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RP-HPLC Method for Simultaneous Estimation of Atorvastatin Calcium and Felodipine from Tablet Dosage Form.

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Abstract

The objective of the present study was to develop a simple, accurate, precise and rapid reversed-phase HPLC method for prepared bilayered tablet of Atorvastatin calcium (ATR) and Felodipine (FEL) and subsequent validation using ICH suggested approach for the estimation of both the drugs in combined dosage form. The chromatographic separation of ATR and FEL was achieved on a KYA TECH HiQ Sil C18HS (250 x 4.6 mm, 5 µm) column using a mobile phase of acetonitrile: water [70:30 %V/V]. The flow rate was 1 $mlmin^{-1}$ with detection at 238 nm. The retention times were 1.86 and 11.46 min for ATR and FEL respectively. The proposed method provided linear responses within the concentration range of 5-30 μgml^{-1} for both the drugs with LOD values of 0.09 and 0.12μgml^{-1} for ATR and FEL respectively. Correlation coefficients (*r*) of the regression equations were greater than 0.999 in all sets. The precision of the method was demonstrated using intra- and inter-day assay R.S.D. values which were less than 2% in all instances. No interference from any components of pharmaceutical dosage forms or degradation products was observed. The percentage recoveries obtained for ATR and FEL ranges from 99.21 to 100.74 %. Method validation results showed that, the proposed method was found to be specific, accurate and precise. It could be applied to the quantitative analysis of these drugs in tablets containing ATR-FEL or ATR-FEL binary mixture.

Key Words

RP-HPLC, Atorvastatin calcium, Felodipine and Ezetimibe.

Introduction

Atorvastatin calcium (ATR) is chemically described as [R-(R*, R*)]-2-(4-fluorophenyl)-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino) carbonyl]-1Hpyrrole-1-heptanoic acid calcium salt trihydrate is an antihyper lipoproteinemic agent act by inhibiting HMG-CoA reductase. FEL is a dihydropyridine derivative that is chemically described as ± ethyl methyl4-(2,3-dichlorophenyl)-1,4-dihydro-2,6 dimethyl-3,5-pyridinedicarboxylate3,5-pyridine dicarboxylic acid, 4-(2,3-dichlorophenyl)-1,4-dihydro 2,6-Dimethyl-,ethyl methyl ester is a dihydropyridine calcium-channel blocker. Literature survey reveals that spectrophotometric and chromatographic methods, and a stability-indicating LC method, have been reported for determination of ATR in pharmaceutical preparations in combination with other drugs [1-8] and there is no method was reported for the simultaneous estimation of ATR with FEL in their combined dosage form.

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In present work RP-HPLC method was developed for simultaneous quantitative determination of the two drugs in tablet dosage forms.

Materials and reagents

Atorvastatin calcium, felodipine and ezetimibe were kindly supplied by Cipla Ltd Goa, India. ATR and FEL in their combined tablet dosage form was formulated in the laboratory and used for analysis. Each tablet contained 10 mg of ATR and 10 mg of FEL. For HPLC work double distilled water was prepared in laboratory. Acetonitrile (Loba Chemie Pvt. Ltd. Mumbai, India) of HPLC grade was used.

Instrumentation

The HPLC system used was a PC based Jasco series comprising of a pump PU-2080 and a UV-2070 detector. Manual injections were carried out using a Rheodyne injector with a fixed 20 µl external loop. The chromatographic separations were performed on a 5 µm KYA TECH HiQ Sil C18 HS column (250 mm x 4.6 mm i.d.), operating at ambient

temperature. All experiments were employed in the isocratic mode.

Chromatographic conditions

Method was developed using a stated instrumentation. Mobile phase used was acetonitrile: water [70:30 %V/V]. This phase was filtered through a 0.45 µm membrane filter and degassed by ultrasonication, prior to use. Solvent introduction in to the system was employed at flow rate of 1 $mlmin^{-1}$. Detection of the analytes was carried out at 238 nm. Injection volume used was 20 µl.

Preparation of bilayered tablet

Bilayered tablets weighing 300mg each were prepared by initially adding FEL granules to die of RIMEK minipress (Karnavati engineering, Gujarat, India) and compressed, ATR blend was poured over it and the final compression was applied to prepare the bilayered tablet using 8 mm flat faced punches .The prepared bilayered tablet contained 150mg immediate release layer of ATR and 150mg of sustained release layer of FEL (each layer containing 10mg of API). ATR layer contained sodium starch glycollate, lactose, magnesium stearate, talc and polyvinylpyrrolidone K-30 whereas FEL layer contained talc, HPMC 50cps, microcrystalline cellulose, aerosil, magnesium stearate.

Standard stock solutions and construction of Calibration curves

Standard stock solution containing ATR and FEL were prepared by dissolving 5 mg of each drug in 20 ml of mobile phase separately. It was then sonicated for 10 min and then final volume of the solutions were made up to 50 ml with mobile phase to get stock solutions containing 100 μgml^{-1} of ATR and FEL each in two different 50 ml volumetric flasks. For each drug, appropriate aliquots were pipetted out from each standard stock solution into a series of 10 ml volumetric flasks and to each flask 0.5 ml of internal standard solution of EZT (50 μgml^{-1}) was added and then final volume of the solutions were made up to 10 ml with mobile phase to get set of dilutions having concentration range of 5-30 μgml^{-1} for ATR and FEL each. Six replicate injections were made for each concentration. The calibration curves for ATR–FEL tablet formulation were constructed by plotting the peak area ratio of the drug to that of internal standard, against the drug concentration.

Analysis of the formulation

From the triturate of 20 tablets, an amount equivalent to 10 mg of ATR and 10 mg of FEL was weighed and dissolved in 30 ml of mobile phase, sonicated for 10 min. The solution was filtered through 0.45 µ PVDF syringe filter and then final volume of the solution was made up to 100 ml with mobile phase. Appropriate aliquots were taken and analyzed by the proposed method using the procedure described earlier. Characteristic of ATR and FEL plot shown in Table no 1. The amounts of ATR-FEL in tablet dosage form were calculated using the related linear regression equations. The results are given in Table no 2. Typical chromatogram of ATR and FEL present in the tablet formulation is given in Fig. 1.

Results and Discussion

Optimization of the chromatographic conditions

The aim of this work was to develop a new, rapid and sensitive liquid chromatographic method for the quality control analysis of ATR-FEL tablet dosage form. As these two compounds separated with good stability, ezetimibe was selected as internal standard for the quantitation. The present work describes the RP-HPLC methods for simultaneous estimation of ATR and FEL in tablet formulation. All the drugs were resolved on a KYA TECH HiQ Sil C18HS (250 x 4.6 mm, 5 µm) column. The different ratios of organic solvents like methanol, acetonitrile were used with water but the satisfactory results were obtained using a mobile phase of acetonitrile: water [70:30 %V/V]. The flow rate of 1.0 $mlmin^{-1}$ was employed and the detection wavelength was set at 238 nm.

Validation of HPLC method

The method validation was done to confirm that the present method was suitable for its intended purpose as described in ICH guidelines Q2A and Q2B [19]. After validation, the developed method has been applied to pharmaceutical dosage forms containing ATR-FEL. The described method has been extensively validated in following terms.

Specificity

The specificity of the RP-HPLC method was determined by comparison of the chromatogram of mixed standards and sample solutions. The parameters like retention time (t_R), resolution (R_S) and symmetry factor (T_f) were calculated. Good correlation was found between the results of mixed

standards and sample solutions; it indicates that the proposed method was not affected by interferences from excipients used in formulations.

Linearity

The calibration curves for ATR and FEL in formulation were constructed by plotting the ratio of the peak area of ATR or FEL to peak area of internal standard (EZT) against the concentration i.e. response factor. Linearity data were obtained using standard solutions containing ATR and FEL at six different concentrations ranging from 5-30 μgml^{-1}, whilst keeping the concentration of the EZT (IS) constant at $25\mu gml^{-1}$. ATR and FEL showed good linearity, correlation co-efficient ('*r*' value) for ATR and FEL was found to be 0.9997 and 0.9991 respectively.

Precision

The precision of the proposed method was checked as repeatability and intermediate precision performing five replicate injections of three different sample solutions at low, medium and high concentrations, which were freshly prepared and analyzed daily (Table no 3). Precision study was performed to find out intra-day and inter-day variations. The % relative standard deviation (RSD) for intra-day precision was 0.67% and 1.6366 % for ATR and FEL respectively and for inter-day precision was 0.9666%, and 1.3966% for ATR and FEL respectively which is less than 2% indicating high degree of precision.

Accuracy

Accuracy was demonstrated by performing recovery studies experiments using standard addition method. This study was performed by standard addition method at three levels. Known amounts of standard ATR and FEL were added to pre-analyzed samples and they were subjected to proposed RP-HPLC method. The results obtained of recovery studies are shown in Table no 4. The % RSD for the tablet analysis and recovery studies was less than 2% indicating high degree of accuracy.

Limit of detection (LOD) and limit of quantitation (LOQ)

In the present study, the LOD and LOQ were based on the use of standard deviation of the response and the slope of the calibration curve and were calculated according to the $3.3\sigma/s$ and $10\sigma/s$ criterions, respectively; where σ is the standard deviation of the peak area ratios and s is the slope of the corresponding calibration curve. The LOD and LOQ value of the developed method was found to be 0.09 and 0.27 for ATR and 0.12 and 0.36 for FEL respectively.

Robustness

The robustness study was done by making small changes in the optimized method parameters, ±1% change in mobile phase ratio, ± 0.1 $mlmin^{-1}$ change in flow rate. It was found that there were no significant impacts on the retention time.

Ruggedness

The ruggedness study was done by changing the source of chemicals. The % RSD for source-I was 0.3406% and 0.3063% for ATR and FEL respectively and % RSD for source -II was found to be 0.3394% and 0.6009% for ATR and FEL respectively.

System suitability

System suitability tests are an integral part of a HPLC method, and they were used to verify that whether the proposed method was able to produce good resolution between the peaks of interest with high reproducibility. The system suitability was determined by making six replicate injections from freshly prepared standard solutions and analyzing each solute for their peak area, theoretical plates (*N*), resolution (*R*) and symmetry factor (*T*). System suitability requirements for ATR and FEL are, % R. S. D. of peak areas and retention times less than 1%, peak resolution (*R*) greater than 2.0 between two adjacent peaks for three analytes, theoretical plate numbers (*N*) at least 2000 for each peaks and symmetry factors (*T*) greater than 1.2. The results of system suitability test in comparison with the required limits are represented in Table no 5. According to the results presented, the proposed method fulfils these requirements within the accepted limits.

Conclusion

The validated RP-HPLC for simultaneous estimation of ATR and FEL from prepared bilayered tablet had been successfully developed. Method had been proved to be simple, precise, rapid and reliable. The proposed method provides a good resolution between ATR and FEL. The developed method reported herein was validated by evaluation of the validation parameters as described in ICH-Q2B guideline. System suitability, specificity, linearity, LOD, LOQ values, inter- and intra-day precision and accuracy of the proposed techniques were obtained

during the validation studies. Thus the proposed method is suitable for the screening of formulated samples in routine quality control applications.

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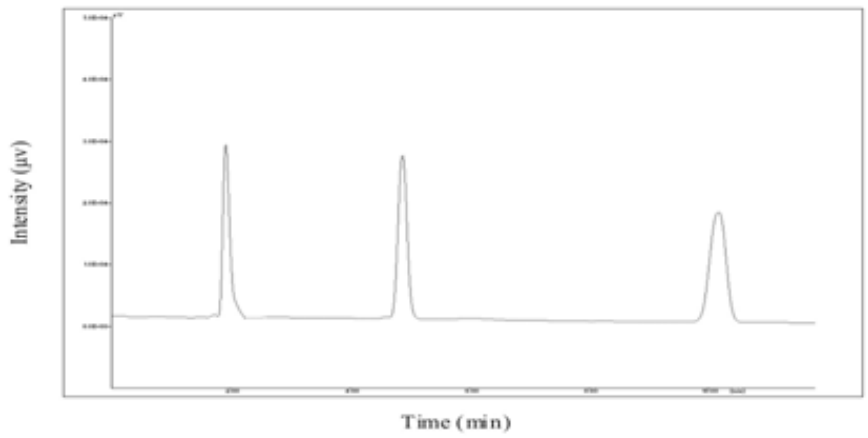

Fig. 1: Chromatogram of ATR (RT=1.82 min), EZT (5.2) and FEL (RT=11.46 min) in tablet formulation.

Table 1: Characteristics of ATR and FEL calibration plots.

Parameter	ATR	FEL
Linearity range (µgml^{-1})	5-30	5-30
Slope	0.0874	0.94711
Intercept	1.247	1.8220
Correlation coefficient (*r*)	0.9997	0.9991
R.S.D.% of slope	0.14	0.51
R.S.D.% of intercept	1.25	1.48
Limit of detection (µgml^{-1})	0.09	0.27
Limit of quantification (µgml^{-1})	1.12	0.36

Table 2: Result of Laboratory formulation analysis.

Formulation	Drug	Label claim (mg/ tablet)	Estimated % of label claim ± SD*	% Recovery ± SD		
				80	100	120
Laboratory	ATR	10	98.6± 0.46	99.95±0.35	100.09±0.74	100.10 ± 0.86
formulation	FEL	10	102.2±0.76	99.59±0.74	99.45 ± 0.52	100.04 ± 0.32

Table 3: Summary of recovery study and intra-day (repeatability) and inter-day (intermediate precision) variability data for simultaneous determination of ATR and FEL standards.

Compound	Theoretical concentration (µgml^{-1}	Intra-day measured concentration (µgml^{-1})[a]		Inter-day measured concentration (µgml^{-1})[b]	
		Mean	R.S.D.%	Mean	R.S.D.%
ATR	8	8.02	0.34	8.04	1.11
	10	10.90	0.78	10.08	1.45
	12	12.23	0.89	12.03	0.34
FEL	8	8.17	1.45	8.17	1.71
	10	10.71	1.78	10.22	1.12
	12	12.99	1.68	12.25	1.36

[a] Mean values represent five different sample standards for each concentration.

[b] Inter-day precision was determined from five different runs over a 2-week period.

Table no 4: Accuracy data for simultaneous estimation of ATR and FEL.

Drug	Label claimed (mg)	Amount recovered(mg)	% Recovery estimated	% RSD
ATR	10	9.628	96.28	0.67
FEL	10	9.861	98.61	0.49

Table 5: System suitability results of the proposed method.

Compound	*N*	*R*	*T*	R.S.D. of	
				*t*R	Peak area
ATR	3566	-	1.98	0.36	0.84
EZT	4146	3.556	1.49	0.24	0.68
FEL	7430	5.56	1.26	0.51	0.49
Required limits	*N*> 20	*R*>2	*T* > 1.2	R.S.D. < 1%	

N: theoretical number of plates; *R*: resolution; *T*: symmetry factor; *t*R: retention time; R.S.D.: relative standard deviation for retention time or peak areas obtained from six replicate injections from six replicate injections (instrument precision).

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