Asian Journal of Chemistry Vol. 20, No. 1 (2008), 155-160

Colorimetric Estimation of Ezetimibe and Simultaneous Spectrophotometric Estimation of Ezetimibe with Atorvastatin Calcium in Tablet Formulation

D.D. DESHMUKH, N.M. BHATIA*, H.N. MORE and M.S. BHATIA
Department of Pharmaceutical Chemistry,
Bharati Vidyapeeth College of Pharmacy, Kolhapur-416 013, India
E-mail: neelabhatia@yahoo.co.uk

A simple, sensitive and rapid colorimetric method for estimation of ezetimibe and spectrophotometric method for simultaneous estimation of atorvastatin calcium and ezetimibe in tablet formulations have been developed. Colorimetric method for the estimation of ezetimibe was based on the formation of ion pair complex of drug with dye. The method was based on the formation of bluish-green coloured complex with patent blue-V and hydrochloric acid. The coloured complex showed absorbance maxima at 636 nm and obeyed Beer's law in the concentration range of 20-50 µg/mL. Multi-wavelength method was employed for simultaneous estimation of atorvastatin calcium and ezetimibe from tablet formulation. Multi-wavelength method was used to eliminate interference due to absorbance of ezetimibe at the sampling wavelength for atorvastatin calcium and absorbance of atorvastatin calcium at the sampling wavelength for ezetimibe. Atorvastatin calcium and ezetimibe showed absorbance maxima at 245 and 232 nm, respectively. Both the drugs obeyed Beer's law in the concentration range of 2.5-20 µg/mL. Results of analysis for both the methods were validated statistically and by recovery studies. Both the methods were found to be precise and accurate and can be adopted for routine analysis of drugs in formulation. Both the methods involve no extraction or separation process for the estimation of two drugs.

Key Words: UV-Visible Spectrophotometry, Ezetimibe, Atorvastatin calcium.

INTRODUCTION

Atorvastatin calcium, chemically [R-(R*,R*)]-2-(4-Flurophenyl)-β-δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4[(phenylamino)carbonyl]-1H-pyrrole-1-hepatonic acid, calcium salt (2:1) trihydrate is an HMG-CoA reductase inhibitor. Several HPLC[1], RP-HPLC[2-4], HPTLC[5], HPLC-MS[6] and UV-visible spectrophotometric methods[7] have been reported for the estimation of atorvastatin. Ezetimibe, chemically 1-(4-flurophenyl)-3(R)-

[3-(4-flurophenyl)-3(S)-hydroxypropyl]-4(S)-(4-hydroxyphenyl)-2-azetidione is a selective inhibitor of intestinal cholesterol. RP-HPLC[8,9], HPLC[10], LC-MS/MS[11] and colorimetric method[12] have been reported for the estimation of ezetimibe. The proposed methods are suitable for routine laboratory analysis as well as for marketed preparation and were found to yield accurate and precise results.

EXPERIMENTAL

The instrument used for the present study was PC based Jasco V-530 UV-visible double beam spectrophotometer with 1 cm matched pair quartz cells and spectral bandwidth of 2 nm. HPLC grade methanol was used for the simultaneous estimation of atorvastatin calcium and ezetimibe and Loba grade patent blue-V was used for colorimetric method.

Standard stock solution of ezetimibe was prepared by dissolving 10 mg of ezetimibe in 40 mL of methanol and then final volume was made up to 100 mL with methanol to get stock solution containing 100 µg/mL of ezetimibe. Solutions of patent blue-V (100 µg/mL) and hydrochloric acid (0.1 N) were freshly prepared in distilled water. In to a series of 10 mL volumetric flasks, 0.5 to 5 mL of ezetimibe solution (100 µg/mL) was pipetted separately and to each flask 1 mL of (100 µg/mL) patent blue-V was added. Then 1 mL of 0.1 N hydrochloric acid was added and then final volume was made up to 10 mL with distilled water. The absorbance of bluish-green colour developed was measured against the reagent blank. The coloured complex showed absorbance maxima at 636 nm and obeyed Beer's law in the concentration range of 20-50 µg/mL against the different concentrations of the drug. Overlain spectra of all the concentrations of drug used for calibration curve are shown in Fig. 1. Using quantitative modes of the instrument slope, intercept and correlation coefficient values for calibration curve were obtained and the concentration of ezetimibe in sample solution was calculated by using equation $Abs = A + B \times C$, where A = -0.0288 and B = 0.0150, C = concentration of ezetimibe at 636 nm and the correlation coefficient value was 0.9983.

From the triturate of 20 tablets, an amount equivalent to 10 mg of ezetimibe was weighed and dissolved in 40 mL of methanol and the solution was filtered through Whatmann filter paper no. 41 and then final volume of the solution was made up to 100 mL with methanol to get a stock solution containing 100 µg/mL of ezetimibe. After appropriate dilutions and addition of reagents as per procedure described for calibration curve absorbances were measured at 636 nm and the concentration of each analyte was determined with the equations generated. The statistical data obtained after replicate determinations (n = 6) are shown in Table-1.

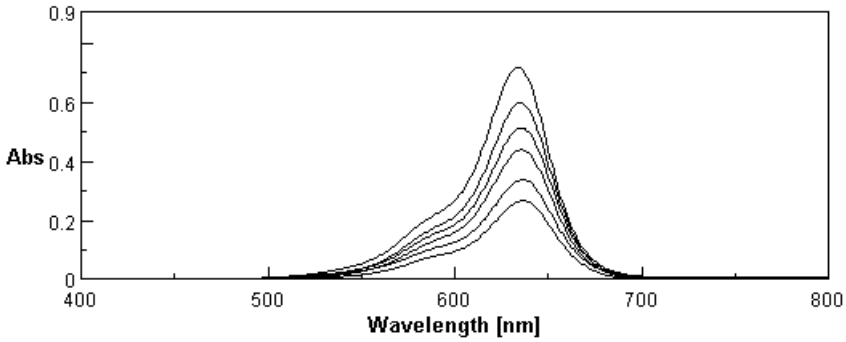

Fig. 1. Overlain spectra of complex of ezetimibe with patent blue-V (all calibration concentrations)

TABLE-1
ANALYSIS OF TABLET FORMULATION

Formulation	Analyte	Label claim (mg)	% Label claim estimated* (Mean ± SD)	Coefficient of variance
Tablet-1	Ezetimibe	10	99.58 ± 1.30	1.305
Tablet-2	Ezetimibe	10	99.31 ± 1.73	1.724

*Average of six determinations.

Accuracy of analysis was determined by performing recovery studies by using spiked concentrations of pure drug in the pre-analyzed tablet sample solution. Absorbance was measured at 636 nm and the concentration of each analyte was determined with the equations generated. The statistical data obtained after replicate determinations (n = 6) are shown in Table-2.

TABLE-2
RECOVERY STUDIES DATA

Formulation	Analyte	Label claim (mg)	% Recovery estimated* (Mean ± SD)	Coefficient of variance
Tablet-1	Ezetimibe	10	100.47 ± 1.290	1.283
Tablet-2	Ezetimibe	10	99.14 ± 0.953	0.961

*Average of six determinations.

Temperature of the reaction, quantity, concentration and addition of various reagents were optimized after several experiments. The optimum quantity and concentration of patent blue-V, hydrochloric acid were found

to be 1 mL, 1 mL and 100 µg/mL, 0.1 N, respectively. The complex was found to be stable for 2 h. The method involves no extraction or heating process.

Simultaneous spectrophotometric method

Standard stock solutions containing atorvastatin calcium and ezetimibe were prepared by dissolving 10 mg of atorvastatin calcium and ezetimibe separately in 40 mL of methanol and then final volume of both the solutions was made up to 100 mL with methanol to get stock solutions containing 100 µg/mL of atorvastatin calcium and ezetimibe. From these stock solutions, working standard solutions of the drugs containing 10 µg/mL of atorvastatin calcium and ezetimibe were scanned separately in the range of 200-400 nm to determine the wavelength of maximum absorption. Atorvastatin calcium and ezetimibe showed absorbance maxima at 245 and 232 nm, respectively. Multi-wavelength method was used to eliminate interference due to absorbance of ezetimibe at the sampling wavelength for atorvastatin calcium and absorbance of atorvastatin calcium at the sampling wavelength for ezetimibe. In the multi-wavelength method developed for simultaneous estimation of atorvastatin calcium and ezetimibe the wavelengths were selected from the overlain spectra as shown in Fig. 2. For atorvastatin calcium the two wavelengths selected were 245 and 225 nm and for ezetimibe were 232 and 257 nm. After appropriate dilutions eight mixed standards containing 2.5-20 µg/mL of atorvastatin calcium and ezetimibe were analyzed and the calibration curve for atorvastatin calcium was constructed by recording absorbance difference at the two selected wavelengths (245 and 225 nm). Similarly, calibration curve for ezetimibe was constructed by recording absorbance difference at the two selected wavelength (232 and 257 nm). Both the drugs obeyed Beer's law in the concentration range of 2.5-20 µg/mL. By using quantitative modes of instrument slope, intercept and correlation coefficient values for calibration curves were obtained for both the drugs. For atorvastatin

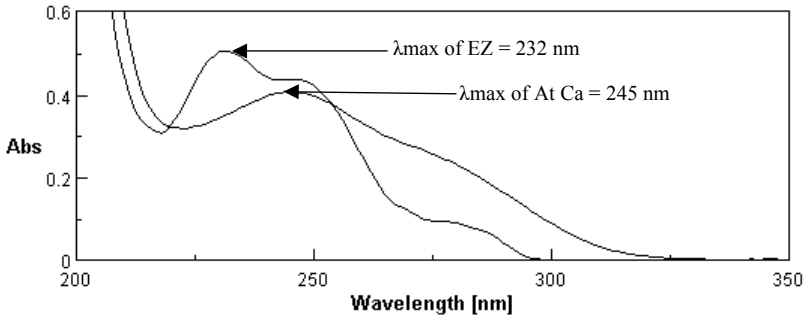

Fig. 2. Overlain spectra of atorvastatin calcium and ezetimibe

calcium the concentration in sample solution was calculated by using equation Abs = A + B × C, where A = -0.0063 and B = 0.0197, C = concentration of atorvastatin calcium. For ezetimibe the concentration in sample solution was calculated by using equation Abs = A + B × C, where A = -0.0078, B = 0.0146, C = concentration of ezetimibe. The correlation coefficient for atorvastatin calcium and ezetimibe was found to be 0.998 and 0.999, respectively.

The commercially available tablet formulations of two manufacturers containing 10 mg of atorvastatin calcium and ezetimibe were analyzed using this method. From the triturate of 20 tablets, an amount equivalent to 10 mg of atorvastatin calcium and 10 mg of ezetimibe was weighed and dissolved in 40 mL of methanol. The solution was then filtered through Whatmann filter paper no. 41 and then final volume of the solution was made up to 100 mL with methanol to get a stock solutions containing 100 μg/mL of atorvastatin calcium and ezetimibe. After appropriate dilutions, the absorbances were measured and the concentration of each analyte was determined with the equations generated in the method. The statistical data obtained after replicate determinations (n = 6) are shown in Table-3.

TABLE-3
ANALYSIS OF TABLET FORMULATION

Formulation	Analyte	Label claim (mg)	% Label claim estimated* (Mean ± SD)	Coefficient of variance
Tablet-1	Atorvastatin calcium	10	100.13 ± 1.830	1.820
	Ezetimibe	10	99.42 ± 1.292	1.297
Tablet-2	Atorvastatin calcium	10	98.27 ± 1.431	1.456
	Ezetimibe	10	99.53 ± 1.573	1.580

*Average of six determinations.

The accuracy of the analysis was determined by performing recovery studies by spiking different concentrations of pure drug in the pre-analyzed tablet sample. Results of recovery studies indicated that the method is rapid, accurate and reproducible shown in Table-4.

RESULTS AND DISCUSSION

The proposed colorimetric method for estimation of ezetimibe and the simultaneous spectrophotometric method for estimation of atorvastatin calcium and ezetimibe in tablet formulations were found to be simple, accurate and reproducible for routine analysis of formulations containing these drugs. The recovery data obtained in both the methods (Tables 2 and 4) does not differ significantly from 100 % and thus confirms accuracy and

TABLE-4
RECOVERY STUDIES DATA

Formulation	Analyte	Label claim (mg)	%Recovery estimated* (Mean ± SD)	Coefficient of variance
Tablet-1	Atorvastatin calcium	10	99.11 ± 1.734	1.745
	Ezetimibe	10	101.17 ± 1.262	1.247
Tablet-2	Atorvastatin calcium	10	99.57 ± 1.517	1.518
	Ezetimibe	10	98.13 ± 1.373	1.399

*Average of six determinations.

sensitivity of the methods. The results of analysis of formulation (Tables 1 and 3) indicate that there is no interference from the common excipients used in the formulation and the methods developed are reproducible.

ACKNOWLEDGEMENT

The authors are thankful to Lupin pharmaceuticals, Mumbai for providing gift samples of atorvastatin calcium and ezetimibe to carry out this work.

REFERENCES

1. S. Eturk, E.S. Aktas, L. Ersoy and S. Ficicioglu, *J. Pharm. Biomed. Anal.*, **33**, 1017 (2003).
2. P. Shanmugapandiyan, K. Manoj and S. Anbazhagan, *Indian Drugs*, **41**, 284 (2004).
3. G. Bahrami, B. Mohammadi, S. Mirzaeei and A. Kiani, *J. Chromatogr. B*, **826**, 41 (2005).
4. K.R Rajeswari, G.G. Sankar, A.L. Rao and J.V.L.N. Seshagirirao, *Indian J. Pharm. Sci.*, **68**, 275 (2006).
5. S.S. Yadav, D.V. Mhaske, A.B. Kakad, B.D. Patil, S.S. Kadam and S.R. Dhaneshwar, *Indian J. Pharm. Sci.*, **67**, 182 (2005).
6. W.W. Bullen, A.M. Ronald and R.N. Hayes, *J. Am. Soc. Mass Spectrom.*, **10**, 55 (1999).
7. R. Shahu and V.B. Patel, *Indian Drugs*, **43**, 160 (2006).
8. R. Sistla, V.S.S.K. Tata, Y.V. Kashyap, D. Chandrasekar and P.V. Diwan, *J. Pharm. Biomed. Anal.*, **39**, 517 (2005).
9. D.G. Sankar and D.V.S.P. Kumar, *Asian J. Chem.*, **18**, 823 (2006).
10. S. Singh, B. Singh, R. Bahuguna, L. Wadhwa and R. Saxena, *J. Pharm. Biomed. Anal.*, **41**, 1037 (2006).
11. S. Oswald, E. Scheuch, I. Cascorbi and W. Siegmund, *J. Chromatogr. B*, **830**, 143 (2006).
12. D.G. Sankar, S.K. Sumanth, A.K.M. Pawar and P.V.M. Latha, *Asian J. Chem.*, **18**, 1526 (2006).

(*Received*: 29 August 2006; *Accepted*: 13 August 2007) AJC-5801