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Development and validation of stability indicating HPLC method for ezetimibe and atorvastatin calcium in pharmaceutical dosage form

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ABSTRACT

A sensitive, selective, precise and stability indicating method of Ezetimibe and Atorvastatin Calcium in pharmaceutical dosage form was developed and validated. Reverse phase isocratic -HPLC with scanning by photo-diode array detection technique was used to analyze samples formed during stress testing and to determine the peak purity of active. The separation was achieved on a Hypersil BDS C18 column, 150 mm × 4.6 mm, 5µm using mobile phase 400 ml of MeCN with 600 ml of 0.1M buffer at a flow rate of 1.0 mL min^{-1}. The column was maintained at 40^0C and detection wavelength set to 240 nm. The injection volume was 20µL. The linear regression analysis data for the calibration plots showed good linear relationship with r^2 = 0.9989 (for Ezetimibe) and 0.9997 (for Atorvastatin Calcium) in the concentration range of 10 to 200µg mL^{-1}. The method was validated in terms of accuracy, precision, and linearity, limit of detection and quantitation and robustness. This method has been successively applied to pharmaceutical formulation and no interference from the tablet excipients was found. Ezetimibe and Atorvastatin Calcium their combination drug product were exposed to acid, base and neutral hydrolysis, oxidation, dry heat and photolytic stress conditions and the stressed samples were analyzed by the proposed method. All the peaks of degraded product were resolved from the standard drug with significantly high resolution. This indicates that the method is reproducible and selective for the estimation of the said drug and can be used as stability-indicating method.

KEYWORDS

Ezetimibe;
Atorvastatin calcium;
Stability indicating;
Validation;
Liquid chromatography.

INTRODUCTION

Atorvastatin (ATC) is 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) a selective HMG-CoA reductase inhibitor[1], causes decrease in intracellular cholesterol levels and an increased clearance of LDL cholesterol in plasma. Several methods for its estimation using HPLC[2-3], HPTLC[4], GC-MS[5], LC-MS[6] and HPLC-Electro spray tandem mass spectrometry[7] are reported.

Ezetimibe (EZ) chemically 1-(-4-flurophenyl)-(3S)-hydroxypropyl]-(4S)-(4-hydroxyphenyl)-2-azeti-

dinone[8] is a selective cholesterol absorption inhibitor, which potently and selectively prevents absorption of cholesterol through the intestinal wall. Very few methods are reported as a validated RP –HPLC[9], spectrophotometric method using floin-Ciocalteu reagent[10].

ATC when used in combination with EZ causes manifold reduction in LDL cholesterol levels as compared to double the dose of the individual drug when used alone. Both these drugs in combination are not official in Indian Pharmacopoeia, British Pharmacopoeia, United States and European Pharmacopoeia. At present few HPLC and UV spectrophotometric methods are reported for the simultaneous estimation of ATC and EZ in tablet formulation[11-12]. These analytical procedures however are not reported as stability indicating.

Safety and efficacy of pharmaceuticals are fundamental issues of importance in drug therapy. Instability of pharmaceuticals may be caused by change in physical, chemical, pharmacological and toxicological properties. The objective of the present study was to develop and validated stability-indicating method for Ezetimibe and Atorvastatin Calcium in pharmaceutical dosage form- tablet.

EXPERIMENTAL

Chemical and reagents

ATC and EZ working standard was supplied by Ipca laboratories ltd, Mumbai India. All chemicals and reagents used were of analytical grade and were purchased from Merck Chemicals, India. The pharmaceutical dosage form of ATC and EZ, ATOCOR-E® film coated tablets, a formulation with a 10mg label claim each, was manufactured by Dr. Reddy's Laboratories limited.

Instrument and equipment

The LC system used for method development, Forced degradation studies and method validation was a Waters 2695 separation module equipped with a Photo diode array detector. The output signal was monitored and processed using Empower software. Photo stability studies were carried out in Newtronic chambers equipped with an illumination of two UV and four fluorescent lamps in accordance to Q1B Option 2.[13].

Chromatographic conditions

The chromatographic separation was achieved on a Hypersil BDS C18 column, 150 mm × 4.6 mm, 5 μm (Chromatopak, India) in isocratic mode using Mobile phase 400 ml of MeCN with 600 ml of buffer (6.8 g of potassium dihydrogen phosphate dissolve in 1000 ml water; 0.1 M) at a flow rate of 1.0 mL min^{-1}. Before delivering into the system the mobile phase was filtered through 0.45μ membrane filter and degassed using vacuum and ultrasonication. The column was maintained at 40^0C and λ detection set to 240 nm. The injection volume was 20μL.

RESULTS AND DISCUSSION

Method development

A variety of mobile phases were investigated in the development of an HPLC SIM suitable for analysis of EZ and ATC in tablet dosage form. Preliminary trials using mobile phases of different composition of water: acetonitrile resulted in poor peak shape. Since both drug showed wide difference of pKa value EZ being 9.55 and of ATC 4.5 did not help much for deciding pH range to be worked on. When phosphate buffer and acetonitrile combination were tried using 25 cm × 4.6 mm, 5μm C18 column longer retention of the EZ was observed. To decrease the retention column oven at 40^0C was used, these gave good resolution between EZ and ATC; however acidic hydrolysis of ATC resulted in long eluting degradant with chromatographic setup. Hence it was thought worthwhile to replace 25 cm length column with smaller length 15 cm × 4.6 mm, 5 μm Hypersil C18. This resulted in decrease runtime of 20 min within which all degradant were eluted without compromising resolution between EZ and ATC. Both the drug substance was easily extracted from the pharmaceutical dosage form by use of diluent water: methanol in ratio (60:40 % v/v). Detection wavelength was set to 240 nm based on UV spectra of both the drugs. (Figures 1,2).

Method validation

The method was found to be linear over the range of 10 μg mL^{-1} to 20μg mL^{-1} for both EZ and ATC hav-

ing correlation coefficient as 0.9989 and 0.9997 respectively. Accuracy was studied as % recovery and was found to be 100.6 % for EZ and 100.8 % for ATC. Precision of the method was studied as repeatability and intermediate precision the mean assay values (n = 12) for EZ was 100.1 % with 0.7 % RSD between the results and ATC was 99.2% with 0.9 % RSD between the result. These values suggest high precision of the method. Also the method was found to be robust for small and deliberate changes in pH of mobile phase composition, column oven temperature and flow rate TABLE 1.

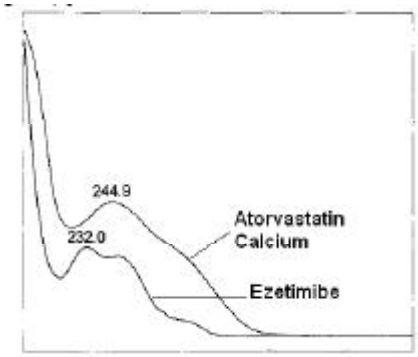

Figure 1 : Spectral overlay of both the drugs

In order to establish whether the proposed method was stability-indicating, pure API of EZ, ATC, combination EZ and ATC, tablet was stressed under various conditions such as exposure to oxidation (3 % H_2O_2), thermal (7days under dry heat) and Photo stability degradation (UV exposure), hydrolysis of individual active solution, in combination and sample to acid (0.5 M HCl), base (0.5 M NaOH) , throughout the forced degradation studies peak of the active where found to be pure,

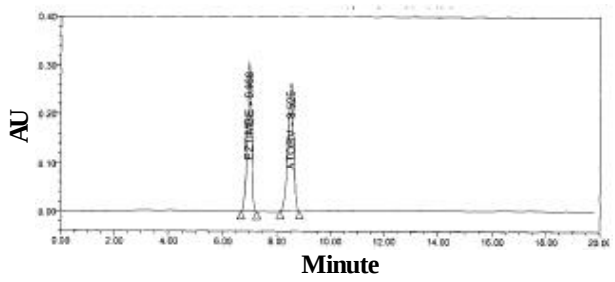

Figure 2: Chromatogram of standard showing resolution between the two drugs

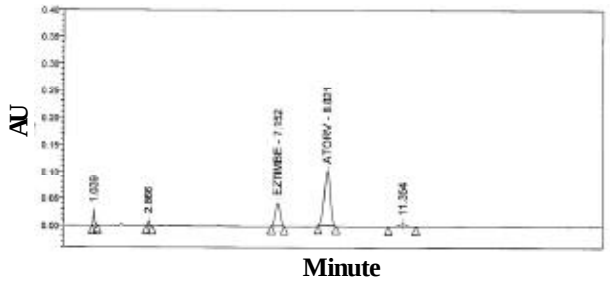

Figure 3: Chromatogram of tablet exposed to alkali hydrolysis for 20 mins

TABLE 1 : Robustness study

Robustness parameters	% Assay		Tailing factor		Resolution (USP) between EZ and ATC
	EZ	ATC	EZ	ATC	
Change in temperature					
(a) 38^{0}C	(a) 99.8	(a) 99.2	(a) 1.3	(a) 1.5	(a) 3.5
(b) 42^{0}C	(b) 99.4	(b) 99.9	(b) 0.9	(b) 1.1	(b) 5.1
Change in column (Waters Symmetry C18)	99.9	100.1	0.9	1.5	5.1
Change in acetonitrile composition					
(a) 8 % v/v	(a) 100.4	(a) 99.6	(a) 1.1	(a) 1.5	(a) 5.4
(b) 12 % v/v	(b) 100.8	(b) 99.4	(b) 1.2	(b) 1.1	(b) 2.9
Change in flow rate					
(a) 0.8 mL min $^{-1}$	(a) 99.6	(a) 100.3	(a) 1.1	(a) 1.5	(a) 4.5
(b) 1.2 mL min $^{-1}$	(b) 99.1	(b) 99.1	(b) 1.1	(b) 1.2	(b) 4.4

TABLE 2 : Results from forced degradation study

Stress conditions	Time	(%) Assay of active		% Total impurities		Mass Balance (% assay + % Total impurities)	
		EZ	ATC	EZ	ATC	EZ	ATC
Base/0.5 N NaOH/75^{0}C	20 min	69.2	74.5	31.4	25.1	100.6	99.6
Acid/0.5 N HCl /75^{0}C	20 min	37.0	97.3	63.9	3.0	99.9	100.3
Oxidation/ 3 % H_2O_2/60^{0}C	60 min	67.4	97.7	32.8	1.6	100.2	99.3
Photostability / illumination of UV and fluorescent light	1.2 million lux h	100.2	96.3	0.1	3.4	100.3	99.7

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TABLE 3 : Summarized validation parameters of proposed RP-HPLC method

Parameters	EZ	ATC
Retention time (min)	7.14	8.85
Tailing factor	0.92	0.77
Resolution (USP)	-	4.95
Linearity range (μg mL^{-1})	10- 200	10- 200
LOD (μg mL^{-1})	0.025	0.01
LOQ (μg mL^{-1})	0.06	0.05
Regression equation (y*) Slope (b)	2922.1	32308.3
Intercept (a)	+155865.2	+ 151147.8
Correlation coefficient (r)	0.9989	0.9997
Method precision Mean % Assay	100.1	99.2
(CV, %) (n = 12)	0.7	0.9
Accuracy /Recovery at 80% level		
(a) Recovery	(a) 100.7	(a) 100.9
(b) % RSD	(b) 0.34	(b) 0.48
At 100 % level (a) Recovery	(a) 100.5	(a) 100.9
(b) % RSD	(b) 0.78	(b) 0.32
At 120 % level (a) Recovery	(a) 100.6	(a) 100.7
(b) % RSD	(b) 0.39	(b) 0.4

y* = a + bc, where c is the concentration

confirming stability indicating nature of the method TABLE 2 and figure 3 shows the typical chromatogram of tablet exposed to Alkali hydrolysis for 20 min.

CONCLUSION

Cost effective stability indicating method for Ezetimibe and Atorvastatin Calcium was developed and validated to deliver safe and efficacious product into the market along with fulfilling medical needs.

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