Int. J. Chem. Sci.: 7(3), 2009, 1793-1801

A RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ATORVASTATIN AND EZETIMIBE IN PHARMACEUTICAL FORMULATION

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ABSTRACT

A simple, selective, rapid and precise RP-HPLC method has been developed for the simultaneous estimation of ezetimibe and atorvastatin from dosage forms. An ODS C-18 (Intersile 4.6 mm x 25 cm, 10 µm) column was used for the separation. The mobile phase was methanol and water (90 : 10 v/v) at the flow rate of 1 mL/min with the detection at 236 nm. The retention time of atorvastatin and ezetimibe were 1.9 min. and 3.46 min. respectively. The developed method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation and solution stability. The proposed method can be used for the estimation of these drugs in combined dosage forms

Key words : RP-HPLC, Ezetimibe, Atorvastatin, Pharmaceutical.

INTRODUCTION

Atorvastatin[1] is chemically [βR, δR]-2-(4-fluorophenyl)-beta, delta-dihydroxy 5-(1-methylethyl)-3-phenyl 4 [(phenyl amino) carbonyl]-1H- pyrrole-1- heptanoic acid. Its molecular formula is $C_{13}H_{34}FN_2O_5$ with a molecular weight of 558.64.Ezetimibe[1] is **(**3*R*, 4S)-1-(4-fluorophenyl)-3-((3*S*)-3-(4-fluorophenyl)-3-hydroxypropyl)-4-(4-hydroxyphenyl) -2-azetidinone. Its molecular formula is $C_{24}H_{21}NF_2O_3$ with a molecular weight of 409.4.Ezetimibe and atorvastatin are used as antilipidemic agents. Literature survey reveals that several methods such as HPLC[2-6] and electrochemical[7] have been reported for estimation of atorvastatin in pharmaceutical dosage form and from human plasma. Similarly, survey of literature for ezetimibe revealed certain methods based on HPLC[8, 9] for estimation of ezetimibe in pharmaceutical dosage form. However, no method has been developed for estimation of these drugs in combined dosage form. Fixed dose combination

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containing atorvastatin 10 mg and ezetimibe 10 mg is available in tablet form in the market. The aim of this work was to develop a RP-HPLC method with ultraviolet detection for the simultaneous estimation of atorvastatin and ezetimibe in pharmaceutical dosage form. The present RP-HPLC method was validated following the ICH guidelines [10,11].

EXPERIMENTAL

Materials and methods

Reagents and chemicals

Methanol HPLC grade was procured from Sisco Research Laboratories Pvt. Limited, Mumbai. Water HPLC grade was obtained from Merck Specialities Private Limited, Mumbai. Reference standards of atorvastatin and ezetimibe were procured from Glenmark Pharmaceuticals, Mumbai and Bal Pharma, Bangalore.

Chromatographic conditions

Chromatographic separation was performed on a Jasco HPLC system consisting of Jasco PU-2080 pump, Jasco UV 2010 photo diode array detector, Rheodyne injection syringe with 20 µL loop volume and windows based chrompass software. An ODS C_{18} RP-Column (Intersile 4.6 mm x 25 cm, 10 µm) was used for separation. The elution was carried out isocratically at flow rate of 1 mL/min using methanol : water (90 : 10 v/v) mobile phase. The detector was set at a wavelength of 236 nm. Under these conditions, the retention time of atorvastatin and ezetimibe were 1.9 min and 3.46 min, respectively.

Preparation of standard solutions

The standard stock solution 1 mg/mL of atorvastatin and ezetimibe were prepared separately using a mixture of methanol and water (90 : 10 v/v). From the standard stock solution, mixed standard solution was prepared to contain 10 µg/mL of atorvastatin and 10 µg/mL of ezetimibe.

Preparation of sample solutions

Accurately weighed quantity of tablet powder equivalent to 50 mg of atorvastatin and 50 mg of ezetimibe (0.0503 g) were transferred to 50 mL volumetric flask, diluted with mixture of methanol and water (90 : 10 v/v) up to the mark. It was filtered through 0.45µ membrane filter. From this solution, 10 mL was pipetted and transferred to 100 mL volumetric flask and the volume was adjusted with the mobile phase to get concentration of 100 µg/mL of atorvastatin and 100 µg/mL of ezetimibe. Further dilutions were made to

get concentration of 10 µg/mL of atorvastatin and 10 µg/mL of ezetimibe and this solution was used for estimation.

Assay method

With the optimized chromatographic conditions, a steady baseline was recorded. The mixed standard solution was injected and the chromatogram was recorded. The retention time of atorvastatin and ezetimibe were 1.9 min and 3.46 min, respectively. This procedure was repeated for the sample solution obtained from the formulation. From the peak area of standard drugs of atorvastatin and ezetimibe, amount of drugs in samples were computed. The values are given in Table 2.

RESULTS AND DISCUSSION

Estimation of atorvastatin and ezetimibe in dosage forms

Estimation of atorvastatin and ezetimibe in dosage forms by RP-HPLC method was carried out using optimized chromatographic conditions. The typical chromatogram of atorvastatin and ezetimibe are given in Fig. 1. The overlaid UV spectrum of atorvastatin and ezetimibe is given in Fig. 2.

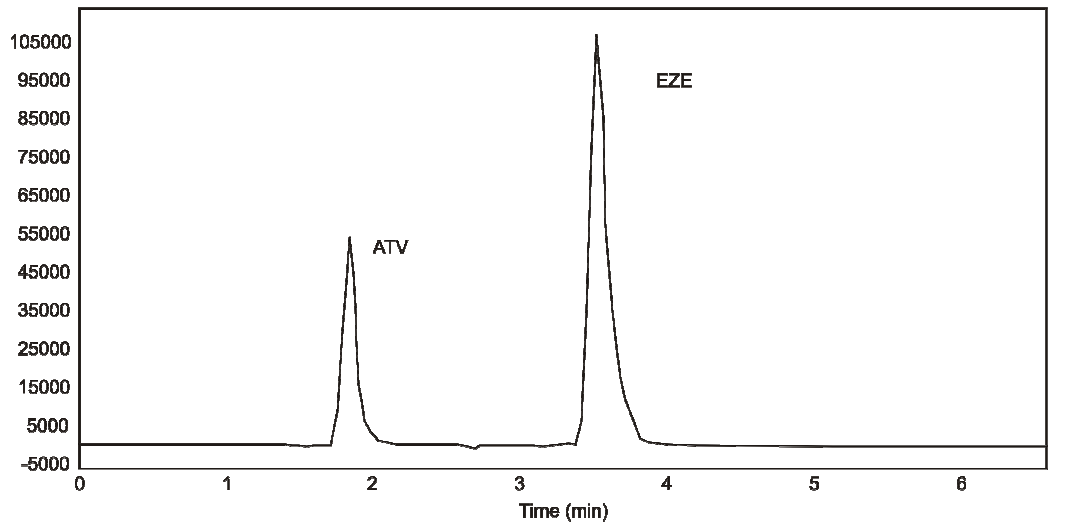

Fig. 1 : Chromatogram of atorvastatin (ATV) and ezetimibe (EZE)

The assay procedure was repeated five times and from the peak area of standard drugs of atorvastatin and ezetimibe, the percentage of individual drugs found in

formulations, standard deviation, % RSD and standard error in formulations were calculated and presented in Table 1.The results of analysis show that the amounts of drugs were in good agreement with the label claim of formulations.

Table 1 : Results of analysis of formulation

Drug	Labeled amount[a] (mg/tab)	Amount found (mg/tab) (n = 5)	% Label claim	SD	% R. S. D.	SE
Atorvastatin	10	9.97	99.45	0.168	0.169	0.075
Ezetimibe	10	10.25	102.2	0.308	0.308	0.137

[a] Atorlip- EZ tab, Cipla Ltd., Mumbai, India

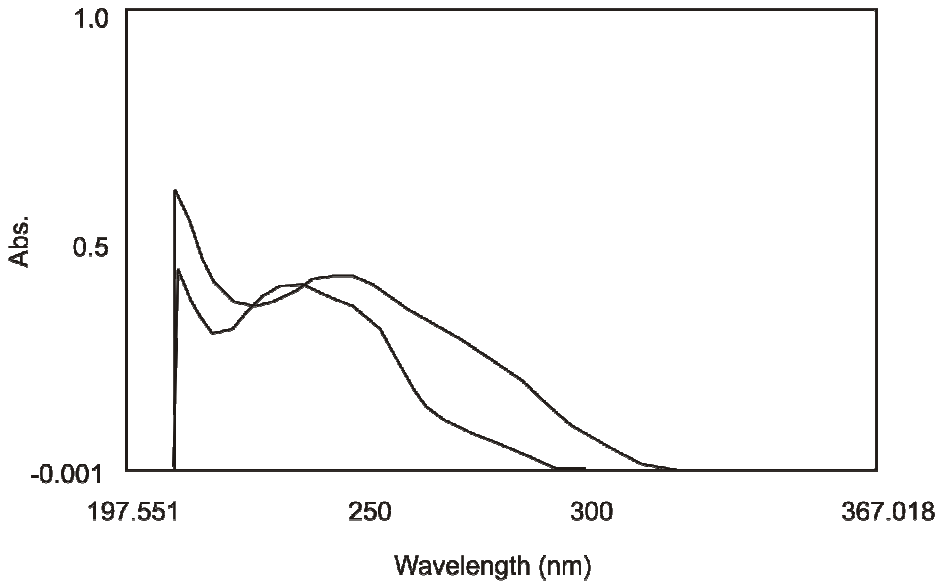

Fig. 2 : Overlain UV spectrum of atorvastatin and ezetimibe

Method validation

Accuracy and precision

The accuracy of the method was determined by recovery experiment. The recovery studies were carried out three times and the percentage recovery and standard deviation, %

RSD and standard error of the percentage recovery were calculated and presented in Table 2.From the data obtained, the developed HPLC method was found to be accurate.

The precision of the method was demonstrated by intraday and interday variation studies. In the intraday studies, six repeated injections of standard and sample solutions were made and the area of drug peaks and percentage RSD were calculated and presented in Table 3. In the interday variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and area of the drug peaks and percentage RSD were calculated and are presented in Table 2.From the data obtained, the developed HPLC method was found to be precise.

Table 2 : Results of recovery studies

Drug	Amount added[a] (mg/tab)	Amount found (mg/tab) (n = 3)	Recovery (%) ± Standard deviation	% R. S. D.	Standard error
Atorvastatin	50	49.85	99.7 ± 0.1376	0.136	0.079
Ezetimibe	50	51.25	102.5 ± 0.3214	0.899	0.515

[a]Atorlip- EZ tab, Cipla Ltd., Mumbai, India

Table 3 : Intra and interday precision studies

Area (μv) (Atorvastatin)	Mean ± % RSD[a] (Intraday)	Area (μv) (Ezetimibe)	Mean ± % RSD[a] (Intraday)
6672	6673.8 ± 0.025	10426.5	10426.7 ± 0.031
6675.2		10430.1	
6674.3		10423.5	
		I day	
6634.3	6639.56 ± 0.02	10696.0	10675.85 ± 0.003
6639.5		10630.0	
6642.1		10635.2	
6642.1		10697.0	

Cont…

Area (μv) (Atorvastatin)	Mean ± % RSD[a] (Intraday)	Area (μv) (Ezetimibe)	Mean ± % RSD[a] (Intraday)
6640.2		10698.0	
6639.2		10698.6	
	II day		
6633.8	6639.6 ± 0.02	10694.5	10687.8 ± 0.003
6641.5		10666.3	
6639.2		10691.2	
6641.2		10692.3	
6642		10690.3	
6640		10692.3	
	III day		
6645	6642.7 ± 0.02	10674.5	10680.3 ± 0.003
6643.3		10681.2	
6644.4		10677.9	
6642.2		10678.0	
6641.2		10687.0	
6640.2		10682.2	

[a] % Relative standard deviation

Linearity and range

The linearity of the method was ranging from 5 to 30 μg/mL for atorvastatin and ezetimibe, respectively and presented in Table 4.The calibration curve was constructed by plotting area against concentration of drugs. The slope and intercept value for calibration curve was $y = 639.03x + 458.8$ ($R^2 = 0.9985$) for atorvastatin and $y = 1011.3x + 418.81$ ($R^2 = 0.9991$) for ezetimibe. The results show that an excellent correlation exists between area and concentration of drugs within the concentration range.

Table 4 : Linearity and range

Atorvastatin concentration (μg/mL)	Area (μv)	Ezetimibe concentration (μg/mL)	Area (μv)
5	3637.3	5	5360.2
10	6672.0	10	10426.5
15	10183.4	15	16028.6
20	13255.3	20	20672.6
25	16776.2	25	25316.7
30	19326.3	30	30892.8

Limit of detection and limit of quantification

Limit of detection (LOD) and limit of quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of standard solutions using the developed RP-HPLC method. The LOD of atorvastin and ezetimibe was found to be 5 ng/mL and 10 ng/mL, respectively. The LOQ was 10 ng/mL and 20 ng/mL for Atorvastin and Ezetimibe, respectively and the results are presented in Table 5.

Table 5 : System suitability studies

Parameters	Atorvastatin	Ezetimibe
Theoretical plates	1972.95	4220.44
Resolution factor	---	0.89
Asymmetric factor	1.42	2.18
LOD (ng/mL)	5	10
LOQ (ng/mL)	10	20

Ruggedness and robustness

Ruggedness is a measure of the reproducibility of a result under expected operating conditions from instrument to instrument and from analyst to analyst. The results of the studies revealed that the developed method is rugged. Robustness is a measure of the

capacity of the method to remain unaffected by small but deliberate variations in method conditions and is an indication of reliability of the method. The results of studies revealed that the developed method is robust.

Solution stability

In order to demonstrate stability of both; standard and sample solutions during analysis, both solutions were analyzed over a period of 5 h at room temperature. The results show that for both solutions, the retention time and peak area of atorvastin and ezetimibe remained almost unchanged (% RSD less than 2.0) and no significant degradation was there within the indicated period. Thus, it indicated that both solutions were stable for 5 h, which was sufficient to complete the whole analytical process.

System suitability studies

The column efficiency, resolution and peak asymmetry were calculated for the standard solutions and the results are presented in Table 5.The values obtained demonstrated the suitability of the system for the analysis of this drug combination.

The proposed RP-HPLC method for the simultaneous estimation of atorvastin and ezetimibe in combined dosage forms is accurate, precise, linear, rugged, robust, simple, rapid and selective. It can, therefore, be conveniently adopted for routine quality control (QC) analysis of raw materials, formulations and dissolution studies.

ACKNOWLEDGEMENT

The authors are thankful to M/s Glenmark Pharmaceuticals, Mumbai and Bal Pharma, Bangalore for providing gift samples of atorvastatin and Ezetimibe, respectively.

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Accepted : 13.05.2009