International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.2, No.1, pp 404-410, Jan-Mar 2010

SIMULTANEOUS ESTIMATION OF ROSUVASTATIN AND EZETIMIBE BY RATIO SPECTRA DERIVATIVE SPECTROPHOTOMETRY METHOD IN THEIR FIXED DOSAGE FORMS

GAJJAR ANURADHA K* ,SHAH VISHAL D.

Department of Pharmaceutical Chemistry, Institute of Pharmacy, Nirma University,

Sarkhej-Gandhinagar Highway, Ahmedabad-382481,Gujarat State, India

***E-mail: anuradha.gajjar@nirmauni.ac.in**
Mobile. No.: +91-9925140156

ABSTRACT: Rosuvastatin is an HMG Co-A inhibitor and Ezetimibe is an intestinal cholesterol absorption inhibitor. The combination formulation is used for the treatment of hypercholestrolemia. The Ratiospectra derivative spectrophotometric method was developed for the simultaneous determination of Rosuvastatin (RSV) and Ezetimibe (EZE) in their fixed dosage forms. The method depends on the use of the first derivative of the ratio-spectra obtained by dividing the absorption spectrum of binary mixtures by a standard spectrum of one of the compounds. The first derivative amplitudes at 237.4 and 223.4 nm were selected for the determination of RSV and EZE respectively. The wavelength interval ($\Delta\lambda$) was selected as 4 nm. Methanol was used as the solvent. Both the drugs showed linearity in the range of 2.5-15µg mL^{-1}. The method was validated statistically and recovery studies were carried out. It was found to be accurate, precise and reproducible. The method was applied to the assay of the drugs in marketed formulation, which were found in the range of 98.0% to 102.0% of the labeled value for both Rosuvastatin and Ezetimibe. Hence, the method herein described can be successfully applied in quality control of combined pharmaceutical dosage forms.
Keywords: Rosuvastatin, Ezetimibe, Ratio Spectra Derivative Spectrophotometry.

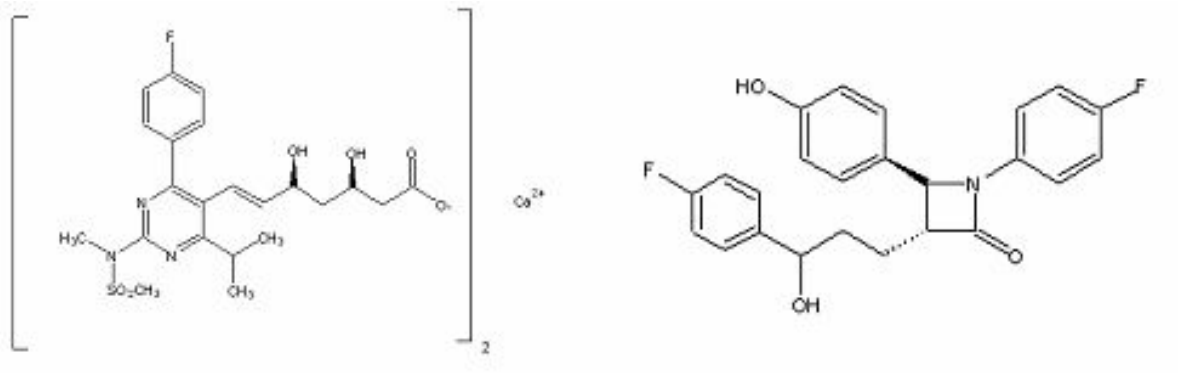
1.INTRODUCTION

Rosuvastatin (RSV) is the calcium salt of (E)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl (methylsulfonyl)amino]pyrimidin-5-yl](3R,5S)-3,5-dihydroxyhept-6-enoic acid. RSV is a selective and competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme that converts 3-hydroxy-3-methylglutaryl coenzyme A to Mevalonate, a precursor of cholesterol.[1]

Ezetimibe (EZE), a selective inhibitor of intestinal cholesterol and related phytosterol absorption, is designated as 1-(4-fluorophenyl)-3(R)-[3-(4-fluorophenyl)-3(S) -hydroxy propyl] - 4(S) - (4 -hydroxy phenyl) -2-azetidinone. It selectively prevents the absorption of cholesterol from dietary and biliary sources by blocking the transport of cholesterol through the intestinal wall.[2]

EZE co-administered with HMG-CoA reductase inhibitors (statins) is licensed for the treatment of primary hypercholesterolemia in patients, poorly controlled with a statin alone, and for homozygous familial hypercholesterolemia.[3] The chemical structures of RSV and EZE are shown in Fig.-1.

A detailed survey of analytical literature for RSV revealed several methods based on varied techniques, viz, HPLC[4-6]; Capillary Zone Electrophoresis[7]; Spectrophotometry[8]; High-Performance Thin- Layer Chromatography (HPTLC)[9]. Similarly, a survey of the analytical literature for EZE revealed methods based on HPLC[10-13] for determination in pharmaceuticals, LC/tandem MS (LC/MS/MS)[14,15] for determination in human plasma and serum, UV-spectrophotometric determination in



(A) **(B)**

Figure-1: Chemical structures of (A) Rosuvastatin and (B) Ezetimibe

combination with other drug[16] and stability-indicating LC[17,18] for determination in combination drug products with other drugs.

A spectrophotometric method based on the use of the first derivative of the ratio spectra was first developed by Salinas et al.[19,20], for resolving binary mixtures. The objective of this work was to develop simple, precise and rapid ratio spectra derivative spectrophotometric method for combination drug products containing RSV and EZE.

2. EXPERIMENTAL

2.1 Materials and Reagents

Reference standards of Rosuvastatin Calcium and Ezetimibe were supplied by Torrent Research Center, Gandhinagar, India with purity of 98.5% and 99.9% respectively. Tablet formulation containing 10 mg of RSV and 10 mg of EZE was procured from the local pharmacy. Methanol (HPLC grade) was purchased from Spectrochem (Mumbai, India). Nylon syringe filters (0.45 µm) were purchased from Millex-HN, Millipore (Mumbai, India).

2.2 Instrumentation

A double-beam Shimadzu (Kyoto, Japan) UV-Visible spectrophotometer, Model UV-2450 PC, equipped with 1 cm quartz cells, with a fixed slit width (1 nm), wavelength accuracy of $\pm$0.5 nm (with automatic wavelength correction) was connected to IBM-PC compatible computer loaded with UV Probe software, version 2.0 (Shimadzu). For scanning, the wavelength range selected was from 400 nm to 200 nm with medium scanning speed.

2.3 Standard and Test Solutions

2.3.1 Preparation of Standard Solution

The standard stock solutions containing 50µg mL^{-1} each of RSV and EZE were prepared separately by dissolving reference standards in Methanol and diluting with the same diluent. Standard solutions of both the drugs were prepared individually by dilution of the standard stock solutions with Methanol to obtain the concentration range of 2.5-15 µg mL^{-1} for each of the drugs.

2.3.2 Preparation of Test Solution

Twenty tablets were weighed and finely powdered in a mortar. A tablet powder equivalent to 10 mg each of RSV and EZE was accurately weighed and transferred to a 200 mL calibrated volumetric flask. Around 150 mL of Methanol was added, and the solution was sonicated for 30 min. Volume was made up to the mark with the same solvent. The solution was filtered through 0.45 µm nylon syringe filter. The resultant solution contained 50µg mL^{-1} each of RSV and EZE. The solution was further diluted with

Methanol to get concentration of 10µg mL^{-1} of both drugs.

2.4 Ratio Spectra Derivative Method

This method works on two mechanisms viz. (1) Ratio and (2) Derivatization. In this method, the mixture spectra are divided with the divisor and first derivative spectra of these ratio spectra are generated. The main advantage of the ratio-spectra derivative spectrophotometry is the chance of doing easy measurements in correspondence of peaks so it permits the use of the wavelength of highest value of analytical signals (a maximum or a minimum). Moreover, the presence of a lot of maxima and minima is another advantage by the fact that these wavelengths give an opportunity for the determination of active compounds in the presence of other compounds and excipients which possibly interferes the assay.[21]

For the determination of RSV, the spectra of RSV at increasing concentrations in methanol were divided by previously stored absorption spectrum standard solution of EZE (10 µg ml^{-1}) to obtain the corresponding ratio spectra. Then the first derivative of the obtained ratio spectra were traced with interval of □□ = 4 nm. In the binary mixtures, content of RSV was determined by measuring the first derivative amplitude at 237.4 nm, where there is no contribution or interference from EZE.

On the other hand, for the determination of EZE, an analogous procedure was followed. The spectra of EZE at increasing concentrations were divided by previously stored spectrum of 10 µg mL^{-1} solution of RSV and the first derivative of the developed ratio spectra were traced with □□ = 4 nm. In the binary mixtures, content EZE was determined by measuring the first derivative amplitude at 223.4 nm, where there is no contribution or interference from RSV.

First-derivative technique (D_1) traced with $\Delta\lambda$= 4 nm was used to resolve the spectral overlapping. The calibration curves were checked for linearity and linear behavior was observed in the concentration range of 2.5-15 µg mL^{-1}, for each of the drugs.

2.5 Method Validation

The method was validated as per ICH guidelines[22] for parameters like Linearity, Accuracy and Precision. The accuracy studies were carried out at different concentrations by spiking a known concentration of standard drug to the pre-analyzed sample and contents were reanalyzed by the developed method. Precision was studied by analyzing six replicates of sample solutions. Intermediate precision was determined in a similar manner on the next day using a different instrument.

3. RESULTS AND DISCUSSION

Zero-order absorption spectra of 10 µg mL^{-1} of each of RSV and EZE showed overlapping peaks that interfere with the simultaneous determination of this formulation as shown in Figure-2. So it was thought of interest to develop the ratio spectra derivative spectrophotometry method for the simultaneous estimation of RSV and EZE in commercially available tablet dosage forms. Methanol was used as the solvent since both the drugs exhibit good solubility in it and no interference due to excipients of the tablet formulation were observed.

3.1 Ratio spectra Derivative Spectrophotometry Method

The absorption spectra of RSV prepared at increasing concentrations in Methanol were recorded in the spectral region of 200.0-300.0 nm and divided by the previously stored spectrum of 10.0µg ml^{-1} EZE in the same solvent and their ratio spectra were obtained as seen in the Figure-3a. Then, the first derivatives of ratio-spectra were recorded as shown in Fig. 3b which were plotted with the interval of □□□□nm and the values of the derivatives were measured at suitably selected wavelength for the determination of RSV. The influence of the □□ for obtaining the first derivative was tested and □□=4 nm was considered as suitable. The concentration of divisor can be modified, and different calibration graphs are then obtained. A concentration of 10.0 µg ml^{-1} of EZE was considered as suitable. The calibration graph was established by measuring at the amplitude at 237.4 nm corresponding to a maximum wavelength.

For determining EZE, an analogous procedure was followed. The ratio spectra were obtained by dividing the spectra of EZE with previously stored spectrum of a 10 µg ml^{-1} RSV solution as shown in figure-4a and their first derivatives were calculated with the interval of □□=4 nm as shown in figure-4b. The values of the derivatives were measured at suitably selected wavelength for the determination of RSV. A concentration of 10.0 µg ml^{-1} of RSV was considered as suitable. The calibration graph was established by measuring at the amplitude at 223.4 nm corresponding to a maximum wavelength.

3.2 Method validation

The developed method was validated for parameters like linearity, precision and accuracy. The method was found to be linear in the range of 2.5-15 µg mL^{-1} for both the drugs with correlation coefficient of 0.99995 and 0.99977 for RSV and EZE respectively. The data for linearity and precision are presented in the Table-1. The data for recovery study are shown in the Table-2. The low value of %R.S.D. indicates that the method is precise and accurate.

3.3 Application of method to Tablet dosage form

The proposed method after validation was applied to the simultaneous estimation of RSV and EZE in tablet dosage forms available commercially. The results obtained show the high reliability and reproducibility of the method. The results of the study are presented in Table-3.

Table-1: Data showing linearity and precision of the developed method

Parameters \ Method	Ratiospectra Derivative Method	
	RSV	EZE
Linearity Range	2.5-15 µg mL^{-1}	2.5-15 µg mL^{-1}
Slope	0.0349	0.0485
Intercept	0.0073	0.0495
Correlation Co-efficient	0.99995	0.99977
Intraday Precision (% assay)[a]	100.3	100.0
Intraday Precision (% R.S.D.)[b]	0.17	0.91
Interday Precision (% assay)[a]	100.4	99.8
Interday Precision (% R.S.D.)[b]	0.85	0.37

[a] Average of six determinations
[b] Estimated on six determinations

Table-2: Data showing recovery of the developed method[c]

Parameters / Method	RSV µg mL^{-1}	EZE µg mL^{-1}	STD. RSV added µg mL^{-1}	STD. EZE added µg mL^{-1}	RSV found µg mL^{-1}	EZE found µg mL^{-1}	% RSV recovered	% EZE recovered
Ratiospectra Derivative	10	10	2.52	2.53	12.63	12.51	100.9	99.8
Spectro-photometry	10	10	5.04	5.06	14.93	14.89	99.3	98.9

[c] Average of three determinations

Table-3: Results of analysis of tablet dosage forms containing RSV and EZE

Method / Parameters	Ratiospectra Derivative Method	
	RSV	EZE
%Assay*	99.7	99.2
SD*	0.35	0.49
%RSD	0.35	0.49

*Average of six determinations
SD: Standard Deviation
RSD: Relative Standard Deviation

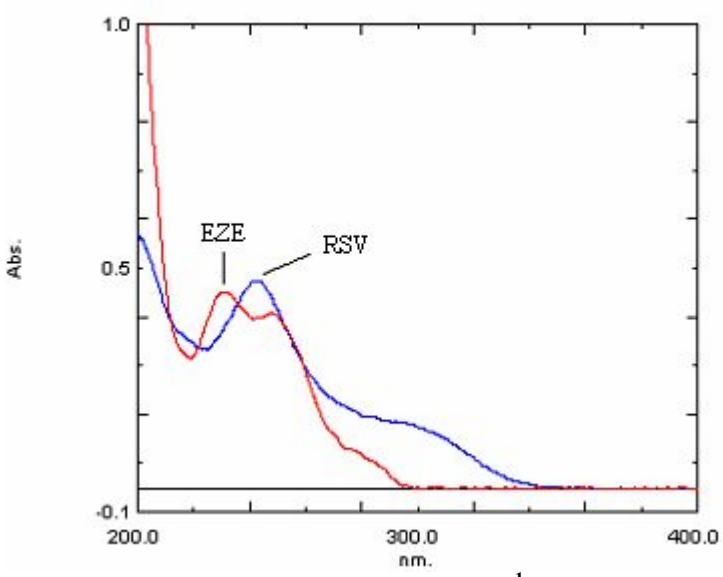


Figure-2: Overlain spectrum of 10 µg mL^{-1} of RSV and EZE

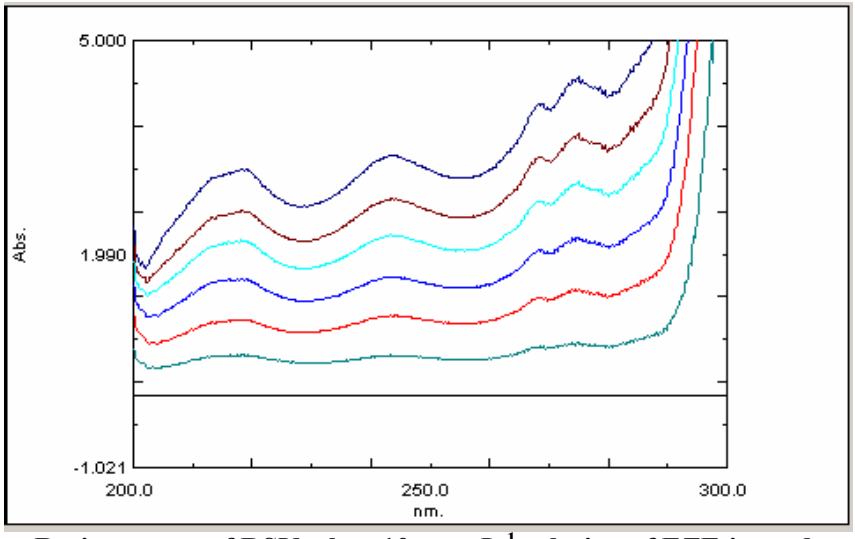


Figure-3a: Ratio spectra of RSV when 10 µg mL^{-1} solution of EZE is used as a divisor

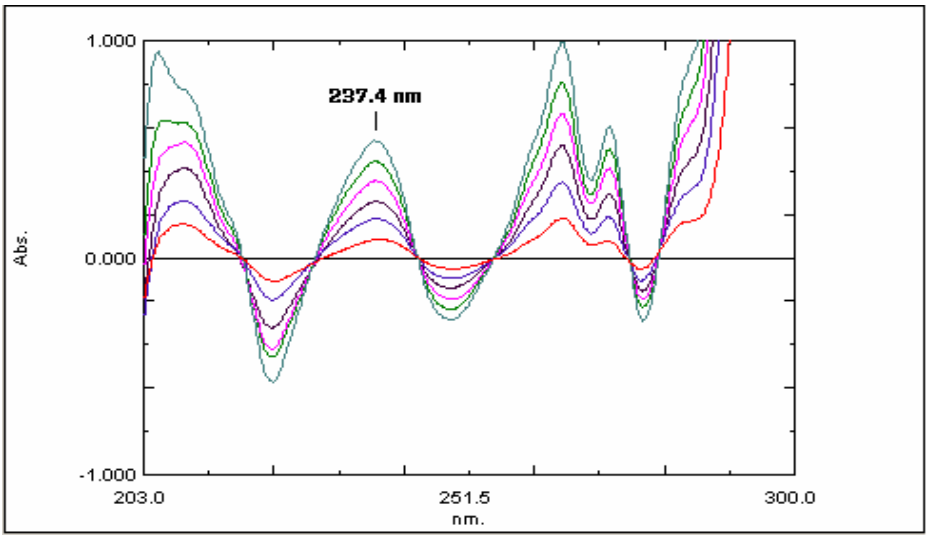


Figure-3b: First Derivative Ratio spectra for RSV

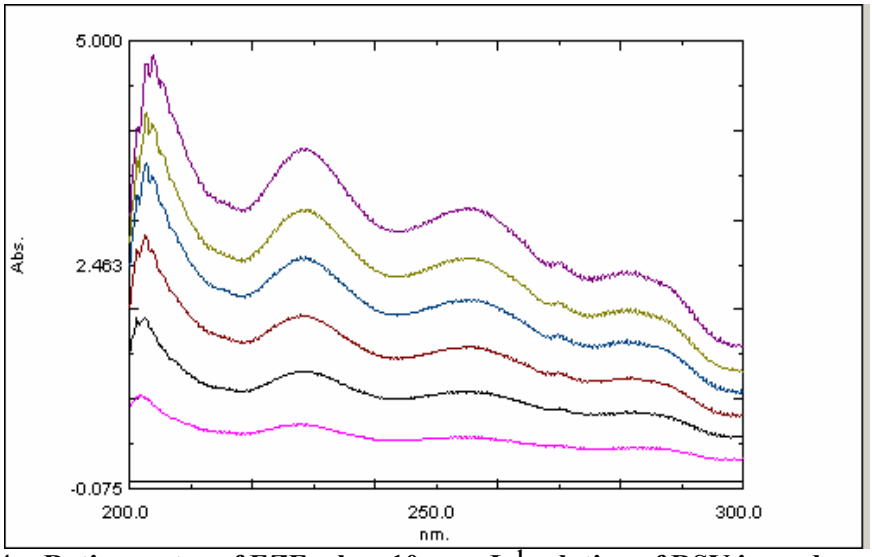


Figure-4a: Ratio spectra of EZE when 10 µg mL^{-1} solution of RSV is used as a divisor

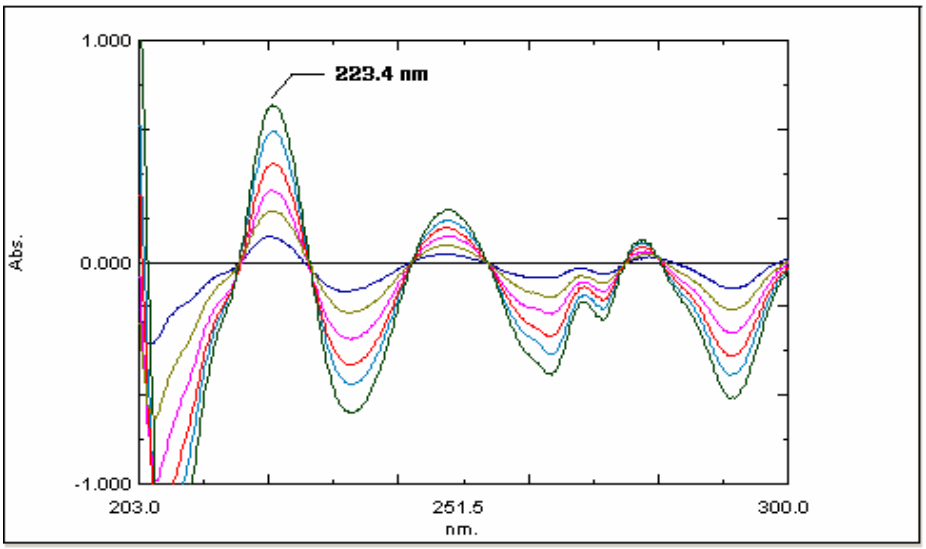


Figure-4b: First Derivative Ratio spectra for EZE

4. ACKNOWLEDGEMENT

The authors are grateful to Torrent Research Center, Gandhinagar, India, for providing free drug standards of RSV and EZE.

5. REFERENCES

1. Lennernas H. and Fager G., Pharmacodynamic and pharmacokinetics of the HMG-Co-A reductase inhibitors, Clin. Pharmacokinet., 1997, 32, 403-425.
2. Leitersdorf E., Selective cholesterol absorption inhibition: a novel strategy in lipid-lowering management, Int. J. Clin. Pract., 2002, 56, 116-119.
3. Heek M.V., Farley C., Compton D., Hoos L. and Davis H.R., Ezetimibe selectively inhibits intestinal cholesterol absorption in rodents in the presence and absence of exocrine pancreatic function, Br. J. Phamacol., 2001, 134(2), 409-417.
4. Kumar T.R., Shitut N.R., Kumar P.K., Vinu M.C., Kumar V.V. and Mullangi R., Determination of Rosuvastatin in rat plasma by HPLC: Validation and its application to pharmacokinetic studies, Biomed. Chromatogr., 2006, 20, 881-887.
5. Vittal S., Kumar T.R., Shitut N.R., Vinu M.C., Kumar V.V. and Mullangi R., Simultaneous quantitation of Rosuvastatin and Gemfibrozil in human plasma by high-performance liquid chromatography and its application to a pharmacokinetic study, Biomed. Chromatogr., 2006, 20, 1252-1259.
6. Mehta T.N., Patel A.K., Kulkarni G.M. and Suubbaiah G., Determination of Rosuvastatin in the presence of its degradation products by a stability-indicating LC method, J. AOAC Int., 2005, 88(4), 1142-1147.
7. Suslu I., Celebier M. and Altinoz S., Determination of Rosuvastatin in Pharmaceutical

Formulations by Capillary Zone Electrophoresis, Chromatographia, 2007, 66, 65-72.
8. Uyar B., Celebier M. and Altinoz S., Spectrophotometric determination of Rosuvastatin calcium in tablets, Pharmazie, 2007, 62, 411-413.
9. Sane R.T., Kamat S.S., Menon S.N., Inamdar S.R. and Mote M.R., Determination of Rosuvastatin calcium in its bulk drug and pharmaceutical preparations by high-performance thin-layer chromatography, J. Planar Chromatogr., 2007, 18, 194-198.
10. Sistla R., Tata V.S., Kashyap Y.V., Chandrasekar D. and Diwan P.V., Development and validation of a reversed-phase HPLC method for the determination of Ezetimibe in pharmaceutical dosage forms, J. Pharm. Biomed. Anal., 2005, 39, 517-522.
11. Akmar S.K., Kothapalli L., Thomas A., Jangam S. and Deshpande A.D., Reverse Phase HPLC method for the estimation of Ezetimibe in bulk and pharmaceutical formulations, Indian J. Pharm. Sci., 2007, 69(5), 695-697.
12. Singh S., Singh B., Bahuguna R., Wadhwa L. and Saxena R., Stress degradation studies on Ezetimibe and development of a validated stability-indicating HPLC assay, J. Pharm. Biomed. Anal., 2006, 41, 1037-1040.
13. Doshi A.S., Kachhadia P.K. and Joshi H., Validation of a stability-indicating LC method for assay of Ezetimibe in tablets and for determination of content uniformity, Chromatographia 2008, 67, 137-142.
14. Oswald S., Scheuch E., Cascorbi I. and Siegmund W., A LC–MS/MS method to quantify the novel cholesterol lowering drug Ezetimibe in human serum, urine and feces in healthy subjects genotyped for SLCO1B1, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci., 2006, 830, 143-150.
15. Li S., Liu G., Jia J., Li X. and Yu C.J., Liquid chromatography–negative ion electrospray tandem mass spectrometry method for the quantification of Ezetimibe in human plasma, J. Pharm. Biomed. Anal., 2006, 40(4), 987-992.
16. Jain N., Jain R., Swami H., Pandey S. and Jain D.K., Spectrophotometric method for simultaneous estimation of Simvastatin and Ezetimibe in bulk drug and its combined dosage form, Int. J. Pharm. Pharm. Sci., 2009, 1(1), 170-175.
17. Chaudhari B.G., Patel M.N., Shah P.B., Patel L.J. and Patel V.P., Stability-indicating reversed-phase liquid chromatography method for simultaneous determination of Atrovastatin and Ezetimibe from their combination drug products, J. AOAC Int., 2007, 90(6), 1539-1546.
18. Chaudhari B.G., Patel N.M. and Shah P.B., Stability-indicating reversed-phase liquid chromatographic method for simultaneous determination of Simvastatin and Ezetimibe from their combination drug products, J. AOAC Int., 2007, 90(5), 1242-49.
19. Salinas F., Nevado J.J. and Espinosa M.A., A new spectrophotometric method for quantitative multicomponent analysis resolution of mixtures of Salicylic and Salicyluric acids, Talanta, 1990, 37(3), 347-351.
20. Nevado J.J., Guiberteau C.C. and Salinas F., Spectrophotometric resolution of ternary mixtures of salicylaldehyde, 3-hydroxybenzaldehyde and 4-hydroxybenzaldehyde by the derivative ratio spectrum-zero crossing method, Talanta, 1992, 39(5), 547-553.
21. El-Gindy A., Ahmed A., Laila A.F. and Shabana M.M., Spectrophotometric determination of benazepril hydrochloride and hydrochlorothiazide in binary mixture using second derivative, second derivative of the ratio spectra and chemometric methods, J. Pharm. Biomed. Anal., 2001, 25(2), 299-307.
22. ICH: Proceedings of the International Conference on Harmonisation of Technical Requirement of Registration of Pharmaceuticals for Human Use (ICH Harmonised Tripartite Guidelines), Validation of Analytical Procedures: Methodology, Q_2B, Geneva, Switzerland, 1996.

*****