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SIMULTANEOUS QUANTITATIVE DETERMINATION OF ATORVASTATIN CALCIUM AND EZETIMIBE BY REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

Atorvastatin calcium and Ezetimibe both active ingredients are not official in any pharmacopoeia. The aim of our present work is to develop a precise and validated RP-HPLC method for the simultaneous determination of Atorvastatin and Ezetimibe in tablet formulation. The quantification was carried out by using Kromasil C-18 (250X4.6 mm), 5µm column in isocratic mode with mobile phase, Buffer: Acetonitrile (50:50). The flow rate was 1.2 ml/min. The peak purity of Atorvastatin and Ezetimibe were 1.000 and 1.000 respectively. Ruggedness and robustness of method were performed and the percentage relative standard deviation (RSD) was found below 2.0%. The percentage recovery was found in the range of 98% to 102% at three different levels. Calibration curves were linear over studies ranges with correlation co-efficient found between the range of 0.99 to 1.00. Sample and standard solution stability study was performed over 22 h at room temperature and found stable. The percentage deviation was below 2.0%. All the validation parameters were within the acceptance range according to ICH norms. The developed method was successfully applied to estimate the amount of both the components simultaneously in commercial tablet dosage form.

Keywords: Atorvastatin; Ezetimibe; Reversed-phase HPLC; HMGCo-A reductase.

INTRODUCTION

IN ATORVA-E tablets a fixed dose combination of two lipids lowering drugs is present i.e. Atorvastatin Calcium and Ezetimibe. Atorvastatin is used to treat Hyperlipidemia [1]. It is a synthetic lipid-lowering agent, which inhibits the enzyme 3-hydroxy3-methylglutaryl-coenzymeA (HMGCo-A) reductase. This enzyme catalyzes the conversion of HMGCo-A to

Mevalonate, an early and rate determining step in Cholesterol synthesis [2-3]. On the other hand Ezetimibe is in a class of lipid lowering compound that selectively inhibits the intestinal absorption of Cholesterol & related phytosterol. Ezetimibe does inhibit Cholesterol synthesis in liver but localize and appear to act at the brush border of the small intestine and inhibit the absorption of Cholesterol, which leads to decrease in the delivery of intestinal Cholesterol to the liver [4-7]. Atorvastatin and Ezetimibe tablet in combination are indicated as an adjunctive therapy to diet for the reduction of elevated total Cholesterol, LDL-C, and TG and also to decrease the hepatic Cholesterol stored and increase in clearance of Cholesterol from blood. Also co-administration of Statin with Ezetimibe could significantly reduce the risk of coronary heart disease event in patient with Hypercholesterolemia [8,9].For the quantitative determination of individual component RP-HPLC method has been reported in literature [10,11] and also for atrovastatin in combination with amlodipine [12]. But literature survey did not reveal any reported method for the simultaneous estimation of Atorvastatin Calcium and Ezetimibe in combination. Hence, there is an immense need to develop a sensitive, specific and validated analytical method for the routine analysis of the active drug in Pharmaceutical dosage forms. Therefore, in the proposed work, a successful attempt has been made to develop and validate [13,14] analytical method with due consideration of accuracy, sensitivity, rapidity, economy and simplicity.

LDL-C: - Low-density Lipoprotein – cholesterol.

TG: - Triglycerides.

Apo-B: - Apolipoprotein -B

MATERIAL AND METHODS

Chemicals and Materials:

Cadila Healthcare Limited, Ankleshwar and Dr. Reddy's Laboratories supplied Atorvastatin calcium and Ezetimibe respectively. Acetonitrile (HPLC grade) and Ammonium acetate were purchased from Spectrochem and E-Merck limited respectively. In-house purified water (USPgrade) was used throughout the study.

Instrumentation: The chromatographic separations were performed using Shimadzu LC 2010C integrated system equipped with quaternary gradient pump, 2010C UV-VIS detector, 2010C Column Oven and 2010C programmable auto sampler controlled by CLASS-VP software. The Kromasil C-18 (250X4.6 mm), 5µm was used as a stationary phase. The system suitability result displayed in Table I was evaluated throughout the study.

Table 1.
SYSTEM SUITABILITY AND SYSTEM PRECISION

Compound	Retention time (Mean ± SEM)	n	k'	R	T	α	
Atorvastatin			3.62 ± 0.0016				
Ezetimibe	9.20 ± 0.00	14456	4.10	9.26	1.03	4.06	

n= Theoritical plates
k'= Capacity Factor
R= Resolution
T= Asymetry
α = Selectivity

HPLC Condition:

Column	Kromasil C-18 (250X4.6 mm), 5µm
Detector	238 nm
Injection volume	20 µl
Flow rate	1.2 ml/min
Temperature	30°
Run time	20 min
Mobile phase (50:50)	Buffer: Acetonitrile

Buffer preparation:

Weigh 0.8-g Ammonium acetate in to 1.0 l volumetric flask. Then add 200-ml HPLC grade water, shake well and make volume up to mark with HPLC grade water.

Diluent:

Use Water: Acetonitrile (30:70) as a diluent

Standard preparation:

Standard stock solutions were prepared in diluent and further for second dilution, dilute it with diluent to make final concentration Atorvastatin 10 µg and Ezetimibe 10 µg respectively.

Sample preparation:

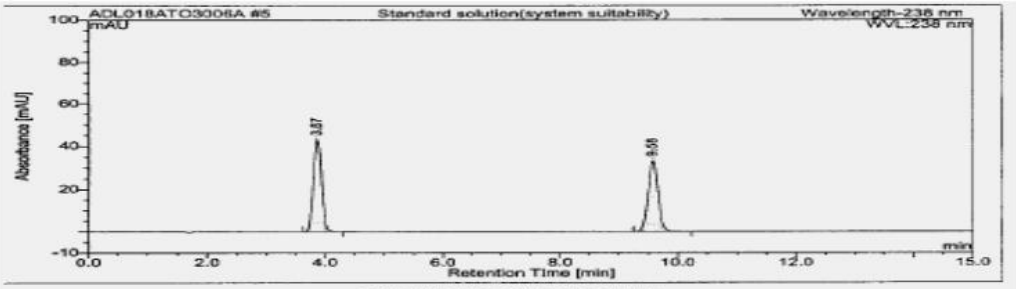
Weigh accurately tablets powdered equivalent to about 50 mg of Atorvastatin and Ezetimibe in to 200-ml volumetric flask. Add about 125-ml diluent and sonicate it for 30 minute to dissolve. Filtered it through 0.45 µ HVLP nylon filter and made further dilution 2.0 ml to 50.0 ml with diluent.

RESULTS

The detection wavelength was chosen at 238 nm because the Atorvastatin and Ezetimibe in tablet dosage form have better absorption and sensitivity at this wavelength. Atorvastatin tablets [8]. However, to achieve the better separation of Atorvastatin and Ezetimibe in the present combination, the mobile phase chromatogram was shown in Fig. 1(a), (b) and (c), which illustrate the separation of both active ingredients in this system. The isocratic program throughout HPLC method was adopted to analyze both components in a short single run time. The proposed method is simple and

economic, which don't require extraction or separation of the analyte.

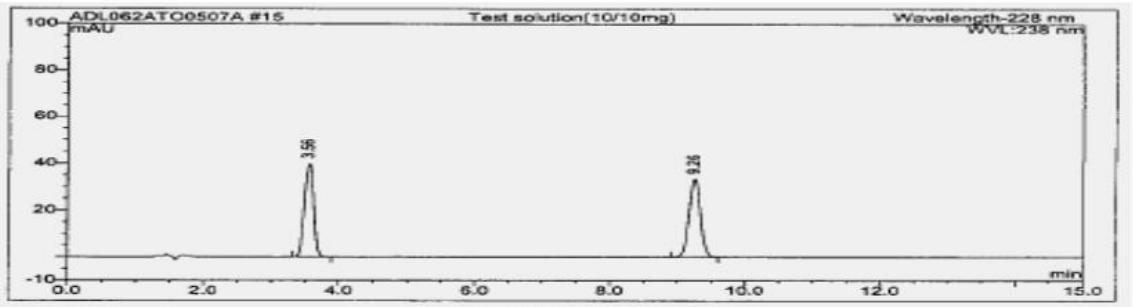
Fig. 1(a).



SYSTEM SUITABILITY RESULT

No.	Ret.Time min	Peak Name	Type	Plates USP	Tailing USP	Resolution USP	K' USP	Area mAU*sec
1	3.87	Atorvastatin	BMB	3915	1.05	n.a.	n.a.	400.557
2	9.58	Ezetimibe	BMB	14456	1.03	20.16	n.a.	397.601
Total:								798.158

Fig. 1(b).



PEAK RESULT

No.	Ret.Time min	Peak Name	Type	Height mAU	Area mAU*sec	Rel.Area %
1	3.56	Atorvastatin	BMB	39.908	369.122	49.26
2	9.26	Ezetimibe	BMB	33.334	380.226	50.74
Total:					749.348	100.00

Linearity and calibration curve:

The linearity of the calibration curve was determined by weighed (1/c) least square regression analysis. The correlation coefficient was found to be 0.99 to 1.00. A linear relationship was found for all components. The results of linearity, limit of detection and limit of quantification were presented in table 2.

Table 2. CHARACTERISTICS OF THE ANALYTICAL METHOD DERIVED FROM THE STANDARD CALIBRATION CURVE

Compound	LOD	LOQ	Linearity µg/ml µg/ml	Correlation range	Residual std. co-efficient	Slope of regression
Atorvastatin	0.038	0.095	5 to 14.5	0.99998	1.00016	41.94419
Ezetimibe	0.083	0.207	5 to 15	1.00000	0.45232	39.75519

LOD= Limit of detection *LOQ= Limit of quantification*

Specificity:

There was no interference from sample placebo and peak purity of Atorvastatin and Ezetimibe were 1.0000 and 1.0000. It showed that developed analytical method was specific for the analysis of Atorvastatin and Ezetimibe in tablet dosage form.

Standard and sample solution stability:

Standard and sample solution stability was evaluated at room temperature for 22 hrs. The relative standard deviation was found below 2.0%. It showed that both standard and sample solution was stable up to 22 h at room temperature.

Method precision:

The precision of the method was established by carrying out the analysis of the analyte (n=6) using the proposed method. The low value of standard deviation showed that the method was precise. The results obtained were presented in table 3.

Table 3. METHOD PRECISION

Compound	Concentration µg/ml (n=6)	Retention time Mean ± SEM (n=6)	% Assay Mean ± SEM (n=6)	% RSD of Assay
Atorvastatin	10	3.62 ± 0.0016	95.3 ± 0.1282	0.3
Ezetimibe	10	9.20 ± 0.0000	100.2 ± 0.1536	0.4

Recovery/Accuracy:

To ensure the reliability and accuracy of the method recovery studies were carried out at three different levels. The results of recovery studies were presented in table 4.

Table 4. METHOD ACCURACY

Level	Drug Added (mg)	Drug recovered (n=3)	% Assay (Mean ± SEM)	Assay (n=3)	% RSD of
For Atorvastatin					
50%	24.89	24.65	99.0 ± 0.0472		0.3
100%	49.46	49.05	99.1 ± 0.0199		0.2
150%					74.20
For Ezetimibe					
50%	25.05	25.24	100.8 ± 0.0693	0.9	
100%	50.00	50.29	100.6 ± 0.1033	0.3	
150%					

Method robustness:

Robustness of the method was determined by small deliberate changes in flow rate, mobile phase ratio and column oven temperature. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was robust. The results of robustness were presented in table 5.

Intermediate precision:

Intermediate precision test was determined between two different analysts, instruments and columns. The value of percentage RSD was below 2.0%, showed ruggedness of developed analytical method. The results of ruggedness were presented in table 6 [1], [2] and [3].

Table 5. METHOD ROBUSTNESS

Compound	**% RSD in Normal and Changed condition (n=5)**			
Temperature	**% RSD Normal**	**% RSD (-5°C)**	**% RSD (+5°C)**	
Atorvastatin	0.5		0.3	0.1
Ezetimibe	0.02		0.1	0.1
Mobile phase ratio	**% RSD Normal**	**% RSD (-0.2 unit)**	**% RSD (+0.2 unit)**	
Atorvastatin	0.5		0.02	1.4
Ezetimibe	0.02		0.03	0.9
Flow Rate	**% RSD Normal**	**% RSD (-10%)**	**% RSD (+10%)**	
Atorvastatin	0.5		0.05	0.1
Ezetimibe	0.02		0.03	0.1

Table 6. INTERMEDIATE PRECISION

Compound	**% Assay Mean ± SEM (n=6)**	**% RSD of Assay (n=6)**
Day 1	**Analyst-1, Instrument-1 & Column-1**	
Atorvastatin	95.3 ± 0.1282	0.3
Ezetimibe	100.2 ± 0.1192	0.4
Day 2	**Analyst-2, Instrument-2 & Column-2**	
Atorvastatin	95.6 ± 0.1536	0.3
Ezetimibe	101.4 ± 0.0816	

DISCUSSION

The method described enables to the quantification of Atorvastatin and Ezetimibe in film-coated tablets. The advantages lie in the simplicity of sample preparation and the low costs of reagents used. The proposed HPLC conditions ensure sufficient resolution and the precise quantification of the compounds.

Results from statistical analysis of the experimental results were indicative of satisfactory precision and reproducibility. Hence, this HPLC method can be used for analysis of commercial formulation.

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