



SIMULTANEOUS ESTIMATION OF ATORVASTATIN CALCIUM, EZETIMIBE AND FENOFIBRATE IN PURE AND COMBINED TABLET DOSAGE FORM BY UV SPECTROPHOTOMETRY

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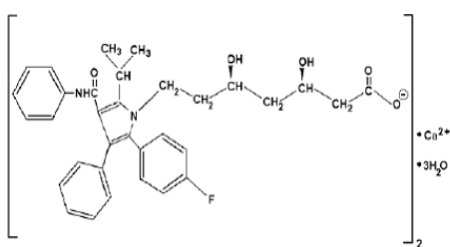
ABSTRACT

A simple, precise, accurate, rapid and economical spectrophotometric method requiring no prior separation have been developed for simultaneous estimation of Atorvastatin calcium, Ezetimibe and Fenofibrate in pure and in combined tablet dosage form have been developed. The method employs formation and solving of simultaneous equations using analytical wavelengths selected for simultaneous equation method were 247 nm for Atorvastatin calcium, 233 nm for Ezetimibe and 287 nm for Fenofibrate. These three drugs shows Beer's Law limit in the range of 5-30 mcg/mL.

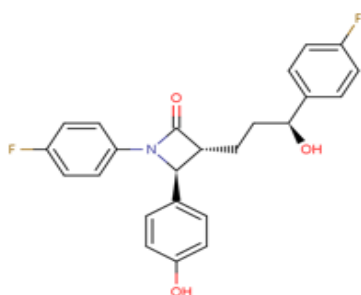
Keywords: Atorvastatin calcium, Ezetimibe, Fenofibrate, UV spectrophotometry, Simultaneous equation method.

INTRODUCTION

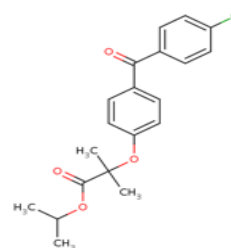
Atorvastatin calcium (ATR) HMG–CoA reductase inhibitor shows major effect by reduction of LDL levels. Chemically, ATR is [R-(R*, R*)]-2-(4-fluorophenyl)-β, δ-dihydroxy-5(1-methylethyl)-3-phenyl-4-[(phenylamino) carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate. Ezetimibe (EZE) is an azetidione-based cholesterol absorption inhibitor that blocks the intestinal absorption of cholesterol. Chemically, EZE is (3R, 4S)-1-(4-fluorophenyl)-3-[(3S)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl) azetidion-2-one. Fenofibrate (FEN) is a fibric acid derivative which has greater LDL cholesterol lowering action. Chemically, FEN is propan-2-yl 2-[4-(4-chlorobenzoyl) phenoxy]-2-methylpropanoate.¹⁻³ Figure 1 represents the molecular structure of three drugs individually.



Atorvastatin calcium



Ezetimibe



Fenofibrate

Figure 1: Chemical structures of Atorvastatin calcium (ATR), Ezetimibe (EZE) and Fenofibrate (FEN).

Literature survey revealed that few methods were available for estimation of ATR, EZE and FEN individually as well as with other combinations⁴⁻⁷. So far no method was available for estimation of ATR, EZE and FEN by UV Spectrophotometric method in combined dosage forms. Hence, an attempt has been made to develop simple, precise, accurate, rapid and economical Spectrophotometric method for the simultaneous estimation of ATR, EZE and FEN in pure and in tablet dosage form by simultaneous equation method.

MATERIALS AND METHODS

Instruments

- (1) Shimadzu 1700 Double Beam UV- Visible spectrophotometer with spectral band width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cells.
- (2) Shimadzu AUX 220 digital balance
- (3) Sonicator sonica ultrasonic cleaner model 2200 MH

Chemicals and reagents

Methanol (AR grade) was procured from Loba Chemie India Limited.



Drug samples

Standard ATR, EZE and FEN samples were obtained as gift samples from Lee Pharma Ltd., Hyderabad.

Marketed Formulations

Fibator^R EZ tablets containing Atorvastatin calcium equivalent to 10mg of Atorvastatin, 10 mg of Ezetimibe and 160mg of Fenofibrate was procured from local pharmacy which belongs to Sun Pharmaceutical industries.

PROCEDURE

Simultaneous equation method

Pure drug sample of ATR, EZE and FEN were dissolved separately in methanol so as to give several dilutions in the concentration of 5-30 mcg/mL of ATR, EZE and FEN. The dilutions were scanned in the wave-length range of 400-200 nm. Figure 2 represents the overlain spectra of these three drugs. Wave-lengths selected for the formation of simultaneous equations were 233, 247 and 287 nm (λ_{\max} of individual drugs respectively) and absorptivity (a) values determined for ATR at 233, 247 and 287 nm were 25.75, 40.5 and 16.5 while respective values for EZE were 35.16, 28.75 and 10.0 and for FEN were 23.50, 24.75 and 44.91 at 233, 247 and 287 nm respectively. These values are the mean of six independent determinations. The simultaneous equations formed were

At λ_1

$$A_1 = a_{x_1}bc_x + a_{y_1}bc_y + a_{z_1}bc_z \quad (1)$$

At λ_2

$$A_2 = a_{x_2}bc_x + a_{y_2}bc_y + a_{z_2}bc_z \quad (2)$$

At λ_3

$$A_3 = a_{x_3}bc_x + a_{y_3}bc_y + a_{z_3}bc_z \quad (3)$$

Where A_1 , A_2 and A_3 are the absorbances of sample solution at 233, 247 and 287 nm respectively. C_x , C_y and C_z were the concentrations (mcg/mL) of ATR, EZE and FEN respectively in sample solution. The absorbances (A_1 , A_2 and A_3) of the sample solutions were recorded at 233, 247 and 287 nm respectively and concentration of these three drugs were calculated using above mentioned equations (1), (2) and (3).

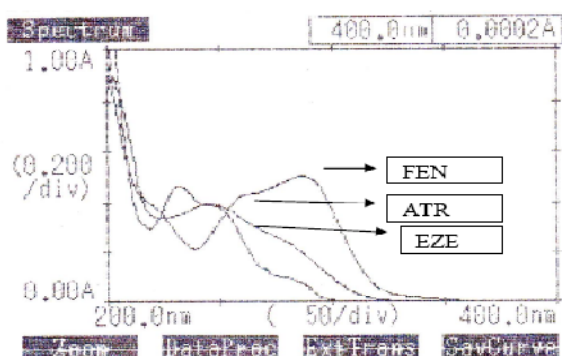


Figure 2: Overlain Spectrum of Atorvastatin calcium, Ezetimibe and Fenofibrate in Methanol (10µg/ml)

Procedure for Analysis of Tablet Formulation

Twenty tablets of formulation were weighed accurately, the average weight of each tablet was determined and then ground to a fine powder. A quantity equivalent to 50 mg of FEN was transferred to 100 ml volumetric flask. Accurately weighed 47.1 mg of standard drug sample of ATR and EZE was transferred to same volumetric flask. The contents were shaken with methanol to dissolved the tablet powder and made to volume with same (500 mcg/mL) and sonicated for 15 min and filtered through Whatmann filter paper No. 41. From the clear solution, further dilutions were made with methanol to give the dilution (12 mcg/mL) within the Beer's Law limit. Absorbances of this solution were measured at 233, 247 and 287 nm respectively and concentrations of three drugs in the sample solution were determined using equations (1), (2) and (3). Results of the analysis of tablet formulation were reported in Table I.

Validation of the Method according to ICH Guidelines^{8,9}

Validation of the method was done according to ICH guidelines for Simultaneous Equation method.

Linearity

The linearity of the method is its ability to elicit test results that are directly proportional to the concentration of the analyte in the samples. ATR was linear with the concentration range of 5 – 30 mcg/mL at 247 nm. EZE showed the linearity in the range of 5 – 30 mcg/mL at 233 nm and FEN was linear in the concentration range of 5 – 30 mcg/mL at 287 nm.

Precision

The repeatability of the method was confirmed by the analysis of formulation was repeated for 6 times with the same concentration. The amount of each drug present in the tablet formulation was calculated. The percentage RSD was calculated. The intermediate precision of the method was confirmed by intraday and inter day analysis i.e. the analysis of formulation was repeated three times in the same day and on three successive days. The concentrations of sample solution were analyzed and percentage RSD also calculated. % RSD was found to be less than 2%.

Ruggedness

Ruggedness of the method was confirmed by the analysis of formulation was done by the different analysts and instruments. The amount and % RSD were calculated.

LOD and LOQ

The linearity study was carried out for six times. The LOD and LOQ were calculated by using the average of slope and standard deviation of intercept.

Accuracy (Recovery studies)

Accuracy of the method was confirmed by recovery studies. To the 50% preanalyzed formulation, a known quantity of raw materials of ATR, EZE and FEN were



added and the procedure was followed as per the analysis of formulation. The amount of each drug recovered was calculated. This procedure was repeated for three times for each concentration. The % RSD was calculated.

RESULTS AND DISCUSSION

In Simultaneous equation method, three wavelengths of respective absorbance maxima i.e. 247 nm for ATR, 233 nm for EZE and 287 nm for FEN were used for the analysis of analytes. The criteria for obtaining maximum precision, by this method were calculated and found to be outside the range of 0.1- 2. The proposed method was found to

be simple, sensitive, accurate, precise, economical and rapid for the routine simultaneous estimation of ATR, EZE and FEN.

The percentage label claim present in tablet formulation was found to be 99.51, 99.85 and 99.18 for ATR, EZE and FEN, respectively. The amount present in tablet formulation was in good concord with the label claim and the % RSD values were found to be 0.53326, 0.82524 and 0.41936 for ATR, EZE and FEN, respectively. The low % RSD values indicate that the method has good precision. The results of analysis are shown in Table I.

Table I: Analysis of Tablet Formulation (Fibator^R EZ) by Simultaneous Equation Method

Drug	Sample No.	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained*	Average (%)	S.D	% R.S.D.	S.E.
ATR	1	10	10.0	100.00	99.51	0.53065	0.53326	0.21663
	2	10	9.90	99.00				
	3	10	10.0	100.00				
	4	10	9.90	99.00				
	5	10	9.91	99.10				
	6	10	10.0	100.00				
EZE	1	10	9.90	99.00	99.85	0.82401	0.82524	0.33639
	2	10	9.90	99.00				
	3	10	10.08	100.8				
	4	10	9.95	99.5				
	5	10	10.0	100.00				
	6	10	10.08	100.8				
FEN	1	160	158.2	98.87	99.18	0.41593	0.41936	0.16980
	2	160	158.5	99.06				
	3	160	158.4	99.00				
	4	160	158.6	99.12				
	5	160	158.5	99.06				
	6	160	160.0	100.00				

* Mean of six observations

Table II: Intra day and Inter day Analysis of Formulation (Fibator^R EZ) by Simultaneous Equation Method

Drug	Sample No.	Labeled amount (mg/tab)	Percentage obtained*		S.D		% R.S.D.	
			Intra day	Inter day	Intra day	Inter day	Intra day	Inter day
ATR	1	10	99.9	100.0	0.50537	0.50732	0.50745	0.507022
	2	10	98.9	99.87				
	3	10	99.87	99.60				
	4	10	98.99	99.90				
	5	10	100.01	99.96				
	6	10	99.89	101.06				
	Mean		99.59	100.06				
EZE	1	10	98.85	98.8	0.71390	0.87294	0.71850	0.87042
	2	10	98.18	101.0				
	3	10	99.8	101.12				
	4	10	99.50	100.06				
	5	10	99.87	100.00				
	6	10	100.01	100.8				
	Mean		99.36	100.29				
FEN	1	160	100.0	100.8	0.55045	0.57173	0.55238	0.57224
	2	160	99.0	99.89				
	3	160	100.0	99.0				
	4	160	99.1	100.02				
	5	160	100.0	99.87				
	6	160	99.0	99.90				
	Mean		99.65	99.91				

* Mean of Six Observations



Further the precision of the method was confirmed by Intraday and Inter day analysis. The analysis of formulation was carried out for three times in the same day and one time in the three consecutive days. The % RSD value of intraday and inter day analysis were found to be 0.50745 and 0.57022 for ATR, 0.71850 and 0.870424 for EZE and 0.55238 and 0.572249 for FEN. The results of analysis are shown in Table II.

The developed method was validated for Ruggedness. In the present work it was confirmed by different analysts. The % RSD value by analyst 1 and analyst 2 were found to be 0.6370 and 0.9271 for ATR, 0.7523 and 0.9047 for EZE and 1.5528 and 0.7113 for FEN, respectively. The % RSD

value by instrument1 and instrument 2 were found to be 0.3181 and 0.4185 for ATR, 0.2754 and 0.2410 for EZE and 0.3939 and 0.4385 for FEN, respectively. The low % RSD values indicate that the developed method was more rugged. The results are shown in Table III.

The accuracy of the method was performed by recovery studies. The absorbance of the solutions was measured and the percentage recovery was calculated. The percentage recovery was found to be in the range of 101.5 - 101.9% for ATR, 96.49 - 100.16% for EZE and 100.63 - 101.05% for FEN. The low % RSD value for three drugs indicates that this method is very accurate. The recovery data is shown in Table IV.

Table III: Ruggedness Study (Fibator^R EZ) by Simultaneous Equation Method

Drug	Condition	Average* % Obtained	S.D	% R.S.D	S.E.
ATR	Analyst 1	100.22	0.6384	0.6370	0.3685
	Analyst 2	99.53	0.9228	0.9271	0.5327
	Instrument 1	100.58	0.3200	0.3181	0.1306
	Instrument 2	100.92	0.4223	0.4185	0.1723
EZE	Analyst 1	99.57	0.7490	0.7523	0.4324
	Analyst 2	99.47	0.9000	0.9047	0.5196
	Instrument 1	100.55	0.2756	0.2754	0.1125
	Instrument 2	100.66	0.2426	0.2410	0.0972
FEN	Analyst 1	99.87	1.5509	1.5528	0.8954
	Analyst 2	99.47	0.7076	0.7113	0.4085
	Instrument 1	100.87	0.3973	0.3939	0.1622
	Instrument 2	100.58	0.3505	0.4385	0.1431

* Mean of three observations

Table IV: Recovery Study Data of Formulation (Fibator^R EZ) by Simultaneous Equation Method

Drug	Percentage	Amount present* (mcg/mL)	Amount added (mcg/mL)	Amount estimated* (mcg/mL)	Amount recovered* (mcg/mL)	% Recovery*	S.D	% R.S.D	S.E.
ATR	25	5.955	3.0	9.013	3.06	101.9	0.4808	0.4718	0.2776
	50	5.941	6.0	12.026	6.093	101.5	0.4636	0.4568	0.2667
EZE	25	5.946	3.0	8.8416	2.895	96.49	1.7616	1.8257	1.0171
	50	5.946	6.0	11.956	6.01	100.16	1.0850	1.0832	0.6264
FEN	25	5.9483	3.0	8.98	3.031	101.05	1.3489	1.3349	0.7788
	50	5.9483	6.0	11.98	6.038	100.63	0.9190	0.9133	0.5306

* Mean of Three Observations

CONCLUSION

The developed UV spectroscopic method was validated and the statistical validation was performed with the simplicity and ease of operation. The method described in this paper for the simultaneous estimation of ATR, EZE and FEN was found to be simple, sensitive, accurate, precise, rapid and economical. The validated method can successfully used for routine analysis of ATR, EZE and FEN in bulk and tablet dosage formulation.

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