



ORIGINAL ARTICLE

Two spectrophotometric methods for simultaneous determination of some antihyperlipidemic drugs

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Received 13 December 2011; accepted 13 February 2012

Available online 21 February 2012

KEYWORDSAtorvastatin;
Ezetimibe;
Dual wavelength
method;
Mean centering of ratio
spectra;
Spectrophotometry

Abstract Two simple, accurate, precise and economic spectrophotometric methods have been developed for simultaneous determination of Atorvastatin calcium (ATR) and Ezetimibe (EZ) in their bulk powder and pharmaceutical dosage form. Method (I) is based on dual wavelength analysis while method (II) is the mean centering of ratio spectra spectrophotometric (MCR) method. In method (I), two wavelengths were selected for each drug in such a way that the difference in absorbance was zero for the second drug. At wavelengths 226.6 and 244 nm EZ had equal absorbance values; therefore, these two wavelengths have been used to determine ATR; on a similar basis 228.6 and 262.8 nm were selected to determine EZ in their binary mixtures. In method II, the absorption spectra of both ATR and EZ with different concentrations were recorded over the range 200–350, divided by the spectrum of suitable divisor of both ATR and EZ and then the obtained ratio spectra were mean centered. The concentrations of active components were then determined from the calibration graphs obtained by measuring the amplitudes at 215–260 nm (peak to peak) for both ATR and EZ. Accuracy and precision of the developed methods have been tested; in addition recovery studies have been carried out in order to confirm their accuracy. On the other hand, selectivities of the methods were tested by application for determination of different synthetic mixtures containing different ratios of the studied drugs. The developed methods have been successfully used for determination of ATR and EZ in their combined dosage form and statistical comparison of the developed methods with the reported spectrophotometric one using *F* and Student's *t*-tests showed no significant difference regarding both accuracy and precision.

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Peer review under responsibility of Xi'an Jiaotong University.

doi:10.1016/j.jpha.2012.02.002

1. Introduction

Atorvastatin calcium (ATR) has the IUPAC name [R-(R*,R*)]-2-(4-fluorophenyl)-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1Hpyrrole-1-heptanoic acid, calcium salt trihydrate [1,2]. ATR belongs to a class known as statins and it is a specific inhibitor of 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-coA) reductase (the enzyme that catalyzes the conversion of HMG-coA to mevalonate which is the rate limiting step in the biosynthesis of cholesterol) [3–5]. So ATR is mainly used for lowering blood



cholesterol [6]. Ezetimibe (EZ) has the IUPAC name (1-(4-fluorophenyl)-3(R)-[(3S)-(4-fluorophenyl)-3-hydroxy propyl]-4(S) (4-hydroxyphenyl) azetidin-2-one) [7–9]. It blocks the intestinal absorption of dietary and biliary cholesterol, without affecting the uptake of triglycerides or fat soluble vitamins. It reduces total cholesterol, LDL, and triglycerides, and increases HDL in patients with hypercholesterolemia [10]. So it is used for the treatment of hypercholesterolemia and homozygous sitosterolemia [11–13].

Combination of ATR and EZ has the advantages of greater therapeutic effects than with either drug alone. This combination causes manifold reduction in LDL cholesterol level as compared to double dose of the individual drug when used alone [14]. Moreover, using of EZ with ATR enhances the effects of statins at lower doses and reduces the associated side effects [15–17].

Different methods have been reported for simultaneous determination of ATR and EZ in their binary mixtures such as HPLC [14], HPTLC [15], Q-spectrophotometry [16] and derivative ratio spectrophotometric methods [17].

Due to the pharmaceutical importance of ATR and EZ combinations, this work concerns with development and validation of two simple, sensitive and selective spectrophotometric methods for determination of the proposed drugs in their pure forms and combined dosage form. The developed methods do not need any derivatization steps and so signal to noise ratio is enhanced; also they can be considered as time and cost effective compared to the published chromatographic methods. The developed dual wavelength analysis method is simple and easy to be understood and applied but it can be used only for determination of binary mixtures. On the other hand, the developed MCR method is more selective than the developed dual wavelength analysis and the published spectrophotometric methods and can be used as a stability indicating assay method [18–20].

2. Experimental

2.1. Instruments

A double beam UV–visible spectrophotometer (SHIMADZU, Japan) model UV-1601 PC with quartz cell of 1 cm and UV-PC personal software version 3.7 was used. The spectral band is 2 nm and scanning speed is 2800 nm/min with 0.1 nm interval. Matlab[®] version 6.5 [21] was used for the proposed mean centering of ratio spectra (MCR) spectrophotometric method.

2.2. Materials

2.2.1. Pure standards

Standard ATR was kindly supplied by Marcyrl Pharmaceutical Industries, El-Obour City, Egypt, while standard EZ was kindly supplied by Egyptian Co. for Chemicals and Pharmaceuticals, ADWIA CO, 10th of Ramadan City, Egypt.

2.2.2. Pharmaceutical dosage form

Atoreza[®] tablets (10/10)(B.N. 1031061) labeled to contain Atorvastatin calcium equivalent to 10 mg ATR and 10 mg EZ were manufactured by Marcyrl Pharmaceutical Industries, El-Obour City, Egypt.

2.2.3. Solvents

Methanol HPLC grade (CHROMASOLVE[®], Sigma-Aldrich Chemie GmbH, Germany) was used for the two methods.

2.2.4. Standard solutions

- Standard stock solutions of ATR and EZ were prepared in methanol at the concentration of 1 mg/mL.
- Standard working solutions of ATR and EZ were prepared in methanol at the concentration of 0.1 mg/mL.

2.3. Procedure

2.3.1. Spectral characteristics and wavelengths selection

The absorption spectra of 16 µg/mL each of ATR and EZ were recorded over the range 200–350 nm using methanol as blank. The overlaid spectra were observed for selection of the suitable wavelengths for dual wavelength spectrophotometric method (Fig. 1).

2.3.2. Linearity

2.3.2.1. Dual wavelength analysis method. Different aliquots equivalent to 60–260 and 80–400 µg/mL of ATR and EZ, were separately transferred from their respective standard working solutions (0.1 mg/mL) into two separate series of 10-mL volumetric flasks and then the volume was completed using methanol. The prepared solutions were scanned in the range of 200–350 nm and the absorbance values at 226.6 and 244 nm (for ATR) and at 228.6 and 262.8 nm (for EZ) were measured. ATR was determined by plotting the difference in absorbance values at 226.6 and 244 nm (difference is zero for EZ) against its corresponding concentration. Similarly for determination of EZ, the difference in absorbance values at 228.6 and 262.8 nm (difference is zero for ATR) was plotted against the corresponding concentrations.

2.3.2.2. Mean centering of ratio spectra (MCR) method. Aliquots of ATR equivalent to 60–260 µg/mL were accurately transferred from its standard working solution (0.1 mg/mL) into a set of 10 mL measuring flasks and the volume was adjusted using methanol. The absorption spectra of the prepared solutions were recorded in the range of 200–290 nm, were divided by the standard spectrum of 40 µg/mL of EZ and then the obtained ratio spectra mean centered.

By the same way the spectra of different concentrations of standard solutions of EZ in the range of 4–40 µg/mL were recorded. The stored spectra were divided by the standard spectrum of 16 µg/mL of ATR to obtain the ratio spectra which were then mean centered. Calibration curves for both ATR and EZ were constructed by plotting the amplitude values of their respective mean centered ratio spectra from 215 to 260 nm (peak to peak) against their corresponding concentrations.

2.3.3. Analysis of laboratory prepared mixtures

Zero order absorption spectra of different laboratory prepared mixtures containing different ratios of ATR and EZ were recorded using methanol as blank and the procedure under linearity for each method was then followed. Concentrations

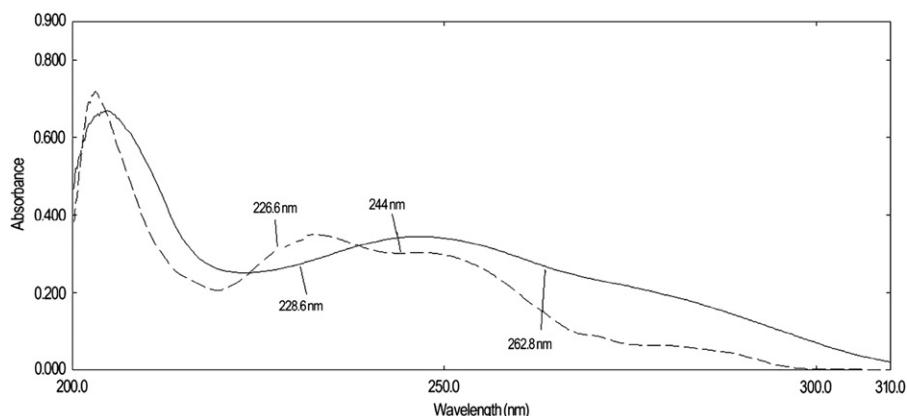


Figure 1 Zero-order absorption spectra of 8 µg/mL each of ATR (—) and EZ (---) using methanol as a solvent.

of ATR and EZ in the prepared samples were calculated from the computed regression equations.

2.3.4. Analysis of the pharmaceutical dosage form

Fourteen tablets of Atoreza[®] tablets were powdered and mixed well. Accurately weighed amount of the powdered tablets equivalent to 100 mg of ATR and EZ was transferred to a 100-mL volumetric flask and 75 mL of methanol was added. The prepared solution was sonicated for 15 min, cooled and the volume was completed to obtain 1 mg/mL stock solution and then the solution was filtered. Appropriate dilutions of the prepared solution were made to prepare its working solution (0.1 mg/mL) and the procedures under linearity were followed.

2.3.4.1. Recovery studies. To study the accuracy of the proposed methods, recovery studies were carried out by application of the standard addition technique. Known amounts of the studied drugs were separately added to a definite amount of the powdered tablet; the prepared samples were then analyzed as under linearity and the percentage recoveries were then calculated.

3. Results and discussion

Atorvastatine and EZ act as antihyperlipidemic drugs which have been used in the treatment of some serious diseases such as heart diseases; hence it is very important to develop analytical methods which are not only accurate, precise, and rapid but also simple and economic for determination of the studied drugs in their pharmaceutical dosage form and this is the main task of the developed spectrophotometric methods.

Since UV-spectrophotometric methods have the advantages of saving time and cost when compared to the HPLC technique, this work concerns with the development and validation of two spectrophotometric methods, dual wavelength and mean centering of ratio spectra spectrophotometric methods, for determination of the suggested drugs. Moreover, the suggested methods provide a simple, rapid, sensitive and accurate way for simultaneous analysis of ATR and EZ in their combined dosage without derivatization steps.

3.1. Dual wavelength method

UV-absorption spectra of ATR and EZ, Fig. 1, display severe overlap that made their direct determination in their binary mixtures very difficult. The developed dual wavelength method provides a simple method for selective determination of both ATR and EZ using their zero order absorption spectra. The principle of this method is that the absorbance difference at two points on the spectra is directly proportional to the component of interest, independent of the interfering component. The pre-requisite for this method is the selection of two wavelengths where the interfering component shows the same absorbance value while the component of interest shows significant difference in absorbance with concentration.

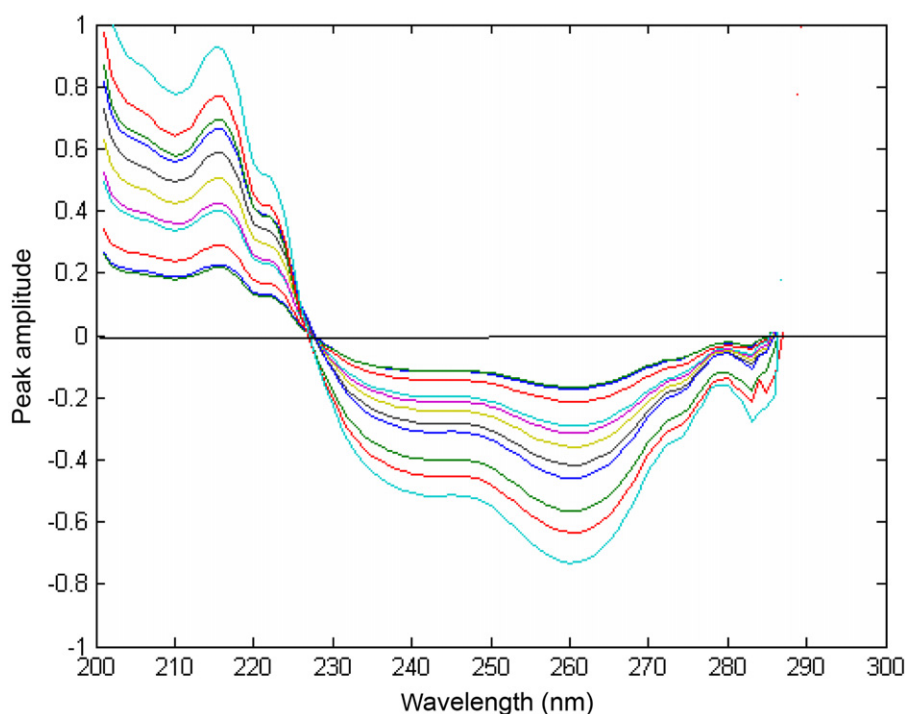
Selection of the suitable wavelengths plays an important role; hence different wavelengths were tried such as 221.4, 258.8 and 225.4, 252.6 nm for ATR and 233.8, 260.6 and 215, 250 nm for EZ. Using the absorbance values at 226.6 and 244 nm (where EZ has the same absorbance) gave the best selectivity when used for determination of ATR. On the other hand absorbance values at 228.6 and 262.8 nm were chosen for determination of EZ where the best results were obtained. Calibration curves for ATR and EZ were constructed by plotting the difference in absorbance values at the selected wavelengths for each drug against their corresponding concentrations. ATR and EZ obeyed Beer Lambert's law in the concentration ranges of 6–26 and 8–40 µg/mL for ATR and EZ, respectively with good correlation coefficients. Regression equation parameters are given in Table 1.

3.2. Mean centering of ratio spectra (MCR) spectrophotometric method

To optimize the developed MCR method, different parameters were tested. Since the wavelength range taken has a great effect on the obtained mean centered ratio spectra, different wavelength ranges were tested and the best results were obtained when using the wavelength range from 200 to 290 nm for both ATR and EZ. The effect of divisor concentration on the selectivity was checked by testing several concentrations each of EZ (normalized spectrum, 10, 20, 25 and 40 µg/mL) and ATR (normalized spectrum, 8, 10, 12 and 16 µg/mL). The best results

Table 1 Linear regression and analytical parameters of the proposed methods for determination of ATR and EZ.

Parameters	Dual wavelength method		MCR method	
	ATR	EZ	ATR	EZ
Linearity range ($\mu\text{g/mL}$)	6–26	8–40	6–26	4–40
Slope	0.0092	0.0202	0.0644	0.0552
Intercept	0.0097	0.009	-0.0243	0.0274
Correlation coefficient	0.9997	0.9999	0.9996	0.9999
Precision				
Repeatability	0.924	1.071	1.754	1.033
Intermediate precision	1.273	1.145	0.945	1.156
Accuracy	100	101.66	99.68	100.4
Specificity (%)	99.89 \pm 1.594	100.87 \pm 1.292	101.81 \pm 1.199	100.45 \pm 1.067

**Figure 2** Mean centered ratio spectra of ATR (6–26 $\mu\text{g/mL}$) using 40 $\mu\text{g/mL}$ of EZ as a divisor and methanol as a solvent.

regarding sensitivity and selectivity were obtained using 40 and 16 $\mu\text{g/mL}$ each of ATR and EZ, respectively as divisors.

To construct the calibration curves of the proposed method, the absorption spectra of the standard solutions of ATR with different concentrations were recorded in the wavelength range of 200–290 nm and divided by the standard spectrum of EZ (40 $\mu\text{g/mL}$). Then mean centering of the resulted ratio spectra has been obtained and the concentrations of ATR were determined by measuring the amplitude values of the mean centered ratio spectra from 215 to 260 nm (peak to peak) as shown in Fig. 2. By the same way different standard solutions of EZ with different concentrations were recorded and divided by the standard spectrum of ATR (16 $\mu\text{g/mL}$) and the ratio spectra were obtained which were then mean centered. The amplitude values from 215 to 260 nm (peak to peak) in the obtained mean centered ratio spectra were used for determination of EZ as shown in Fig. 3. The computed

regression equation parameters for each of the studied drugs are given in Table 1.

The specificity of the proposed methods was assessed by analysis of different laboratory prepared mixtures containing different ratios of the suggested drugs, where satisfactory results were obtained and are given in Table 1. The developed spectrophotometric methods have been also applied for determination of ATR and EZ in Atoreza[®] tablets and the results obtained were acceptable with small RSD% values. The validity of the methods was further assessed by applying the standard addition technique which also confirmed the accuracy of the methods (Table 2). The results obtained by applying the proposed methods for determination of Atoreza[®] tablets were statistically compared with those obtained by applying the reported first derivative of ratio spectra spectrophotometric method [17] and no significant differences were obtained between them (Table 2).

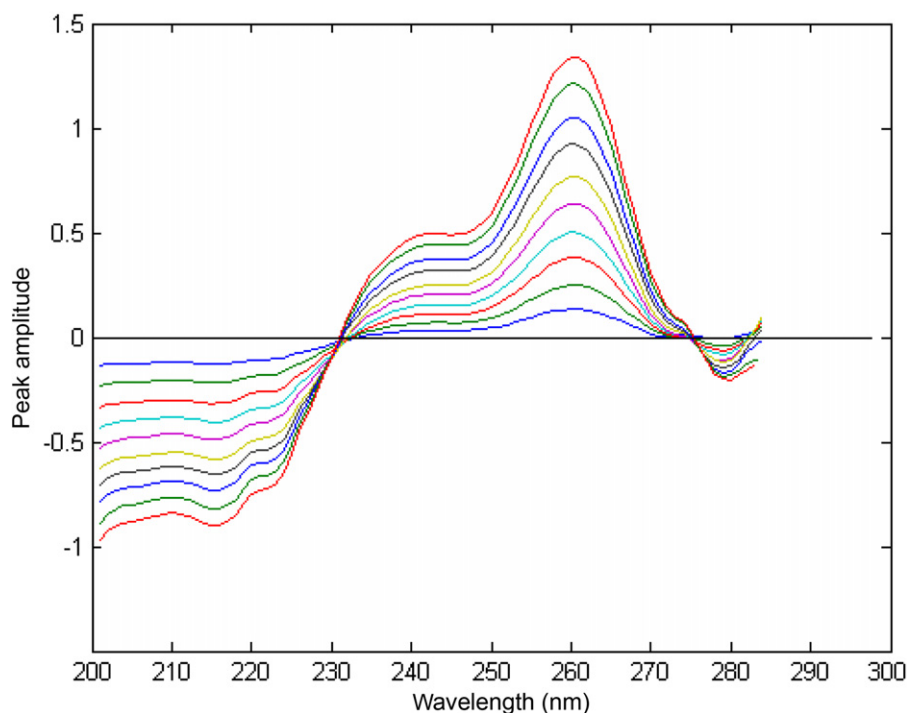


Figure 3 Mean centered ratio spectra of EZ (4–40 µg/mL) using 16 µg/mL of ATR as a divisor and methanol as a solvent.

Table 2 Determination of the studied drugs in the laboratory prepared mixtures (L.P.), pharmaceutical preparations by the proposed methods and statistical comparison with the reported spectrophotometric method.

Sample form	Dual wavelength method		MCR method		Reported method [17]	
	ATR (%)	EZ (%)	ATR (%)	EZ (%)	ATR (%)	EZ (%)
Atoreza [®] tablets ^a (B.N.1031061)	99.97 ±1.80	101.54 ±1.06	100.41 ±1.05	102.58 ±0.97	99.98 ±1.05	102.33 ±1.12
Standard addition ^a	100.6 ±1.02	101.17 ±1.85	100.18 ±1.69	99.95 ±2.17		
<i>F</i> -test (6.388) ^b	2.491	1.124	1.000	1.324		
Student's <i>t</i> -test (2.306) ^b	0.006	1.147	0.642	0.376		

^aAverage of six determination.

^bThe values in the parenthesis are the corresponding theoretical values at $p=0.05$.

3.3. Method validation

Validation of the method has been carried out according to ICH recommendations [22].

3.3.1. Linearity and range

The calibration range for ATR and EZ was established through considerations of the practical range necessary according to adherence to Beer–Lambert's law and the concentrations of ATR and EZ present in the pharmaceutical dosage form to give accurate, precise and linear results. Linearity ranges of both ATR and EZ are shown in Table 1.

3.3.2. Accuracy

The accuracy of the results was checked by applying the proposed methods for determination of different blind samples of ATR and EZ and the concentrations were obtained

from the corresponding regression equations. Good percentage recoveries were obtained and are presented in Table 1. Accuracy of the methods was further assured by applying the standard addition technique where good percentage recoveries were obtained, confirming the accuracy of the proposed methods (Table 2).

3.3.3. Precision

3.3.3.1. Repeatability. Three concentrations of ATR and EZ (12, 16, 20 µg/mL) were analyzed three times intra-daily using the proposed methods. Good percentage recoveries were obtained, confirming the repeatability of the methods (Table 1).

3.3.3.2. Intermediate precision. The previous procedures were repeated inter-daily on three different days for the analysis of the three chosen concentrations. The obtained percentage recoveries were within the acceptable range (Table 1).

3.3.4. Specificity

Specificity of the methods was achieved by analysis of different laboratory prepared mixtures of ATR and EZ within their linearity ranges. Satisfactory results are shown in Table 1.

4. Conclusion

The developed dual wavelength and mean centering of ratio spectra (MCR) spectrophotometric methods have been successfully applied for simultaneous determination of ATR and EZ in their combined marketed sample; they are found to be rapid, simple, accurate and easy to be understood and applied. On the developed dual wavelength method once the equations were constructed, analysis required only measuring the absorbance values of the sample solution at the selected wavelengths followed by a few simple calculations. On the other hand the MCR method does not need derivatization steps or complex algorithms. When the suggested methods were completely validated they showed satisfactory data for all the method validation parameters tested. Recovery studies indicated that practically there was no interference from the tablets additives, so these methods can be easily and conveniently adopted for routine quality control analysis of ATR and EZ.

References

- [1] A.J. Jani, A.A. Mehta, B. Dosandi, et al., Liquid chromatographic–MS/MS determination of atorvastatin and metabolites in human plasma., *Eurasian J. Anal. Chem.* 5 (2010) 46–52.
- [2] D. Liu, J. Jiang, H. Zhou, et al., Quantitative determination of atorvastatin and para-hydroxy atorvastatin in human plasma by LC–MS–MS, *J. Chromatogr. Sci.* 46 (2008) 862–866.
- [3] B. Stanisz, L. Kania, Validation of HPLC method for determination of atorvastatin in tablets and for monitoring stability in solid phase, *J. Acta Pol. Pharm.* 63 (2006) 471–476.
- [4] A.A. Shirkhedkar, S.J. Surana, Development and validation of a reversed-phase high-performance thin-layer chromatography–densitometric method for determination of Atorvastatin calcium in bulk drug and tablets, *J. AOAC Int.* 93 (2010) 798–803.
- [5] D. Guille'n, F. Cofa'n, E. Ros, et al., Determination of atorvastatin and its metabolite ortho-hydroxyatorvastatin in human plasma by on-line anion-exchange solid-phase extraction and liquid chromatography tandem mass spectrometry, *J. Anal. Bioanal. Chem.* 394 (2009) 1687–1696.
- [6] D. Skorda, C.G. Kontoyannis, Identification and quantitative determination of atorvastatin calcium polymorph in tablets using FT-Raman spectroscopy, *Talanta* 74 (2008) 1066–1070.
- [7] M. Sharma, D.V. Mhaske, M. Mahadik, et al., UV and three derivative spectrophotometric methods for determination of ezetimibe in tablet formulation, *Indian J. Pharma. Sci.* 70 (2008) 258–260.
- [8] E. Ucakturk, N. Ozaltin, B. Kaya., Quantitative analysis of ezetimibe in human plasma by gas chromatography–mass spectrometry, *J. Sep. Sci.* 32 (2009) 1868–1874.
- [9] R. Sistla, V.S.S.K. Tata, Y.V. Kashyap, et al., Development and validation of a RP–HPLC method for the determination of ezetimibe in pharmaceutical dosage forms, *J. Pharm. Biomed. Sci.* 39 (2005) 517–522.
- [10] S.L. Dalmora, P.R. Oliveira, T. Barth, et al., Development and validation of a stability-indicating micellar electrokinetic chromatography method for the determination of ezetimibe in pharmaceutical formulations, *J. Anal. Sci.* 24 (2008) 499–503.
- [11] C.R. Barhate, K. Mohanraj, What is the degradation product of ezetimibe?, *J. Pharm. Biomed. Sci.* 55 (2011) 1237–1238.
- [12] A.K. Gajjar, V.L.D. Shah, Isolation and structure elucidation of major alkaline degradant of Ezetimibe, *J. Pharm. Biomed. Sci.* 55 (2011) 225–229.
- [13] S.J.S. Basha, S.A.I. Naveed, N.K. Tiwari, et al., Concurrent determination of ezetimibe and its phase-I and II metabolites by HPLC with UV detection: quantitative application to various in vitro metabolic stability studies and for qualitative estimation in bile, *J. Chromatogr. B.* 853 (2007) 88–96.
- [14] B.G. Chaudhari, N.M. Patel, P.B. Shah, et al., Stability-indicating reversed-phase liquid chromatographic method for simultaneous determination of atorvastatin and ezetimibe from their combination drug products, *J. AOAC Int.* 90 (2007) 1539–1546.
- [15] B.G. Chaudhari, N.M. Patel, P.B. Shah, et al., Development and validation of a HPTLC method for the simultaneous estimation of atorvastatin calcium and ezetimibe, *Indian J. Pharm. Sci.* 68 (2006) 793–796.
- [16] S.S. Sonawane, A.A. Shirkhedkar, R.A. Fursule, et al., Simultaneous spectrophotometric estimation of atorvastatin calcium and ezetimibe in tablets, *Indian J. Pharm. Sci.* 69 (2007) 683–684.
- [17] H.M. Maher, R.M. Youssef, E.M. Hassan, et al., Enhanced spectrophotometric determination of two antihyperlipidemic mixtures containing ezetimibe in pharmaceutical preparations, *J. Drug Test. Anal.* 3 (2011) 97–105.
- [18] A. Afkhami, M. Bahram, Mean centering of ratio kinetic profiles as a novel spectrophotometric method for the simultaneous kinetic analysis of binary mixtures, *Anal. Chim. Acta* 526 (2) (2004) 211–218.
- [19] A. Afkhami, M. Bahram, A novel spectrophotometric method for the simultaneous kinetic analysis of ternary mixtures by mean centering of ratio kinetic profiles, *Talanta* 68 (4) (2006) 1148–1155.
- [20] A. Afkhami, M. Bahram, Mean centering of ratio spectra as a new spectrophotometric method for the analysis of binary and ternary mixtures, *Talanta* 66 (3) (2005) 712–720.
- [21] Matlab Ver. 6.5; 1999, Mathworks Inc.
- [22] International Conference on Harmonization (ICH), Q2B Validation of Analytical Procedures: Methodology, vol. 62, 1997.