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RP-HPLC Method Development and Validation for the Simultaneous Estimation of Atorvastatin, Fenofibrate and Ezetimibe in a Pharmaceutical Dosage Form

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Abstract

A simple and selective LC method is described for the determination of Atorvastatin, Ezetimibe and Fenofibrate in tablet dosage forms. Chromatographic separation was achieved on a reversed-phase C18 column (Inertsil ODS 3 V, 5 μ, 250 mm × 4.6 mm) using mobile phase of a mixture of 80 volumes of Methanol: 10 volumes of Acetonitrile and 10 volumes of Water with detection of 256 nm. Linearity was observed in the range 3-7 μg/ml for Atorvastatin (r^2=0.9985), 3-7 μg /ml for Ezetimibe (r^2=0.9971) and 48-112 μg /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 results show that the method was found to be specific, simple, accurate, precise, sensitive and validated. The accuracy of the methods was assessed by recovery studies at three different levels. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. The method was successfully applied for the determination of three drugs in combined tablet dosage form.

Keywords: Atorvastatin calcium; Ezetimibe; Fenofibrate; Gradient; Reverse phase HPLC; Photodiode array (PDA)

Introduction

Atorvastatin 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 [1].

Decreased hepatic cholesterol levels increases hepatic uptake of cholesterol and reduces plasma cholesterol levels. It is chemically 7-[2-(4-fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-(propan-2-yl)-1H-pyrrol-1-yl]-3,5-dihydroxyheptanoate.

It is used to reduce the risk of myocardial infarction, stroke and angina 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. It is chemically (3R,4S)-1-(4-fluorophenyl)-3-[(3S)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)azetidin-2-one. Ez is used in adjunctive therapy to diet for the reduction of elevated total-C, LDL-C, and Apo B in patients with primary (heterozygous familial and non-familial) hypercholesterolemia [2].

Fenofibrate is an antilipemic agent which reduces both cholesterol and triglycerides in the blood. It is chemically propan-2-yl 2-{4-[(4-chlorophenyl) carbonyl]phenoxy}-2-methylpropanoate. It is used in For use as adjunctive therapy to diet to reduce elevated LDL-C, Total-C, Triglycerides and Apo B, and to increase HDL-C in adult patients with primary hypercholesterolemia or mixed dyslipidemia (Fredrickson Types IIa and IIb) (Figure 1) [3,4].
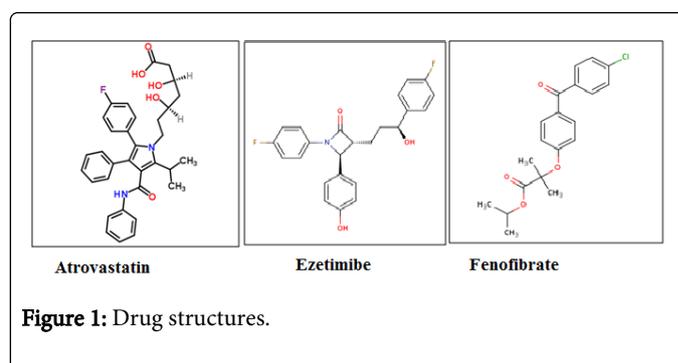

Figure 1: Drug structures.

Material and Methods

Chemical and reagents

AT calcium, EZ and FE were supplied by a Research centre. The reagents like Sodium di hydrogen ortho phosphate, Acetonitrile, Methanol were of AR, HPLC Grade.

HPLC instrumentation and conditions

HPLC used was Shimadzu (LC 20 AT VP), Agilent 1200 series. A reversed-phase C18 column (Inertsil ODS 3 V, 5 μ, 250 mm × 4.6 mm)of drugs using mobile phase of a mixture of 80 volumes of Methanol: 10 volumes of Acetonitrile and 10 volumes of Water with detection of 256 nm using PDA detector at a wavelength of 256 nm. The column was maintained at room temperature and injection volume of 20 μl was used for a run time of 8 mins. The mobile phase was filtered using 0.45-micron syringe filter [5-8].

Preparation of stock and standard solutions

Standard stock solutions of Atorvastatin, Ezetimibe 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 5 min and dilute to 100 ml with mobile phase (stock). Further dilutions are prepared in 5 replicates of of 5 µg/mL of Atorvastatin, 5 µg/mL of Ezitimibe and 80 µg/mL of Fenofibrate was made by adding 1 ml of stock solution to 10 ml of mobile phase.

Preparation of tablets 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 5 min and dilute to 100 ml with mobile phase (stock). Further dilutions are prepared in 5 replicates of of 5 µg/mL of Atorvastatin, 5 µg/mL of Ezitimibe and 80 µg/mL of Fenofibrate was made by adding 1 ml of stock solution to 10 ml of mobile phase

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

Result and Discussion

HPLC method development and optimization

Reversed-phase C18 column (Inertsil ODS 3 V, 5 µ, 250 mm × 4.6 mm) maintained at room temperature was used for the separation. The composition, pH of the mobile phase were changed to optimize the separation using main contens of the three compounds. A mobile phase consisting of various concentrations of Methanol-Acetonitrile-Water with gradient elution (Table 1) were selected.

Calculation

%Assay = AT/AS X WS/DS X DT/WT X P/100 X AW/LC X100

Where,

AS: Average peak area due to standard preparation

AT: Peak area due to assay preparation

WS: Weight of At, Ez and Fe in mg

WT: Weight of sample in assay preparation

DT: Dilution of assay preparation

P: % Purity of working standard

AW: Average Weight

LC: Label Claim

DS: Dilution of standard solution

Trial-1	Ammonium.Acetate Buffer+Methanol(50:50)
Trial-2	Phosphate Buffer+ACN(50:50)
Trial-3	Phosphate Buffer+ACN+Methanol(20:40:40)
Trial-4	Phosphate Buffer+ACN(40:60)
Trial-5	Methanol+ACN (80:20)
Trial-6	Methanol+ACN+Water
(Optimized trial)	(80:10:10)

Table 1: Method development.

The chromatogram obtained below is the optimized trial which was obtained at Ph 4.5 were there is a good resolution between the peaks and tailing was within the limits (Figure 2).
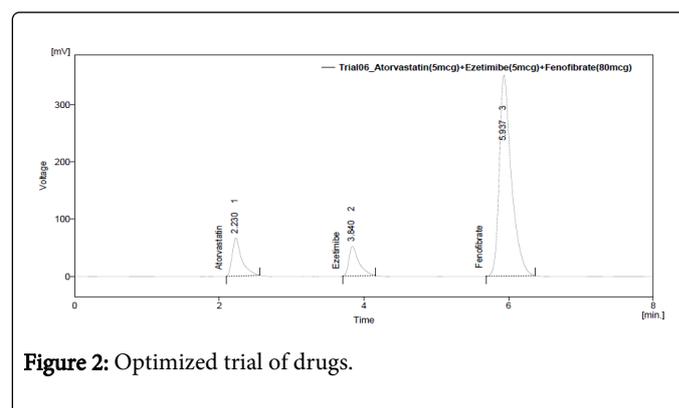

Figure 2: Optimized trial of drugs.

Validation

The analytical method was validated with respect to parameters such as linearity & range, limit of quantification, limit of detection (LOD), precision, accuracy, and robustness [9-13].

Linearity and Range

Linearity was observed in the range 3-7 µg/ml for Atorvastatin (r^2=0.9985), 3-7 µg/ml for Ezetimibe (r^2=0.9971) and 48-112 µg/ml for Fenofibrate (r^2=0.9964).

The relationship between the concentration (%) of Atorvastatin, Ezitimibe and Fenofibrate and area of Atorvastatin, Ezitimibe and Fenofibrate should be linear in the specified range and the correlation should not be less than 0.99 (Figure 3).
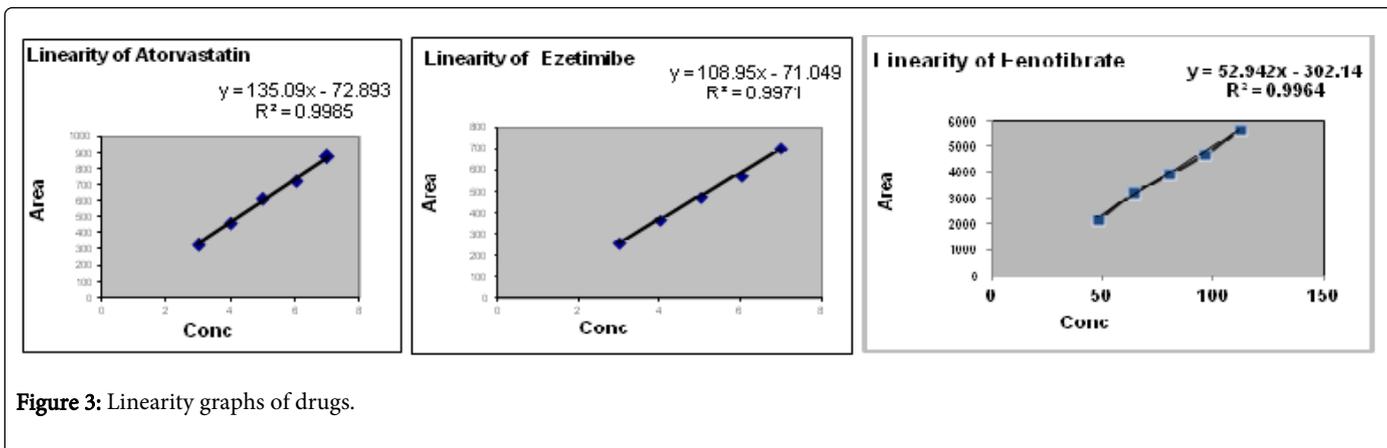
Figure 3: Linearity graphs of drugs.

Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (preanalysed 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.

To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 80%, 100%, 120% by adding 5% of standard drug solution in each level (Table 2).

Drug	Amount Taken(mcg/ml)	Amount Recovered	% Recovery	Avg % Recovery
Atorvastatin	5	5.09	101.78	100.79%
	6	5.92	98.73	
	7	7.13	101.87	
Ezetimibe	5	5.03	100.51	100.52%
	6	5.96	99.34	
	7	7.12	101.71	
Fenofibrate	80	81.55	101.94	100.58%
	96	94.44	98.38	
	112	113.61	101.43	

Table 2: Accuracy percentage of drugs.

Precision

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

The % Relative standard deviation of Assay preparations of Atorvastatin, Ezitimibe and Fenofibrate should be not more than 2.0% (Table 3) [14].

Drug	Average		Std Deviation		% RSD	
Atorvastatin	Rt	Area	Rt	Area	Rt	Area
	2.1565	576.29	0.0186	2.507	0.86	0.43
Ezetimibe	Rt	Area	Rt	Area	Rt	Area
	3.836	463.68	0.008	1.174	0.22	0.25
Fenofibrate	Rt	Area	Rt	Area	Rt	Area
	5.941	4051.8	0.014	12.683	0.23	0.31

Table 3: Precision of drugs.

Limit of Detection

LOD = $3.3\sigma/S$

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. The LOD for this method was found to be 0.04 µg/ml & area 5.22 for Atorvastatin and 0.05 µg/ml & area 5.23 for Ezitimibe, 1.58 µg/ml and area 83.64 for Fenofibrate (Table 4) [15].

Drug	Std Deviation	Slope
Atorvastatin	1.62	135.1
Ezetimibe	1.58	108.95
Fenofibrate	25.29	52.94

Table 4: Limits of detection.

Limit of Quantification

LOQ = $10\sigma/S$

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.

The LOQ for this method was found to be 0.12 µg/ml & area 15.82 for Atorvastatin and 0.15 µg/ml & area 15.83 for Ezitimibe, 4.78 µg/ml & area 253.45 for Fenofibrate.

Robustness

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.

The system suitability should pass as per the test method at variable conditions.

Ruggedness

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

The % Relative standard deviation of Assay values between two analysts should be not more than 2.0%.

Conclusion

"Development and validation of RP-HPLC method for the simultaneous estimation of Atorvastatin, Ezetimibe and Fenofibrate in bulk and Pharmaceutical dosage forms" with the facilities and the results are incorporated in this paper [16].

In conclusion a validated RP-HPLC method has been developed for determination of Atorvastatin, Ezetimibe and Fenofibrate in 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.

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