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Enhanced spectrophotometric determination of two antihyperlipidemic mixtures containing ezetimibe in pharmaceutical preparations

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Two spectrophotometric methods are presented for the simultaneous determination of ezetimibe/simvastatin and ezetimibe/atorvastatin binary mixtures in combined pharmaceutical dosage forms without prior separation. The first is the derivative ratio method where the amplitudes of the first derivative of the ratio spectra (^{1}DD) at 299.5 and 242.5 nm were found to be linear with ezetimibe and simvastatin concentrations in the ranges 0.5–20 μgml^{-1} and 1–40 μgml^{-1}, respectively, whereas the amplitudes of the first derivative of the ratio spectra (^{1}DD) at 289.5 and 288 nm were selected to determine ezetimibe and atorvastatin in the concentration ranges 5–50 μgml^{-1} and 1–40 μgml^{-1}, respectively. The second is the H-point standard additions method; absorbances at the two pairs of wavelengths, 228 and 242 nm or 238 and 248 nm, were monitored while adding standard solutions of ezetimibe or simvastatin, respectively. For the analysis of ezetimibe/atorvastatin mixture, absorbance values at 226 and 248 nm or 212 and 272 nm were monitored while adding standard solutions of ezetimibe or atorvastatin, respectively. Moreover, differential spectrophotometry was applied for the determination of ezetimibe in the two mixtures without any interference from the co-existing drug. This was performed by measurement of the difference absorptivities (ΔA) of ezetimibe in 0.07 M 30% methanolic NaOH relative to that of an equimolar solution in 0.07 M 30% methanolic HCl at 246 nm. The described methods are simple, rapid, precise and accurate for the determination of these combinations in synthetic mixtures and dosage forms.

Keywords: ezetimibe/simvastatin; ezetimibe/atorvastatin; pharmaceutical preparations; derivative ratio spectrophotometry; H-point standard additions method

Introduction

Resolving complex multicomponent systems with no prior separation of the constituent analytes is rather a difficult task. In the last few years, the development of methods for the resolution of such systems has grown dramatically, probably as a result of the increasing affordability of powerful instrumentation and the availability of graphical and numerical methods.[1] Derivative spectrophotometry has been used for the analysis of binary mixtures of compounds with overlapping spectra by zero-crossing measurements.[2,3] However, sometimes the derivative technique cannot cope with the level of interference, especially when the spectra are strongly overlapped. Derivative ratio spectrum (^{1}DD) is able to resolve the strong overlapping of spectra.[4,5] In this method, the absorption spectrum of the mixture is recorded and divided, amplitude-by-amplitude, by the absorption spectrum of a standard solution of one of the components, and then the first derivative of the ratio spectrum is obtained. The concentration of the other component is then determined from a calibration graph.

The H-Point Standard Additions Method (HPSAM)[6] is based on the principle of dual wavelength spectrophotometry and the standard additions methods. The method uses the analytical signal data at two accurately selected wavelengths (it only requires working at two wavelengths where the analytical signal due to the interferent is constant). By plotting the analytical signal versus added analyte concentration, two straight lines are obtained that have a common point with coordinates H (-C_H, A_H), where -C_H is the unknown analyte concentration and A_H the analytical signal due to the interferent. The method permits both proportional and constant errors produced by the matrix of the sample to be corrected directly and also the unbiased analyte concentration to be determined when a direct interferent is present and even the interferent concentration to be determined when it is known to be present. So, HPSAM can determine the two components simultaneously. The method has been applied for the analysis of several binary mixtures.[1,7–9]

Simvastatin (SIM; 2,2-dimethylbutanoic acid (1*S*,3*R*,7*S*,8*S*,8a*R*,)-1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-[2-[(2*R*,4*R*)-tetrahydro-4-hydroxy-6-oxo-2*H*-pyran-2yl]ethyl]-1-naphthalenyl ester) and atorvastatin (ATO; [R-(R*,R*)-2-(4-flurophenyl)-β -dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptonic acid-calcium salt) are selective 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitor. They are widely used for the treatment of hypercholesterolemia.[10] However, administration of the highest approved statin dose offers only limited additional lowering of LDL cholesterol at the expense of an increased incidence of side effects.[11] A

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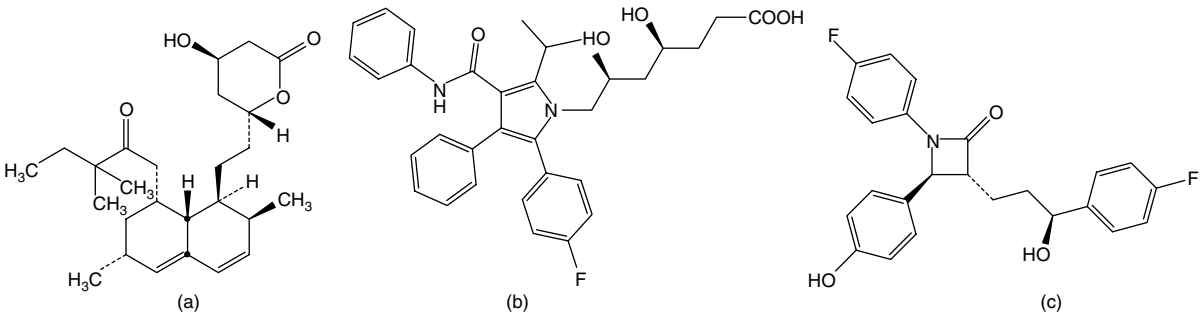

Figure 1. Chemical structures of SIM (a), ATO, (b) and EZE (c).

recently introduced compound, ezetimibe, (EZE; 1-(4-fluorophenyl)-(3R)-[3-(4- fluorophenyl)-(3S)-hydroxypropyl]-(4S)- (4 hydroxyphenyl)-2-azetidinone), is an effective option for treating patients with primary hypercholesterolemia, reducing the risk of coronary heart disease.[12] Ezetimibe complements the lipid lowering effects of other therapies, such as statins. The chemical structures of the three drugs are illustrated in Figure 1.

A survey of the literature revealed that few analytical techniques concerned with the determination of ezetimibe (EZE)/simvastatin (SIM) mixture have been reported including high performance liquid chromatography (HPLC),[13–17] high performance thin-layer liquid chromatography (HPTLC),[18] micellar electrokinetic capillary chromatography[19] and spectrophotometry.[20–22] Again, the literature review revealed few methods for the analysis of ezetimibe (EZE)/atorvastatin (ATO) mixture including HPTLC,[23] HPLC,[24–27] and spectrophotometry.[24,28]

In this work, different methods (derivative ratio spectrophotometry and HPSAM) are proposed as selective, sensitive, simple, and low-cost procedures for the simultaneous determination of ezetimibe/simvastatin (EZE/SIM) – mix I; and ezetimibe/atorvastatin (EZE/ATO) – mix II; binary mixtures in synthetic mixtures and pharmaceutical preparations. A difference spectrophotometric method for the assay of ezetimibe content in the two mixtures would also be of some interest.

The proposed methods are more applicable than the published chemometric method[21] that needs a special computer program which may not be widely accessible. In addition, the proposed derivative ratio method is more sensitive than the published spectrophotometric ones.[20,22,24,28]

Experimental

Materials and reagents

Pharmaceutical grade of EZE and ATO were supplied as a gift sample by Pharonia Pharmaceuticals (Alexandria, Egypt) whereas SIM was obtained from El-Amria Pharmaceutical Co. (Alexandria, Egypt). All reagents used were of analytical grade. Methanol and hydrochloric acid (BDH Laboratory Suppliers, Poole, UK.) were used while sodium hydroxide was obtained from El-Nasr Chemical Ind. Co. Alexandria (Egypt).

Apparatus

All spectrophotometric measurements were performed using a Perkin-Elmer Lambda EZ201 UV-visible spectrophotometer (USA), with matched 1-cm quartz cells. The instrument is connected to a Panasonic impact dot matrix printer KX-P3626.

Pharmaceutical formulations

Two commercial products, namely Inegy® and Atoreza® tablets, were studied. Inegy® tablets produced by Global Napi Pharmaceuticals 6th of October (Egypt) under license from Merck & Co. Inc./Schering-Plough Co. Whitehouse Station, NJ USA (Batch No. NL03510) labeled to contain 10 mg EZE and 10 mg SIM per tablet. Atoreza® tablets produced by Marcyrl Pharmaceutical Industries (El Obour City Egypt) (Batch No. 93568) containing 10 mg EZE and 10 mg ATO per tablet.

Standard solutions and synthetic mixtures

Stock solutions of 1 mgml^{-1} of EZE, SIM, and ATO were prepared in methanol. These stock solutions were further diluted with 0.1 M NaOH to obtain working standard solutions of suitable concentrations (corresponding to the linearity range stated in Table 1). Accurate volumes of each of EZE, SIM, and ATO stock solutions were transferred into 10-ml volumetric flasks and diluted to volume with 0.1 M NaOH to prepare synthetic mixtures within the concentration range of each compound (Table 2). For difference spectrophotometry, the solutions were prepared as follows. Accurate volumes of standard EZE were transferred into two sets of 10-ml flasks (to give final concentrations within the linearity ranges stated in Table 1) then the volume was completed to 3 ml with methanol. One set was diluted to volume with 0.1 M NaOH and the other set was diluted to volume with 0.1 M HCl.

General Procedure

Construction of calibration curves

Derivative ratio method

The absorption spectra of the working solutions prepared for both mixtures were recorded against 0.1M NaOH and stored. The stored spectra were divided (amplitude at each wavelength) by the spectrum of a standard solution of 5 μgml^{-1} SIM or ATO (for EZE) in both mixtures and 3 μgml^{-1} EZE for SIM (mixI) or ATO (mix II). The absolute values of the first derivative of the ratio spectra for each drug at the selected $\Delta\lambda$ and wavelengths (Table 1) were plotted against the corresponding concentrations.

HPSAM

Absorbance of the prepared working standard solutions of EZE, SIM, and ATO was measured at the appropriate pairs of wavelengths stated in Table 1B.

Table 1. Regression and statistical parameters for the determination of the two mixtures using the proposed methods

(A) Derivative ratio and derivative-difference spectrophotometry

	Derivative ratio				Difference spectrophotometry
	EZE/SIM mixture		EZE/ATO mixture		
	EZE	SIM	EZE	ATO	EZE
Parameter	$^1DD_{299.5}$ ($\Delta\lambda = 4$ nm)	$^1DD_{242.5}$ ($\Delta\lambda = 6$ nm)	$^1DD_{289.5}$ ($\Delta\lambda = 6$ nm)	$^1DD_{288}$ ($\Delta\lambda = 4$ nm)	ΔA_{246}
Linearity range ($\mu g ml^{-1}$)	0.5–20	1–40	5–50	1–40	5–50
LOQ ($\mu g ml^{-1}$)	0.38	0.76	2.17	0.52	3.14
LOD ($\mu g ml^{-1}$)	0.11	0.23	0.65	0.16	0.94
Intercept	3.32×10^{-1}	-3.20×10^{-3}	-1.34×10^{-2}	-6.16×10^{-2}	-2.76×10^{-3}
Slope	11.51	7.78×10^{-1}	8.20×10^{-2}	6.10×10^{-1}	2.77×10^{-2}
Correlation coefficient	0.9999	0.9999	0.9997	0.9998	0.9999
S_a	8.33×10^{-1}	6.99×10^{-2}	8.68×10^{-3}	4.28×10^{-2}	7.46×10^{-3}
S_b	7.44×10^{-2}	3.11×10^{-3}	2.86×10^{-4}	1.91×10^{-3}	2.66×10^{-4}
$S_{y/x}$	1.31	1.06×10^{-1}	1.11×10^{-2}	6.48×10^{-2}	9.53×10^{-3}

(B) HPSAM

	EZE/SIM mixture				EZE/ATO mixture			
	EZE		SIM		EZE		ATO	
Parameter	A_{238}	A_{248}	A_{228}	A_{242}	A_{212}	A_{272}	A_{226}	A_{248}
Linearity range ($\mu g ml^{-1}$)	5–25	5–25	5–25	5–25	5–25	5–25	5–25	5–25
LOQ ($\mu g ml^{-1}$)	2.56	3.15	3.78	2.84	4.21	4.17	3.56	2.89
LOD ($\mu g ml^{-1}$)	0.77	0.95	1.13	0.85	1.26	1.25	1.07	0.87
Intercept	-6.50×10^{-3}	-2.00×10^{-3}	-4.00×10^{-3}	4.00×10^{-4}	2.00×10^{-4}	-1.00×10^{-4}	-6.10×10^{-3}	2.30×10^{-3}
Slope	5.56×10^{-2}	5.48×10^{-2}	4.64×10^{-2}	4.62×10^{-2}	1.34×10^{-2}	1.37×10^{-2}	3.65×10^{-2}	3.57×10^{-2}
Correlation coefficient	0.9998	0.9997	0.9999	0.9997	0.9998	0.9999	0.9997	0.9996
S_a	1.06×10^{-2}	1.15×10^{-2}	3.63×10^{-3}	1.01×10^{-2}	1.99×10^{-3}	1.47×10^{-3}	7.64×10^{-3}	9.92×10^{-3}
S_b	6.41×10^{-4}	6.93×10^{-4}	2.19×10^{-4}	6.12×10^{-4}	1.20×10^{-4}	8.87×10^{-5}	4.61×10^{-4}	5.98×10^{-4}
$S_{y/x}$	1.01×10^{-2}	1.10×10^{-2}	3.46×10^{-3}	9.68×10^{-3}	1.90×10^{-3}	1.40×10^{-3}	7.28×10^{-3}	9.48×10^{-3}

S_a is standard deviation of intercept, S_b is standard deviation of slope, and $S_{y/x}$ is standard deviation of residuals.

Table 2. Accuracy and precision for the determination of EZE/SIM and EZE/ATO mixtures using derivative ratio and difference spectrophotometry

	EZE/SIM			EZE/ATO		
	EZE		SIM	EZE		ATO
Parameter	ΔA_{246}	$^1DD_{299.5}$	$^1DD_{242.5}$	ΔA_{246}	$^1DD_{289.5}$	$^1DD_{288}$
Mean % recovery	101.28	100.86	100.66	100.42	101.24	99.90
± SD[a]	±0.30	±0.65	±0.84	±1.09	±0.45	±0.73
RSD %[b]	0.29	0.64	0.83	1.08	0.44	0.73
E_r (%)[c]	1.28	0.86	0.66	0.42	1.24	−0.10

[a] Mean ± standard deviation for five determinations.
[b] Percentage relative standard deviation.
[c] Percentage relative error.

Difference spectrophotometric method

The spectra of EZE in 0.07 M methanolic NaOH (methanol:0.1M NaOH, 30 : 70 v/v) were recorded against the corresponding equimolar solutions in 0.07 M methanolic HCl (methanol:0.1M HCl, 30 : 70 v/v) as a blank. The absolute amplitudes of ΔA at 246 nm were plotted versus the concentrations of EZE.

Analysis of synthetic mixtures

Derivative ratio method

The absorption spectra of the prepared synthetic mixtures were recorded against 0.1M NaOH and stored. The stored spectra were then processed as under construction of the calibration curves.

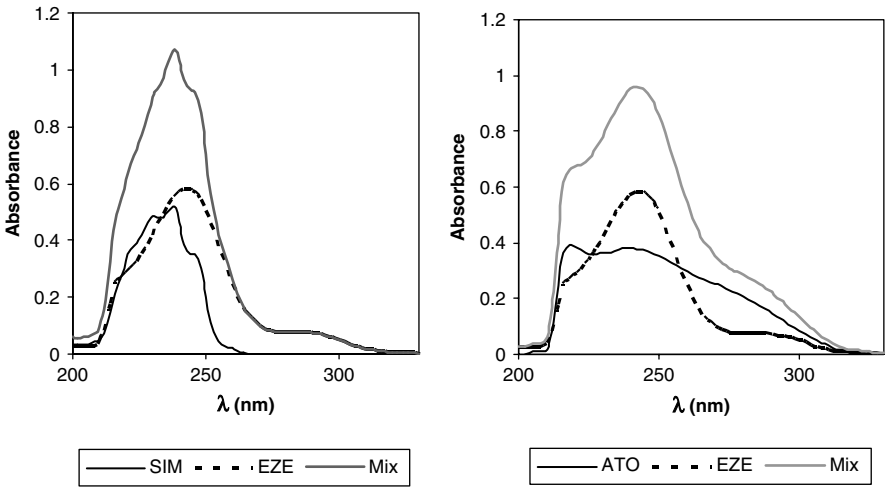

Figure 2. Absorption spectra of standard EZE, SIM (10 μgml^{-1} each) and their corresponding mixture (a) and standard EZE, ATO (10 μgml^{-1} each) and their corresponding mixture (b) in 0.1M NaOH.

HPSAM

For both mixtures I and II, synthetic samples containing different concentration ratios were prepared and standard additions of EZE or SIM; mix I; or EZE or ATO; mix II; (up to 20 μgml^{-1}, n=5) was made in 10-ml volumetric flasks and completed to the mark with 0.1M NaOH. Simultaneous determination with HPSAM was performed by measuring the absorbance values at 238 and 248 nm for each sample solution (when SIM standard solutions were added) or at 228 and 242 nm for each sample solution (when EZE standard solutions were added) against blank. Also the absorbances were recorded at 212 and 272 nm for the determination of EZE (when standard ATO solutions were added) or at 226 and 248 nm for the determination of ATO (when standard EZE solutions were added) against blank.

At each wavelength pair (λ_1 and λ_2), two addition lines were obtained from which the concentration of the drug considered as the analyte was calculated (Appendix A, Equation 3). On the other hand, the concentration of the drug considered as the interferent was calculated from a calibration graph at λ_1 (or λ_2) for pure interferent.

Difference spectrophotometric method

Accurate volumes of standard EZE and SIM (mix I) or EZE and ATO (mix II) were transferred into two sets of 10-ml volumetric flasks then the volume was completed to 3 ml with methanol. One set was diluted to volume with 0.1 M NaOH and the other set was diluted to volume with 0.1 M HCl. The spectra of the mixture in 0.07 M methanolic NaOH were recorded against the corresponding equimolar solutions in 0.07 M methanolic HCl as a blank. The absolute amplitudes of ΔA at 246 nm were measured

Analysis of tablet solutions

An accurately weighed portion of the mixed contents of 7 Inegy® tablets equivalent to 25 mg EZE and 25 mg SIM or of 7 Atoreza® tablets containing the equivalent of 25 mg EZE and 25 mg ATO was transferred into a 25-ml volumetric flask using about 15 ml methanol, shaking for 10 min and completed to volume with methanol. The contents of the flask were mixed well and filtered. Aliquots of the filtrate were diluted with methanol to obtain final concentration within the ranges mentioned in Table 1 and then proceeded as mentioned in analysis of synthetic mixtures.

Results and Discussion

Spectrophotometric characteristics

Figure 2 represents the completely overlapped spectra of the components of each mixture. This spectral overlapping is sufficient to demonstrate the resolving power of the proposed spectrophotometric methods. The univariate methods cannot be directly applied to the simultaneous determination of the two drugs in each mixture without prior separation. In contrast, the proposed methods can resolve bands overlapping, without physical separation.

Derivative-ratio method

The main instrumental parameters that affect the shape of the derivative-ratio spectra are the wavelength scanning speed, the concentration of the standard solution used as a divisor, the wavelength increment over which the derivative is obtained (Δλ) and the smoothing function.[29] Medium scan speed (200 nm/min) was chosen for all measurements.

A study was carried out to test the effect of the divisor concentration on the calibration graphs of EZE, SIM, and ATO. It was found that derivative ratios are inversely proportional to the divisor concentration, although the position of maxima and minima remains unaffected by changing the divisor concentration. The best results in terms of signal-to-noise ratio, sensitivity and repeatability were obtained by using 5 μgml^{-1} SIM (for EZE) and 3 μgml^{-1} EZE (for SIM) as divisors in the determination of EZE/SIM mixture. Also 5 μgml^{-1} ATO (for EZE) and 3 μgml^{-1} EZE (for ATO) were chosen as divisors in the determination of EZE/ATO mixture.

To study the influence of Δλ on the ratio spectra, the absorbance values of standards and their mixture were differentiated with respect to wavelength at different Δλ intervals. The optimum Δλ values were chosen according to the best recoveries obtained from these results, Δλ = 4 nm was considered suitable for the determination of EZE and Δλ = 6 was chosen for the determination of SIM. Meanwhile, a 6 nm interval was chosen

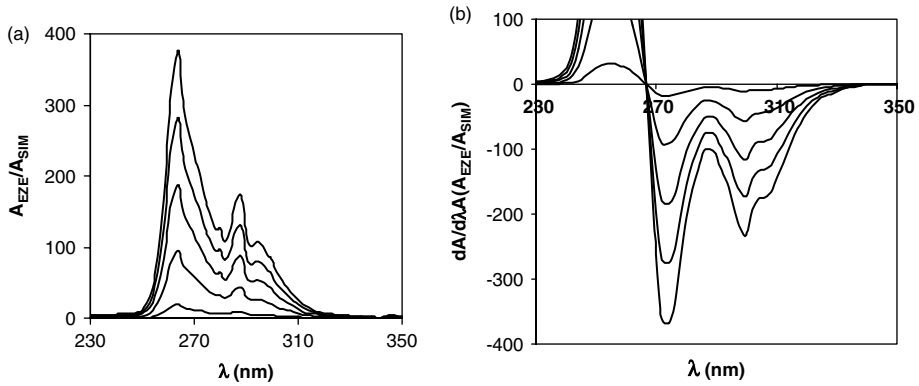

Figure 3. Ratio spectra (a) and first derivative of the ratio spectra (b) of 1,5,10,15 and 20 μgml^{-1} EZE using 5 μgml^{-1} SIM in 0.1M NaOH as divisor ($\Delta\lambda = 4$ nm).

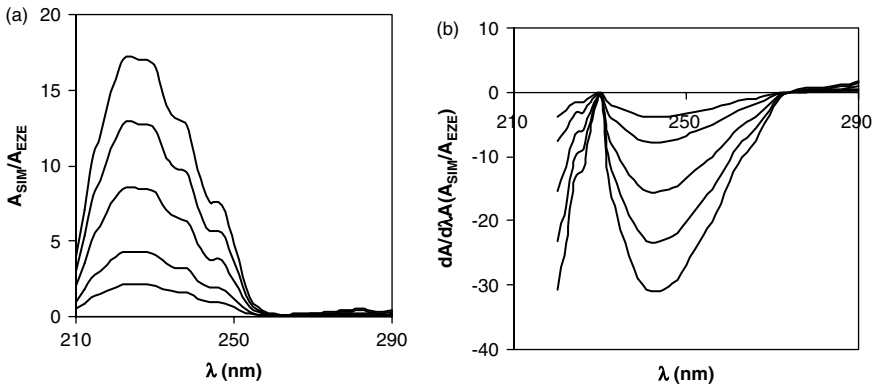

Figure 4. Ratio spectra (a) and first derivative of the ratio spectra (b) of 5,10,20,30 and 40 μgml^{-1} SIM using 3 μgml^{-1} EZE in 0.1M NaOH as divisor ($\Delta\lambda = 6$ nm).

for the determination of EZE in its mixture with ATO and a 4 nm interval was optimum for the determination of ATO.

For EZE/SIM mixture, the ratio spectra of different EZE standards at increasing concentrations in 0.1M NaOH and the first derivative of these ratio spectra are shown in Figure 3. The trough amplitudes at 299.5 nm on the first derivative curves were proportional to EZE concentration. For the determination of SIM, similar procedure was followed (Figure 4) and the first derivative values at 242.5 nm were found to be proportional to SIM concentration.

Similar pattern of results were obtained with EZE/ATO mixture. The first derivative values at 289.5 and 288 nm were measured and found to be proportional to EZE and ATO concentrations, respectively, (Figures 5 and 6).

HPSAM

Figure 2 shows the absorption spectra for the two components of each binary mixture. To select the appropriate wavelength pair for HPSAM, the following principles were applied. At the selected wavelengths, the analyte signals had to be linear while the interferent signal is remaining constant with the changing analyte concentration. In addition, the analytical signal obtained from a mixture of the analyte and the interferent should be equal to the sum of the individual signals of the two species. Also, the difference in slopes of the two straight lines measured at the two wavelengths selected (λ_1 and λ_2) should be as large as possible in order to reach good accuracy and sensitivity.[30]

When EZE was selected as the analyte in its mixture with SIM, selecting several pairs of wavelengths was possible. Considering that the higher the value of the slope increment, the smaller the error of the analyte concentration, 228 and 242 nm were chosen as the best wavelength pair. Consequently, SIM concentration could be determined as an interferent from the regression line at either 228 or 242 nm. Upon considering SIM as the analyte, 238 and 248 nm were chosen as the working pair. Also EZE could be determined as an interferent at 238 or 248 nm.

On the other hand, for EZE/ATO mixture, 226 and 248 nm or 212 and 272 nm wavelength pairs were considered optimum for the determination of EZE and ATO, respectively. In each case, the other drug could be determined as an interferent from the regression lines obtained at any of the two working wavelength pairs.

Difference spectrophotometric method

EZE, being a phenolic compound, showed bathochromatically shifted curve upon rendering the medium alkaline (Figure 7). Such a phenomenon was used for its determination in presence of SIM or ATO without any interference from SIM or ATO at $\Delta A = 246$ nm (Figure 8). Upon preparing EZE in aqueous 0.1M HCl, precipitation of the drug occurs, so methanolic solutions of both HCl and NaOH were used instead. The least methanol ratio for complete solubilization of EZE was optimized and found to be (methanol:0.1M HCl or 0.1M NaOH, 30 : 70 v/v). Figure 7 shows the zero order spectra of 20μgml^{-1} EZE in 0.07 M methanolic HCl and 0.07 M methanolic NaOH. The differential absorption spectra

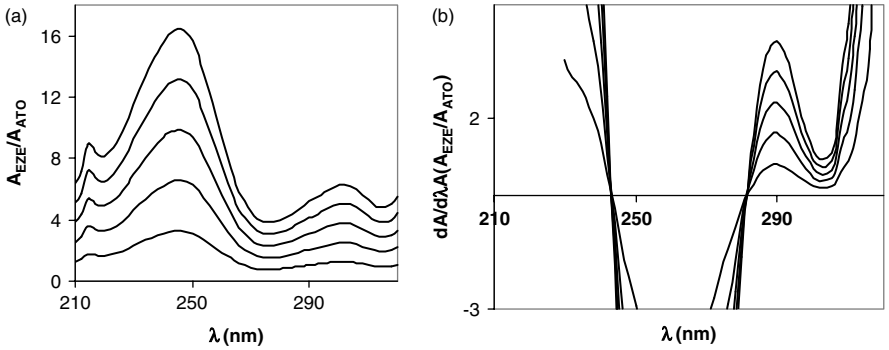

Figure 5. Ratio spectra (a) and first derivative of the ratio spectra (b) of 10,20,30,40 and 50 μgml^{-1} EZE using 5 μgml^{-1} ATO in 0.1M NaOH as divisor ($\Delta\lambda = 6$ nm).

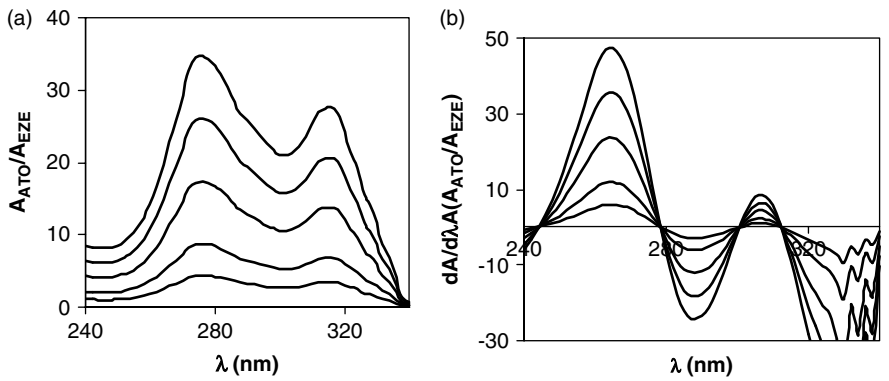

Figure 6. Ratio spectra (a) and first derivative of the ratio spectra (b) of 5,10,20,30 and 40 μgml^{-1} ATO using 3 μgml^{-1} EZE in 0.1M NaOH as divisor ($\Delta\lambda = 4$ nm).

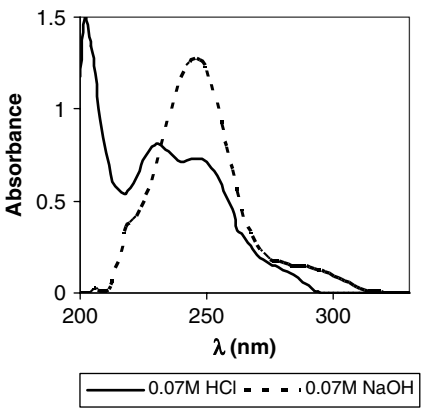

Figure 7. Zero order spectra of 20 μgml^{-1} EZE in 0.07 M methanolic HCl and 0.07 M methanolic NaOH.

of EZE and SIM or EZE and ATO (20 μgml^{-1} each) are shown in Figure 8.

Validation

Linearity

Under the described experimental conditions, standard calibration curves were constructed for ATO, EZE, and SIM in the two mixtures *versus* concentration. The statistical parameters, regression equations calculated from the calibration graphs, along with the standard deviation of the intercept (S_a) and the slope (S_b) and the standard deviation of residuals ($S_{y/x}$) are shown in Table 1. The results obtained show that linearity of the calibration graphs and the compliance with Beer's Law were validated, as illustrated by the high values of correlation coefficients of regression equations and the small values of intercepts together with the high F-values.

Limits of detection (LOD) and limits of quantification (LOQ)

In accordance with the formulae given by Miller,[31] LOD = 3 S/b and LOQ = 10 S/b, where S is the standard deviation of replicate blank responses (under the same conditions as for sample analysis) and b is the sensitivity, namely the slope of the calibration graph. The detection and quantification limits were calculated and presented in Table 1.

Accuracy and precision

The accuracy and precision were assessed through analysis of five synthetic mixtures containing EZE with SIM or ATO in combinations with different ratios as follows (3 : 1, 2 : 1, 1 : 1, 1 : 2 and 3 : 8; EZE : SIM or ATO). The concentrations obtained for EZE, SIM, and ATO, (both as analyte and interferent for HPSAM), are given in Tables 2 and 3. The results show good accuracy expressed as percentage relative error and acceptable precision expressed as percentage relative standard deviation (Tables 2 and 3).

Interferences

It was shown that the excipients such as starch, lactose, and magnesium stearate, which are commonly formulated in tablet

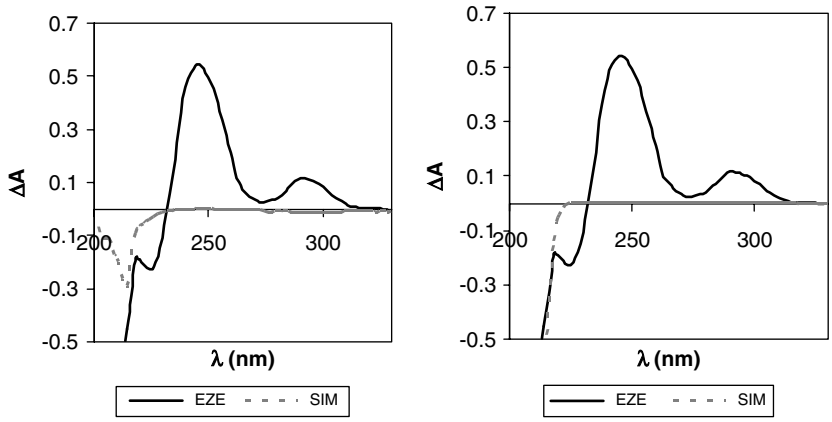

Figure 8. Difference absorption spectra of 20 μgml^{-1} EZE and 20 μg ml^{-1} SIM (a) or 20 μgml^{-1} EZE and 20 μgml^{-1} ATO (b) 0.07M methanolic NaOH against 0.07M methanolic HCl.

Table 3. Accuracy and precision for the determination of EZE/SIM and EZE/ATO mixtures using HPSAM

	EZE/SIM				EZE/ATO			
Parameter	EZE		SIM		EZE		ATO	
λ (nm)	228/242	238	238/248	228	226/248	212	212/272	226
Mean % recovery	98.88	99.43	99.96	101.01	100.98	98.74	100.46	99.13
± SD[a]	±1.10	±1.01	±1.13	±1.33	±1.43	±1.35	±1.37	±1.10
RSD %[b]	1.11	1.02	1.13	1.32	1.42	1.37	1.36	1.11
E_r (%)[c]	−1.12	−0.57	−0.04	1.01	0.98	−1.26	0.46	−0.87

[a] Mean ± standard deviation for five determinations.
[b] Percentage relative standard deviation.
[c] Percentage relative error.

dosage form, do not interfere with the proposed methods. Application of the proposed methods for the determination of the two drugs in dosage forms gave good recoveries indicating no interferences from the aforementioned excipients (Table 4).

Stability in solutions

The stability of EZE, SIM, and ATO in their solutions during the analytical procedures was studied. Standard solutions of the three drugs were prepared and kept at room temperature for 2 h. They were then analyzed using the proposed methods. Since no significant changes in A, ΔA or ^{1}DD values were obtained throughout the analysis time, the three drugs are considered stable in solutions for at least 2 h.

Assay of pharmaceutical preparations

Inegy® and Atoreza® tablets were assayed for the simultaneous determination of EZE with SIM or ATO, respectively. The results obtained were satisfactory and in good agreement with the label claim (Table 4). The RSD (%) and E_r (%) for the assay results showed the high reproducibility and accuracy of the proposed methods. Results of the proposed methods were compared with those obtained using first order derivative zero-crossing method[20] (for EZE/SIM mixture) or using simultaneous equation method[24] (for EZE/ATO mixture). More or less concordant results were obtained with respect to their precision (F-test) and accuracy (*t*-test). The calculated values did not exceed the theoretical ones for either of the two mixtures, indicating that there is no significant difference between the proposed methods (Table 4).

Conclusion

The proposed methods provide simple, rapid, accurate and reproducible quantitative analysis for the determination of EZE with SIM or ATO as binary mixtures in dosage forms, without any interference from excipients. The proposed methods are simple as there is no need for solvent extraction. They are also direct since they estimate each drug independently. In addition, they offer the advantage of time and cost saving as well as simplicity of reagents and apparatus. The methods were validated and found suitable for quality control laboratories, where economy and time are essential.

Appendix A. H-Point Standard Additions Method[1]

The HPSAM allows the determination of two species X and Y in a mixture even if their analytical signals are extensively overlapping and their maxima are coincident. The method is based on measurements of a standard addition line at two wavelengths λ_1 and λ_2 where the interferent Y shows the same absorbance. The absorbance of the analyte X at λ_1 and λ_2 should be different. The two standard addition lines intersect at the so-called H-point with co-ordinates ($-C_H$, A_H), where C_H is the concentration of the analyte and A_H the analytical signal due to the interferent (Figure 9). The addition lines obtained at the wavelengths λ_1 and

Table 4. Assay of EZE/SIM and EZE/ATO in tablets using the proposed methods

(A) EZE/SIM

	EZE					SIM			
			HPSAM		Reference		HPSAM		Reference
Inegy® tablets	ΔA_{246}	$^1DD_{299.5}$	228/242	A_{238}	Method[20]	$^1DD_{242.5}$	238/248	A_{228}	Method[20]
Mean % recovery ± SD[a]	100.96	100.01	99.82	99.46	100.18	100.12	101.26	101.87	100.93
	±0.80	±1.44	±0.73	±0.78	±0.84	±0.63	±1.03	±1.17	±1.05
RSD (%)	0.79	1.44	0.73	0.78	0.84	0.63	1.01	1.14	1.04
E_r (%)	0.96	0.01	−0.18	−0.54	0.18	0.12	1.26	1.87	0.93
t[b]	1.52	0.22	0.72	1.40	–	1.48	0.50	1.34	–
F[b]	1.14	2.93	1.32	1.18	–	2.75	1.06	1.23	–

(B) EZE/ATO

	EZE					ATO			
			HPSAM		Reference		HPSAM		Reference
Atoreza® tablets	ΔA_{246}	$^1DD_{289.5}$	226/248	A_{212}	Method[24]	$^1DD_{288}$	212/272	A_{226}	Method[24]
Mean % recovery ± SD[a]	101.15	100.07	100.13	100.74	99.59	100.62	100.59	99.57	99.78
	±0.75	±0.93	±0.92	±1.07	±1.41	±0.65	±1.25	±0.77	±1.61
RSD (%)	0.74	0.93	0.92	1.06	1.42	0.65	1.24	0.77	1.62
Er (%)	1.15	0.07	0.13	0.74	−0.41	0.62	0.59	−0.43	−0.22
t[b]	2.18	1.40	0.71	1.45	–	1.08	0.88	0.26	–
F[b]	3.51	2.28	2.34	1.70	–	6.07	1.67	4.39	–

[a] Mean ± standard deviation of five determinations.
[b] Theoretical values of t and F are 2.31 and 6.39, respectively, at 95% confidence limit (n=5).

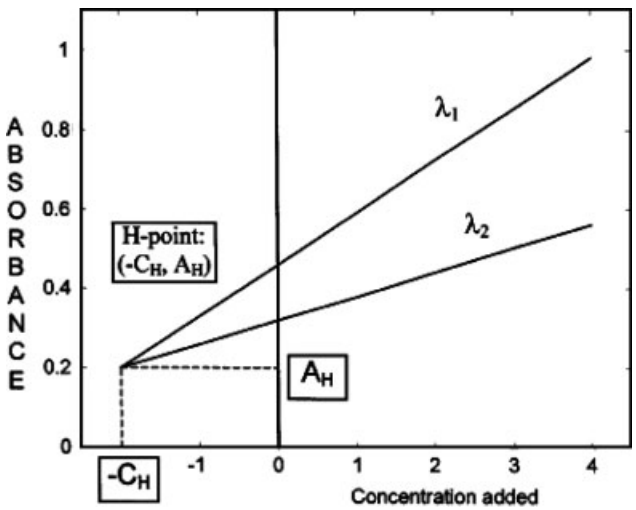

Figure 9. H-point standard addition lines.

λ_2 are given by:

$$A(\lambda_1) = B + A_1 + M(\lambda_1) \cdot C^I_X \quad (1)$$

$$A(\lambda_2) = B + A_2 + M(\lambda_2) \cdot C^I_X \quad (2)$$

A_1 and A_2 denote the absorbances of the analyte in the sample at λ_1 and λ_2, respectively, B is the absorbance of the interferent which is the same at λ_1 and λ_2, $M(\lambda_1)$ and $M(\lambda_2)$ are the slopes of the addition lines, and C^I_X is the concentration of the analyte added.

The addition lines intersect at the H-point, $(-C_H, A_H)$, given by:

$$A_1 + M(\lambda_1) \cdot (-C_H) = A_2 + M(\lambda_2) \cdot (-C_H)$$

This can also be written as:

$$-C_H = \frac{(A_2 - A_1)}{M(\lambda_1) - M(\lambda_2)} = \frac{C^O_X \cdot (M(\lambda_2) - M(\lambda_1))}{M(\lambda_1) - M(\lambda_2)} = -C^O_X \quad (3)$$

The unknown analyte concentration C^o_X therefore corresponds to C_H. The absorbance at the H-point, A_H, corresponds to the absorbance of the interferent since from Equation (1):

$$A(\lambda_1) = B + A_1 M(\lambda_1) \cdot (-C_H) = B + M(\lambda_1) \cdot C^O_X + M(\lambda_1) \cdot (-C_H) = B \quad (4)$$

If required, the concentration of the interferent can then be calculated from a calibration line for the pure interferent at λ_1 (or λ_2).

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