Fenofibrate were obtained as gift sample from Pharmadeep Remedies Hyderabad

PHARMACEUTICAL FORMULATION

The tablet dosage form LIPIKIND tablets (claim: 10mg Atorvastatin and 160 mg of Fenofibrate) was procured from local market.

2.2 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 drugs to be detected is to be selected. From the UV spectra obtained for drugs, 254 nm was selected as the wavelength for study. Spectra of standard drugs was shown in fig 3.

2.3 CHROMATOGRAPHIC CONDITIONS

Chromatographic separation was performed on shimadzu HPLC with Thermo Scientific BDS C18 column (250 × 4.6 mm i.d 5m) and constant flow pump. Rheodyne injector with 20 µl loop. The composition of the mobile phase was in the ratio of 25mMSodium acetate (pH adjusted to 5.0 With 1.0 M Glacial acetic acid): Acetonitraile 10:90 % v/v and was delivered at a flow rate of 1.0 ml /min. The mobile phase was filtered through a 0.45 µ membrane filter and sonicated for 5 min. Analysis was performed at ambient temperature. Chromatogram of standard drugs was shown in fig 4. Optimized chromatographic conditions are listed in table 1.

2.5. Preparation of Mobile Phase

The buffer solution of 25 mM of Sodium Acetate was prepared by dissolving 340 mg of dried Sodium Acetate in 100 ml volumetric flask containing 25 ml of HPLC water then shake it until it dissolve. Finally make up the volume with water to 100 ml. It gives 25 mM. Sodium acetate buffer solution P^H 5.0 was adjusted with Glacial Acetic The buffer and acetonitrile were mixed in the ratio of 10:90 v/v.

2.6 Preparation of standard Atorvastatin and Atorvastatin, solutions

Stock solutions were prepared by dissolving 10 mg of Atorvastatin, 10 mg of Fenofibrate in 10 ml of Acetonitrile separately. Aliquots of the standard stock solutions 1ml of Atorvastatin and Fenofibrate were transferred into 25 ml volumetric flasks and solution was made up to the volume to yield required concentrations of Atorvastatin and Fenofibrate., were prepared by suitable dilution of the stock solution with mobile phase

2.7. Preparation of sample solution

Twenty LIPIKIND tablets each containing 10 mg of Atorvastatin and 160 mg of Fenofibrate were weighed, average weight was calculated and powdered. Above weighed powder to add 15 mg of ATOR to made A quantity equivalent to 16mg of Atorvastatin and 16mg of Fenofibrate was weighed and transferred into 100 ml volumetric flask. It is extracted with acetonitrile. The volumetric flask was sonicated for 20 minutes to affect the complete dissolution of the drugs and the solution was made up to the volume with Acetonitrile and filtered.. Suitable aliquots of formulation solution were prepared and injected to HPLC to obtain concentration in the linearity range

2.8 Analysis of formulation

The amount of drug present in the pharmaceutical formulation was calculated through peak area by making use of the standard calibration curve (Concentration in µg/ml on x-axis and peak area on Y-axis) the results were shown in table-2.

3.0 RESULTS AND DISCUSSIONS

Once the HPLC method development was over, the method was validated in terms of parameters like linearity, precision, LOD, LOQ, recovery studies etc,. The proposed HPLC method was validated as per ICH guidelines [19].

3.1. Linearity and Range

Linearity was established by least squares linear regression analysis of the calibration curve. The calibration curves were linear over the concentration range of 1-5 µg/ml for Atorvastatin and 1-5 µg/ml for Fenofibrtate, Fig. 5-6 and table 2-3. Peak areas were plotted versus respective concentrations and linear regression analysis was performed on the resultant curves

3.2. Precision

The precision of the method was ascertained separately from the peak areas obtained by actual determination of three replicates of a fixed amount of drug. The intraday, inter-day and Repeatability variation in the peak areas of the drug solution was calculated in terms of percent RSD and the results are presented in table 4, 5, 6

3.3. Recovery Studies

Recovery studies of the drug were carried out for determining accuracy parameter. It was done by mixing known quantity of standard drugs with the analyzed sample formulation and the contents were reanalyzed by the proposed method. This was carried out at 50 and 100% levels. Results of recovery are shown in Table 7

3.4. Limit of Detection and Limit of Quantification

The limit of Detection (LOD) and limit of Quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. LOD and LOQ were calculated mathematically. The LOD of Atorvastatin and Fenofibrate were found to be 0.155 µg/ml and 0.144 µg/ml respectively.. The. The LOQ of Atorvastatin and Fenofibrate were found to be 0.471µg/ml and 0.438 µg/ml respectively.

3.5 System suitability studies

System suitability parameters like Retention time, number of theoretical plates (N), Tailing factor, resolution (Rs) etc., were studied, and results are given in Table 8

3.6. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness of the method was investigated under a variety of conditions including changes of composition of buffer in the mobile phase and flow rate. % RSD of assay was calculated for each condition. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters has proven that the method is robust.

3.7. Ruggedness

The ruggedness of the method was assessed by comparison of the intra-day and inter-day assay results for Atorvastatin and Fenofibrate that has been performed by two analysts. The % RSD values for assays performed in the same laboratory by two analysts did not exceed 2, indicating the ruggedness of the method.

4. CONCLUSION

The proposed RP-HPLC for the estimation of the Anti-Hyperlipidamic drugs (Atorvastatin and Fenofibrate) in the pharmaceutical dosage form were simple, reliable, sensitive and selective providing satisfactory accuracy and precision with lower limits of detection and quantification. The recoveries achieved were good by both the methods. The methods can be recommended for routine and quality control analysis of these drugs in the pharmaceutical dosage forms.

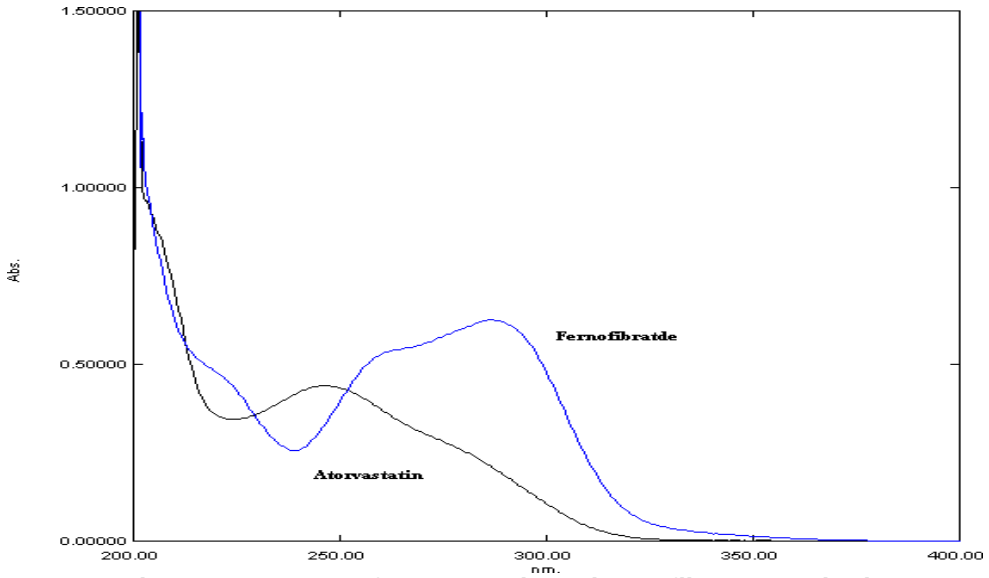

Fig. 3: UV spectra of Atorvastatin and Fenofibrate standards

Table 1: Optimized Chromatographic Conditions

Parameters	Method
Stationary phase (Column)	Thermo Scientific BDS C_{18} column (250 × 4.6 mm i.d 5m)
Mobile phase	25mMSodium acetate (pH adjusted to 5.0 With 1.0 M Glacial acetic acid): Acetonitraile (10:90 % v/v)
Flow rate (ml/min)	1
Pressure (kgf)	40
Run time (min)	10
Column temperature(^{0}c)	Ambient
Detection wavelength (nm)	254
Drugs Retention time (min)	2.672±0.05 min (Atorvastatin) 4.971±0.07 min (Fenofibrate)

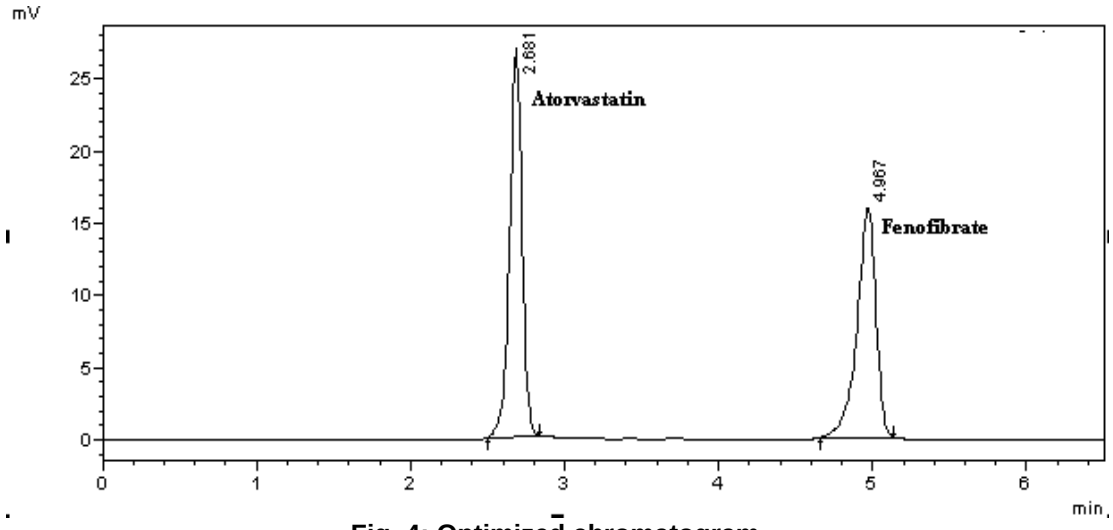

Fig. 4: Optimized chromatogram

Table 2: Linearity Response of Atorvastatin

S.NO	Concentration(µg/ml)	Peak Area
1	0	0
2	1	53767
3	2	99183
4	3	151197
5	4	195908
6	5	241067

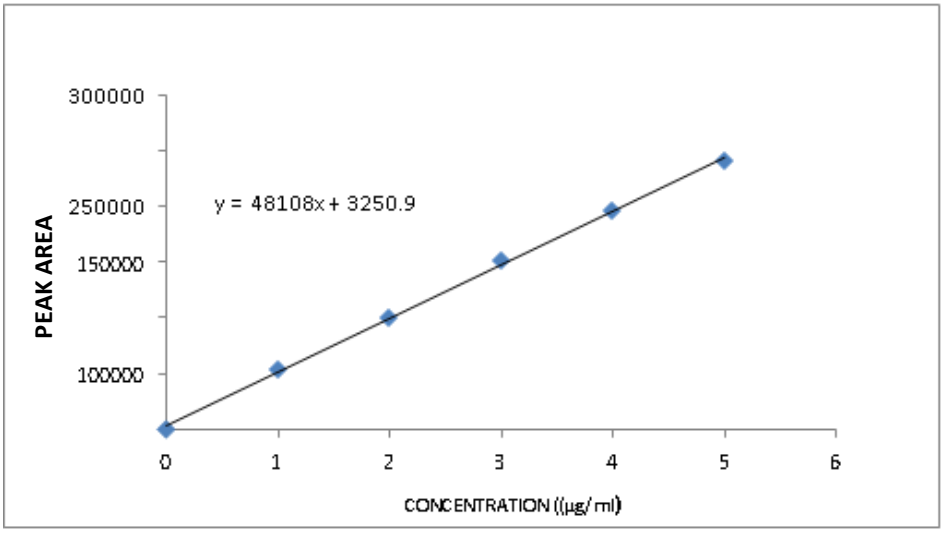

Fig. 5: Calibration Graph of Atorvastatin by RP-HPLC

Table 3: Linearity Response of Fenofibrate

S.NO	Concentration(µg/ml)	Absorbance
1	0	0
2	1	47482
3	2	89494
4	3	135928
5	4	181712
6	5	221940

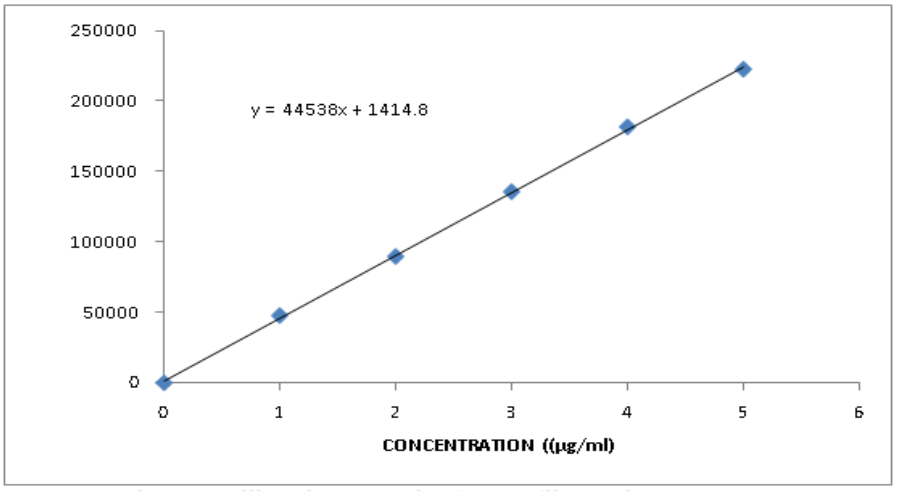

Fig. 6: Calibration Graph of Fenofibrate by RP-HPLC

Table 4: Intraday Precision

S.NO	Concentration ((µg/ml)		Peak area		%RSD	
	ATOR	FENO	ATOR	FENO	ATOR	FENO
1	2	2	99463	89201	0.358	0.773
			99615	89411		
			99539	89306		
2	3	3	151646	137488	0.301	0.281
			152713	136809		
			151179	137153		
3	4	4	193055	180855	0.225	0.028
			195544	181483		
			195908	181169		

ATOR-Atorvastatin- , FENO- Fenofibrate

Table 5: Inter day precision

S.NO	Concentration ((µg/ml)		Peak area		%RSD	
	ATOR	FENO	ATOR	FENO	ATOR	FENO
1	2	2	2435983	1479796	0.490	0.772
			2459959	1457109		
			2446758	1467847		
2	3	3	2767646	1873240	0.548	0.489
			2797618	1879230		
			2787664	1861230		
3	4	4	3157395	2361278	0.394	0.214
			3157395	2351180		
			3132674	2356120		

ATOR-Atorvastatin- , FENO- Fenofibrate

Table 6 : Repeatability of injection

Concentration ((µg/ml)		Injection	Peak area		%RSD	
ATOR	FENO		ATOR	FENO	ATOR	FENO
4	4	1	195908	181712	0.419	0.297
		2	195875	180855		
		3	195544	181483		
		4	194299	181710		
		5	195086	180969		
		6	19486	181169		

ATOR-Atorvastatin- , FENO- Fenofibrate

Table 7: System suitability studies

Drug	Theoretical plates	Asymmetry	Tailing factor
ATOR	4539	1.0	0.91
FENO	7546	0.99	0.82

ATOR-Atorvastatin- , FENO- Fenofibrate

Table 8: Robustness parameters results

Chromatographic conditions	Normal	Variation	Assay (mean)	%RSD	Fig.no
Mobile phase	10:90	15:85	100.1	0.89	6.2.21
		20:80	101.2	0.98	
Flow rate	1.0	1.2	100.5	1.51	6.2.22
		0.8	100.4	1.23	

Table 9: Recovery Studies

Drugs	Amount taken (µg/ml) for both methods	Amount added (µg/ml) for both methods	Total amount found (µg/ ml)	%Recovery	%*RSD
ATOR	4	2	1.988	99.61	0.382
		4	3.993	99.82	0.561
FENO	4	2	2.012	100.60	0.256
		4	4.1	102.50	0.146

* mean of six observations, ATOR- Atorvastatin, FENO- Fenofibrte

Table 10: Analysis of marketed formulation

Drugs	Labelled amount, mcg/ tablet	Amount to be taken , mcg/tablet	% Label claim	% *RSD
ATOR	10	4	99.82	0.095
FENO	160	4	102 .50	0.215

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