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Method development and validation for the estimation of Atorvastatin, Ezitimibe and Fenofibrate in bulk and pharmaceutical dosage forms by RP-HPLC method

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ABSTRACT

A Simple, specific and sensitive an isocratic Estimation by RP-HPLC analytical Method were developed and validated for the quantification Atorvastatin, Ezitimibe and fenofibrate in bulk and Pharmaceutical dosage forms. Quantification was achieved by using the mobile phase (A mixture of 80 volumes of Methanol: 10 volumes of Acetonitrile and 10 volumes of Water.). Inertsil ODS 3V column (250×4.6mm× 5µ) was used as stationary phase. The flow rate was 1.0 ml/min. Measurements were made at a wavelength of 256nm. The average retention times for about 2.230min for Atorvastatin, 3.840 for Ezitimibe and 5.937min for Fenofibrate.and Internal standard was found to be 4.37& 6.70 min. The proposed method was validated for selectivity, precision, linearity and accuracy. The assay methods were found to be linear from 50-150µg/ml. All validation parameters were within the acceptable range. The developed methods were successfully applied to estimate the amount of Atorvastatin, Ezitimibe and fenofibrate

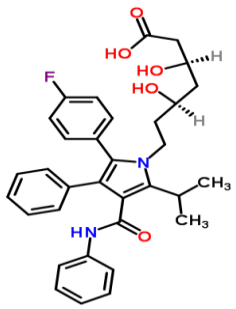
Keywords: Atorvastatin, Ezitimibe Fenofibrate, RP-HPLC method, Inertsil ODS 3V column, and Methanol, Acetonotrile, Water and Validation.

INTRODUCTION

Atorvastatin

Atorvastatin (Lipitor) is a member of the drug class known as statins. It is used for lowering cholesterol. Atorvastatin is a competitive inhibitor of hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-determining enzyme in cholesterol biosynthesis via the mevalonate pathway. HMG-CoA reductase catalyzes the conversion of HMG-CoA to mevalonate. Atorvastatin acts primarily in the liver. Decreased hepatic cholesterol levels increases hepatic uptake of cholesterol and reduces plasma cholesterol levels [1].

Structure of atorvastatin



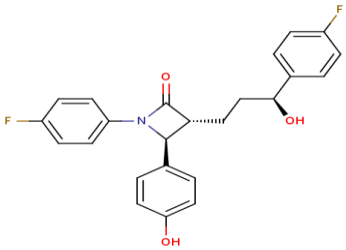
IUPAC Name

7-[2-(4-fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-(propan-2-yl)-1H-pyrrol-1-yl]-3,5-dihydroxyheptanoate.

Description

Ezetimibe is an anti-hyperlipidemic medication which is used to lower cholesterol levels. Specifically, it appears to bind to a critical mediator of cholesterol absorption, the Niemann-Pick C1-Like 1 (NPC1L1) protein on the gastrointestinal tract epithelial cells as well as in hepatocytes [2].

Structure of Eztimibe



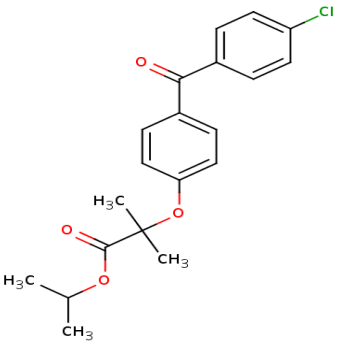
IUPAC name

(3R,4S)-1-(4-fluorophenyl)-3-[(3S)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl) azetidin-2-one

Description

An antilipemic agent who reduces both cholesterol and triglycerides in the blood.

Structure of Fenofibrate



IUPAC Name

Propan-2-yl 2-{4-[(4-chlorophenyl) carbonyl] phenoxy}-2-methylpropanoate

MATERIALS AND METHODS

Instruments The chromatographic technique performed on a Shimadzu (LC 20 AT VP) Liquid chromatography with SPD-20A prominence UV-visible detector and Spinchrom software, reversed phase C18 column (Inertsil ODS 3V column (250×4.6mm× 5µ)) as stationary phase, Electron corporation Nicolet evolution 100, Citizen, Digital Ultrasonic Cleaner, Shimadzu analytical balance [3], Vacuum micro filtration unit with 0.45µ membrane filter was used in the study. Pharmaceutically pure sample of Atorvastatin were obtained as gift samples from Chandra lab, Prashanthinagar, Kukatpally, Hyderabad, India [4]. The purity of the drug was evaluated by obtaining its melting point and ultraviolet (UV) and infrared (IR) spectra. No impurities were found. The drug was used without further purification. HPLC-grade acetonitrile and Methanol from standard reagents Pvt. Ltd.

Determination of working wavelength (Λmax)

In simultaneous estimation of two drugs isobestic wavelength is used. Isobestic point is the wavelength where the molar absorptivity is the same for two substances that are interconvertible. So this wavelength is used in simultaneous estimation to estimate both drugs accurately [5].

RESULTS

The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The resulting spectra are shown in the figures.

Observation

The isosbestic point was found to be 256 nm for atorvastatin and ezitimibe and fenofibrate in combination and was shown in figure [6].

Preparation of mobile phase

A mixture of Methanol Acetonitrile and Water in the ratios of 80:10:10 was prepared. The mobile phase was sonicated for 10min to remove gases.

Analysis of formulation

Preparation of samples for assay

Standard sample

Standard stock solutions of Atorvastatin, Eztimibe and Fenofibrate (microgram/ml) were prepared by dissolving 5 mg of Atorvastatin, 5 mg of Ezitimibe and 80 mg of Fenofibrate dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5min and dilute to 100 ml with mobile phase (stock). Further dilutions are prepared in 5 replicates of of 5µg/mL of Atorvastatin, 5µg/mL of Ezitimibe and 80µg/mL of Fenofibrate was made by adding 1 ml of stock solution to 10 ml of mobile phase.

Tablet sample

10tablets (each tablet contains Atorvastatin-10 mg, Fenofibrate-160 mg and Ezetimibe-10 mg) were weighed and taken into a mortar uniformly mixed. Test stock solutions of 5µg/mL of Atorvastatin, 5µg/mL of Ezitimibe and 80µg/mL of Fenofibrate were prepared by dissolving weight equivalent to5 mg of Atorvastatin, 5 mg of Ezitimibe and 80 mg of Fenofibrate and dissolved in sufficient mobile phase [7]. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 100ml with mobile phase. Further dilutions are prepared in 5 replicates of 5µg/mL of Atorvastatin, 5µg/mL of Ezitimibe and 80µg/mL of Fenofibrate was made by adding 1 ml of stock solution to 10 ml of mobile phase.

Calculation

The amount of theAtorvastatin, Eztimibe and Fenofibrate present in the formulation by using the formula given below, and results shown in above table:

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AW}{LC} \times 100$$

Where,

AS: Average peak area due to standard preparation

AT: Peak area due to assay preparation

WS: Weight of Atorvastatin, Eztimibe and Fenofibratein mg
WT: Weight of sample in assay preparation
DT: Dilution of assay preparation

METHOD VALIDATION

Linearity and range

Preparation of standard stock solution

Standard stock solutions of Atorvastatin, Ezitimibe and Fenofibrate (microgram/ml) were prepared by dissolving 5mg of Atorvastatin and 5mg of Ezitimibe and 80 mg of Fenofibrate dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5min and dilute to 100 ml with mobile phase and further dilutions were given in the table 1.

Observation

The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of Atorvastatin, Ezitimibe and Fenofibrate is 0.9985, 0.9971 and 0.9964 respectively. The relationship between the concentration of Atorvastatin, Ezitimibe and Fenofibrate and area of Atorvastatin, Ezitimibe and Fenofibrate is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits [8].

Precision

Method precision

Prepared sample preparations of Atorvastatin, Ezitimibe and Fenofibrate as per test method are injected 6 times in to the column [9].

Limit of detection

$$\text{LOD} = \frac{3.3\sigma}{S}$$

Where, σ = the standard deviation of the response
S = the slope of the calibration curve
The slope S may be estimated from the calibration curve of the analyte.

Limit of Quantification

$$\text{LOQ} = \frac{10\sigma}{S}$$

Where,
σ = the standard deviation of the response
S = the slope of the calibration curve
The slope S may be estimated from the calibration curve of the analyte.

Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (pre analysed sample), the reference standards of the drugs were added at the level of 80%, 100%, 120%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in table. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analysed sample solution at three different levels 80%, 100%, 120% by adding 5% of standard drug solution in each level [10].

Specificity by direct comparison method

There is no interference of mobile phase, solvent and placebo with the analyte peak and also the peak purity of analyte peak which indicate that the method is specific for the analysis of analytes in their dosage form.

System suitability

Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters like theoretical plates, resolution and asymmetric factor were evaluated.

Robustness

Chromatographic conditions variation

To demonstrate the robustness of the method, prepared solution as per test method injected at different variable conditions like using different conditions like flow rate and temperature. System suitability parameters were compared with that of method precision.

Ruggedness

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts [11].

RESULTS AND DISCUSSION

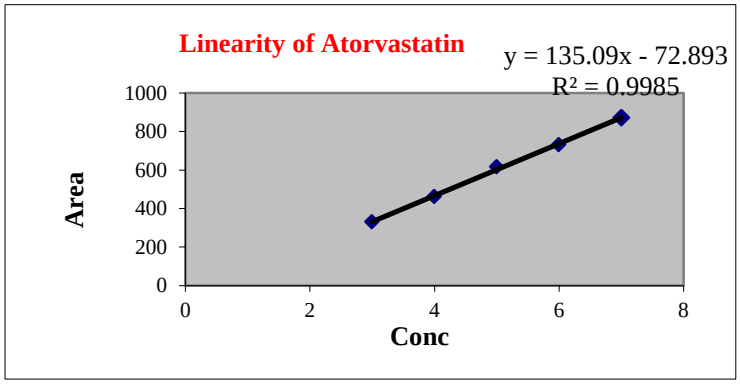
Discussion

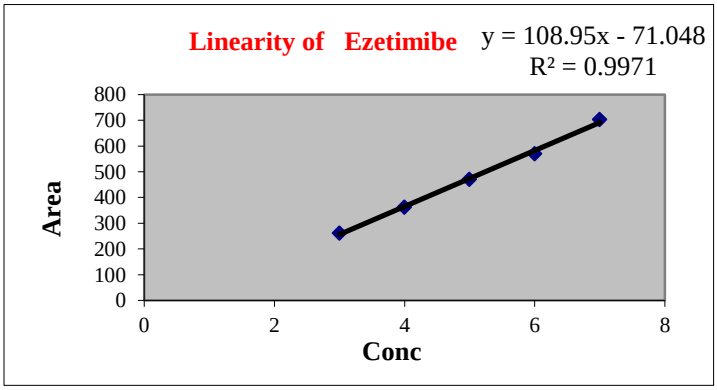
A simple and selective LC method is described for the determination of Atorvastatin, Ezitimibe and fenofibratein tablet dosage forms. Chromatographic separation was achieved on a reversed-phase C18column (Inertsil ODS 3V, 5µ, 250 mm×4.6mm) using mobile phase of a mixture of 80 volumes of Methanol: 10 volumes of Acetonitrile and 10 volumes of Waterwith detection of 256 nm. Linearity was observed in the range 3-7µg /ml for Atorvastatin (r^2 =0.9985), 3-7 µg /ml for Ezitimibe (r^2 =0.9971) and 48-112µg /ml for Fenofibrate (r^2 =0.9964) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim.

The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

Table 1: Linearity Preparations

Preparations	Volume from standard stock transferred in ml	Volume made up in ml (with mobile phase)	Concentration of solution(µg /ml)		
			Atorvastatin	Ezitimibe	Fenofibrate
Preparation 1	0.6	10	3	3	48
Preparation 2	0.8	10	4	4	64
Preparation 3	1.0	10	5	5	80
Preparation 4	1.2	10	6	6	96
Preparation 5	1.4	10	7	7	112





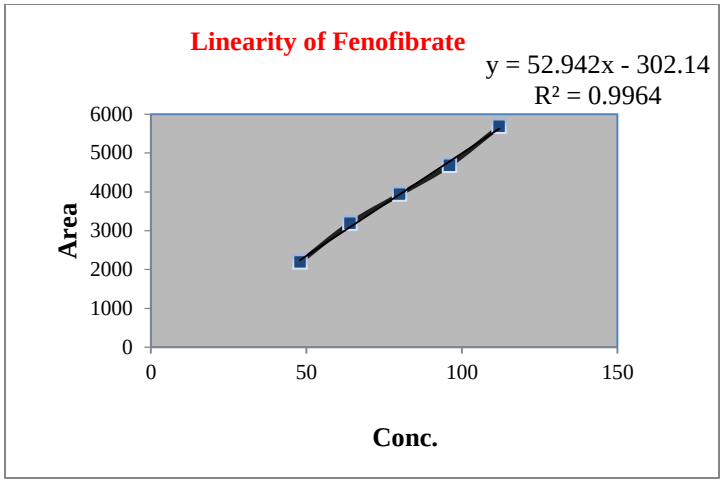


Table 2: LOD &LOQ data for Atorvastatin, Ezetimibe and Fenofibrate

Parameter	mcg (µg/ml)			Area		
	Atorvastatin	Ezetimibe	Fenofibrate	Atorvastatin	Ezetimibe	Fenofibrate
LOD	0.04	0.05	1.58	5.22	5.23	83.64
LOQ	0.12	0.15	4.78	15.82	15.83	253.85

Table 3: Recovery data for Atorvastatin, Ezetimibe and Fenofibrate

Recovery level	Accuracy Atorvastatin					Average % Recovery
	Amount taken(mcg/ml)	Area	Average area	Amount recovered(mcg/ml)	%Recovery	
80%	5	590.141	587.259	5.09	101.78	
	5	594.825				
	5	576.811				
100%	6	738.837	730.117	5.92	98.73	
	6	727.979				100.79%
	6	723.535				
120%	7	867.159	868.710	7.13	101.87	
	7	866.597				
	7	872.373				

Recovery level	Accuracy Ezitimibe					Average % Recovery
	Amount taken(mcg/ml)	Area	Average area	Amount recovered(mcg/ml)	%Recovery	
80%	5	444.321	455.020	5.03	100.51	
	5	456.927				
	5	463.812				
100%	6	559.362	561.839	5.96	99.34	
	6	572.922				
	6	553.232				100.52%

120%	7	674.588	676.231	7.12	101.71	
	7	671.065				
	7	683.040				

Recovery level	**Accuracy Fenofibrate**					**Average % Recovery**
	Amount taken(mcg/ml)	**Area**	**Average area**	**Amount recovered(mcg/ml)**	**%Recovery**	
80%	80	4035.746	4062.386	81.5517	101.94	
	80	4087.727				
	80	4063.686				
100%	96	4607.767	4643.225	94.44111	98.38	
	96	4641.250				
	96	4680.659				100.58%
120%	112	5497.645	5536.840	113.6103	101.43	
	112	5534.686				
	112	5578.188				

Table 4: Results of Robustness study

Parameter	**Atorvastatin**		**Ezitimibe**		**Fenofibrate**	
	Retention time(min)	**Tailing factor**	**Retention time(min)**	**Tailing factor**	**Retention time(min)**	**Tailing factor**
Flow Rate						
0.8 ml/min	2.570	1.117	4.280	1.483	6.613	1.800
1.0 ml/min	2.133	1.152	3.827	1.459	5.930	1.821
1.2 ml/min	1.730	1.108	2.883	1.487	4.447	1.867
Wavelength						
254nm	2.290	1.139	3.823	1.459	5.900	1.873
256nm	2.133	1.152	3.827	1.459	5.930	1.821
258nm	2.290	1.124	3.820	1.393	5.897	1.873

Table 5: Ruggedness data of Atorvastatin, Ezitimibe and Fenofibrate

Ruggedness		Atorvastatin	Ezitimibe	Fenofibrate
% RSD		1.26	0.27	0.35
Assay	Analyst-1	100.26	98.88	97.69
	Analyst-2	98.62	98.35	98.35

Table 6: Assay Results of Atorvastin, Ezitimibe & Finofibreate

Atorvastatin		
	Standard Area	**Sample Area**
Injection-1	600.684	583.390
Injection-2	598.337	593.001
Injection-3	592.210	618.630

Injection-4	590.073	600.684
Injection-5	586.890	610.211
Average Area	593.639	601.183
Tablet average weight	240.2	
Standard weight	5	
Sample weight	120.1	
Label amount	10	
std. purity	98.6	
Amount found in mg	9.99	
Assay(%purity)	99.85	

Ezitimibe		
	Standard Area	**Sample Area**
Injection-1	481.379	508.444
Injection-2	489.799	479.379
Injection-3	471.455	497.165
Injection-4	501.290	481.379
Injection-5	478.759	499.282
Average Area	484.5364	493.1298
Tablet average weight	240.2	
Standard weight	5	
Sample weight	120.1	
Label amount	10	
std. purity	98.7	
Amount found in mg	10.05	
Assay(%purity)	100.45	

Fenofibrate		
	Standard Area	**Sample Area**
Injection-1	4058.912	4066.285
Injection-2	4059.882	4054.258
Injection-3	4069.353	4143.728
Injection-4	4071.134	4058.912
Injection-5	4054.814	4066.391
Average Area	4062.819	4077.915
Tablet average weight	240.2	
Standard weight	80	
Sample weight	120.1	

Label amount	160
std. purity	98.7
Amount found in mg	158.51
Assay(%purity)	99.07

Acceptance criteria

The amount of Atorvastatin, Ezitimibe and Fenofibratepresent in the taken dosage form was found to be 99.85 %, 100.45 and 99.07% respectively.

Table 7: Method precision of Atorvastatin,Ezitimibe and Fenofibrate

Atorvastatin		
S.No.	**Rt**	**Area**
1	2.133	573.541
2	2.140	575.530
3	2.153	573.809
4	2.160	576.811
5	2.170	578.138
6	2.183	579.956
Avg	2.1565	576.298
SD	0.0186	2.507
%RSD	**0.86**	**0.43**

Ezetimibe		
S.No.	**Rt**	**Area**
1	3.827	461.814
2	3.827	463.922
3	3.833	465.456
4	3.837	463.812
5	3.843	463.272
6	3.847	463.807
Avg	3.836	463.681
SD	0.008	1.174
% RSD	**0.22**	**0.25**

Fenofibrate		
S.No.	**Rt**	**Area**
1	5.930	4041.508
2	5.927	4043.788
3	5.930	4044.079

4	5.943	4063.686
5	5.953	4071.839
6	5.960	4046.164
Avg	5.941	4051.844
SD	0.014	12.683
% RSD	**0.23**	**0.31**

Table 8: Validation parameters of evaluated method

S. No	Parameter	Value Obtained of Atorvastatin	Value Obtained of Ezetimibe	Value Obtained of Finofibrate
1.	**Accuracy (%Recovery)**	100.79%	100.52%	100.58”%
2.	**Linearity concentrations Range (mcg/mL)**	3-7	3-7	48-112
	Regression coefficient (R2 value)	0.998	0.997	0.996
3.	**LOD (mcg/mL)**	0.04	0.05	1.58
4.	**LOQ (mcg/mL)**	0.12	0.15	4.78
3.	**Precision (% RSD)**	0.0-0.86	0.0-0.22	
	Method precision(Repeatability) (%RSD, n = 6)			0.0-0.23
4.	**Robustness**	Met with system suitability criteria	Met with system suitability criteria	Met with system suitability criteria
5.	**Ruggedness(%RSD analyst to analyst variation)**	1.26	0.27	0.35

LOD = Limit of detection, LOQ = Limit of quantification, %RSD = Relative standard deviation.

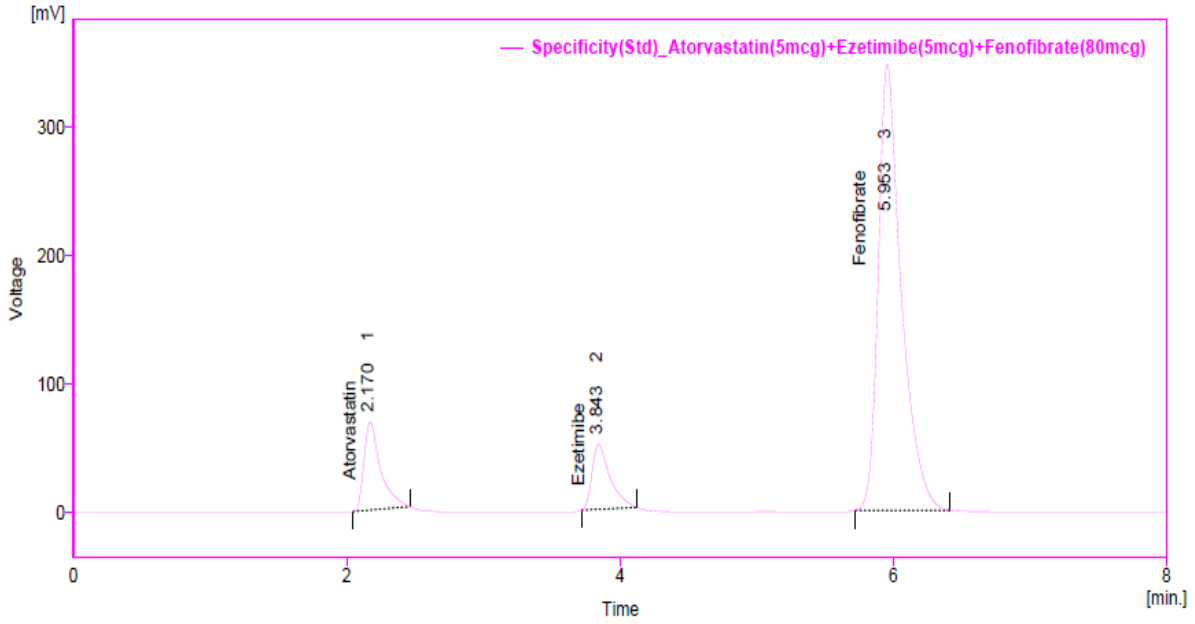


Fig 1: Chromatogram for standard of Atorvastatin, Ezitimibe and Fenofibrate

CONCLUSION

A validated RP-HPLC method has been developed for determination of Atorvastatin, Ezitimibe and fenofibratein their bulk and combined tablet dosage forms. The results show that the method was found to be specific, simple, accurate, precise and sensitive. The method was successfully applied for the determination of both drugs in combined tablet dosage form. In the future, this method may be applied for routine analysis of both the drugs in API and in tablet formulation.

Acknowledgement

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