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SIMULTANEOUS DETERMINATION OF ATORVASTATIN AND EZETIMIBE BY RP-HPLC IN PURE AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, precise and rapid RP-HPLC method was developed for the simultaneous determination of atorvastatin and ezetimibe in combined pharmaceutical dosage forms. The method was carried out on a Shim-pack, RP-C18 column using a mixture of acetonitrile : 0.1% v/v orthophosphoric acid and detection was done at 239 nm using external standard method as quantitation. The linearity range of atorvastatin and ezetimibe were 0.1 to 25 µg/mL. The intra-day and inter-day precision were in the range of 0.55-0.91 and 0.62-0.87 for atorvastatin and 0.48-0.53 and 0.67-1.24 for ezetimibe.

Key word: Atorvastatin, Ezetimibe, Tablets, RP-HPLC.

INTRODUCTION

Atorvastatin[1] (Fig. 1) is a lipid-lowering agent, HMG-CoA reductase inhibitor. Chemically, atorvastatin is [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) trihydrate. A number of methods have been developed for the analysis of this drug, which include liquid chromatography tandem mass spectrometry[2-4] and HPLC with gradient elution[5].

Ezetimibe[1] (Fig. 1), a selective inhibitor of intestinal cholesterol and related phytosterol absorption, is designated as 1-(4-fluorophenyl)-3(R)-[3-(4-fluorophenyl)-3(S)-

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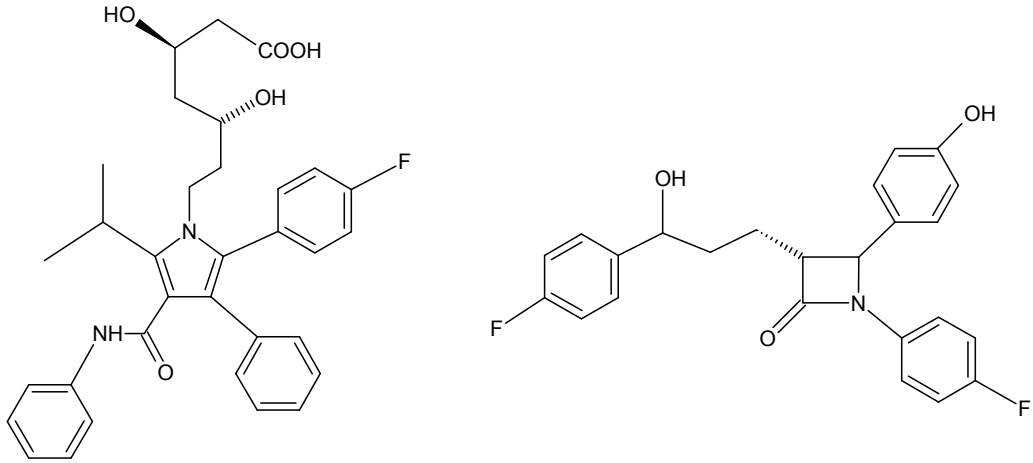
hydroxypropyl]-4(S)-(4-hydroxyphenyl)-2-azetidinone. Few HPLC methods for the determination of ezetimibe were reported in literature[6,7].



Atrovastatin Ezetimibe

Fig. 1: Chemical structures of atrovastatin and ezetimide

There are very few HPLC[8,9] methods for the simultaneous estimation of the atorvastatin and ezetimibe. The availability of a HPLC method with high sensitivity and selectivity will be very useful for the simultaneous determination of atorvastatin and ezetimibe in combined dosage forms. The developed method can be successfully applied to quality control and other analytical purposes.

EXPERIMENTAL

Reagents

Atorvastatin (assigned purity, 99.8%) and ezetimibe (assigned purity, 99.9%) were gift samples from Ranbaxy Labs, India. Acetonitrile of HPLC grade (E. Merck, India), ortho phosphoric acid and HPLC grade water (Qualigen Chemicals, India) were used in the study. These commercially available combined dosage forms claimed to contain 10 mg each of atorvastatin and ezetimibe, respectively and were procured from the local market.

Apparatus

Quantitative HPLC was performed on a gradient high pressure liquid chromatograph (Shimadzu HPLC Class 10A series) with two LC-10AT pumps, a fixed wavelength programmable UV/VIS detector (SPD-10A), a guard column (Shimadzu TM,

ODS 2 cm, Shim-pack) and RP C-18 column (250 mm x 4.6 mm i.d., particle size 5 μ) was used. The HPLC system was equipped with the software Class LC-10AT series version 5.03 (Shimadzu).

HPLC conditions

The contents of mobile phase were acetonitrile and 0.1% v/v orthophosphoric acid in the ratio 50 : 50 v/v. They were filtered before use through a 0.45 μm membrane filter, degassed with a helium spurge for 15 min and pumped from the respective solvent reservoirs to the column at a flow rate of 1 mL/min, which yielded a column back pressure of 130-140 kg/cm^2. The run time was set at 10 min and the column temperature was maintained at 30^{0}C. The volume of injection loop was 20 μL. Prior to injection of the drug solutions, the column was equilibrated for at least 30 min with the mobile phase flowing through the systems. The eluents were monitored at 239 nm and the data were acquired, stored and analyzed with the software Class LC-10AT series version Shimadzu.

Recommended procedure

A stock solution of atorvastatin and ezetimibe was prepared by dissolving 25 mg of atorvastatin and 25 mg of ezetimibe in 25 mL of volumetric flask containing 15 mL of acetonitrile (HPLC grade) sonicated for about 15 min and then made up to volume with acetonitrile. Daily working standard solutions of atorvastatin and ezetimibe were prepared by suitable dilution of stock solution with the diluent (50% acetonitrile). Five sets of the drug solution were prepared in 50% acetonitrile, containing atorvastatin and ezetimibe in the concentration of 0.1-25 μg/ mL. Each of these drug solutions (20 μL) was injected six times into the column and the peak area and retention times were recorded.

Procedure for pharmaceutical formulations

Not fewer than twenty tablets were weighed to obtain the average tablet weight and were then powdered. A sample of the powdered tablets, claimed to contain 25 mg of atorvastatin and 25 mg of ezetimibe were taken in a 25 mL volumetric flask. Then contents were dissolved in the volume that was made up with 50% acetonitrile. This mixture was shaken well and was then filtered through a 0.45 μm membrane filter. An aliquot of this solution (0.1 mL) was transferred to a volumetric flask and made up to a sufficient volume with 50% acetonitrile to get a concentration of 100 μg/ mL. From this, 1 μg/ mL of the solution was prepared. Various volumes of this aliquot were diluted to get concentrations between 80-120% with diluent (50% acetonitrile). All determinations were conducted in triplicate. The same procedure was used to estimate the concentration of the drug in two different strengths of tablets.

RESULTS AND DISCUSSION

The development of HPLC methods for the simultaneous determination of drugs has received considerable attention in recent years because of their importance in the quality control of drugs and drug products.

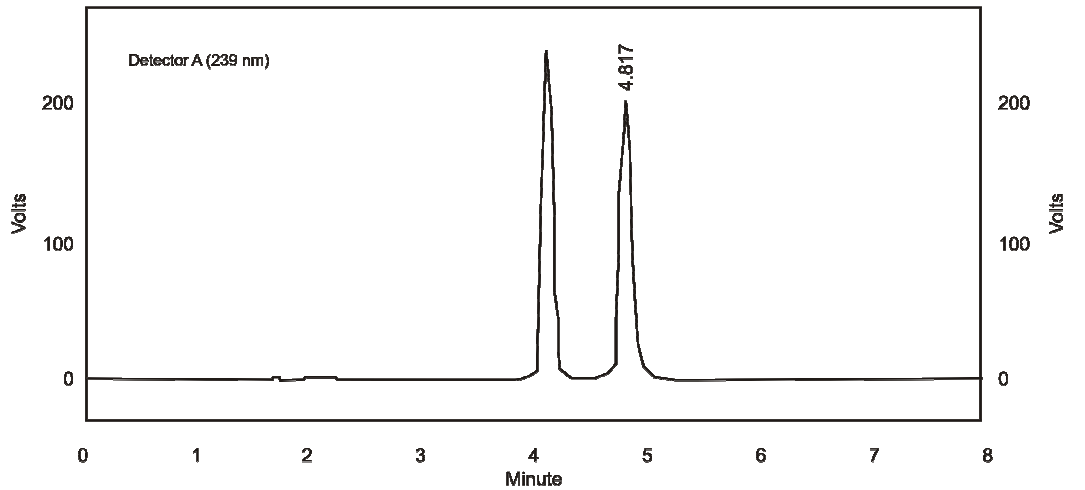


Fig. 2: A typical chromatogram of atrovastatin and ezetimibe reference substance

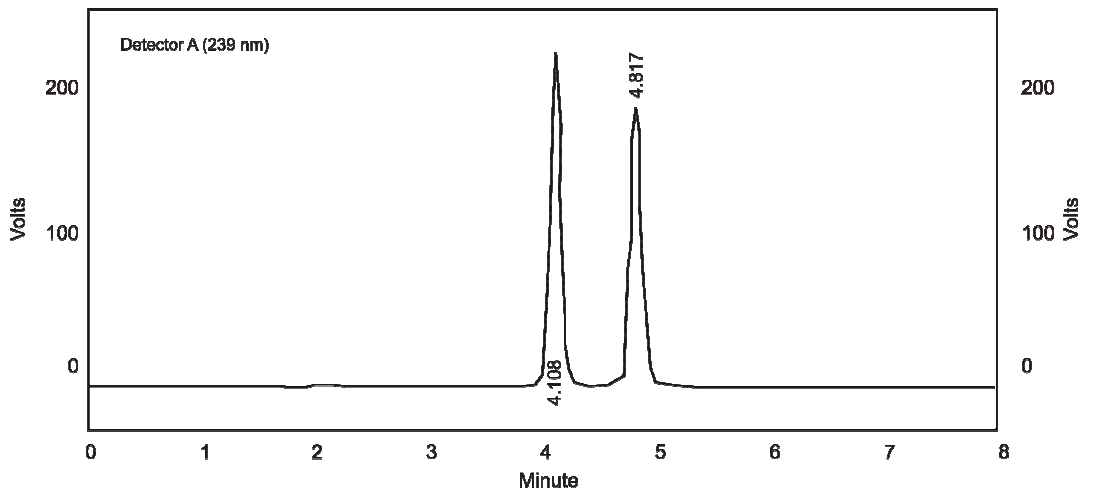


Fig. 3: A typical chromatogram of atrovastatin and ezetimibe tablets

The goal of this study was to develop a rapid HPLC method for the analysis of

atorvastatin and ezetimibe in its combined pharmaceutical dosage form, using the most commonly employed RP C-18 column with UV detection. The retention time of atorvastatin and ezetimibe were found to be 4.81 and 4.10 min, respectively. Typical chromatograms of atorvastatin and ezetimibe were shown in Fig. 2 and 3.

The calibration curve of atorvastatin and ezetimibe was constructed by plotting the peak area of drug (Y) to the concentration (X). It was found to be linear with a correlation coefficient of 0.9994 and 0.9942, the representative linear regression equation being Y= 27584.19X + 9216.25 and Y= 22901.02X + 18627.36, respectively. This method was validated for its intra- and inter- day precision. In the range of 0.1-25 µg/ mL, the relative standard deviations of five triplicate injections were found to be 0.5 and 0.4%, respectively (Table 1).

Table 1. Calibration of the proposed HPLC method

Concentraton (µg/mL)		Peak area	
Atorvastatin	**Ezetimibe**	**Atorvastatin**	**Ezetimibe**
0.1	0.1	28101.48	2901.86
1	1	28221.86	29115.54
5	5	140507.25	145259.80
10	10	281824.59	290156.84
15	15	421522.34	348759.14
25	25	702593.25	580318.69

Table 2. Intra- and Inter- day precision for atorvastatin and ezetimibe assay in combined pharmaceutical dosage forms by proposed HPLC method

Concentration (µg/mL)		Observed concentration (µg/mL)							
		Intra-day				Inter-day			
		Mean (n = 5)		% RSD		Mean (n = 5)		% RSD	
EZM	ATV	EZM	ATV	EZM	ATV	EZM	ATV	EZM	ATV
5	5	4.93	4.97	0.53	0.55	5.04	4.78	0.67	0.62
10	10	10.06	10.09	0.48	0.91	10.02	9.98	1.24	0.87

EZM – Ezetimibe: ATV – Atorvastatin

The inter assay precision (3 days, n = 6) was expressed as relative standard deviation and range between 0.62%-0.87% and 0.67%-1.24% for atorvastatin and ezetimibe, respectively (Table 2).

The HPLC method developed in the present study was used to quantify atorvastatin and ezetimibe in tablet dosage forms. The obtained results are given in Table 3.

Table 3: Mean (± S.D) amount of atorvastatin and ezetimibe in tablet dosage forms by proposed HPLC method

Labeled amount		Observed amount		% purity	
Atorvastatin	**Ezetimibe**	**Atorvastatin**	**Ezetimibe**	**Atorvastatin**	**Ezetimibe**
10	10	9.97±0.20	9.98±0.46	99.7±0.01	99.8±0.03
10	10	9.98±0.24	9.97±0.64	99.87±0.82	99.7±0.15

Table 4: Recovery values of atorvastatin and ezetimibe by proposed method

Concentration(µg/mL)		% Recovery	
Atorvastatin	**Ezetimibe**	**Atorvastatin**	**Ezetimibe**
50% sample 1	50% sample 1	99.76	100.3
50% sample 2	50% sample 2	99.23	99.2
50% sample 3	50% sample 3	98.82	99.8
100% sample 1	100% sample 1	99.63	99.8
100% sample 2	100% sample 2	98.29	99.5
100% sample 3	100% sample 3	101.05	100.3
150% sample 1	150% sample 1	99.08	99.8
150% sample 2	150% sample 2	99.43	100.7
150% sample 3	150% sample 3	98.07	99.6

No interfering peaks were found in the chromatogram, indicating that the tablet excipients did not interfere with the estimation of the drug by the proposed HPLC method. Also, when a known amount of the drug solution was added to a powdered sample of the tablet dosage form and subjected to an estimation of the drug by the proposed method, there was a high recovery (Table 4) of atorvastain and ezetimibe, indicating that the

proposed procedure for the determination of atorvastatin and ezetimibe in combined tablet dosage forms is highly accurate.

The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate. It will be useful for the determination of atorvastatin and ezetimibe in its combined pharmaceutical dosage forms.

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REFERENCES

1. www. rxlist. com.
2. W. W. Bullen, R. A. Miller and R. N. Hayes, J. Am. Soc. Mass Spectom, **10,** 55 (1999).
3. M. Jemal, Z. Ouyang, B. C. Chen and D. Teitz, Rapid Commun. Mass Spectrom., **13**, 1003 (1999).
4. M. Hermann, H. Christensen and J. L. Reubsaet, Anal. Bioanal. Chem., **382**, 1242 (2005).
5. S. Erturk, E. Sevinc Aktas, L. Ersoy and S. Ficicioglu, J. Pharm. Biomed. Anal., **33**, 1017 (2003).
6. S. Singh, B. Singh, R. Bahuguna, L. Wadhwa and R. Saxena, J. Pharm. Biomed. Anal., **41**, 1037 (2006).
7. R. Sistla, V. S. Tata, Y. V. Kashyap, D. Chandrasekar and P. V. Diwan, J. Pharm. Biomed. Anal., **39**, 517 (2005).
8. B. G. Chaudhari, N. M. Patel, P. B. Shah and K. P. Modi, Indian J. Pharm. Sci., **68(6),** 793 (2006).
9. S. S. Qutab, S. N. Razzaq, I. U. Khan, M. Ashfaq and Z. A. Shuja, J. Food Drug Anal., **15(2)**, 139 (2007).

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