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Simultaneous Spectrophotometric Determination of Atorvastatin Calcium and Ezetimibe in Tablet Dosage Form

Baldha R. G.[1], Patel Vandana. B.[2*] and Mayank Bapna[2]

[1]Pharmaceutical Quality Assurance Laboratory, Pharmacy Department, Faculty of Technology and Engineering,The Maharaja Sayajirao University of Baroda,Vadodara 390 001,Gujarat ,India
[2] Baroda College of Pharmacy,P.O. Limda, Ta. Waghodia,Dist. Vadodara, Pin 391 760 Gujarat, India
E-mail: vbpatel04@yahoo.com

ABSTRACT : Rapid, accurate and precise UV spectrophotometric method using dual wavelength measurement was developed for the simultaneous determination of Atorvastatin Calcium and Ezetimibe in tablet dosage form. In the proposed method, Atorvastatin Calcium was determined by plotting the difference in absorbance at 227 and 246.5 nm (difference is zero for Ezetimibe) against the concentration of Atorvastatin Calcium. Similarly for the determination of Ezetimibe, the difference in absorbance at 235 and 258.2 nm (difference is zero for atorvastatin calcium) was plotted against the concentration of Ezetimibe. Linearity was observed in the concentration range of 5-30 mcg/ml for both the drugs. The method was validated statistically and recovery study was performed to confirm the accuracy of the method. Commercially available tablet formulation was successfully analyzed using the developed method.
KEY WORDS: Atorvastatin, Ezetimibe, Spectrophotometry, Pharmaceutical formulations

INTRODUCTION

Atorvastatin Calcium[1] (ATVC), (βR,δR)-2-(4-fluorophenyl)-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-lH-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate; is a synthetic cholesterol-lowering agent, called HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) reductase inhibitor. Ezetimibe[2] (EZTB), (3R,4S)-1-(4-fluorophenyl)-3-[(3S)-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)-2-azetidinone; is a class of lipid-lowering compound that selectively inhibits the intestinal absorption of cholesterol and related phytosterols. Combination therapy of ATVC and EZTB is used for the treatment of primary (heterozygous familial and non-familial) hypercholesterolemia. Analytical methods have been developed by one of the authors for estimation of ATVC with other antihypertensive agent[3-4]. Various analytical methods like spectrophotometric[5], HPLC[6-7], HPTLC[8] have been reported for the determination of ATVC and EZTB in binary mixture. The aim of this work is to develop a new UV spectrophotometric method using dual wavelength measurement for simultaneous determination of both these drugs.

EXPERIMENTAL

APPARATUS

Spectrophotometric analysis was carried out on a Shimadzu 1601 double beam spectrophotometer with a fixed slit width (2 nm) using a pair of 1 cm matched quartz cells. The system software of the instrument was used for obtaining the spectra. Pure drug sample of ATVC and EZTB was kindly gifted by M/s. Zydus Cadila Ltd., Ahmedabad and M/s. Sun Pharmaceuticals Ltd., Salvassa respectively. Methanol was procured from Allied Chemical Corporation, Vadodara. Commercial pharmaceutical preparation (Zatistat-10®, Torrent, Indrad) was procured from commercial source. All weighings were performed on an electronic single pan balance (Precisa 205A SCS).

PREPARATION OF STANDARD STOCK SOLUTIONS

ATVC and EZTB, 50 mg each, were accurately weighed and dissolved separately in 50 ml of methanol. Five ml of the above solutions were diluted separately to 50 ml with methanol to produce 100 mcg/ml standard stock solutions of ATVC and EZTB in methanol.

PREPARATION OF WORKING STANDARD SOLUTION AND BINARY SOLUTIONS

Suitable aliquots of standard stock solutions were diluted separately with methanol to obtain 15 mcg/ml of working standard solution of ATVC and EZTB. Also, suitable aliquots of standard stock solutions of both the drugs

were mixed and diluted with methanol to prepare binary solutions containing ATVC and EZTB in the concentration range of 5:5 mcg/ml to 30:30 mcg/ml.

PREPARATION OF CALIBRATION CURVE

The working standard solutions of ATVC and EZTB were scanned in the entire range from 200 to 400 nm to select the wavelength for estimation of drugs. The difference in absorbance at 227 and 246.5 nm was found to be zero for EZTB. Hence these two wavelengths were selected for the determination of ATVC. Similarly, 235 and 258.2 nm were selected for the determination of EZTB, where the difference in absorbance was found to be zero for ATVC.

Different binary solutions of ATVC and EZTB were run in the entire range from 200 to 400 nm. The difference in absorbances at 227 and 246.5 nm were plotted against the concentration of ATVC and that at 235 and 258.2 nm were plotted against the concentration of EZTB to construct two separate calibration curves for ATVC and EZTB. The method shows good linearity in concentration range of 5-30 mcg/ml for both ATVC and EZTB. The data are shown in Table I.

Table I Calibration data for ATVC and EZTB

Parameters	**For ATVC** *	**For EZTB****
Beer's law limit (µg/ml)	5 – 30	5 – 30
Correlation coefficient (r^2)	0.9992	0.9986
Slope	0.0085	0.0154
Intercept	+0.0023	+ 0.01
Intraday precision (% RSD)	2.049	0.896
Interday precision (% RSD)	2.180	1.253
Reproducibility (% RSD)	2.505	2.106

* **Difference in absorbance at 227 and 246.5 nm,**

** **Difference in absorbance at 235 and 258.2 nm**

VALIDATION OF THE METHOD

The developed method was validated in terms of accuracy, precision and robustness. Accuracy of the method was determined by performing recovery studies by standard addition method in which pre-analyzed samples were taken and standard drug was added at five different levels from 80 % to 120 % of the label claim. Intraday and interday precision of the method were confirmed by repeating the calibration curve five times in a day and also on five different days. Reproducibility of the method was confirmed by repeating the calibration curve with the use of methanol from three different manufacturers (Allied Chemical Corporation, Vadodara, S.D. fine Chemicals, Mumbai and Qualigens, Mumbai). The results of validation studies are shown in Table II and III.

ANALYSIS OF TABLETS

A total of twenty tablets were accurately weighed and crushed to fine powder. An amount equivalent to one tablet (containing 10 mg of ATVC and 10 mg of EZTB) was taken and dissolved in 10 ml of methanol by magnetically stirring it for five minutes. About 10 ml of methanol was added and stirred for further 5 minutes. The mixture was transferred to centrifuge tubes and centrifuged at 1000 rpm for 5 minutes. The supernatant was transferred to a 100 ml volumetric flask through a Whatman No. 40 filter paper. The residue was washed thrice with methanol and the combined filtrate and washings were made up to the mark with methanol. The sample solution thus prepared was diluted with methanol to get the solutions containing ATVC: EZTB in 1:1 mcg/ml proportions. The above solution was analyzed for the content of ATVC and EZTB using the method described in preparation of calibration curve. The results of analysis are shown in Table IV.

RESULTS AND DISCUSSION

The proposed method for simultaneous determination of ATVC and EZTB in their combined dosage form was found to be simple, accurate, economical and rapid. The interference of other drug was nullified by carefully selecting the wavelengths at which the other drug shows zero absorbance. The difference in absorbance at 227 and 246.5 nm was selected for ATVC (difference is zero for EZTB) whereas 235 and 258.2 nm was selected for the determination of EZTB (difference is zero for ATVC) .

The method shows good linearity in concentration range of 5-30 mcg/ml for both ATVC and EZTB with correlation coefficients, 0.9992 and 0.9986, slopes, 0.0085 and 0.0154, and intercepts, +0.0023 and +0.01 respectively as shown in Table I. The method was validated statistically and the results are summarized in Tables II and III. The average relative standard deviation (% RSD) for intraday precision was found to be 2.049 for ATVC and 0.896 for EZTB and the values for interday precision were found to be 2.180 for ATVC and 1.253 for EZTB. The average % RSD, 2.565 for ATVC and 2.106 for EZTB proves the reproducibility of the method. The % recovery±SD was in the range of 98.10±0.68 to 100.5±0.95 for ATVC and 98.50±0.65 to 100.9±0.49 for EZTB, which justifies the accuracy of the method. The commercially available tablet formulation (Zetistat-10®) was successfully analyzed by the proposed method. The value of % recovery±SD, 101.8±1.03 for ATVC and 102.4±1.18 for EZTB as shown in Table IV revealed the suitability of the proposed method for the analysis of tablet formulation.

Table II Recovery study of proposed method

% Standard addition	Sample conc. ATVC: EZTB (µg/ml)	Difference in amplitude 227 and 246.5 nm	Difference in amplitude 235 and 258.2 nm	% Recovery of ATVC	% Recovery of EZTB
80	10: 10	0.114	0.211	100.4 ± 0.725	98.80 ± 0.483
90	10: 10	0.121	0.228	98.10 ± 0.680	100.7 ± 0.679
100	10: 10	0.129	0.241	98.70 ± 0.526	98.50 ± 0.651
110	10: 10	0.140	0.258	100.5 ± 0.950	99.70 ± 1.421
120	10: 10	0.148	0.275	100.1 ± 1.121	100.9 ± 0.492

Table III Precision and reproducibility data for ATVC and EZTB

Conc. (µg/ml)	*Difference in amplitude		% RSD1*		% RSD2*		% RSD3*	
	at 227 and 246.5 nm for ATVC	at 235 and 258.2 nm for EZTB	ATVC	EZTB	ATVC	EZTB	ATVC	EZTB
5	0.044	0.083	4.670	1.791	3.466	2.227	3.756	4.819
10	0.085	0.163	2.853	1.003	2.661	1.864	2.086	2.77
15	0.131	0.243	1.819	0.985	2.409	1.403	3.580	1.489
20	0.175	0.322	1.098	0.561	1.925	0.958	2.591	1.552
25	0.215	0.400	0.899	0.453	1.431	0.598	1.820	1.012
30	0.254	0.463	0.954	0.584	1.188	0.469	1.556	0.991
Average % RSD			**2.049**	**0.896**	**2.180**	**1.253**	**2.565**	**2.106**

***** Average of five experiments, **% RSD1=** Intraday precision, **% RSD2** = Inter day precision,
% RSD3= Reproducibility with methanol from three different manufacturers

Table IV Analysis of tablet formulation

Formulation	Label claim (mg)		Conc. found* (mg)		% Recovery± SD	
	ATVC	EZTB	ATVC	EZTB	ATVC	EZTB
Zatistat-10®	10	10	10.05	9.92	100.5± 0.489	99.17± 0.317

CONCLUSION

The proposed dual wavelength measurement method for simultaneous determination of ATVC and EZTB is a suitable technique for reliable analysis of the commercial formulation containing combination of these drugs and may be successfully applied in control laboratories for their determination in combined dosage form. There was no interference from the excipients used in the tablet formulations and hence the methods are suitable for analysis of tablets. The results of validation show that the proposed methods are simple, linear, precise, accurate and selective and can be employed in routine assay of ATVC and EZTB in tablets.

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