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Determination of Atorvastatin and Fenofibrate in A Fixed Dose Combination by High Pressure Liquid Chromatography

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ABSTRACT

An accurate and reproducible liquid chromatographic assay method was developed and validated for the determination of Atorvastatin and Fenofibrate in capsule formulation. Water (pH 4.0) and acetonitrile (20:80, v/v) was used for reverse-phase liquid chromatography at detection wavelength 254 nm to determine the contents of Atorvastatin and Fenofibrate in combination capsule dosage form. The method was validated by determining parameters such as specificity, linearity, LOD and LOQ, precision, accuracy, ruggedness and robustness. The method was found to be specific against placebo interference. Linearity was evaluated over the concentration range of 1.0 to 6.0 µg/mL and 5.0-50.0 µg/mL for both Atorvastatin and Fenofibrate (the correlation of coefficient obtained was 0.999 for both). The intraday and interday precision values of the system and method were determined. The accuracy of the method ranged from 99.99 to 102.24% for Atorvastatin from 100.45 to 101.22% for Fenofibrate. The proposed method was found to be robust when slight but deliberate changes were made in analytical conditions. The developed method will be suitable for the assay of Atorvastatin and Fenofibrate in raw materials, capsule formulation as well in other forms of combined dosage forms.

INTRODUCTION

The combined dosage form of any pharmaceuticals is developed for the synergistic effect or to give longer time effect. In present study Atorvastatin and Fenofibrate combination is used as antihypertensive. Atorvastatin calcium (ATV) is chemically [R-(R*,R*)]-2-(4-flurophenyl)-β, δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt trihydrate. Atorvastatin calcium is an inhibitor of 3-hydroxy-3methyl glutaryl coenzyme A (HMG-Co A) reductase. This enzyme catalyses the conversion of HMG-CoA to mevalonate, an early and rate-limiting step in cholesterol biosynthesis. Fenofibrate (FEB) is chemically Isopropyl 2,4,4 chlorobenzoyl phenoxy 2 methylpropionate. Fenofibrate a fibric acid derivative is a lipid regulating drug with actions on plasma lipids similar to those of bezafibrate. It is used to reduce low density lipoprotein (LDL) cholesterol, total cholesterol, triglycerides, and apolipoprotein B, and to increase high density lipoprotein (HDL) cholesterol, in the management of hyperlipidaemias, including type IIa, type IIb, type III, type IV, and type V hyperlipoproteinaemias.

A literature review reveals that only a few methods have been developed for the quantification of individual drug Atorvastatin and Fenofibrate. The chemical structure is as shown in **Figure 2.**

MATERIALS AND METHODS

Experimental

HPLC Instrumentation

The chromatographic separation was performed on the Waters liquid chromatographic system equipped with (Waters-1515) isocratic solvent delivery system pump with (Waters-2487) dual wavelength absorbance UV-detector and Rheodyne 7725i injector with 50 µL loop volume. Breeze 3.3 data station was applied for data collecting and processing. A phenomenox C_{18} column (25cm x 4.6mm i.d., 5µm particle size) was used for the separation. The mobile phase consisted of a mixture of water (pH 4.0) and acetonitrile in the ratio of (20:80, v/v). The mobile phase was prepared daily, filtered, sonicated before use and delivered at the flow rate of 1.0 mL/min. The detection wavelength fixed at 254 nm an isobestic point for both drugs as shown in **Figure 1**.

Chemicals and reagents

The pharmaceutical grade gift reference and working standards of Atorvastatin calcium (99.77%) procured from HAB Pharmaceutical & Research Ltd (Thane, India) and Fenofibrate (99.56%) from Cadila pharmaceutical Ltd (Ankleshwer, India). The chemical structure of samples will be as Shown in **Figure 2**. Internal standard Diclofenac sodium (99.92%), gift sample was procured from Apex pharmaceuticals (Medak, India). The solvents acetonitrile and methanol used were of HPLC grade; all analytical grade chemicals and solvents were obtained from E Merck (India) Ltd, Mumbai. Ammonium sulphate AR grade were procured from Qualigens fine chemicals, Mumbai. Water HPLC grade was obtained from a Milli-QRO water purification system.

By UV Spectrophotometer

Solvent	--	Methanol and Water	(50: 50 v/v)
λ_{max}	--	Atorvastatin	246 nm
		Fenofibrate	288.8 nm
Overlay	--	Isobestic point	254 nm
Linearity and range	--	Atorvastatin	1.0 - 6.0 µg/ml
		Fenofibrate	5.0 -50 µg/ml

Procedure for UV Calibration

Above mentioned Spectra shown in **Figure 1** and Linearity in **Table no. 1.** The selection of wavelength is carried out and Linearity is confirmed by UV-Spectrophotometry along with IS selected.

Standard solutions and calibration curves by HPLC

Standard stock solutions were prepared at concentration of 1 mg/mL of ATV and FEB separately using a mixture of Water (pH 4.0) and acetonitrile (20:80, v/v). The working standard solutions were prepared of different concentrations ranging from 1.0 to 6.0 µg/mL and 5.0 to 50.0 µg/mL for ATV and FEB respectively, by maintaining the concentration of Diclofenac sodium (IS) at a constant level of 50µg/mL. From the above each mixture 50µL was injected in triplicate for the

estimation of standard drugs, under the optimized chromatographic conditions, a steady baseline was recorded; the typical chromatogram was recorded for standard ATV and FEB with internal standard as shown in **Figure 3**. The retention times of standard and internal standard were found to be 3.58, 5.68 and 8.85 min, respectively. The calibration curve was obtained by simple linear regression of concentration of drug to the response factor as denoted in **Table no. 2.**

Analysis of formulation by HPLC

Twenty tablets each containing 10 mg of Atorvastatin and 200 mg of Fenofibrate were weighed and the amount equivalent to one tablet content was weighed, powdered and dissolved in acetonitrile: water (50:50 v/v) with appropriate amount of IS was added in mixture. The drugs were extracted with same solvent mixture, filtered and further dilutions were made by mobile phase to get a concentration of 2 µg/ml of Atorvastatin, 40µg/ml of Fenofibrate and 50 µg/ml of Diclofenac sodium as internal standard. These solutions were injected for the estimation. The retention times obtained for Atorvastatin, Diclofenac sodium (IS), and Fenofibrate were at 3.56, 4.66 and 8.75 min respectively as in **Figure 4**.

RESULTS AND DISCUSSION

Method development

The aim of present work was to develop simple RP-HPLC with ultraviolet detection for the simultaneous determination of Atorvastatin and Fenofibrate in solid pharmaceutical dosage forms. As the solubility of Atorvastatin and w Fenofibrate as sparingly soluble in water therefore mixture of acetonitrile and water (1:1, v/v) was used as solvent for preparation of all standard and sample solutions.

Chromatographic conditions for separation of analytes chromatographic conditions for Atorvastatin and Fenofibrate

Stationary phase	:	Phenomenex C_{18} (250 x 4.6 mm i.d., 5µ)
Mobile phase	:	Water (pH 4.0): Acetonitrile
Mobile phase ratio	:	20:80 % v/v

Detection wavelength	:	254 nm
Flow rate	:	1 ml / min
Sample size	:	50 µl
Temperature	:	Room temperature
Internal standard (IS)	:	Diclofenac sodium

Several attempts were performed in order to get satisfactory resolution of ATV and FEB in different mobile phases with various ratios of organic phases and buffers by using C_{18} column. Initially the mobile phase used was mixture of water and methanol followed by water and acetonitrile in different ratios. Other mobile phase tried was acetonitrile-ammonium acetate buffer (pH 5.5) in ratio (60:40, v/v) by isocratic elution which could not give satisfactory resolution. Acetonitrile and 30mM ammonium sulphate buffer (pH 4) in ratio of (80:20, v/v) mobile phase was used by isocratic elution to obtain satisfactory and good resolution with internal standard Diclofenic Sodium. The effect of solvent composition by changing the ratio of acetonitrile-ammonium sulphate buffer in ratio of (80:20, v/v) was shown satisfactory resolution. Therefore this method was sensitive to mobile phase ratio. The effect of change in pH of mobile phase by ± 0.2 does not show significant change in retention time of each analyte. The retention time of ATV and FEB with Diclofenic Sodium (IS) on C_{18} column was found satisfactory with above mobile phase at a flow rate of 1.0 mL/min. The resolution of standard and sample solution for ATV and FEB and hydrochlorothiazide found reproducible and satisfactory.

Selection of UV wavelength and internal standard

The detector wavelength of the present study was selected on the basis of higher sensitivity. The internal standard was selected due to its suitable retention time, recovery and lack of interference with endogenous peaks and also not much affected by the mobile phase pH. These phenomena helped their good separation with other peaks.

Method validation

Linearity and range

The linearity and range HPLC method was determined at six concentration levels for ATV and FEB. The linearity and range of ATV and FEB were found 0.5-5.0 µg/mL and 0.5-5.0 µg/mL respectively. The calibration curve was constructed by plotting response factor against concentration of drugs. The slope and intercept values of calibration curve for ATV y = 0.011X-0.0025 (R^2= 0.9984) and for FEB y = 0.0132 X-0.0067 (R^2= 0.9989) where Y represents the ratio of peak area ratio of analyte to IS and X represents analyte concentration.

Accuracy and precision

The accuracy of the developed method was determined using a mixture ATV and FEB solutions containing three concentration levels of standard drugs corresponding to 80%, 100% and 120% and determining the recovery of the added drug, at each concentration six determinations were performed.

The precision of the method was assessed by replicate analysis of pharmaceutical preparations. The precision and accuracy of HPLC method was obtained by analyze on the same day (intra-day accuracy) and analyze on the different days by triplicate analysis (inter-day accuracy) and expressed as relative standard deviation percentage (R.S.D. %). The correlation coefficient & the data of precision and accuracy are reported in **Table 4.**

LOD and LOQ

The sensitivity of ATV and FEB was estimated as limit of detection (LOD) and limit of quantification (LOQ), they were calculated by the use of the equations LOD = 3.3 × N/B and LOQ = 10 × N/B, where N is the standard deviation of the peak areas of the drugs (n = 3), taken as a measure of the noise, and B is the slope of the corresponding calibration plot. The LOD and LOQ Values were reported in **Table 3.**

Recovery and stability

The accuracy of the method was determined by the method of standard addition at three different levels. The recovery studies were carried out for capsules by spiking standard of each drugs

equivalent to 80%, 100%, and 120% to the original amounts present in each drug formulations. The average recoveries were as reported in **Table 4.**

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24 h at room temperature. The retention time and peak area of ATV and FEB remained almost similar (% R.S.D. less than 2.0) and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 24 h, which was sufficient time to complete the whole analytical process.

In conclusion, a novel HPLC method was developed and validated for the simultaneous determination of ATV and FEB in solid dosage form. It assured the satisfactory precision and accuracy and has high analytical potential. The proposed method was found to be simple, accurate, economical and reproducible and can be applied for routine analysis in laboratories. RP-HPLC method is suitable for the quality control of the raw materials, formulations, dissolution studies and can be employed for bioequivalence studies for the same formulation.

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FIGURES

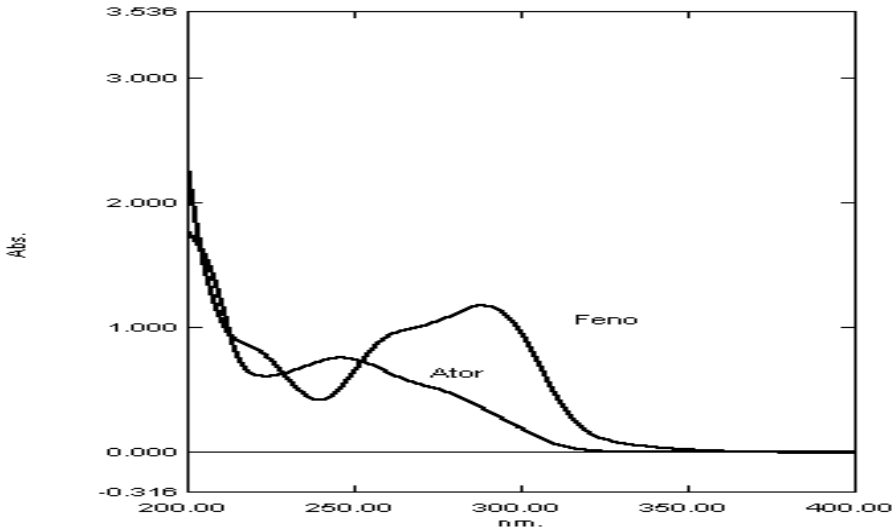
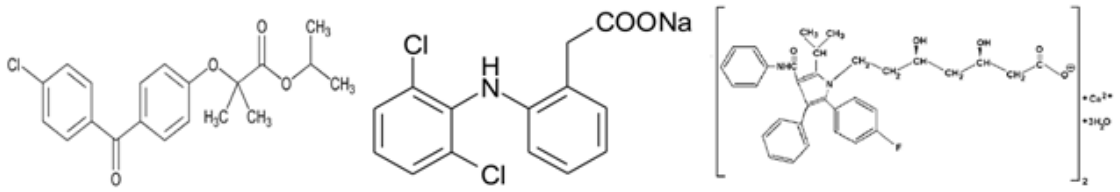


Figure 1. Overlay UV spectra of Atorvastatin and Fenofibrate



Fenofibrate **Diclofenac sodium** **Atorvastatin calcium**

Figure 2. Structure of Atorvastatin, Diclofenac (IS) and Fenofibrate

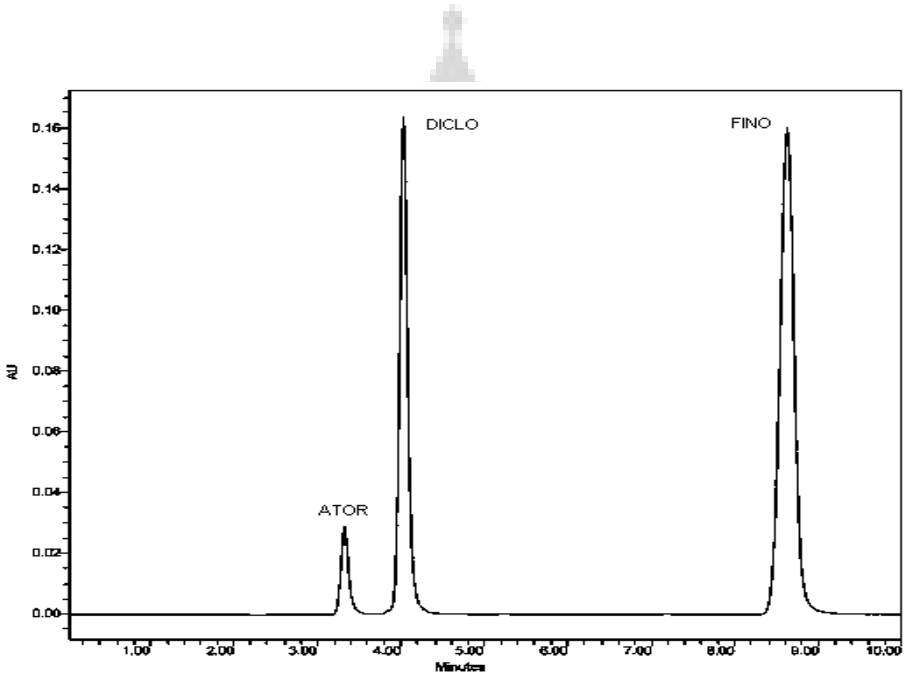


Figure 3. Typical chromatogram of standard solution of Atorvastatin, Diclofenac (IS) and Fenofibrate

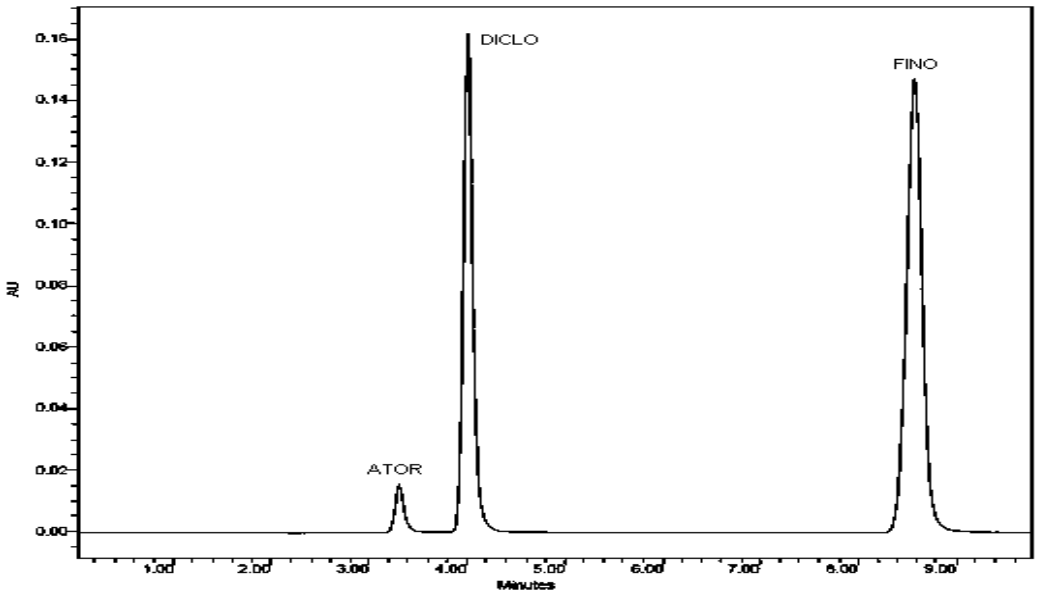


Figure 4. Typical chromatogram of sample solution of Atorvastatin, Diclofenac (IS) and Fenofibrate

TABLES

Table 1. Linearity of Atorvastatin and Fenofibrate by UV

Atorvastatin	**Fenofibrate**	**Diclofenac sodium**	**Water: Acetonitrile**
1.0 µg/ml	10 µg/ml	50 µg/ml	(50:50 v/v)
2.0 µg/ml	20 µg/ml		
3.0 µg/ml	30 µg/ml		
4.0 µg/ml	40 µg/ml		
5.0 µg/ml	50 µg/ml		
6.0 µg/ml	60 µg/ml		

Table 2. Linearity of Atorvastatin and Fenofibrate by HPLC

Atorvastatin				Fenofibrate			
IS* Peak area	**Conc. µg/ml**	**Peak area**	**Response factor**	**IS* Peak area**	**Conc. µg/ml**	**Peak area**	**Response factor**
	1	45620	0.0452		5	552254	0.5474
	2	90150	0.0893		10	818428	0.8112
1008835	3	141178	0.1399	1008835	20	1302230	1.2908
	4	183423	0.1818		30	1800425	1.7846
	5	235116	0.2330		40	2209688	2.1903
	6	280512	0.2780		50	2706398	2.6826

IS* =Internal standard used Diclofenac sodium (50 µg/ml)

Table 3. System suitability parameters of Atorvastatin and Fenofibrate

S.No.	Parameters	Atorvastatin	Fenofibrate
1	Retention time (minutes)	3.35 ± 0.02	8.74± 0.08
2	Theoretical plates	5567	11654
3	Resolution	10.78	
4	Asymmetry factor	1.00	1.01
5	Calibration range (µg/ml)	1-6	10-60
6	Correlation coefficient (r)	0.999	0.999
7	LOD (ng/ml)	6	18
8	LOQ (ng/ml)	20	175

Table 4. Result of Analysis Tablet formulation by HPLC

Drug	Amount (mg/tablet)		% Label claim*	% Recovery*	Precision (% RSD)*	
	Labeled	Found*			Interday	Intraday
Atorvastatin	10	9.88	100.04±0.921	100.20±1.686	0.452	0.983
Fenofibrate	200	199.5	99.87±1.450	99.96±1.844	0.410	0.831