

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Pharmaceutical Sciences

Research Article.....!!!

Received: 07-12-2011; Accepted: 25-12-2011

DEVELOPMENT AND VALIDATION OF A REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR THE DETERMINATION OF ATORVASTATIN CALCIUM AND EZETIMIBE IN PHARMACEUTICAL DOSAGE FORMS

Maitreyi Zaveri^{1*}, Bhavita Dhru¹, Amit Khandhar²

1. Department of Pharmacognosy, K.B. Institute of Pharmaceutical Education and Research, Sector-23, GH-6, Gandhinagar-382023, Gujarat, India.
2. Zydus Cadila Healthcare Limited, Sarkhej Bavla N.H.-8A, Changodar, Ahmedabad, Gujarat, India.

ABSTRACT

Keywords:

Atorvastatin; Ezetimibe;
Reversed-phase HPLC;
Combination Tablets

For Correspondence:

Maitreyi Zaveri

Department of
Pharmacognosy, K.B. Institute
of Pharmaceutical Education
and Research, Sector-23, GH-
6, Gandhinagar-382023,
Gujarat, India

E-mail:

khandharmaitreyi@gmail.com

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 μ 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%.

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. It is a synthetic lipid-lowering agent, which inhibits the enzyme 3-hydroxy3-methylglutaryl-coenzymeA (HMGC_o-A) reductase. This enzyme catalyzes the conversion of HMGC_o-A to Mevalonate, an early and rate determining step in Cholesterol synthesis¹. Ezetimibe is in a class of lipid lowering compound that selectively inhibits the intestinal absorption of Cholesterol & related phytosterol. Atorvastatin also reduces LDL (Low-density Lipoprotein) production and Apo-B (Apolipoprotein -B) in patient with non-familial form of Hypercholesterolemia and mixed Dyslipidemia^{2, 3}. Ezetimibe 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. Atorvastatin is extensively metabolized to ortho and para-hydroxylated derivative and various Beta-oxidation products^{4, 5}. In vitro inhibition of HMGC_oA reductase by this metabolite is equivalent to Atorvastatin. Maximum plasma half-life of Atorvastatin is 14hrs. But the half-life of inhibitory activity for HMGC_oA reductase is 20-30 hrs due to the contribution of active metabolite⁶. Ezetimibe is rapidly metabolized to Ezetimibe-Glucouronide in human. Plasma half-life for Ezetimibe and Ezetimibe-Glucouronide is 22 hrs⁷. Atorvastatin and Ezetimibe tablet in combination are indicated as an adjunctive therapy to diet for the reduction of elevated total Cholesterol, LDL-C (Low-density Lipoprotein – cholesterol), and TG(Triglycerides) and also to decrease the hepatic Cholesterol stored and increase in clearance of Cholesterol from blood^{8,9}. Also co-administration of Statin with Ezetimibe could significantly reduce the risk of coronary heart disease event in patient with Hypercholesterolemia^{10, 11}. Since, there is no official method available for the estimation of Atorvastatin Calcium and Ezetimibe in combination, 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^{12, 13}. In the present study, a rapid, specific and validated HPLC method for the quantitative estimation of Atorvastatin Calcium and Ezetimibe in pharmaceutical dosage forms was reported.

MATERIAL AND METHODS:

Chemicals and Materials: Cadila Healthcare Limited, Ankleshwar supplied Atorvastatin calcium and Dr. Reddy's Laboratories supplied Ezetimibe. Acetonitrile and Ammonium acetate were procured from (Spectrochem and E-Merck Limited).

Instrumentation:

Shimadzu 2010C integrated high performance liquid chromatographic system was used for this experiment. Shimadzu 2010C 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 μm was used as a stationary phase.

HPLC Condition:

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

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 μm 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 single run.

Figure 1: Chromatogram of Atorvastatin and Ezetimibe tablet at 238 nm

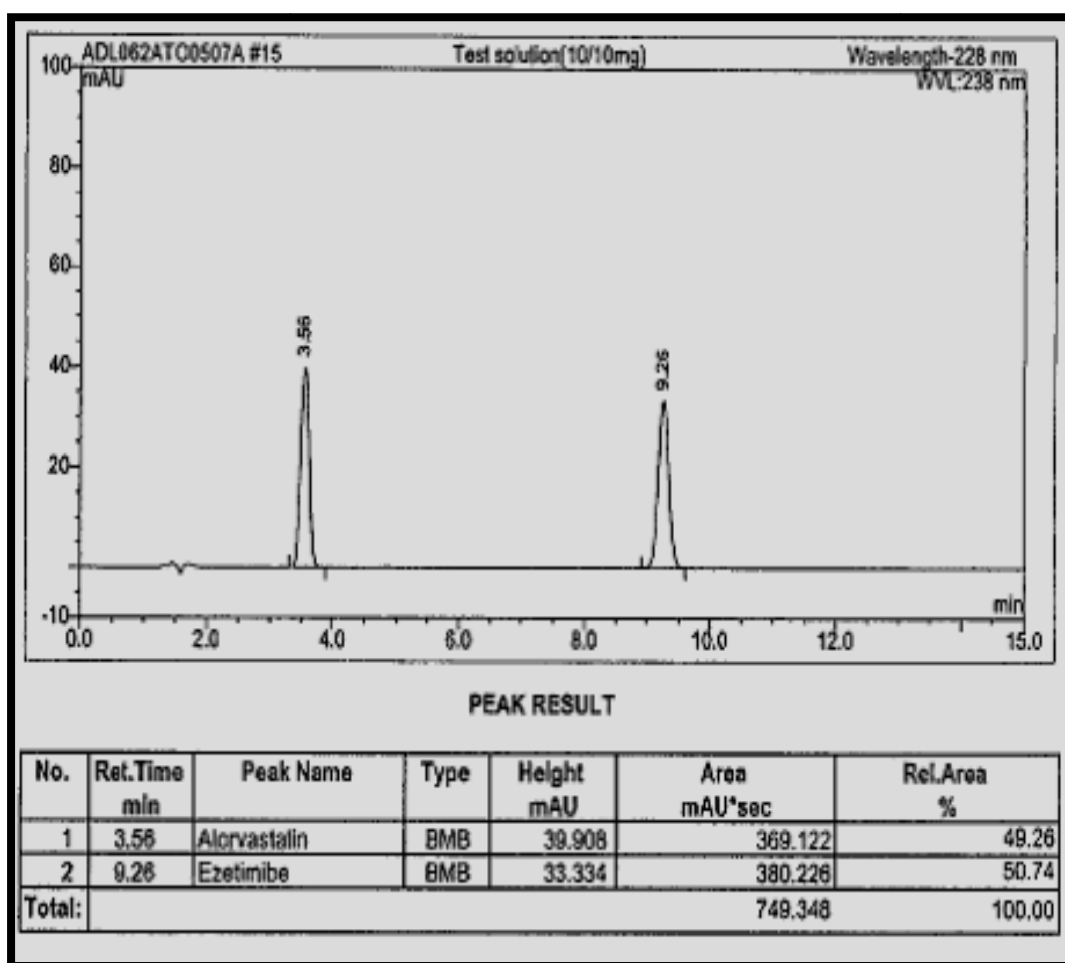


Figure 2 (a) Calibration curve of reference standard Atorvastatin

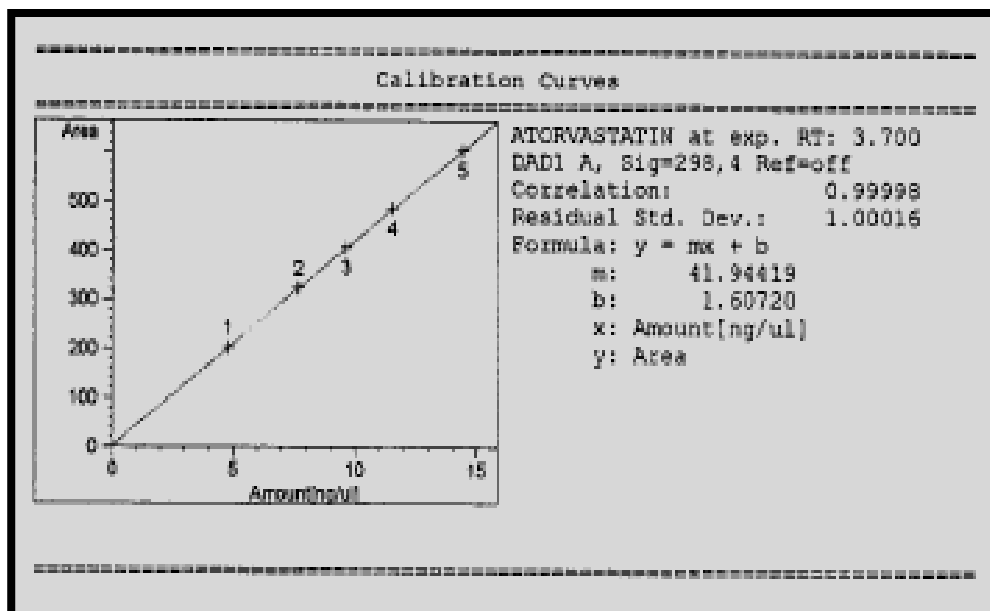
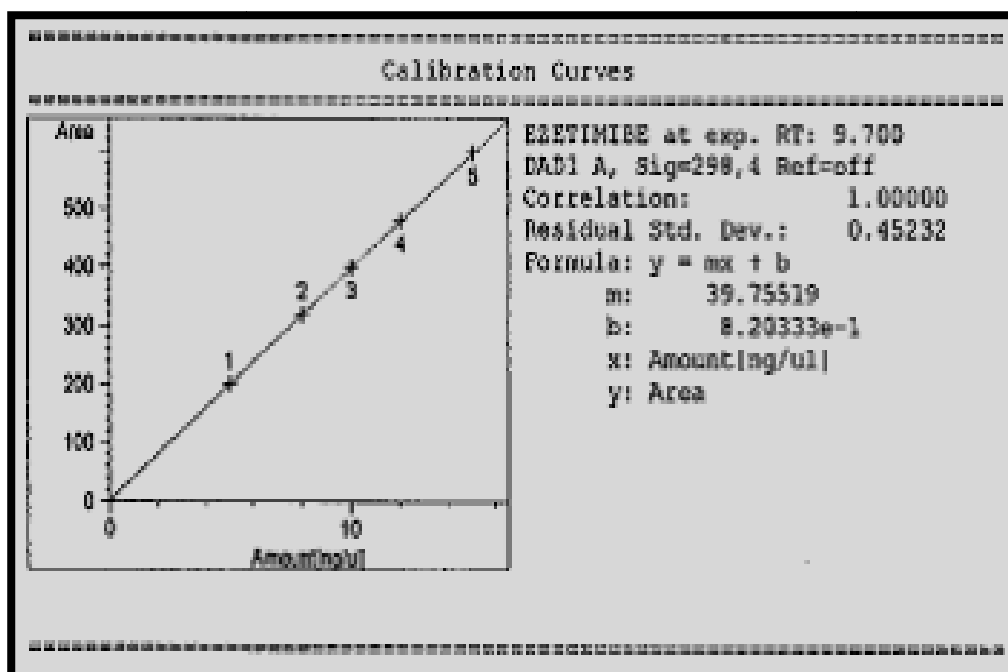


Figure 2 (b) Calibration curve of reference standard Ezetimibe



System suitability and system precision:

System suitability and system precision was daily performed during entire validation of this method. The results of system suitability and system precision were presented in table 1.

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.

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 h. 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.

Method 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.

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.

Method Ruggedness:

Ruggedness 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 1 System suitability and system precision

| Compound | Retention time (Mean ± SEM) | n | k' | R | T | α |
|-----------------|--|----------|-----------|----------|----------|----------|
| Atorvastatin | 3.62 ± 0.0016 | 3915 | 1.01 | - | 1.0 | - |
| Ezetimibe | 9.20 ± 0.00 | 14456 | 4.10 | 9.26 | 1.03 | 4.06 |

n= Theoretical plates

k'= Capacity Factor

R= Resolution

T= Asymetry

α = Selectivity

Table 2 Characteristics of the analytical method derived from the standard calibration curve

| Compound | LOD µg/ml | LOQ µg/ml | Linearity range n=(5) | Correlation co-efficient µg/ml | Residual std. regression σ | Slope of regression S |
|-----------------|----------------------|----------------------|--------------------------------------|---|---|--------------------------------------|
| 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

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 |

Table 4 Method accuracy

| Level | Drug Added (mg) | Drug recovered (mg) | % Assay (Mean ± SEM) (n=3) | % RSD of Assay (n=3) |
|-------------------------|--------------------------------|------------------------------------|---|-------------------------------------|
| 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 | 73.42 | 99.0 ± 0.1978 | 0.5 |
| 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% | 74.95 | 75.38 | 100.6 ± 0.0643 | 0.2 |

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 Method ruggedness

| Compound | % Assay Mean \pm SEM (n=6) | % RSD of Assay (n=6) |
|-----------------|--|---------------------------------|
| Day 1 | Analyst-1, Instrument-1 & Column-1 | |
| Atorvastatin | 95.3 \pm 0.1282 | 0.3 |
| Ezetimibe | 100.2 \pm 0.1192 | 0.4 |
| Day 2 | Analyst-2, Instrument-2 & Column-2 | |
| Atorvastatin | 95.6 \pm 0.1536 | 0.3 |
| Ezetimibe | 101.4 \pm 0.0816 | 0.2 |

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 routine drug analysis.

ACKNOWLEDGEMENTS

The authors are thankful to Zydus Cadila Limited, Ahmedabad, India for providing reference standards and all facilities to complete this research work.

REFERENCES

1. Kosoglou T., Seiberling M., Startkevich P., Cutler D.L., Yang B., Anderson L. Pharmacodynamic interaction between the new selective cholesterol absorption inhibitor ezetimibe and atorvastatin. *J Amer Coll Cardiol* 2001; 37: 229-230.
2. Michael H.D., Darbie M., Michael S. John Storny. Striated muscle. *Amer J Cardiol* 2006; 97(2):223-228.
3. Ballantyne C.M., Houri J., Notarbartolo A. Effect of ezetimibe co administered with atorvastatin in patient with primary Hypercholesterolemia. *Circulation* 2003; 107:2409-2415.
4. Ballantyne C.M., Blazing M.A., King T.R., Brady W.E., Palmisano J. Efficacy and safety of ezetimibe co-administered with simvastatin compared with atorvastatin in adults with Hypercholesterolemia. *Amer J Cardiol* 2003; 91:418-424.
5. Harold E. B., Leiv Ose, Neil F., Diane, Steven R., Donahue. Factorial design study to evaluate the lipid-altering efficacy and safety of the ezetimibe/simvastatin tablet compared with ezetimibe and simvastatin monotherapy in patients with primary Hypercholesterolemia. *Clin Therapeut* 2004; 26 (11):1758-1770.
6. Stone N. Combination Therapy: its rationale and role of ezetimibe, *Euro. Heart J Supplements* 2002;4: 19-22.
7. Omar M.A., Wilson J.P., Cox T.S. Rhabdomyolysis and HMG-CoA reductase inhibitors. *Ann Pharmacother* 2001; 35:1096-1107.

8. Stein E.A. An investigative look: Selective cholesterol absorption inhibitors: embarking on a new standard of care. *Amer J Manag Care* 2002; 8: S36-39.
9. Grundy S.M. HMG-CoA reductase inhibitors for treatment of Hypercholesterolemia. *N Engl J Med*.1988; 319:24-33.
10. Bidlingmeyer B.A., Deming S.N., Price W.P., Sachok B., Pertuse M. *Advances in Chromatography*.14th ed.Marcel Dekker, Houston; 1979; p.435-450
11. Sistla R., Tata V.S., Kashyap Y.V., Chandrasekar D., Diwan P.V. Development and validation of a reversed-phase HPLC method for the determination of ezetimibe in pharmaceutical dosage forms. *J Pharm Bio Med Ana*.2005; 39:517-522.
12. Gholamreza B., Bahareh M., Shahla M., Amir K. Determination of atorvastatin in human serum by reversed-phase high performance liquid chromatography with UV detection. *J Chrom B*. 2005; 826:41-45.
13. Rajeswari K., Sankar G., Rao A.L, Seshagirirao J.V. RP-HPLC method for the simultaneous determination of atorvastatin and amlodipine in tablet dosage form. *Indian J Pharm Sci*. 2006; 68:275-277.