

**Original Article**

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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), 5 $\mu$ m 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 atorvastatin 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 (USP grade) 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), 5 $\mu$ m 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 n (Mean $\pm$ SEM)	k'	R	T	$\alpha$
Atorvastatin		3.62 $\pm$ 0.0016			
Ezetimibe	9.20 $\pm$ 0.00	14456	4.10	9.26	1.03 4.06

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n= Theoretical plates  
k'= Capacity Factor  
R= Resolution

T= Asymetry  
 $\alpha$  = Selectivity

#### HPLC Condition:

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

#### 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  $\mu$  HVLP nylon filter and made further dilution 2.0 ml to 50.0 ml with diluent.

#### 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  $\mu$ g and Ezetimibe 10  $\mu$ g respectively.

#### 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).

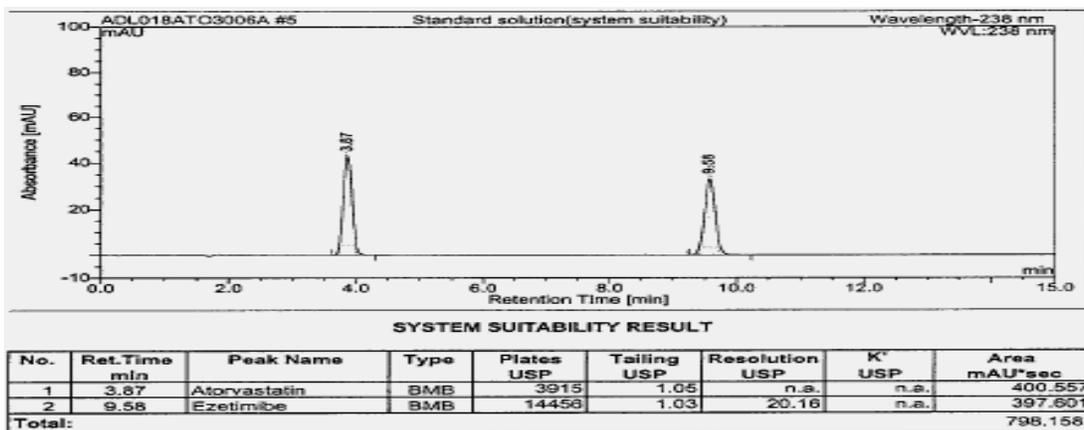
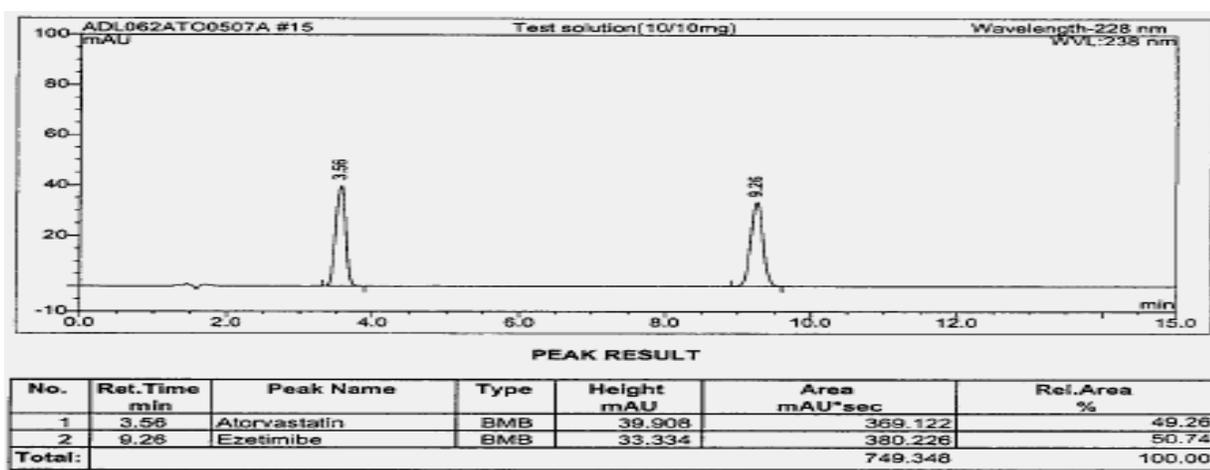


Fig. 1(b).



**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	Correlation µg/ml	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 (mg)	% Assay (Mean ± SEM) (n=3)	% RSD of Assay (n=3)
<b>For Atorvastatin</b>				
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
<b>For Ezetimibe</b>				
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)		
<b>Temperature</b>	<b>% RSD Normal</b>	<b>% RSD (-5°C)</b>	<b>% RSD (+5°C)</b>
Atorvastatin	0.5		0.3      0.1
Ezetimibe	0.02		0.1      0.1
<b>Mobile phase ratio</b>	<b>% RSD Normal</b>	<b>% RSD (-0.2 unit)</b>	<b>% RSD (+0.2 unit)</b>
Atorvastatin	0.5		0.02      1.4
Ezetimibe	0.02		0.03      0.9
<b>Flow Rate</b>	<b>% RSD Normal</b>	<b>% RSD (-10%)</b>	<b>% RSD (+10%)</b>
Atorvastatin	0.5		0.05      0.1
Ezetimibe	0.02		0.03      0.1

**Table 6. INTERMEDIATE PRECISION**

Compound	% Assay Mean $\pm$ SEM (n=6)	% RSD of Assay (n=6)
<b>Day 1</b>	<b>Analyst-1, Instrument-1 &amp; Column-1</b>	
Atorvastatin	95.3 $\pm$ 0.1282	0.3
Ezetimibe	100.2 $\pm$ 0.1192	0.4
<b>Day 2</b>	<b>Analyst-2, Instrument-2 &amp; Column-2</b>	
Atorvastatin	95.6 $\pm$ 0.1536	0.3
Ezetimibe		101.4 $\pm$ 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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### REFERENCES

1. Kosoglou T, Seiberling M, Startkevich P, Cutler DL, Yang B, Anderson L et al. 'Pharmacodynamic interaction between the new selective cholesterol absorption inhibitor ezetimibe and atorvastatin' J Amer Coll Cardiol, 37(suppl), 229, 2001.
2. Michael H.Davidson, Darbie Maccubbin, Michael Stepanavage, John Storny. 'Striated Muscle Safety of *Ezetimibe/Simvastatin* (Vytorin)' Amer J Cardiol, 97(2), 223, 2006.
3. Ballantyne CM, Houri J, Notarbartolo A. Circulation, 'Effect of Ezetimibe Coadministered With Atorvastatin in 628 Patients With Primary Hypercholesterolemia' 2409, 107, 2003.
4. Ballantyne CM, Blazing MA, King TR., Brady WE, Palmisano J. "Efficacy and safety of ezetimibe coadministered with lovastatin in primary hypercholesterolemia", Amer J Cardiol, 91,418, 2003.
5. Harold E. Bays, Leiv Ose, Neil Fraser, Diane, Steven R. Donahue. 'A multicenter, randomized, double-blind, placebo-controlled, factorial design study to evaluate the lipid-altering efficacy and safety profile of the ezetimibe/simvastatin tablet compared with ezetimibe and simvastatin monotherapy in patients with primary hypercholesterolemia' Clin Therapeut, 26 (11), 1758, 2004.
6. N. Stone. 'Combination therapy: its rationale and the role of ezetimibe' Euro. Heart J, 4(suppl), J19 (2002)
7. Omar MA, Wilson JP, Cox TS. Ann Pharmacother, 35, 'Rhabdomyolysis and HMG-CoA reductase inhibitors' 1096 (2001).
8. Stein EA. 'An investigative look: selective cholesterol absorption inhibitors--embarking on a new standard of care.' Amer J Manag Care, 8(suppl), S36, 2002.
9. Grundy SM. 'HMG-CoA reductase inhibitors for treatment of hypercholesterolemia' N Engl J Med., 319, 24, 1988.
10. ICH, Q2B, Validation of analytical procedure: methodology, in: Proceedings of the International Conference on Harmonisation, London, 1995.
11. B.A. Bidlingmeyer, S.N. Deming, W.P. Price, B. Sachok, M.Pertuse. Advances in Chromatography, Marcel Dekker, Houston, 1979; pp.435-450

12. R.Sistla, V.S.S.K. Tata, Y.V. Kashyap, D.Chandersekar, P.V.Diwan. 'Development and validation of a reversed-phase HPLC method for the determination of ezetimibe in pharmaceutical dosage forms' J Pharm Biomed Ana, 39, 517, 2005.
13. Gholamreza Bahrami, Bahareh Mohammad, Shahla Mirzaeei, Amir Kiani. 'Determination of atorvastatin in human serum by reversed-phase high-performance liquid chromatography with UV detection.' J Chrom B, 826,41, 2005.
14. Rajeswari K, Sankar GG, Rao AL, Seshagirrao JVLN. 'A Validated Hplc Method For Analysis Of Atorvastatin Calcium, Ramipril And Aspirin As The Bulk Drug And In Combined Capsule Dosage Forms' Indian J pharm sci., 68, 275,2006.