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Simultaneous Estimation of Atorvastatin and Ezetimibe in Combined Formulation by RP-HPLC

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A simple, selective, robust and sensitive reversed phase high performance liquid chromatography method has been developed and validated for the simultaneous estimation of atorvastatin and ezetimibe in bulk drug and pharmaceutical formulations. The separation was achieved on a phenomenex Gemini C-18 (250 × 4.6 mm, packed with 5 µ) column by using an isocratic mobile phase mixture composed of acetonitrile: ammonium acetate buffer pH 3.0 (50:50, v/v) with 1.2 mL min^{-1} as flow rate and the eluents were monitored at 247 nm. The retention times for atorvastatin, ezetimibe were 3.0, 5.2 min respectively, the linearity for both analytes was found to be 80-120 % with reference to labeled claim (10 mg of each components) and with r = 0.9989 and 0.9979 for atorvastatin and ezetimibe respectively. The method was validated for its system suitability, ruggedness, accuracy, precision and stability. The proposed method was successfully employed for the simultaneous quantification of atorvastatin and ezetimibe in their pharmaceutical formulation.

Key Words: Atorvastatin, Ezetimibe, Simultaneous, RP-HPLC.

INTRODUCTION

Atorvastatin (ATR) is a synthetic lipid-lowering agent is chemically [R-(R*,R*)]-2-(4-flurophenyl) β-dihydroxy-5-1-methyl ethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1*H*-pyrrole-1-heptanoic acid. It lowers the cholesterol level by inhibiting the 3-hydroxy-3-methyl-glutaryl reductase (HMG-CoA reductase)coenzyme. HMG-CoA reductase is responsible for the conversion of HMG-CoA to mevalonate, an early and rate limiting step in the synthesis of cholesterol in liver. Inhibition of cholesterol synthesis in the liver leads to an increase in LDL-catabolism. This also reduces the LDL-production to some extent which results into inhibition of hepatic synthesis of very low density lipoprotein, the precursor of LDL-cholesterol[1,2]. There are numerous methods for estimation atorvastatin alone[3] HPLC and in combination with other drugs such as ramipril, aspirin, telmisartan, fenofibrate were reported[3-12].

Ezetimibe (EZT) is chemically 1-(4-flurophenyl)-3(R)[3-(4-flurophenyl)-3(S)-hydroxypropyl]-4(S)-(4-hydroxyphenyl) -2-azetidione] [Fig. 1(b)] is also a lipid lowering agent. It reduces the blood cholesterol by preventing intestinal absorption of cholesterol without altering absorption of triglycerides, fatty acids, bile acids and fat-soluble vitamins[13-17]. Reports revealed that ezetimibe alone can be estimated by HPLC and LC-MS/MS methods[18,19]. Methods such as spectrophotometric, HPLC

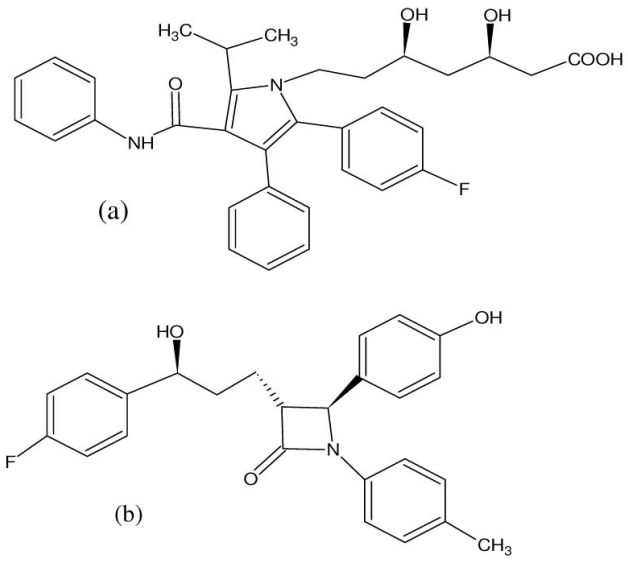

Fig. 1. Structure of (a) atorvastatin (b) ezetimibe

and HPTLC methods were reported for the simultaneous estimation of atorvastatin and ezetimibe[20-22]. Recently, Bhatt *et al.*[23] reported a simultaneous method for the estimation of atorvastatin and ezetimibe. The method was sensitive enough in terms of its limit of detection (LOD) and limit of quantification

(LOQ). It utilizes a large quantity of methanol as one of its mobile phase solvent (> 60 % in total volume), in general methanol is a most poisonous and high viscous solvent which cause high back pressure in column used in HPLC, which leads to reduced column life. Hence present paper portray a highly sensitive, more reproducible and robust HPLC method for the estimation of atorvastatin and ezetimibe using new mobile phase with acetonitrile a high polar, low viscous less hazardous HPLC solvent with ammonium acetate buffer.

EXPERIMENTAL

Atorvastatin (ATR) and ezetimibe (EZT) reference standards were received from Sigma-Aldrich (Germany), acetonitrile HPLC grade from J.T. Baker (Mallinckrodt Baker, Inc., USA), ammonium acetate AR grade (Acros organics, NJ, USA), Milli-Q water obtained from Millipore water system (Billerica, USA). Ammonium acetate AR grade (Acros organics, NJ, USA), all the above chemicals used as received without any further purification. Pharmaceutical tablet formulation containing 10 mg of each ATR and EZT was purchased from local pharmacy (Lipitor).

Chromatographic conditions: Chromatographic separation was performed with a Shimadzu HPLC system LC-10 AT VP isocratic solvent-delivery module (Japan) and Shimadzu SPD-10A UV-visible detector and a Rheodyne model 7125 injection valve with 20-µL fixed volume loop. The analytes were chromatographed on a Phenomenex Gemini C-18 (250 × 4.6 mm, packed with 5 µ) column. The output signal was monitored and integrated using Shimadzu Class -VP version 6.12 SP1 software. 50:50 acetonitrile and (0.05M) ammonium acetate buffer (pH 3.0 ± 0.05, adjusted by addition of 10 % acetic acid) as mobile phase with 1.2 mL/min flow rate and detection wavelength was fixed as 247 nm. The column and HPLC system was kept in ambient temperature throughout the analysis. Before analysis the mobile phase was degassed by use of a Branson Ultrasonics (USA) sonicator and filtered through a 0.2 µm injection filter. The column was equilibrated before each injection.

Standard preparation: A standard stock solution of 1 mg $mL^{-1}$of atorvastatin and ezetimibe in acetonitrile was prepared in volumetric flask. Working solutions of following concentrations 25, 50, 100, 125 and 150 % of the labeled amount atorvastatin 10.0 mg tablets were prepared by diluting the stock solutions with acetonitrile.

Assay preparation for commercial formulations: Powder equivalent to 10 mg of atorvastatin was accurately weighed from the well crushed powder of 20 tablets and transferred into 100 mL volumetric flask. Add little amount of acetonitrile to dissolve the analytes by sonication for 30 min with frequent shaking, make the remaining volume with acetonitrile, a quantity of this was centrifuged at 4000 rpm for 10 min. An aliquot was transferred from the supernatant of above solution into 10 standard flask and diluted to get the concentration within the calibration limit. Then the final solution was filtered using 0.45 µm nylon membrane filter before injection.

RESULTS AND DISCUSSION

The method was validated for system suitability, precision, accuracy, bench top stability, LOD, LOQ, ruggedness and robustness with reference to ICH and USP guidelines for analytical method validation[24].

Method development and optimization: Different trials were conducted to determine the column to be used, solvent selectivity (solvent type), solvent composition (volume fraction of organic solvent(s) in the mobile phase), additive strength detection wavelength and flow rate, which gives the best separation. The mobile phase conditions were optimized so there was no interference with the analytes peak from solvent or excipient peaks. Other parameters such as the total run time required for analysis, assay sensitivity, solvent noise and use of the same solvent system for extraction of the drug from formulation matrices during drug analysis were also considered[25,26]. From the trial it was observed that a better base line resolved peak with good symmetry, less tailing and good theoretical plate for atorvastatin and ezetimibe were obtained with 50:50 acetonitrile and (0.05M) ammonium acetate buffer (pH 3.0 ± 0.05, adjusted by addition of 10 % acetic acid) with flow rate of 1.2 mL (Fig. 2 a-c). This optimized chromatographic condition was used for the validation of the method.

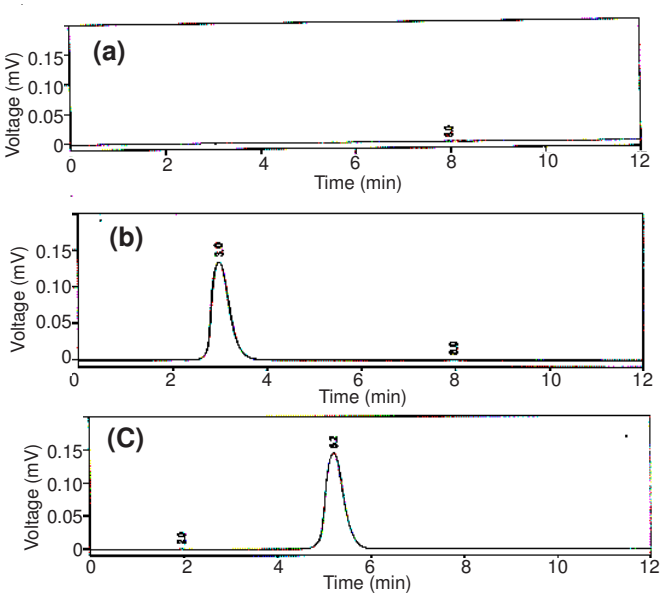

Fig. 2. Chromatograms of (a) blank (b) ATR std (c) AZT std

System suitability: The HPLC system was equilibrated with the initial mobile phase composition, followed by 5 injections of the same standard. These 5 consecutive injections were used to evaluate the system suitability on each day of method validation. The system suitability parameters including resolution (R) = 3.189 tailing factor for atorvastatin, ezetimibe were 1.741, 1.436, which were less than the standard value required for tailing (< 2). All parameters were satisfactory with good specificity for the stability assessment of analytes. Theoretical plates of the column for atorvastatin and ezetimibe were > 3000.

Linearity of the method: Linearity was demonstrated by analyzing five different percentage concentrations of active compound with respect to 10 mg tablets. Peak areas were recorded for all the peaks and calibration plot was constructed by plotting peak area *vs* percentage concentration of atorvastatin and ezetimibe separately, which were found to be linear in the

range of 80 to 120 % for atorvastatin as well as ezetimibe. Correlation coefficient of atorvastatin was 0.9989 and for ezetimibe was 0.9979 (Fig. 3).

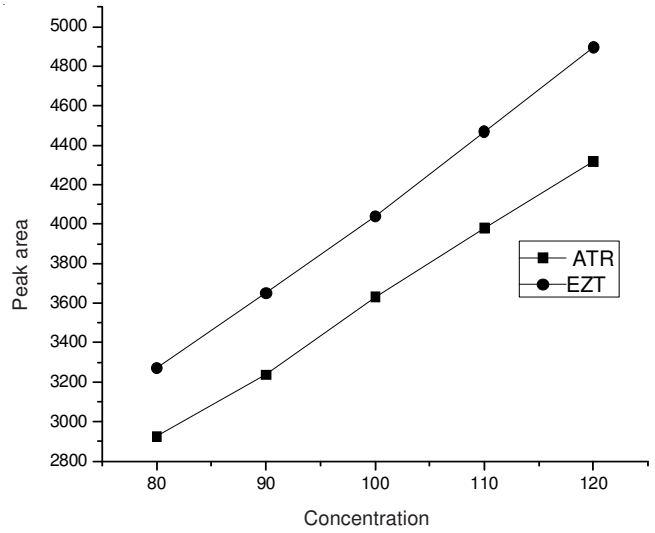

Fig. 3. Calibration curve of ATR and EZT

Specificity: Specificity is the study of suitability of the method for the specific analyte and its freedom from interfering substance either in formulation or in mobile phase. Specificity was accessed by placebo studies and blank run. For the placebo, solution of common excipients used in tablet formulation (starch, talc, lactose) was prepared and injected into the chromatogram. There were no additional peaks or interfering peak were noted near by the retention time of atorvastatin and ezetimibe in the chromatogram obtained using blank run and placebo (Fig. 2a and 4a).

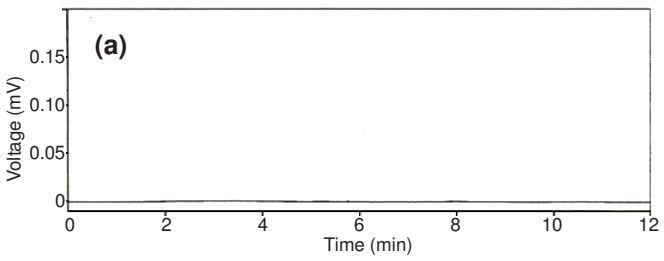

Fig. 4a. Chromatograms of placebo

Limits of detection and limits of quantification: The limit of detection, taken as the lowest absolute concentration of analytes in a sample, which can be detected but not necessary quantified under the stated experimental condition was, 10.5 and 11.4 % for atorvastatin and ezetimibe, respectively. The lowest concentration of analyte in a sample, which can be determined with acceptable precision and accuracy under the experimental condition, is termed as the limit of quantitation, which was found to be 13.0, 13.5 % for atorvastatin, ezetimibe, respectively for the labeled amount 10 mg tablets.

Precision: The precision of a measurement system also called as reproducibility or repeatability is the degree to which repeated measurements under unchanged conditions show the same results. For this solution containing same concentration of the drug was injected repeatedly at different time in the same day (intraday), for 3 days (inter-day). The results of the analysis shows that the relative standard deviation (RSD) of intra-day precision for atorvastatin and ezetimibe at 90 % concentration level were 0.101, 0.0712 (% RSD < 2.0 %) and inter-day precision were 0.201, 0.106 for atorvastatin and ezetimibe (% RSD < 2.0 %) respectively (Table-1) with respect to the labeled amount 10 mg tablets.

Accuracy: Recovery study was done for this purpose, to access this a known quantity of the drug containing solution at three concentrations one above and one below 100 % with respect to to the labeled amount of 10 mg tablets (80, 100 and 120 % with reference to the concentration of pre-analyzed sample solution) were spiked into the pre-analyzed sample and the final solution was injected into the chromatographic system. The results of accuracy were good in agreement with limit given in guidelines for accuracy (Table-1).

Bench top stability: Bench top stability of sample solutions with definite concentration of atorvastatin and ezetimibe were determined by assay after 24 and 48 h at room temperature against fresh standard solutions. It shows that the drug is stable and does not show much variation in the time span up to 48 h (Table-2).

Ruggedness: The study of variation upon the method was evaluated using different HPLC system and by different analyst. This was established by determining atorvastatin and ezetimibe in dosage formulation using the two different chromatographic systems and by two analysts. The percentage RSD % for analyst variation were 0.210-0.481 for atorvastatin and 0.410-0.505 for ezetimibe (limit < 2.0 %).The results of system variation were 0.763 (System 2 Shimadzu 20AT-VP with SPD 10A detector), 0.668 for atorvastatin and 0.683, 0.516 (limit < 2.0 %). This indicates that the method was rugged.

Robustness: Robustness is the study of method's stability towards small, deliberate changes in method parameters like flow rate and detection wavelength on assay of the analyte of interest. Here, the detection wavelength was varied ± 1 nm, whereas the flow rate was varied ± 0.1 mL min^{-1}. The percentage RSD value of assay determined by change in flow rates were 0.760 (ATR), 0.591 (EZT) with 1.2 mL/min and 0.532 (ATR), 0.867 (EZT) (1.3 mL/min). RSD for wavelength change

TABLE-1
RESULTS OF PRECISION AND ACCURACY OF THE METHOD

Precision					Concentration of preanalyzed sample (ATR : EZT)*	Accuracy of the method†	
Concentration*	Inter day†		Intraday† (3 days)			Spike level (%)	Recovered (%)
90%	0.101	0.0712	0.201	0.106	80:80	10	99.83
					100:100	20	99.75
					120:120	30	100.02

ATR: Atorvastatin; EZT: Ezetimibe; *% with respect to labeled amount, †RSD of six determination

TABLE-2
RESULTS OF RUGGEDNESS AND BENCH TOP STABILITY

Ruggedness								Bench top stability							
Analyst*				System*				Initial assay		24 h		48 h		Deviation from the initial assay	
1		2		1		2									
ATR	EZT	ATR	EZT	ATR	EZT	ATR	EZT	ATR	EZT	ATR	EZT	ATR	EZT	ATR	EZT
0.210	0.410	0.481	0.505	0.763	0.683	0.668	0.516	98.9	100.01	98.97	99.78	99.91	99.98	0.07 to 1.08	-0.23 to -0.03

% RSD (limit NMT 2.0%); *Mean of five determinations; ATR: Atorvastatin; EZT: Ezetimibe

TABLE-3
ASSAY RESULTS OF TABLET FORMULATION

Labeled amount (mg/tab)		Assay* (mg/tab)		Assay (%)		RSD* (%)	
ATR	EZT	ATR	EZT	ATR	EZT	ATR	EZT
10	10	9.89	10.01	98.9	100.01	0.752	0.456

ATR: Atorvastatin; EZT: Ezetimibe; *mean of six determinations

0.591 (ATR), 0.658 (EZT) at 246 nm, 0.675 (ATR), 0.799 (EZT) at 248 nm. From that above results were within the limit RSD < 2 % for robustness, which proves that the method is robust over the deliberate change.

Application of the method to formulation: The method was used for the simultaneous estimation of atorvastatin and ezetimibe in tablet formulation as described above. The results obtained (Table-3) showed with high assay values and low percentage RSD were confirms the adaptability of the method for routine simultaneous determination of these components in its pharmaceutical preparation. Typical chromatogram obtained from the analysis of tablet formulation was given in Fig. 4b.

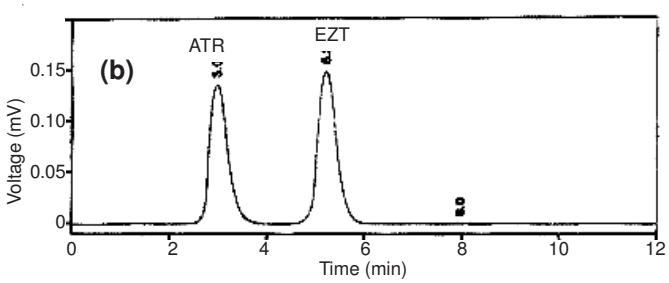

Fig. 4b. Chromatograms of sample

Conclusion

A simple, rapid, sensitive and reliable HPLC method for the simultaneous estimation atorvastatin and ezetimibe in pharmaceutical formulation has been developed and validated with respect to ICH guidelines[26]. However many methods has been reported already, some were less sensitive, longer run time and more tailing in the analytes peak. The recently reported method was more sensitive among the other methods in terms of low range of linearity and detection limit[24] for the simultaneous estimation of atorvastatin and ezetimibe but that too have the disadvantage of using methanol as major solvent (about 60 % of total volume) for separation, which is poisonous and require a strict inventory control for its storage and use further more using methanol cause more column backpressure. This leads to reduction in HPLC pump and column life span due its high viscosity when compared to acetonitrile used in the present method. On the other hand, the methods reported earlier were partially validated. Here in we used a simple mobile phase composed of acetonitrile (popularly used low viscous HPLC solvent) and ammonium acetate in low concentration (0.05 M) both of them were not affect the column life, which makes the method more reliable than other method. Moreover the present methods is comparable with the recently reported method[24] in terms of RT of analytes, shorter runtime, less tailing of component peaks, simple sample preparation further the method reported here is fully validated with respect to ICH guide lines for validation. Hence, it is concluded that present method is suitable as well as an alternative to the existed method for the routine quality control analysis of atorvastatin and ezetimibe in combined tablet formulation without prior extraction of the individual drug components.

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