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A SENSITIVE RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF ATORVASTATIN CALCIUM, FENOFIBRATE, AND ORLISTAT IN TABLET DOSAGE FORM

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ABSTRACT

A simple, sensitive and reproducible method was developed and validated for the simultaneous estimation of Atorvastatin calcium, Fenofibrate, and Orlistat in tablet formulation by reverse phase high performance liquid chromatography by using HPLC Prominance / shimadzu (Isocratic system) with Biochrom – Double beam UV-VISIBLE spectrophotometer at the λmax of 210 nm, using Zorbax C8(250 x 4.6), 5 µm. The mobile phase used as Phosphate buffer (pH 6.8): Acetonitrile: Water: Phosphoric acid (850:150:0.05) with isocratic flow (flow rate 1.0 ml/min) and the pH was adjusted with phosphate buffer. Mobile phase is used as diluent. The compounds Atorvastatin calcium, Fenofibrate, and Orlistat were eluted at 2.78, 4.62 and 10.27 min respectively. The peaks were eluted with better resolution. The sample concentrations are measured on weight basis to avoid the internal standard. The method is validated and shown to be linear. The correlation coefficients for Atorvastatin, Fenofibrate and Orlistat are 0.999, 0.989 and 0.999, respectively. The relative standard deviation for six replicates is always less than 2%. This HPLC method is successfully applied to the simultaneous quantitative analysis of the drugs in tablets.

Keywords: C8 column, Atorvastatin calcium, Fenofibrate, Orlistat, RP-HPLC, Method Validation.

INTRODUCTION

Atorvastatin (AT) calcium; chemically [R-(R*,R*)]-2-(4-fluorophenyl)- β, δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino) carbonyl]- 1H-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate, is a synthetic lipid-lowering agent. AT is an inhibitor of 3-hydroxy-3- methylglutaryl-coenzyme A (HMG-CoA) reductase, the enzyme catalyzes the conversion of HMG-CoA to mevalonate an early and rate-limiting step in cholesterol biosynthesis [1,2]. AT is indicated to reduce the risk of myocardial infarction stroke and reduce the risk for revascularization procedures and angina [3,4]. Bioanalytical, HPLC, HPTLC, UPLC and FT- Raman Spectroscopy methods are reported for its individual determination and in combination with other drugs [5-12].

Fenofibrate chemical name: 2-[4-(4-chlorobenzoyl) phenoxy]-2-methyl-propanoic acid, 1-methylethyl ester. Fenofibrate is a fibric acid derivative. It lowers lipid levels by activating Peroxisome proliferator-activated receptor alpha (PPARα). PPARα activates lipoprotein lipase and reduces apoprotein CIII, which increases lipolysis and elimination of triglyceride-rich particles from plasma.PPARα also increases apoproteins AI and AII, which reduces very low-density lipoprotein (VLDL) and low- density lipoprotein (LDL)

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containing apoprotein B, and increases high-density lipoprotein (HDL) containing apoprotein AI and AII.In addition, by reducing the synthesis and increasing the catabolism of VLDL, fenofibrate increases LDL clearance and reduces small and dense LDL, which are associated with coronary heart disease.

Orlistat Chemical Name: (S)-2-formylamino-4-methyl-pentanoic acid (S)-1-[[(2S, 3S)-3-hexyl-4-oxo-2-oxetanyl] methyl]dodecyl ester. Orlistat is used for the treatment of obesity.[13] Orlistat works by inhibiting gastric and pancreatic lipases, the enzymes that break down triglycerides in the intestine. When lipase activity is blocked, triglycerides from the diet are not hydrolyzed into absorbable free fatty acids, and are excreted undigested instead. Only trace amounts of orlistat are absorbed systemically; the primary effect is local lipase inhibition within the GI tract after an oral dose.

These drugs have good pharmacological actions. Many formulations are marketed individually or combination with other drugs. UV/Vis Spectrophotometric methods, liquid chromatographic (HPTLC) methods [14, 15], are available for estimation of Atorvastatin calcium and Fenofibrate, in formulations individually or in combination with other compounds. The present study aims in is to develop and validate a suitable high precision and accurate analytical method for the simultaneous estimation of Atorvastatin calcium, Fenofibrate, Orlistatin tablet dosage form by reverse phase high performance liquid chromatography (RP-HPLC).

Materials and methods

Drug samples:

Atorvastatin calcium, Fenofibrate and Orlistat raw materials were obtained as gift samples from Spectrum labs, Hyderabad.

Instruments used:

HPLC Prominence/Shimadzu, Column: Zorbax C8(250 x 4.6), 5 µm. Polomo pH meter, Biochrom – Double beam UV-VISIBLEspectrophotometer, shimadzu electronic balance.

Reagents and Chemicals:

Ammonium dihydrogen phosphate (HPLC grade), Potassium dihydrogen phosphate (HPLC grade), Acetonitrile (HPLC grade) and Water (HPLC grade) were used in the present study.
Acetonitrile (HPLC grade)
Water (HPLC grade)
Ortho phosphoric acid (AR Grade)

Preparation of mobile phase:

850 ml of Acetonitrile, 150 ml of Water and 0.05ml of ortho phosphoric acid are mixed and sonicated to remove to degas.

Preparation of diluent

Mobile phase is used as diluent.

Preparation of standard solutions

Atorvastatin calcium (0.1mg/mL): weigh accurately 1.0mg of standard drug substance and dissolve in 10ml of diluent and sonicated. So a working concentration of 0.1mg/ml is obtained.

Fenofibrate (0.2mg/mL): weigh accurately 2mg of standard drug substance and dissolve in 10ml of diluent and sonicated. So a working concentration of 0.2mg/ml is obtained.

Orlistat (5.0mg/mL): weigh accurately 50mg of standard drug substance and dissolve in 10ml of diluent and sonicated. So a working concentration of 5mg/ml is obtained.

Method validation

The HPLC method was validated in terms of precision, accuracy and linearity according to ICH guidelines [16, 17]. Assay method precision was determined using eight-independent test solutions. The intermediate precision of the assay method was also evaluated. Assay method was evaluated with the recovery of the standards from excipients. Three different quantities levels (low, medium and high) of the authentic standards were added to pre analyzed tablet powder. The mixtures were extracted and were analyzed using the developed HPLC method. The LOD and LOQ for analytes were estimated by injecting a series of dilute solutions with known concentration. To determine the robustness of the method, the final experimental conditions were purposely altered and the results were examined. The flow rate was varied by (±) 0.1 mL/min and wavelength.

Validation of method

Precision and accuracy: The precision of the method was determined by performing five replicate analyses of the same working solution. The relative standard deviation (R.S.D.) obtained for Atorvastatin calcium, Fenofibrate, and Orlistat were 1.43, 1.03 and 0.72 %, respectively. The results showed that the precision of the method is good. Accuracy of the method was calculated by recovery studies at three levels by standard addition method. The mean percentage recoveries obtained for Atorvastatin calcium, Fenofibrate, and Orlistat was 100.00, 100.05 and 99.99%, respectively (Table 1).

Linearity: For the construction of calibration curves, seven calibration standard solutions were prepared over the concentration range. Linearity was determined for Atorvastatin calcium in the range of 0.05-0.15, for Fenofibrate, 0.25-0.70 and for orlistat, 2.5–7.5 mg/mL. The correlation coefficient (‘r’) values were >0.98 (n = 6) (Table 2).

System Suitability

The %RSD of the peak area and retention time of three drugs were within 2% indicating system suitability (Table 3).

Sensitivity: LOD and LOQ for the procedure were performed on samples containing very low concentrations of analytes based on calibration curve method. Solutions of Atorvastatin calcium, Fenofibrate, and Orlistat were prepared and injected in triplicate. Average peak area of three analyses was plotted against concentration. LOD and LOQ were calculated by using following equations.

LOD = (3.3 ×Syx)/b LOQ= (10.0 ×Syx)/b

Where Syx is residual variance due to regression; b is slope. The LOD and LOQ values were found to be 0.00538, 0.00905, 3.332 ngm/ml and 0.0163, 0.027, 10.09 ngm/ml for Atorvastatin calcium, Fenofibrate, and Orlistat respectively.

Solution Stability: Solution stability as described in method validation under experimental section was studied. Result of short term, long-term and the auto sampler stability of the Atorvastatin calcium, Fenofibrate, and Orlistat solutions were calculated form (from) nominal concentrations and found concentration. Results of the stability studies were within the acceptable limit (98–102%).

Robustness: Robustness of the method was investigated under a variety of conditions including changes of flow rate and wavelength. The mixed standard solution is injected in four replicates and sample solution of 100% concentration is prepared and injected in triplicate for every 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 (Table 4).

Table 1a. Optimization of the chromatographic conditions

S no	Parameter	Trial 1	Trial 2	Trial 3	Trial 4
1	Column	Hypersil c18(260×4.6),5µm	Hypersil c18(150×3.9),4µm	Zorbax c8(250×4.6),5µm	Zorbax c8(250×4.6),5µm
2	Injection Volume	20µl	10µl	20µl	20µl
3	Run Time	20 min	20 min	20 min	20 min
4	Mode	Isocratic	Isocratic	Isocratic	Isocratic
5	Oven Temperature	40°c	40°c	40°c	40°c
6	Flow Rate	1 ml/min	1 ml/min	1 ml/min	1 ml/min
7	Wavelength	196,210,254,280nm	196,210,254,280nm	196,210,254,280nm	196,210,254,280nm
8	Diluent	Methanol	Methanol	Methanol	Methanol
9	Mobile Phase	ACN: H_2O:H_3PO_4	ACN:CH_3OH	ACN: BUFFER	ACN: H_2O:H_3PO_4

Table 1b. Optimization of the chromatographic conditions

S.No	Parameter	Trial 1	Trial 2	Trial 3	Trial 4
1	Column	Hypersil c18(260×4.6),5µm	Hypersil c18(150×3.9),4µm	Zorbax c8(250×4.6),5µm	Zorbax c8(250×4.6),5µm
2	Injection Volume	20µl	10µl	20µl	20µl
3	Run Time	20 min	20 min	20 min	20 min
4	Mode	Isocratic	Isocratic	Isocratic	Isocratic
5	Oven Temperature	40°c	40°c	40°c	40°c
6	Flow Rate	1 ml/min	1 ml/min	1 ml/min	1 ml/min
7	Wavelength	196,210,254,280nm	196,210,254,280nm	196,210,254,280nm	196,210,254,280nm
8	Diluent	Methanol	Methanol	Methanol	Methanol
9	Mobile Phase	ACN: H_2O:H_3PO_4	ACN:CH_3OH	ACN: BUFFER	ACN: H_2O:H_3PO_4

Table 2a. Results of accuracy

	Level	Amount added (conc. mg/ml)	Area response	Amount found (conc. mg/ml)	% of recovery	Mean % of recovery
	80%	0.08084	2820901	0.08106	100.3	
Atorvastatin	100%	0.10105	3543814	0.10149	100.4	**100.0**
	120%	0.12126	4247620	0.12138	100.1	
	80%	0.16092	4677955	0.16104	100.1	
Fenofibrate	100%	0.20115	5912647	0.20047	99.7	100.00

	120%	0.24138	7219363	0.24219	100.3	
	80%	4.00184	4617957	3.99463	99.82	
Orlistat	100%	5.00021	5780365	5.00676	100.13	**99.9958**
	120%	6.0028	6902044	5.98343	99.68	

Table 2b. Results of accuracy

	Level	**Amount added (conc. mg/ml)**	**Area response**	**Amount found (conc. mg/ml)**	**% of recovery**	**Mean % of recovery**
Atorvastatin	80%	0.08084	2820901	0.08106	100.3	100.0
	100%	0.10105	3543814	0.10149	100.4	
	120%	0.12126	4247620	0.12138	100.1	
Fenofibrate	80%	0.16092	4677955	0.16104	100.1	100.0
	100%	0.20115	5912647	0.20047	99.7	
	120%	0.24138	7219363	0.24219	100.3	
Orlistat	80%	4.00184	4617957	3.99463	99.82	100.0
	100%	5.00021	5780365	5.00676	100.13	
	120%	6.0028	6902044	5.98343	99.68	

Table 3a. Results of linearity

	Atorvastatin		**Fenofibrate**		**Orlistat**	
Level	Concentration (mg/ml)	Average area response	Concentration (mg/ml)	Average area response	Concentration (mg/ml)	Average area response
50%	0.05053	17253990	0.2508	3874584	2.5002	2851748.5
80%	0.08084	2800950	0.40128	5866774	4.0004	4615155
100%	0.10105	3506458.5	0.5016	7227789	5.0005	5761378.5
120%	0.12126	4232396	0.60192	8630163	6.0006	6907564
150%	0.15158	5270842	0.70224	10044006	7.50075	8588672
Correlation coefficient		0.999		0.981		0.999

Table 3b. Results of linearity

	Atorvastatin		**Fenofibrate**		**Orlistat**	
Level	Concentration (mg/ml)	Average area response	Concentration (mg/ml)	Average area response	Concentration (mg/ml)	Average area response
50%	0.05053	17253990	0.25080	3874584	2.5	2851749
80%	0.08084	2800950	0.40128	5866774	4.0	4615155
100%	0.10105	3506458.5	0.50160	7227789	5.0	5761379
120%	0.12126	4232396	0.60192	8630163	6.0	6907564
150%	0.15158	5270842	0.70224	10044006	7.5	8588672
Correlation coefficient		0.999		0.981		0.999

Table 4a. Result of robustness study

S.No.	**Flow rate (ml/min)**	**Retention time (min)**			**Peak area**		
		ATR	**FFB**	**OST**	**ATR**	**FFB**	**OST**
1.	1.2ml/min	2.78	4.59	10.22	5504198	6479542	5326781
2.	1.4ml/min	2.80	4.62	10.19	5510021	6491253	5319887
3.	**1.0ml/min**	**2.77**	**4.60**	**10.20**	**5504234**	**6486576**	**5327552**
4.	1.3ml/min	2.79	4.58	10.23	5503987	6480187	5330873

Table 4b. Result of robustness study

S.No	**$\lambda_{max;}$ (nm)**	**Retention time (min)**			**Peak area**		
		ATR	FFB	OST	ATR	FFB	OST
1	246	2.76	4.58	10.23	5503698	6478742	5324897
2	**210**	**2.77**	**4.60**	**10.20**	**5504234**	**6486576**	**5327552**
3	254	2.81	4.62	10.19	5503914	6481754	5324579
4	196	2.79	4.59	10.21	5503917	6480550	5335 79

Table 4c. Result of robustness study

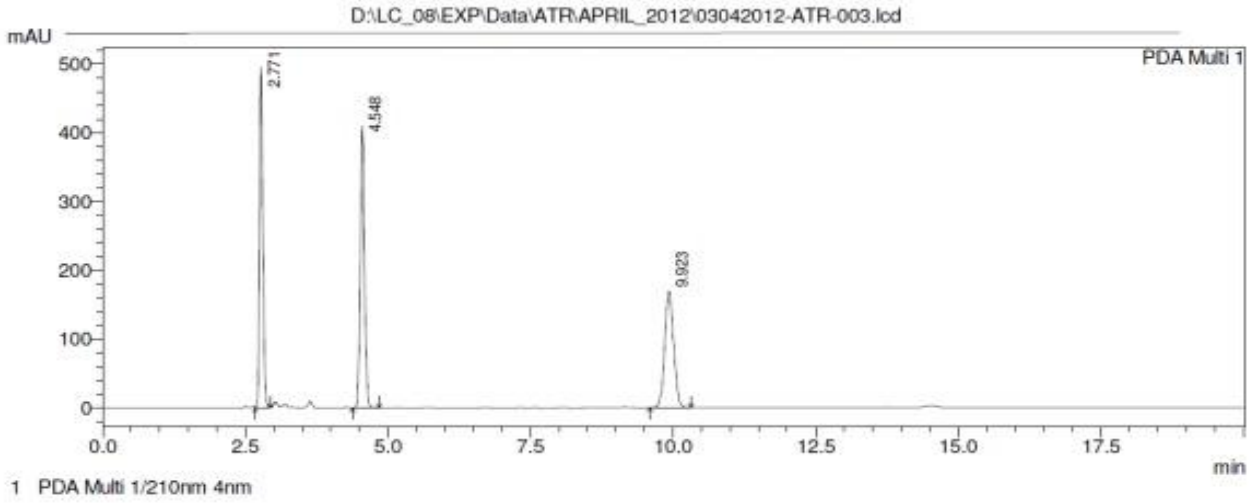
S.No	Flow rate (ml/min)	Retention time (min)			Peak area		
		ATR	FFB	OST	ATR	FFB	OST
1	1.2	2.78	4.59	10.22	5504198	6479542	5326781
2	1.4	2.80	4.62	10.19	5510021	6491253	5319887
3	1.0	2.77	4.60	10.20	5504234	6486576	5327552
4	1.3	2.79	4.58	10.23	5503987	6480187	5330873
Avg		2.79	4.60	10.21	5505610	6484390	5326273
SD		0.01	0.02	0.02	2942.68	5569.19	4612.84
%RSD		0.46	0.37	0.18	0.053	0.09	0.09

Table 4d. Result of robustness study

S.No	$\lambda_{max\ (nm)}$	Retention time			Peak area		
		ATR	FFB	OST	ATR	FFB	OST
1	246	2.76	4.58	10.23	5503698	6478742	5324897
2	210	2.77	4.60	10.20	5504234	6486576	5327552
3	254	2.81	4.62	10.19	5503914	6481754	5324579
4	196	2.79	4.59	10.21	5503917	6480550	5335579
Avg		2.78	4.60	10.21	5503941	6481906	5328152
SD		0.02	0.02	0.02	220.76	3350.70	5127.76
%RSD		0.80	0.37	0.17	0.0040	0.05	0.10

Fig 1. Combination Chromatogram (ATR+FFB+OST):

<Chromatogram>



ATORVASTATIN

Injection Number	Retention Time	Peak Area	Theoretical plates	Tailing factor
Injection – 1	2.79	2036097	8064	1.3
Injection – 2	2.80	2054176	8005	1.3
Injection – 3	2.81	2051433	7978	1.3
Injection – 4	2.80	2064230	8347	1.3
Injection – 5	2.79	2047207	7931	1.3
Injection – 6	2.78	2034145	8175	1.3
Mean	**2.79**	**2047881**	**8084**	1.3
Acceptance	**NMT – 1.0%**	**NMT – 2.0%**	**NLT or equal to 2500**	**NMT – 2.0%**
Result	**Pass (RSD –0.37)**	**Pass (RSD –0.56)**	**Pass**	**Pass**

FENOFIBRATE

Injection Number	Retention Time	Peak Area	Theoretical plates	Tailing factor
Injection – 1	4.63	2150242	14116	1.1
Injection – 2	4.64	2158544	14190	1.1
Injection – 3	4.66	2167411	14266	1.1
Injection – 4	4.64	2156044	14295	1.1
Injection – 5	4.63	2150672	13393	1.1
Injection – 6	4.61	2148777	14019	1.1
Mean	**4.63**	**2155281**	**14047**	**1.1**
Acceptance	**NMT – 1.0%**	**NMT – 2.0%**	**NLT or equal to 2500**	**NMT 2.0%**
Result	**Pass (RSD-0.32)**	**Pass (RSD 0.33)**	**Pass**	**Pass**

ORLISTAT

Injection Number	Retention Time	Peak Area	Theoretical plates	Tailing factor
Injection – 1	10.31	1825406	17960	1.1
Injection – 2	10.33	1849821	17951	1.1
Injection – 3	10.35	1856504	17948	1.1
Injection – 4	10.32	1850457	17924	1.1
Injection – 5	10.29	1840994	17874	1.1
Injection – 6	10.27	1830545	17794	1.1
Mean	**10.31**	**1842288**	**17909**	**1.1**
Acceptance	**NMT – 1.0%**	**NMT – 2.0%**	**NLT or equal to – 2500**	**NMT – 2.5**
Result	**Pass (RSD –0.28)**	**Pass (RSD –0.66)**	**Pass**	**Pass**

Fig 2a. Results of linearity
ATORVASTATIN

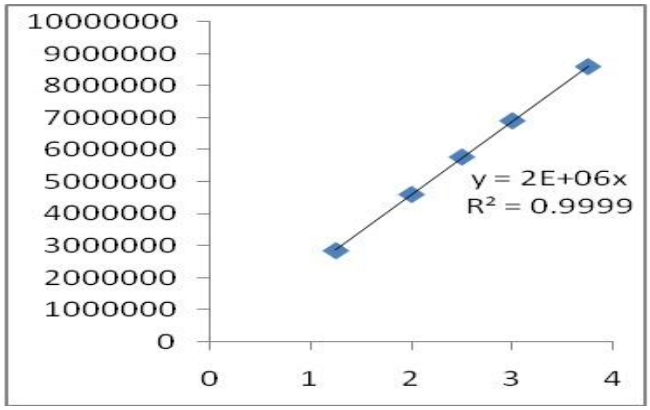


Fig 2b. Results of linearity
FENOFIBRATE

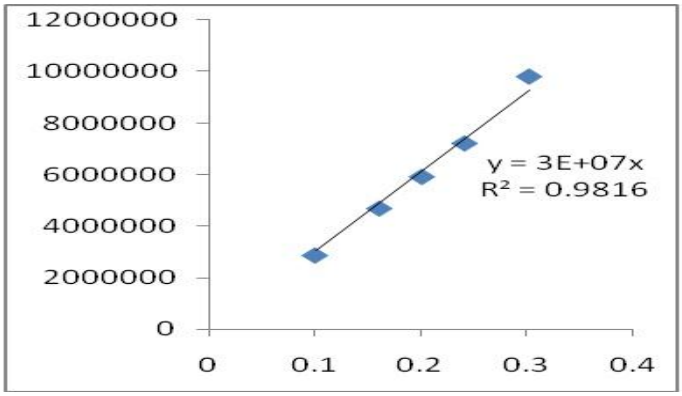
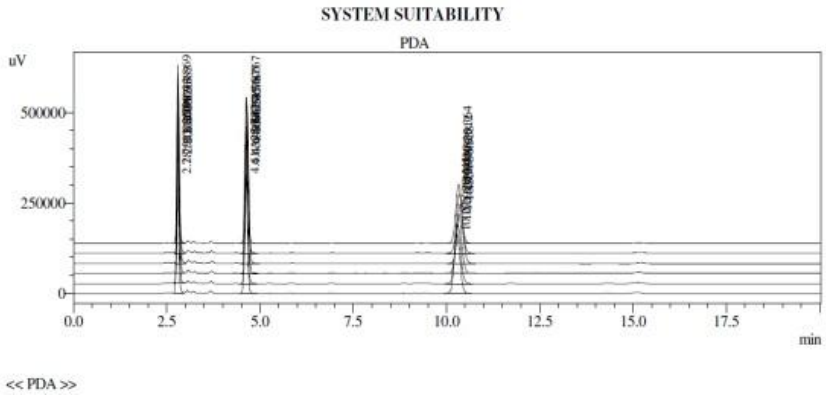


Fig 3. System Suitability



CONCLUSION

The proposed method was found to be simple, sensitive, rapid and economical for the determination of atorvastatin, fenofibrate and orlistat in combined formulation. The method was found to be linear, precise, accurate. The proposed RP HPLC method was simple, precise because of commonly used buffer and shorter run time. The mean percentage recovery above 99% indicates the reproducibility and accuracy of new developed method compared. The simple recoveries in all formulations were in good agreement with their respective label claims and they suggest non-interference of formulation recipients in the estimation. After validating proposed method as per ICH guidelines and correlating obtained values with the standard values, satisfactory results were obtained. Hence the method can easily and conveniently adopted for the routine estimation of combined dosage form of atorvastatin, fenofibrate and Orlistat.

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