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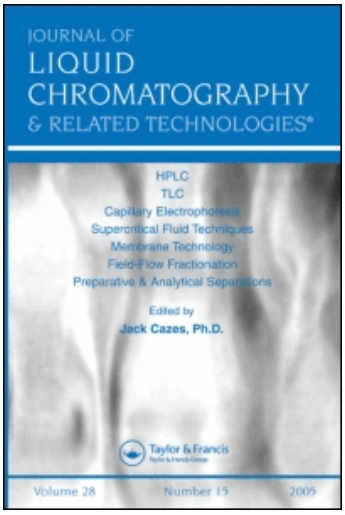

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Unnam Seshachalam[a]; Chandrasekhar B. Kothapally[b]
[a] Quality Assurance, Matrix Laboratories Limited, Secunderabad, Andhra Pradesh, India [b] Department of Chemistry, JNTU College of Engineering, Ananthapur, Andhra Pradesh, India

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HPLC Analysis for Simultaneous Determination of Atorvastatin and Ezetimibe in Pharmaceutical Formulations

Unnam Seshachalam[1] and Chandrasekhar B. Kothapally[2]

[1]Quality Assurance, Matrix Laboratories Limited,Secunderabad, Andhra Pradesh, India
[2]Department of Chemistry, JNTU College of Engineering, Ananthapur, Andhra Pradesh, India

Abstract: A simple, isocratic, and sensitive reverse phase high performance liquid chromatographic (RP-LC) method has been developed, for the first time, for quantitative determination of atorvastatin and ezetimibe in pharmaceutical formulations. Atorvastatin and ezetimibe were chromatographed using 0.01 M ammonium acetate buffer (pH:3.0): Acetonitrile (50:50 v/v) as mobile phase. The detection was monitored at 254 nm. The retention times of ezetimibe and atorvastatin were 15.50 ± 0.07 and 19.30 ± 0.06, respectively. The linearity of the method was studied over the concentration range of 4–400 μg/mL for atorvastatin and 5–500 μg/mL for ezetimibe. The limit of detection for atorvastatin and ezetimibe were found as 1.25 μg/mL and 1.48 μg/mL, respectively. The proposed method was applied for the quantitative determination of atorvastatin and ezetimibe in commercial combination formulations.

Keywords: Column liquid chromatography, Validation, Atorvastatin, Ezetimibe, Formulation

INTRODUCTION

Ezetimibe (EZ) [(3*R*,4*S*)-1-(4-fluorophenyl)-3-[(3*S*)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)-2-azetidinone] (Figure 1) is a selective

Correspondence: Unnam Seshachalam, Quality Assurance, Matrix Laboratories Limited, 1-1-151/1, Sairam Towers, Alexander Road, 500 003, Secunderabad, Andhra Pradesh, India. E-mail: chalam_us@rediffmail.com or chalam@matrixlabsindia.com

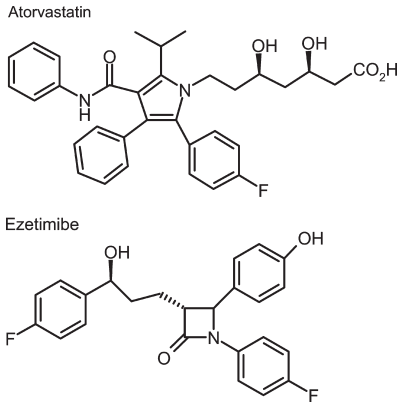

Figure 1. Chemical structures of atorvastatin and ezetimibe.

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cholesterol absorption inhibitor, which potently inhibits the absorption of biliary and dietary cholesterol[1] from the small intestine without affecting the absorption of fat soluble vitamins,[2] triglyceride, or bile acids. Ezetimibe reduces the small intestinal enterocyte uptake and absorption of cholesterol that keeps the cholesterol in the intestinal lumen for excretion.[3] Ezetimibe is rapidly absorbed and primarily metabolized in the small intestine and liver to its glucuronide, both of which undergo enterohepatic recycling in humans.[4,5] Since ezetimibe does not influence the activities of CYP 450 enzymes, significant pharmacokinetic interactions with other medications, including statins, fibrates, digoxin, and warfarin have not been found. Ezetimibe complements the lipid lowering effects of other therapies, such as statins. Clinical studies have shown that coadministration of ezetimibe with statins could provide significant reductions in both the low density lipoproteins (LDL) and the total cholesterol, with slight increases in the high density lipoproteins (HDL).[6–9] Also, coadministration of ezetimibe with statins could significantly reduce the risk of coronary heart disease (CHD) events in patients with hypercholesterolemia.[10]

Atorvastatin (AT) (Figure 1) is a selective, competitive inhibitor of HMG-CoA reductase, the rate limiting enzyme that converts 3-hydroxy-3-methylglutaryl-coenzyme A to mevalonate, a precursor of the sterols, including cholesterol. It is used to reduce LDL cholesterol, apolipoprotein B, and triglycerides, and to increase HDL cholesterol in the treatment of hyperlipidaemias.[11–13]

The combination of atorvastatin (AT) and ezetimibe (EZ) has recently been introduced in the market. There are few analytical methods reported in literature for determination of either AT[14–19] or EZ[20–23] alone, or in combination, of other drugs individually in biological fluids or in pharmaceutical dosage forms. One method was reported in literature for simultaneous determination of AT and EZ through HPTLC.[23] Hence, no publications are available for simultaneous determination of these two products through

HPLC. Therefore, the aim of the current study was to develop an HPLC method for determination of atorvastatin and ezetimibe in pharmaceutical formulations.

EXPERIMENTAL

Reagents

Qualified working standards of atorvastatin calcium and ezetimibe were obtained from the process research laboratory of Matrix Laboratories Limited, Secunderabad, India. HPLC grade acetonitrile (J.T. Baker, USA) and ammonium acetate (Qualigen Chemicals, Mumbai) were used for mobile phase preparation as solvent and buffer. Milli Q water, generated through the Millipore system from the quality control laboratory, was used for preparation of solutions and for making the dilutions. Commercially available combination formulations were procured from a local market.

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Instruments

All analyses were performed with the Waters HPLC system consisting of a 2695 separation module, 2487 dual wavelength UV detector, auto sampler, column heater, degasser, and sample cooler. Waters Empower software was used for data acquiring and calculation of system suitability parameters. The Inertsil ODS-3V column (250 mm $\times$ 4.6 mm, 5 μm) was procured from M/s. GL Sciences. Inc., USA.

Chromatographic Conditions

Chromatographic estimations were performed under the following conditions:

Inertsil ODS-3V column (250 mm $\times$ 4.6 mm, 5 μm) was used at ambient (25 $\pm$ 2°C) temperature. The mobile phase comprised of equal volumes of 10 mM ammonium acetate (pH: 3.0 $\pm$ 0.02):acetonitrile (50:50 v/v) was pumped at a flow rate of 1.0 mL/min. The mobile phase was filtered through a Nylon 0.45 μm membrane filter and was degassed before use. The elution was monitored at 254 nm, keeping an injection volume of 20 μL.

Preparation of Combined Standard Solution of Atorvatatin Calcium and Ezetimibe

Atorvastatin (40 mg) and ezetimibe (50 mg) were weighed accurately and transferred into a 100 mL volumetric flask. The sample was initially

dissolved in 50 mL of mobile phase and diluted further up to mark. The solution contains 400 μg of atorvastatin and 500 μg ezetimibe, respectively, per mL of solution.

Preparation of Calibration Curve

Calibration curves were constructed by plotting peak areas versus concentrations of atorvastatin and ezetimibe and the regression equations were calculated. The calibration curves were plotted over the concentration range 4–400 μg/mL and 5–500 μg/mL for atorvastatin and ezetimibe, respectively. The combined standard solutions prepared above were accurately measured (1.0 mL, 5.0 mL, 10.0 mL, 20.0 mL, and 50.0 mL) and were transferred into a series of 100 mL volumetric flasks and diluted to the mark with mobile phase. Of each solution 20 μL was injected into the chromatograph.

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Preparation of Pharmaceutical Formulation Samples

The contents of 10 tablets were ground to a fine powder. The weight equivalent to 50 mg of each atorvastatin and ezetimibe was transferred to a conical flask and dissolved in mobile phase. The solution was sonicated for about 20 minutes. The extract was filtered through Whatman filter paper No. 41 and the residue was washed with mobile phase. The extract and washing were pooled and transferred to a 100 mL volumetric flask and make up to the mark. The final test solution contains 500 μg each of atorvastatin and ezetimibe per mL. Of this solution, 20 μL was injected into the chromatograph. The contents of atorvastatin and ezetimibe were determined using the weights and corresponding areas of standard solution.

RESULTS AND DISCUSSION

A good separation was achieved with a resolution of more than 6.5. The retention times of ezetimibe and atorvastatin were 15.20 ± 0.07 and 19.30 ± 0.06 (Figure 2). The limit of detection was found to be 1.25 μg/mL for atorvastatin and 1.48 μg/mL for ezetimibe. The limit of quantification was found to be 4 μg/mL for atorvastatin and 4.98 μg/mL for ezetimibe.

The linearity of atorvastatin and ezetimibe were in the range of 4–400 μg/mL and 5–500 μg/mL, respectively, with correlation coefficients of more than 0.99997. The average linear regression equation was represented for atorvastatin as $Y = 276.42X + 2217.45$ and for ezetimibe as $Y = 374.11X + 2196.22$.

The intra-day precision (%RSD) was determined for standard atorvastatin and ezetimibe 3 times on the same day. The inter-day precision (%RSD) was

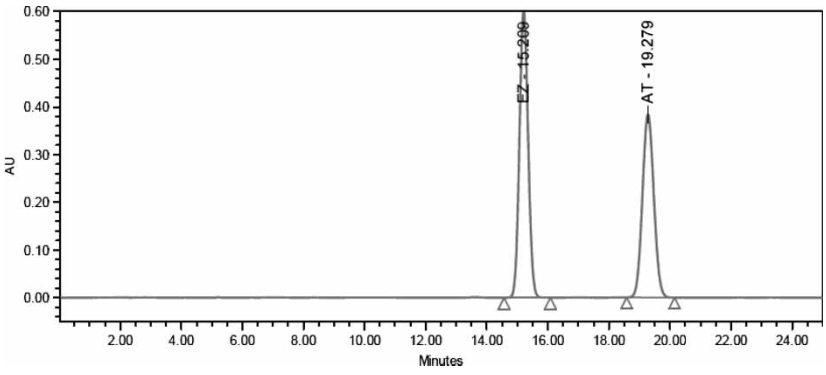

Figure 2. Typical HPLC chromatogram of method specificity.

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calculated for standard atorvastatin and ezetimibe 6 times over a period of one week. The coefficients of variations for intra-day and inter-day for both drugs were found to be in the range of 0.22–0.65% and 0.27–0.73%, respectively. The values suggest that the developed method is precise.

The precision of the instrument was checked by six replicate injections of standard preparation solution (400 μg/mL of atorvastatin and 500 μg/mL of ezetimibe) without disturbing the instrument. The RSD of peak responses and retention times of both drugs were calculated and was found to be in the range of 0.12–0.18% and 0.07–0.09%, respectively.

The accuracy of the method was evaluated by calculating the recovery of atorvastatin and ezetimibe by the standard addition method at three different levels of the calibration range. The percent recovery was found in the range of

Table 1. Summary of validation parameters of the proposed HPLC method

	HPLC method	
Parameters	Atorvastatin	Ezetimibe
Linearity range (μg/mL)	4–400	5–500
Correlation coefficient	0.99997	0.99998
LOD (μg/mL)	1.25	1.48
LOQ (μg/mL)	4.0	4.98
Accuracy	99.06–100.22	99.21–99.87
Precision (%RSD)		
Intra-day (n = 3)	0.32–0.58	0.22–0.65
Inter-day (n = 3)	0.27–0.66	0.55–0.73
Repeatability (n = 6)		
Peak responses (%RSD)	0.18	0.12
Peak retention (%RSD)	0.07	0.09

Table 2. Analysis of commercial formulation of two different brands amount found ± SD (%)

Formulation brand	Atorvastatin	Ezetimibe
Brand–A	99.36% ± 0.27	99.88% ± 0.11
Brand–B	100.07% ± 0.84	100.16 ± 0.73

99.06–100.22% for atorvastatin and 99.21–99.87% for ezetimibe and indicates that the method is sufficiently accurate.

Various validation parameters for the proposed LC method for the simultaneous determination of atorvastatin and ezetimibe are summarized in Table 1. The method was applied for the commercial formulations of two different brands in the market. The content of each drug available in the formulation was determined and the percent of label claim calculated. The label claim of atorvastatin and ezetimibe in both formulations were found to be in the range of 99.36–100.07% and 99.88–100.16%, respectively. The results of percent of label claim against each formulation were presented in Table 2.

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REFERENCES

1. Margaret, V.H.; Constance, F.F.; Douglas, S.C.; Robbie, L.M.; Nathan, P.Y.; Kevin, B.A.; Edmund, J.S.; Davis, H.R. Jr., In vivo metabolism-based discovery of a potent cholesterol absorption inhibitor, SCH58235, in the rat and rhesus monkey through the identification of the active metabolites of SCH48461. Pharmacol. Exp. Ther. **1997**, *283*, 157–163.
2. Knoop, R.H.; Bays, H.; Manion, C.V.; Lipka, L.J.; Melani, L.; LeBeaut, A.P.; Suresh, R.; Veltri, E.P. Proceedings of the 72nd EAS Congress, Posters, P175, p. 90.
3. Davis, H.R. Ezetimibe: first in a new class of cholesterol absorption inhibitors. Intl. Congress Ser. **2004**, *1262*, 243–246.
4. Patrick, J.E.; Kosoglou, T.; Kathe, L.S.; Kevin, B.A.; Stephen, E.M.; Zhu, Y.; Paul, S.; Robert, I.; Swapan, C.; Melton, A.; Mitchell, N.C. Disposition of the selective cholesterol absorption inhibitor ezetimibe in healthy male. Drug Metab. Dispos. **2002**, *30*, 430–437.
5. Heek, M.V.; Farley, C.; Compton, D.; Hoos, L.; Alton, K.; Sybertz, E.; Davis, H. Proceedings of the 12th International Symposium on Atherosclerosis, Tu T3 W16 (Abstract).
6. Christie, M.B.; John, H.; Alberto, N.; Lorenzo, M.; Leslie, J.L.; Ramachandran, S.; Steven, S.; Alexandre, P.L.; Philip, T.S.; Enrico, P.V. Effect of ezetimibe coadministered with atorvastatin in 628 patients with primary hypercholesterolemia: a prospective, randomized, double-blind trial. Circulation **2003**, *107*, 2409–2415.
7. Davidson, M.H.; McGarry, T.; Bettis, R.; Melani, L.; Lipka, L.J.; LeBeaut, A.P.; Suresh, R.; Sun, S.; Veltri, E.P. Ezetimibe coadministered with simvastatin in patients with primary hypercholesterolemia. J. Am. Coll. Cardiol. **2002**, *40*, 2125–2134.

8. Boris, K.; John, C.; Stephan, S.; Lipka, L.J.; Melani, L.; LeBeaut, A.P.; Suresh, R.; Pabak, M.; Veltri, E.P. Efficacy and safety of ezetimibe coadministered with lovastatin in primary hypercholesterolemia. Am. J. Cardiol. **2003**, *91*, 418–424.
9. Melani, L.; Mills, R.; Hassman, D.; Lipetz, R.; Lipka, L.J.; LeBeaut, A.P.; Suresh, R.; Pabak, M.; Veltri, E.P. Efficacy and safety of ezetimibe coadministered with pravastatin in patients with primary hypercholesterolemia: a prospective, randomized, double-blind trial. Eur. Heart J. **2003**, *24*, 717–728.
10. Farnier, M.; Volpe, M.; Massaad, R.; Davies, M.J.; Allen, C. Effect of coadministering ezetimibe with on-going simvastatin treatment on LDL-C goal attainment in hypercholesterolemic patients with coronary heart disease. Int. J. Cardiol. **2005**, *102*, 327–332.
11. McEvoy, G.K. *American Hospital Formulary Service*®; American Society of Health-System Pharmacists Inc.: Bethesda, 2001; pp. 488, 865.
12. Goodman, A.G., Gilman, L.S., Rall, T.W., Nies, A.S., Taylor, P., Eds.; *The Pharmacological Basis of Therapeutics*, 8th edn.; Pergamon Press: Oxford, 1990; p. 774.
13. Sweetman, S.C. *Martindale the Complete Drug Reference*, 34th edn.; Pharmaceutical Press: London, 2005; pp. 862, 866.
14. Bahrami, G.; Mohammadi, B.; Mirzaeei, S.; Kiani, A. Determination of atorvastatin in human serum by reversed-phase high-performance liquid chromatography with UV detection. J. Chromatogr. B **2005**, *826*, 41–45.
15. Sıdıka, E.; Akta, E.S.; Ersoy, L.; Fıçıcıoglu, S. An HPLC method for the determination of atorvastatin and its impurities in bulk drug and tablets. J. Pharm. Biomed. Anal. **2003**, *33*, 1017–1023.
16. Xiu-Sheng, M.; Chris, D.M. Determination of cholesterol-lowering statin drugs in aqueous samples using liquid chromatography–electrospray ionization tandem mass spectrometry. J. Chromatogr. A **2003**, *998*, 133–141.
17. Xiu-Sheng, M.; Chris, D.M. Determination of pharmaceuticals in aqueous samples using positive and negative voltage switching microbore liquid chromatography/electrospray ionization tandem mass spectrometry. J. Mass Spectrom. **2002**, *38*, 27–34.
18. Jemal, M.; Ouyang, Z.; Chen, B.C.; Teitz, D. Quantitation of the acid and lactone forms of atorvastatin and its biotransformation products in human serum by high-performance liquid chromatography with electrospray tandem mass spectrometry. Rapid Commun. Mass Spectrom. **1999**, *13*, 1003–1015.
19. Bullen, W.W.; Miller, R.A.; Hayes, R.N. Development and validation of a high-performance liquid chromatography tandem mass spectrometry assay for atorvastatin, ortho-hydroxy atorvastatin, and para-hydroxy atorvastatin in human, dog and rat plasma. J. Am. Soc. Mass Spectrom. **1999**, *10*, 55–66.
20. Saranjit, S.; Baljinder, S.; Rakesh, B.; Lalit, W.; Rahul, S. Stress degradation studies on ezetimibe and development of a validated stability-indicating HPLC assay. J. Pharm. Biomed. Anal. **2006**, *41*, 1037–1040.
21. Sistla, R.; Tata, V.S.S.K.; 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. Biomed. Anal. **2005**, *39*, 517–522.
22. Ezzet, F.; Krishna, G.; Wexler, D.B.; Statkevich, P.; Kosoglou, T.; Batra, V.K. A population pharmacokinetic model that describes multiple peaks due to enterohepatic recirculation of ezetimibe. Clin. Ther. **2001**, *23*, 871–885.

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23. Chaudhari, B.G.; Patel, N.M.; Shah, P.B.; Modi, K.P. Development and validation of a HPTLC method for the simultaneous estimation of atorvastatin calcium and ezetimibe, Indian. J. Pharm. Sci. **2006**, *68*, 793–796.

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