ELSEVIER

Short communication

Spectrophotometric determination of binary mixtures of pseudoephedrine with some histamine H_1-receptor antagonists using derivative ratio spectrum method

Hoda Mahgoub, Azza A. Gazy, Fawzi A. El-Yazbi *, Mahmoud A. El-Sayed, Rasha M. Youssef

Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, University of Alexandria, EL-Messalah 21521, Egypt

Abstract

A derivative spectrophotometric method is developed for the assay of three binary mixtures of pseudoephedrine with fexofenadine (mix I), cetirizine (mix II) and loratadine (mix III). The method is based on the use of the first derivative of the ratio spectrum. The ratio spectrum was obtained by dividing the absorption spectrum of the mixture by that of one of the components. The concentration of the other component was determined from its respective calibration graph treated similarly. Moreover, the influence of $\Delta\lambda$ for obtaining the first derivative of the ratio spectra and the effect of the divisor concentration on the calibration graphs were studied. The described method was applied for the determination of these combinations in synthetic mixtures and dosage forms. The results obtained were accurate and precise.
© 2003 Elsevier Science B.V. All rights reserved.

1. Introduction

The combinations of pseudoephedrine (PE) with each of fexofenadine (FEX), cetirizine (CET) and loratadine (LOR) are widely used in the treatment of allergic rhinitis [1].

The literature presents HPLC methods for the simultaneous determination of binary mixtures of FEX–PE and LOR–PE [2,3]. For CET–PE binary mixture, both HPLC and HPTLC methods have been described [4,5].

The analysis of multicomponent mixtures without separation of the constituents is rather a difficult task. Derivative spectrophotometry has been used for the analysis of binary mixtures of compounds with overlapping spectra by zero-crossing measurements [6,7]. However, sometimes the derivative technique cannot cope with the level

* Corresponding author. Tel.: +20-3-4871-317; fax: +-20-3-4873-273.
E-mail address: pharmacy_alex@hotmail.com (F.A. El-Yazbi).

of interference especially when the spectra are strongly overlapped.

Recently, the derivative ratio spectrum (^{1}DD) was introduced [8] which is able to resolve the strong overlapping of spectra [9,10]. 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 another component is then determined from a calibration graph.

In this work the derivative ratio spectrum technique was used to develop a spectrophotometric method for the simultaneous determination of the components of three binary mixtures, namely: FEX–PE (mix I), CET–PE (mix II) and LOR–PE (mix III).

2. Experimental

2.1. Apparatus

A Shimadzu UV-1601 PC spectrophotometer, with 1-cm quartz cells and supported with PC and UV PC software ver. 3.91, and a Hewlett Packard Deskjet 610 C printer were used.

2.2. Materials and reagents

All materials and reagents used were of analytical reagent grade. Fexofenadine hydrochloride (FEX) was kindly supplied by Hoechst, Egypt. Cetirizine dihydrochloride (CET) was kindly supplied by Amyria Pharm. Co., Egypt. Loratadine (LOR) and pseudoephedrine hydrochloride (PE) were kindly supplied by Pharaonia Pharmaceuticals, Egypt. Allerga-D tablets (USA) containing 60 mg FEX and 120 mg PE, Cirrus capsules (UCB S.A. Pharm. Co.) containing 5 mg CET and 120 mg PE, Decongess L capsules (Pharaonia Pharmaceuticals) and Clarinase tablets (Medical Union Pharmaceuticals Co., Shering–Plough Corporation USA) each containing 5 mg LOR and 120 mg PE were purchased from the market.

2.3. Reference drug solutions

- Stock solutions of each of FEX, CET and LOR containing 12, 4 and 4 μg ml^{-1}, respectively, were prepared in 0.1 N HCl.
- Srock solution of pseudoephedrine hydrochloride containing 96 μg ml^{-1} was prepared in 0.1 N HCl.
- Further dilutions from each stock solution were made using 0.1 N HCl as a solvent.

2.4. Sample preparation

An accurately weighed amount of powdered tablets or contents of capsules equivalent to 60 mg (FEX), 5 mg (CET) or 5 mg (LOR), respectively and 120 mg of PE was transferred into 50 ml volumetric flask and extracted with 0.1 N HCl by shaking for 30 min. The volume was completed with 0.1 N HCl and filtered. Aliquots of the filtrate, within the specified range, were diluted as described under calibration graphs.

2.5. Calibration graphs

Various aliquots of each standard solution, within the concentration range stated in Table 2, were transferred into four sets of 50 ml volumetric flasks, the solutions were completed to volume with 0.1 N HCl.

2.6. Spectrophotometric measurements

The absorbances of the standard and sample solutions were recorded within the wavelength range 200–300 nm and stored.

3. Results and discussion

3.1. Mix I

This mixture contains FEX and PE. The absorption spectra of the two components are strongly overlapped, that the application of the derivative technique failed to resolve it. This spectral overlapping was sufficiently enough to demonstrate the resolving power of the proposed

derivative ratio spectrum method. For the determination of FEX, the absorption spectra of standard solutions of FEX were divided (amplitude by amplitude at appropriate wavelengths) by absorption spectrum of a standard solution of 480 µg ml^{-1} PE to obtain the corresponding ratio spectra (Fig. 1a). Then the first derivative of the obtained ratio spectra were calculated with $\Delta\lambda = 2$ nm (Fig. 1b). From this figure, FEX can be determined in this mixture by measuring the

amplitude at 225 nm or 230 nm where there is no contribution from PE.

On the other hand, for the determination of PE, an analogous procedure was followed. The absorption spectra of PE were divided by that of a solution of 12 µg ml^{-1} FEX (Fig. 2a), and the first derivative of the developed ratio spectra were calculated with $\Delta\lambda = 2$ nm (Fig. 2b). It was shown from this figure that, PE can be determined by measuring the amplitude at many wavelengths

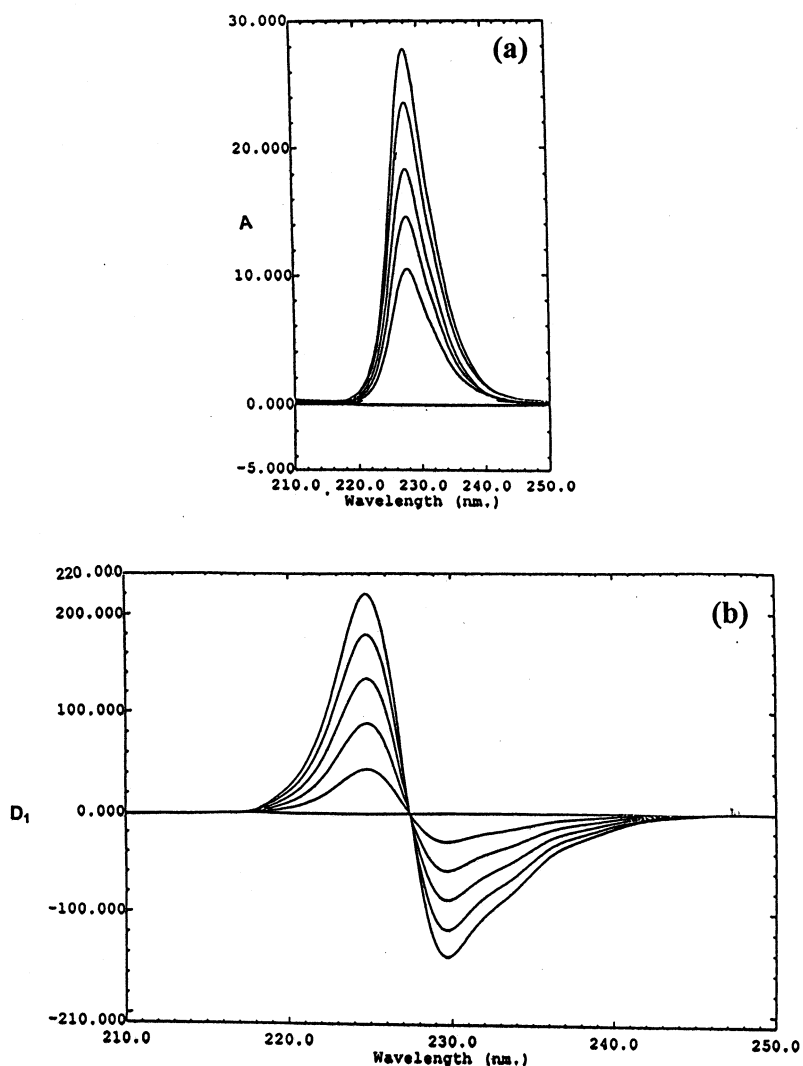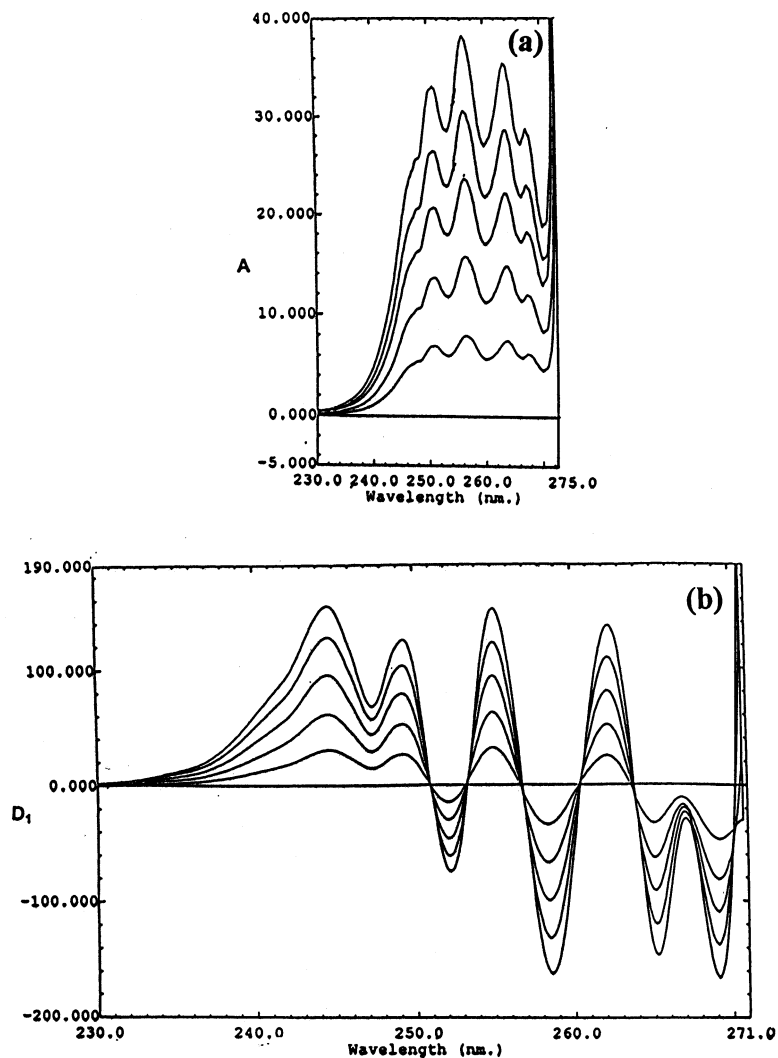Fig. 1. Ratio spectra (a) and first derivative of ratio spectra (b) of FEX 12, 24, 36, 48 and 60 µg ml^{-1} (divisor: 480 µg ml^{-1} PE).

Fig. 2. Ratio spectra (a) and first derivative of ratio spectra (b) of PE 96, 192, 288, 384 and 480 μg ml^{-1} (divisor: 12 μg ml^{-1} FEX).

where FEX has no contribution. It was found that the amplitudes at 255.1 nm gave the best results.

The influence of $\Delta\lambda$ for obtaining the first derivative of the ratio spectra was tested to obtain the optimum wavelength interval; $\Delta\lambda = 2$ nm was considered as suitable.

The effect of the divisor concentration on the calibration graphs was studied. The results obtained from this study indicate that the divisor concentration has no effect on the assay.

3.2. Mix II

Although PE is present as a major component in this mixture, but due to its weak absorptivity in the UV region, its absorption spectrum is completely overlapped by that of CET, meaning that the use of conventional UV methods would give unacceptable results. Thus, the derivative approach was applied in order to achieve resolution and subsequent determination of CET–PE in dosage forms

using the derivative ratio spectrum method. Consequently for the determination of CET, the stored absorption spectra of standard solutions of CET were divided (amplitude by amplitude) by the absorption spectrum of a standard solution of 384 μg ml^{-1} PE to obtain the corresponding ratio spectra, then the first derivative of the ratio spectra were calculated with $\Delta\lambda = 2$ nm. The obtained spectra showed that CET can be determined at three different wavelengths (225.4, 234.5 and 238nm) from which $\lambda_{225.4}$ nm was chosen as it has the greatest response.

On the other hand, for the quantitation of PE, an analogous procedure was followed where the absorption spectra of PE were divided by that of standard solution of CET (12.78 μg ml^{-1}) and the first derivative of the developed ratio spectra were obtained. PE can be measured at several wavelengths where there are no contribution from CET. The response at 268.8 nm was chosen as it gives the best results. The optimum $\Delta\lambda$ for obtaining the first derivative of the ratio spectra was found to be 2 nm. Moreover, the influence of the divisor concentration on the calibration graphs was studied. It was found that, the divisor concentration affects only the slope of the calibration curves.

3.3. Mix III

This mixture represents a combination of a weakly absorbing compound (PE) and a strongly absorbing one (LOR). Although the concentration of PE is greater than that of LOR, its absorption spectrum is completely overlapped by that of LOR, i.e. the use of conventional UV methods would give unacceptable results. The derivative technique was therefore proposed to correct for interference. Thus, the derivative ratio method was chosen to resolve this spectral overlapping and to develop an accurate and precise method of analysis for this mixture. Therefore, for the determination of LOR the amplitudes of standard solutions of LOR were divided by the corresponding amplitudes of a solution of PE (187.48 μg ml^{-1}) and then calculating the first derivative values for the developed ratio spectra. From the obtained

spectra, LOR can be determined by measuring the amplitudes at 273.6 nm where there is no contribution from PE. On the other hand, PE can be assayed by analogous procedure, where the amplitudes of the absorption spectra of PE standard solutions were divided by the corresponding amplitudes of a solution of 7.06 μg ml^{-1} LOR, and then the first derivative values for the developed ratio spectra were obtained. PE can be easily quantified by measuring its response at 255 nm where LOR has no contribution.

From the study of the influence of $\Delta\lambda$ for obtaining the first derivative of the ratio spectra, it was found that $\Delta\lambda$ 4 nm and 2 nm were suitable for LOR and PE, respectively. Again, the divisor concentration affects only the slope of the standard curves while the maxima and minima remain at the same wavelengths.

3.4. Effect of divisor concentration

A study was carried out to test for the effect of the divisor concentration on the calibration graphs. Thus the absorption spectra of standard solutions of different concentrations (Table 1) of FEX, CET, LOR and PE were obtained. The amplitudes of each set of these solutions were divided by the corresponding amplitudes of the standard solutions of the other component in the mixture. The resultant ratio spectra were then differentiated with respect to wavelength using the appropriate $\Delta\lambda$ (Table 1). The derivative values of each component (^{1}DD) were measured, at the specified wavelengths (Table 1), and plotted against its concentrations. The data obtained from the statistical analysis of these graphs using least squares method were shown in Table 1. The results obtained from this study using three different levels of divisor concentration (Table 1) indicate that the divisor concentration has no effect on the assay. If the concentration of the divisor is increased or decreased, the resulting ^{1}DD values are proportionately decreased or increased, respectively, although the maxima and minima remain at the same wavelength.

Table 1
Effect of divisor concentration on the assay of FEX–PE, CET–PE, LOR–PE binary mixtures

	Component conc.	Divisor conc.	$\Delta\lambda$	Selectedλ	Linear regression			CV (%)	Standard deviation	
	(μg/ml)	(μg/ml)	(nm)	(nm)	Intercept (a)	Slope (b)	Corr. coeff. (r)		Intercept (S_a)	Slope ($S_b \times 10^{-2}$)
Mix I	**FEX**	**PE**								
		480.00	2	225.0	0.234	3.706	0.9999	1.08	0.496	1.247
	12–60	530.00			3.640	3.209	0.9995	2.00	1.564	3.920
		560.00			5.184	3.177	0.9997	0.93	1.255	3.150
	PE	**FEX**								
		12.00	2	255.1	0.316	0.319	0.9999	0.90	0.317	0.990
	96–480	24.00			−0.243	0.116	0.9989	1.70	0.694	2.180
		60.00			−0.846	0.067	0.9998	1.77	0.172	0.540
Mix II	**CET**	**PE**								
		384.00	2	225.4	1.257	5.920	0.9997	0.80	1.462	8.091
	8.5–25.5	768.80			0.837	3.870	0.9997	0.81	0.949	5.250
		1537.60			0.464	1.888	0.9997	0.79	0.464	2.567
	PE	**CET**								
		12.78	2	268.8	0.581	0.172	0.9999	0.77	0.620	0.655
	192–1537.6	17.04			0.501	0.124	0.9999	0.79	0.438	0.462
		25.56			0.283	0.071	0.9999	0.79	0.259	0.272
Mix III	**LOR**	**PE**								
		187.48	4	273.6	−0.424	4.965	0.9998	0.73	0.623	4.160
	7–22	749.92			−0.467	4.030	0.9996	1.26	0.873	5.820
		1499.80			−0.252	2.170	0.9996	1.26	0.472	3.150
	PE	**LOR**								
		7.06	2	255.0	0.060	0.012	0.9999	0.43	0.026	0.003
	187–1499	11.41			0.030	0.006	0.9999	0.43	0.013	0.001
		21.10			0.035	0.004	0.9999	1.10	0.021	0.002

3.5. Validation of the method

3.5.1. Linearity

The linearity of the proposed method was evaluated for each drug by analyzing a series of different concentrations of each of FEX, CET, LOR and PE; within the range stated in Table 2 in the absence and presence of a certain concentration of the other component in the mixture. The assay was performed according to the experimental conditions previously established. The [1]DD values for each drug were measured, at the specified wavelengths (Table 2), and plotted against its concentration. A straight line was obtained in each case. The statistical analysis of these graphs using least squares method was made for the slope, intercept and correlation coefficients. The results obtained show that the linearity of calibration graphs and the compliance with Beer′s law were validated, as illustrated by the excellent values of correlation coefficients of the regression equations and the small values of intercepts. Furthermore, the slope of the calibration graph for each drug was independent on the concentration of the other component in the mixture (Table 2).

Moreover, each set of standard solutions of the tested drugs, within the range stated in Table 1, was assayed by the proposed method using three levels of divisor concentration. The regression lines obtained with different divisor concentrations together with their statistical data are compiled in Table 1. The test of significance of the experimental intercepts (a) of the regression lines was done by calculating the quantities $t = a/S_a$ and their comparison with the tabulated data for the Student's t-distribution. The results of these calculations proved that the intercepts of most of regression lines are not different from zero. The divisor concentration that gives t values not exceeding the 95% criterion of the tabulated ones was considered optimum and was selected for further study.

3.5.2. Accuracy

This study was performed by the addition of known amounts of each drug to placebos containing either excipients only or certain concentration of the other component in the mixture. Each analyte was tested at five levels below and above the label claim of each one (Table 3). The resulting mixtures were assayed and the accuracy is then calculated from the test results as the percentage of

Table 2
Assay parameters for the analysis of FEX, CET and LOR in binary mixtures with PE by the proposed method

Component conc.		Divisor conc.	$\Delta\lambda$	Selected λ	Linear regression			CV (%)
(μg ml^{-1})		(μg ml^{-1})	(nm)	(nm)	Intercept (a)	Slope (b)	Corr. coeff. (r)	
FEX	**PE**							
12–60	–	PE 480	2	225.0	0.234	3.706	0.9999	1.08
12–60	96	PE 480	2	225.0	1.286	3.724	0.9999	0.37
–	96–480	FEX 12	2	255.1	0.316	0.319	0.9999	0.90
36	96–480	FEX 12	2	255.1	0.717	0.309	0.9998	1.00
CET	**PE**							
8.5–25.5	–	PE 192.36	2	225.4	1.257	5.920	0.9997	0.80
8.5–25.5	192	PE 192.36	2	225.4	2.576	5.819	0.9999	1.17
–	96.1–769.4	CET 12.78	2	268.8	0.581	0.172	0.9999	0.77
12.78	96.1–769.4	CET 12.78	2	268.8	0.376	0.174	0.9999	1.27
LOR	**PE**							
7–22	–	PE 187.48	4	273.6	−0.424	4.966	0.9998	0.73
7–22	480.0	PE 187.48	4	273.6	0.874	4.860	0.9997	1.31
–	187–1499	LOR 7.06	2	255.0	0.602	0.012	0.9999	0.43
10.59	187–1499	LOR 7.06	2	255.0	0.066	0.012	0.9999	0.62

Table 3
Assay results for the determination of FEX, CET, LOR and PE in synthetic mixtures and dosage forms

Synthetic mixtures					Dosage forms		
	Concentration ($\mu g \ ml^{-1}$)		Mean recovery %$\pm$S.D.[a]			Mean recovery %$\pm$S.D.[a]	
Mix I	**FEX**	**PE**	**FEX**	**PE**		**FEX**	**PE**
	24–72	96	101.1$\pm$0.37	98.3$\pm$1.00	Allerga-D Tablets	100.7$\pm$0.26	98.8$\pm$1.22
	36	24–120	100.4$\pm$0.16	99.2$\pm$1.44			
Mix II	**CET**	**PE**	**CET**	**PE**		**CET**	**PE**
	8.52–25.56	384.0	99.2$\pm$1.17	101.3$\pm$0.40	Cirrus Capsules	100.1$\pm$0.72	100.2$\pm$0.52
	12.78	96.0–480.0	100.5$\pm$0.28	100.8$\pm$1.27			
Mix III	**LOR**	**PE**	**LOR**	**PE**		**LOR**	**PE**
	7.06–21.18	480.0	100.3$\pm$1.30	99.5$\pm$0.07	Decongess L Capsules	99.8$\pm$1.07	99.9$\pm$0.99
	10.59	93.74–656.18	99.1$\pm$0.30	100.1$\pm$0.62	Clarinase Tablets	99.6$\pm$0.58	100.8$\pm$0.40

[a] Standard deviation of five determinations.

analyte recovered by the assay. The excellent recoveries obtained (Table 3) suggest that good accuracy of the proposed method and there is no interference from excipients and other component which are present in dosage forms.

3.5.3. Precision

To test the repeatability of the proposed method, separate determinations at different concentration levels were carried out for each drug either alone or in the presence of certain concentration of the other component. The results obtained (Table 3) show that, the relative standard deviation was less than 2%, which indicates high degree of precision of the proposed method.

3.5.4. Selectivity

Method selectively was achieved by preparing different mixtures of the tested drugs within the linearity range. The mixtures contain varying amounts of one component and constant amount of the other (Table 3). The synthetic mixtures were analyzed according to the previous procedure. The [1]DD values for each component were measured at the specified wavelengths (Table 2). Statistical analysis of the data shows that the slope of the calibration graph for each drug was independent on the concentration of the other component of the mixture. This means that the [1]DD amplitudes of the mixture was only a function of the concentration of the drug at the specified wave-

length. Consequently, the results obtained (Table 3) were good indicating the high selectivity of the proposed method and its potential for the simultaneous determination of these mixtures.

3.6. Dosage forms analysis

The present method was applied for the simultaneous determination of the above mentioned mixtures in their available commercial dosage forms. The results obtained (Table 3) were both precise and accurate. The results show the high reliability, selectivity and repeatability of the method and conform satisfactory to the label claim amounts.

4. Conclusion

The proposed method provides simple, accurate and reproducible quantitative analysis for the determination of FEX, CET, LOR and PE as binary mixtures in dosage forms, without any interference from the excipients. The proposed method is simple as there is no need for solvent extraction and direct as it estimates each drug independent of the other. The method has the advantage of lower cost, rapid and environmental protecting. The proposed method was completely validated and suitable for quality control laboratories, where economy and time are essential.

References

[1] Physicians' Desk Reference Generics, 3rd ed., Medical Economics, New Jersey, 1997, p. 1962.

[2] S.S. Zarapkar, N.P. Bhandari, U.P. Halker, Indian Drugs 37 (9) (2000) 421–425.

[3] G.V. Kanumula, R. Phanu, Indian Drugs 37 (12) (2000) 574–576.

[4] S.S. Zarapkar, U.P. Halkar, S.H. Rane, Indian Drugs 35 (10) (1998) 658–661.

[5] S.N. Makhija, P.R. Vavia, J. Pharm. Biomed. Anal. 25 (3–4) (2001) 663–667.

[6] A.F. Fell, Trends Anal. Chem. 2 (1983) 63.

[7] F.A. El-Yazbi, M.A. Korany, M. Bedair, J. Pharm. Belg. 40 (4) (1985) 244–248.

[8] F. Salinas, J.J. Berzas Nevado, A. Espinosa Mansilla, Talanta 37 (1990) 347–351.

[9] F.A. El-Yazbi, H.H. Abdine, R.A. Shaalan, E.A. Korany, Spectrosc. Lett. 31 (7) (1998) 1403–1414.

[10] F.A. El-Yazbi, K.-A. Kovar, Sci. Pharm. 66 (1998) 325–333.