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Simultaneous RP-HPLC method for the stress degradation studies of atorvastatin calcium and ezetimibe in multicomponent dosage form

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ABSTRACT

A stability-indicating reversed-phase high performance liquid chromatographic (RP-HPLC) method has been developed and validated for simultaneous estimation of atorvastatin calcium and ezetimibe for their multicomponent dosage form. The proposed RP-HPLC method utilizes a 125 mm x 4.6 mm i.d 5 µm Phenomenex C-18 column at ambient temperature; the optimum mobile phase consists of acetonitrile and 0.4% v/v triethylamine (pH adjusted to 5.5 with ortho-phosphoric acid) in the ratio of 55:45, v/v respectively, flow rate of 1.0 ml/min. Measurements were made at a wavelength of 231 nm. Multicomponent dosage form was exposed to thermal, photolytic, hydrolytic and oxidative stress. No co eluting, interfering peaks from excipients, impurities were observed for the degradation products and hence the method was found to be specific. The method was linear in the range of 5-25 µg/ml for atorvastatin calcium and ezetimibe. The mean recoveries were 98.82% and 98.72% for atorvastatin calcium and ezetimibe respectively. The method was validated for linearity, range, precision, accuracy, specificity, selectivity, intermediate precision, ruggedness, robustness, solution stability and suitability.

KEY WORDS: Atorvastatin. Ezetimibe. Degradation. Stress testing. Stability-indicating. Validation.

RESUMEN

Se desarrolló y validó un método estable de cromatografía líquida de alta eficacia de fase reversa (RP-HPLC) para la estimación simultánea de atorvastatina de calcio y ezetimiba en su forma de dosificación multicomponente. El método RP-HPLC propuesto utiliza, a temperatura ambiente, una columna C-18 Phenomenex de 125 mm x 4,6 mm y d.i de 5 µm; la fase móvil óptima consta de acetonitrilo y 0,4% v/v de trietilamina (pH ajustado a 5,5 con ácido ortofosfórico) en una proporción de 55:45, v/v, respectivamente, y una velocidad de flujo de 1,0 ml/min. Las medidas se realizaron a una longitud de onda de 231 nm. La forma de dosificación multicomponente se expuso a estrés oxidativo, hidrolítico, fotolítico y térmico. No se observaron, en la degradación de productos, ni impurezas ni picos de coelución o interferencia por excipientes, y, además, el método resultó ser específico. El método fue linear, en el rango de 5-25 µg/ml para atorvastatina de calcio y ezetimiba. Las recuperaciones medias fueron del 98,82% y 98,72% para atorvastatina de calcio y ezetimiba, respectivamente. El método se validó para linealidad, rango, precisión, exactitud, especificidad, selectividad, precisión intermedia, dureza, robustez, estabilidad de la disolución e idoneidad.

PALABRAS CLAVE: Atorvastatina. Ezitimiba. Degradación. Prueba de estrés. Indicador de estabilidad. Validación

INTRODUCTION

Atorvastatin calcium (ATV) is Chemically [R-(R*, R*)]-2-(4-fluorophenyl)-[beta], dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino) carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate[1]. Ezetimibe (EZE) is (3R,4S)-1-(4-flurophenyl)-3-[(3S)-3-(4-flurophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)-2-azetidinone[2]. Atorvastatin is a synthetic lipid-lowering agent that inhibits [beta]-hydroxy-[beta]-methylglutaryl-coenzyme A (HMG-CoA) reductase, and ezetimibe inhibits the absorption of cholesterol, decreasing the delivery of intestinal cholesterol to the liver. The combined dosage form of ATV and EZE has recently been introduced in the market, where the co administration of EZE with ATV offers a well-tolerated and highly efficient new treatment option for patients with dyslipidemia and may help in prescribing a low dose ATV, which reduce side effects[3].

Detailed survey of analytical literature of ATV revealed several methods viz, LC[4,5] and LC/MS[6,7] for determination in plasma and serum; LC[8] for determination in human serum and pharmaceutical formulations; LC[9,10] for determination in pharmaceuticals in combination with other drugs and high-performance thin-layer chromatography[11] for determination in pharmaceuticals. Similarly, a survey of the analytical literature for EZE revealed methods based on LC[12] for determination in pharmaceuticals and LC/tandem MS[13,14] for determination in human plasma and serum. A stability-indicating LC method with gradient elution has been reported for determination of EZE[15]. None of the reported analytical procedures described a stability-indicating method for simultaneous determination of ATV and EZE in the presence of their degradation products. Hence the present study was aimed to establish inherent stability of ATV and EZE through stress studies under a variety of ICH recommended test conditions and to develop a stability-indicating assay method.

MATERIALS AND METHODS

Instruments and Chromatographic Conditions

Shimadzu HPLC with LC-20AT prominence liquid chromatogram, Rheodyne 7725i with 20 µl loop injector, SPD-M20A Prominence-diode array detector and Sonica ultrasonic cleaner sonicator was used. The output signal was monitored and processed using lab solution software on a HCL computer. Stability studies were carried out in a humidity chamber and photo stability studies were carried out in a photo stability chamber. Thermal stability studies were performed in a dry air oven. The chromatographic column used was a phenomenonx C_{18}, 250 x 4.6 mm i.d. with 5 mm particles. Mobile phase A contains 0.4% v/v triethylamine, pH adjusted to 5.5 using orthophosphoric acid. Mobile phase B contains acetonitrile, in the ratio of 45:55 (v/v). The flow rate of mobile phase was 1.0 ml min^{-1}. The column temperature was maintained at 25°C ± 2°C and the detection was monitored at a wavelength of 231 nm. The injection volume was 20 µl.

Drug and chemicals

ATV and EZE were supplied by Micro Labs (Bangalore, India) and combined dosage form of ATV and EZE was purchased from local pharmacy. The HPLC grade acetonitrile, analytical reagent grade hydrochloric acid, sodium hydroxide and hydrogen peroxide were purchased from Merck, Germany. HPLC grade water was prepared by using Millipore MilliQ plus water purification system.

Preparation of Stock Solutions

A stock solution of ATZ and EZE standard and sample (10 µg/ml) were prepared by dissolving an appropriate amount in mobile phase.

Analysis of Dosage Forms

Twenty tablets were weighed, their mean weight was determined, and they were crushed in a mortar. An amount of powdered mass equivalent to 10 mg each of ATV and EZE was weighed and transferred into a conical flask. The drugs from the powder were dissolved and extracted with 7 ml mobile phase. To ensure complete extraction of the drugs, the solution was sonicated for 30 min. The extract was filtered through Whatmann filter paper No. 41 (Gelman Laboratory, Mumbai, India). The extract was transferred to a 10 ml volumetric flask, and the volume was made up to the line with mobile phase. A 1 ml aliquot from this solution was transferred in a 10 ml volumetric flask, and the volume was adjusted with mobile phase up to the mark (sample stock solution). Aliquot of sample stock solution was suitably diluted with mobile phase to get final concentration of 10 µg/ml for ATV and EZE.

Stress Studies

All stress degradation studies were performed at an initial drug concentration of 10 µg/ml.

Acid hydrolysis

Twenty five mg of the sample was transferred to a round-bottomed flask, and then 10 ml of 1.0 M HCl was added to the above, and refluxed for 2 h in a boiling water bath. At the end of the exposure, the solution was cooled and neutralized with 1.0 M NaOH and transferred into a 250 ml volumetric flask and the volume was made up with mobile phase.

Base hydrolysis

Twenty five mg of the sample was transferred to a round

bottomed flask, and then 10 ml of 1.0 M NaOH was added to the above, and refluxed for 2 h in a boiling water bath. At the end of the exposure the solution was cooled and neutralized with 1.0 M HCl and transferred into a 250 ml volumetric flask and the volume was made up with mobile phase.

Oxidation

Twenty five mg of the sample was transferred to a round-bottomed flask for an exposure of 2 h. Five to 10 ml of 10% hydrogen peroxide was added to the above, and refluxed for 2 h in a boiling water bath. At the end of the exposure, the solution was cooled and transferred into a 250 ml round bottomed flask and the volume was made up with mobile phase.

Validation of the method

System suitability

System suitability of method was performed by calculating the chromatographic parameters namely, column efficiency, resolution peak asymmetry factor and capacity factor on the repetitive of injection of standard solution.

Linearity and range

A stock solution of the drug was prepared at strength of 1 mg/ml. It was diluted to prepare solutions containing 5–25 µg/ml of the drug. The solutions were injected in triplicate into the HPLC column, keeping the injection volume constant (20 µl).

Accuracy

The accuracy of the method was evaluated in triplicate in different concentration levels i.e. 8, 10 and 12 µg/ml in bulk samples of ATV and EZE; the percentage of recoveries were calculated.

Precision

The precision of the method was evaluated in terms of intermediate precision (intra-day and inter-day). Three different concentrations of ATV and EZE were analyzed in six independent series during the same day (intra-day precision) and six consecutive days (inter-day precision); within each series every sample was injected in triplicate. The %RSD values of intra- and inter-day studies for ATV and EZE showed that the precision of the method was satisfactory.

Sensitivity

The sensitivity of the method was determined with respect to LOD, LOQ. The LOD was calculated as 3 times the noise level and LOQ was calculated as 10 times the noise level.

Ruggedness and robustness of the method

Method robustness and ruggedness was determined by analyzing same sample at normal operating conditions and also by changing some operating analytical conditions such as column make, mobile phase composition, flow rate, instrument and analyst.

RESULTS AND DISCUSSION

To develop a precise, accurate, specific and suitable stability-indicating RP-LC method for the simultaneous estimation of ATV and EZE, different mobile phases were used and the proposed chromatographic conditions were found appropriate for quantitative determination in the presence of degradation products and impurities. The optimum mobile phase consisted of 0.4% v/v triethylamine (pH adjusted to 5.5 with ortho phosphoric acid) and acetonitrile; mobile phase flow rate of 1.0 ml min-1; and UV detection at 231 nm. This was selected because it was found to ideally resolve the peaks of ATV (retention time, 4.71 min) and EZE (retention time, 8.15 min) and gave complete separation of their degradation products and impurities and ambient temperature for the column were found to be best for analysis.

Linearity and range

Five working solutions for each analyte in the range of 5-25 µg/ml for ATV and EZE were simultaneously prepared. Each solution was injected in five replicates into the HPLC column, keeping the injection volume constant (20 µl). The linear regressions analysis of ATV and EZE were constructed by plotting the peak area of the analytes (y) versus analytes concentration (µg ml−1) in (x) axis. The calibration curves were linear in the range of 5-25 µg/m for each analyte, with a mean correlation coefficient (r) of more than 0.9999 and the mean of regression equations of $y = 34476 \times C + 21283$ and $y = 29494 \times C - 12490$ for ATV and EZE, respectively. The mean values (± S.D. n = 5) of correlation coefficient, slope, and intercept were calculated.

Precision

The intraday and interday variation of the method was evaluated at 5 different concentration levels (5, 10 and 20 µg/ml). The % R.S.D. values of within-day and day-to-day study were <2% for ATV and EZE revealed that the proposed method is sufficiently precise.

Ruggedness and robustness of the method

As recommended in the ICH Guidelines and the Dutch Pharmacists Guidelines a robustness assessment was performed during the development of the analytical procedure[16]. The ruggedness[17] of the method is assessed by

Table 1: Results of forced degradation studies of ATV and EZE samples using the proposed method

Stress condition/duration/ State	Degradation (%)	
	ATV	EZE
Acidic/0.1 M HCl/24 h/Solution/Room temperature	86.11	9.23
Acidic /0.1 M HCl /2 h/Solution/Elevated temperature (70˚C)	87.83	9.53
Base / 0.1 M NaOH/24 h/ Solution/Room temperature	8.32	88.50
Base / 0.1 M NaOH/2 h/ Solution/ Elevated temperature (70˚C)	8.58	88.69
Oxidative /30% H2O2 /24 h/ Solution/Room temperature	48.1	38.0
Oxidative /30% H2O2 /2 h/ Solution/ Elevated temperature (70˚C)	49.52	44.33
Dry heat/120˚C/2 h/Solid	7.38	2.74
Photolysis/ UV/2 h /Solid	4.51	5.33

Table 2: System Suitability parameters

Parameters	ATV	EZE
Number of theoretical plates/ column efficiency	5174	9304
HETP	32.10	16.34
Tailing factor / Asymmetry factor	1.02	1.08
Capacity factor (k′)	2.759	2.373
Limit of detection LOD (µg/ml)	0.44	0.52
Limit of quantification LOQ (µg/ml)	1.34	1.57
Resolution	3.996	8.234

Table 3: System Suitability parameters

Drug	Level	Amount of standard taken (µg/ml)	Amount of sample taken (µg/ml)	Amount of standard recovered (µg/ml)	Recovery (%)	%RSD
ATV	I	8	10	7.99	99.94	0.0650
	II	10	10	9.96	99.66	0.2172
	III	12	10	11.97	99.81	0.1585
AZE	I	8	10	7.98	99.81	0.1128
	II	10	10	10.32	100.31	0.3018
	III	12	10	11.83	98.60	1.5420

comparison of the intra-day and inter-day assay results for ATV and EZE that has been performed by two analysts. The %RSD values for intra-day and inter-day assays of ATV and EZE in the commercial tablet (Encoded as AE-01) performed in the same laboratory by two analysts did not exceed 0.2% and 0.25% respectively, indicating the method is rugged.

In addition, the robustness of the method was investigated under a variety of conditions including changes of pH of the eluent, flow rate and of buffer composition[18]. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters and by changing analytical operators has proven that the method is robust.

System suitability

System suitability of method was performed by calculating the chromatographic parameters namely; column efficiency, resolution peak asymmetry factor and capacity factor on the repetitive of injection of standard solution were shown in table 2.

Accuracy

Accuracy of the method was checked by a recovery study using the standard addition method at 3 different concentration levels, i.e., a multilevel recovery study. The preanalyzed samples were spiked with an extra 80, 100, and 120% of the standard ATV and EZE, and the mixtures were analyzed by the proposed method. Results of the recovery study were shown in table 3.

Table 4: Assay of ATV and EZE tablet dosage forms by RP-HPLC Method

Formulation	Labeled amount, (mg)		Amount found, (mg)		% Assay		%RSD	
	ATV	EZE	ATV	EZE	ATV	EZE	ATV	EZE
AE-01	10	10	9.88*	9.82*	98.88*	98.82*	0.321	0.312

*average of 5 determinations

Assay of ATV and EZE from Its Tablet Dosage Forms

The assay results of ATV and EZE in tablet dosage forms were comparable with the value claimed on the label. The assay results obtained indicated that the method is suitable for the routine analysis of ATV and EZE in their combined dosage forms (table 4).

Forced degradation studies

The chromatogram of samples degraded with acid, base and hydrogen peroxide showed well separated peaks of pure ATV and EZE as well as some additional peaks at different Rt values. The spots of degraded product were resolved from the drug peak. The chromatograms are shown in figures 1,2,3,4,5 and 6. The number of degradation product with their Rt values listed in table 1.

CONCLUSION

Based on the peak purity study results by the proposed method, it can be concluded that there is no other co eluting peak with the main peaks and hence the method is specific for the estimation of ATV and EZE in the presence of degradation products. Although no attempt was made to identify the degradation products, the described method can be used as stability indicating method for the assay of ATV and EZE in their combined dosage forms. The gradient simultaneous RP-HPLC method proved to be simple, linear, precise, accurate and specific. The method was completely validated showing satisfactory data for all the method validation parameters that were tested. As the described method is capable of separating the drug from its degradation products, it can be employed as a stability

Figure 1: Chromatogram of ATV and EZE standard solution (10 µg/ml)

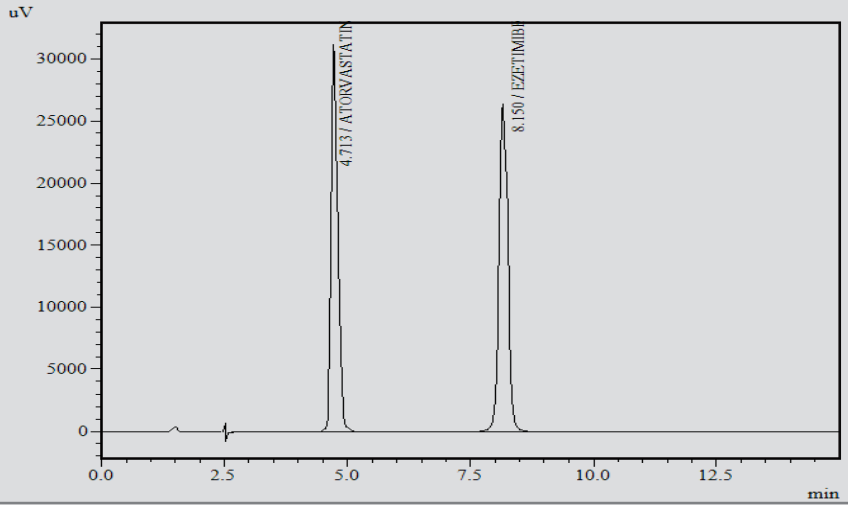


Figure 2: Chromatogram of combined formulation treated with 0.1 M HCl at elevated temperature (70˚C) for 2 h.

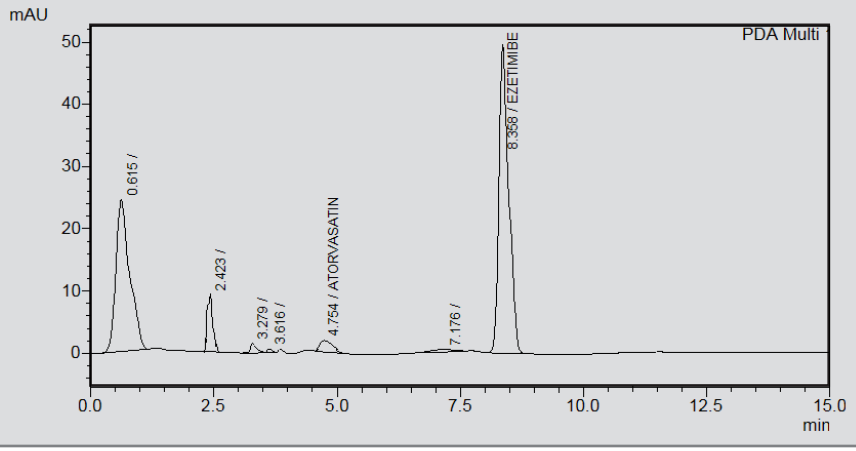


Figure 3: Chromatogram of combined formulation treated with 0.1 M NaOH at elevated temperature (70˚C) for 2 h

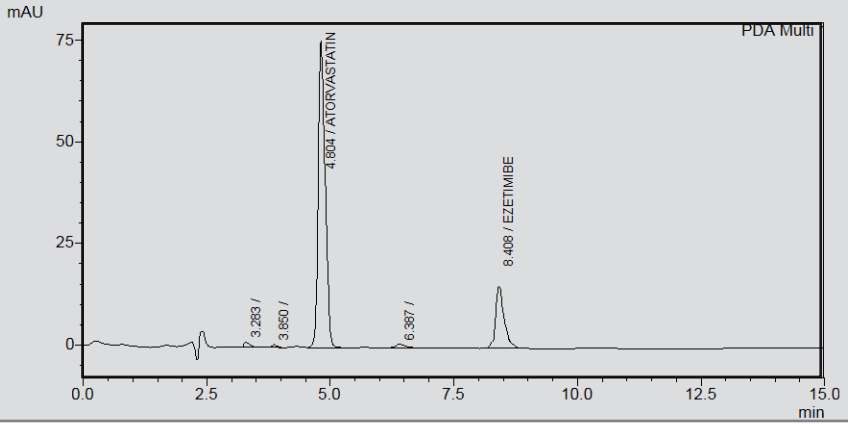


Figure 4: Chromatogram of combined formulation treated with 30% H_2O_2 at elevated temperature (70˚C) for 2 h

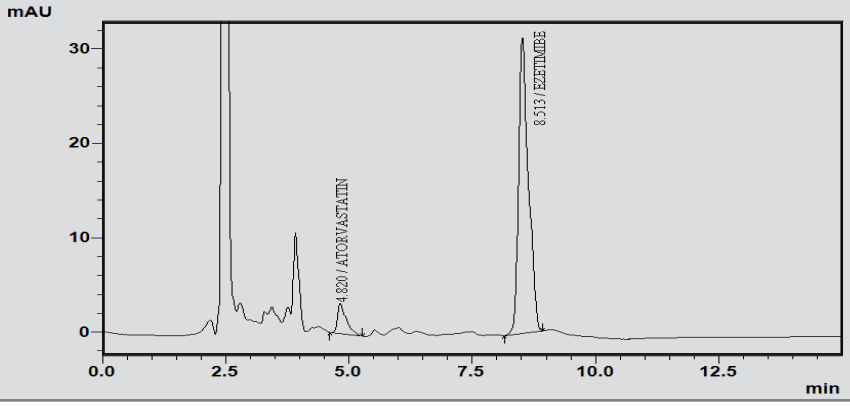


Figure 5: Chromatogram of combined formulation after dry heat (120˚C) degradation for 2 h

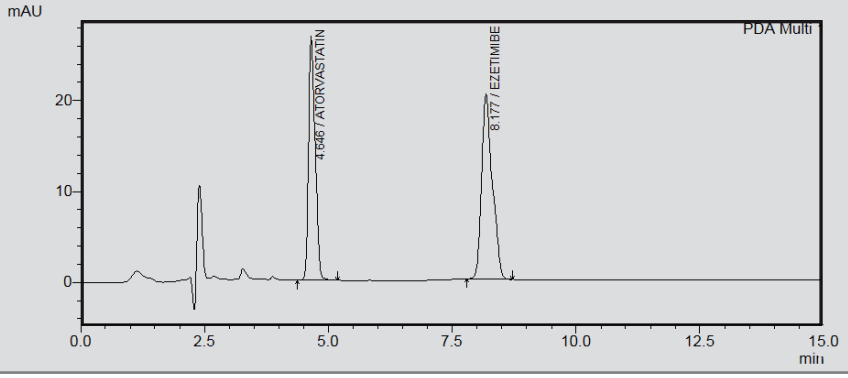
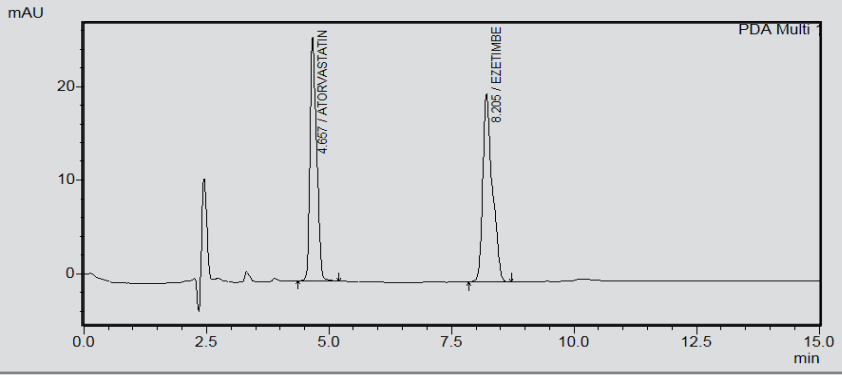


Figure 6: Chromatogram of combined formulation after photolytic degradation for 2 h



indicating one.

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