Spectrophotometric determination of chromium (III) with isatin in the presence of surfactant

Khalid Abdel-Salam M.Attia, Nasr M.El-Abasawy, Ahmed M.Abdelraoof*

Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Al-Azhar University, 11751, Nasr City, Cairo, (EGYPT)

E-mail: Ahmedmeetyazeed79@yahoo.com

ABSTRACT

FiveSimple, rapid, sensitive, accurate and precise stability-indicating spectrophotometric methodswere developed for the determination of Etilefrinehydrochloride (ETF)in bulk powder and in pharmaceutical preparation. Method (A) Ratio derivative method(^{1}DD),Method (B) Ratio difference method, Method (C)Mean centering method, Method (D) Dual wavelength method and Method (E)Area under the curve (AUC). These methodswere used for the determination of Etilefrine hydrochloride in binary mixture with (chlorophenermine maleate)(CPM). These methodswere validated and successfully applied to the determination of Balkis®capsule with an average percent recovery ± RSD% of 100.26±0.622 for method (A), 99.73 ± 0.583 for method (B), 99.15 ± 0.670 for method (C), 99.63 ± 0.862 for method (D) and 99.41 ± 1.225 for method (E). The obtained results werestatistically compared with those of the reported method by applying t-test and F-test at 95% confidence level and no significant difference was observed regarding accuracy and precision.   © 2016 Trade Science Inc. - INDIA

INTRODUCTION

Etilefrine hydrochloride (ETF) is chemically designated as alpha- [(Ethylamino) methyl]-3-hydroxy benzenemethanol hydrochloride[1] (Figure 1). It is a cardiac stimulant used as an anti-hypotensive. It is a sympathomimetic amine of the 3-hydroxyphenylethanolamine series used in treating orthostatic hypotension of neurological, cardiovascular, endocrine or metabolic origin. Reported methods of analysis of etilefrine hydrochloride include spectrophotometric[2-8], chemiluminescence assay[9], HPLC[10], LC-MS[11], glass capillary column GC[12],

and potentiometric method[13].

The present work is concerned with development and validation of simple, rapid and selective five spectrophotometric methods which don't need any special program, so they can be easily applied as alternative to reported LC method which requires
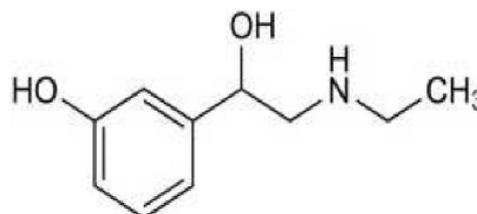

Figure 1 : Structural formula of etilefrine

Full Paper

time, experience, expensive instruments and solvents.

MATERIALS AND METHODS

Apparatus

- Shimadzu UV-Vis. 1650 Spectrophotometer (Japan).
- Jenway, pH meter 3510 (Jenway, USA).
- Hot plate (Torrey pines Scientific, USA).
- rotary evaporator (scilogex, USA)

Materials and reagents

- Pure drug samples of CPM and ETF were kindly supplied by EIPICO Pharmaceuticals, 10th of Ramadan City, Egypt. Their purity was checked and found to be 99.42 ± 0.662 % and 99.09 ± 0.771 % according to the BP [24] for CPM and ETF, respectively.
- Balkis® capsules (EIPICO Pharmaceuticals) Batch No. 1108630, labeled to contain 6 mg chlorophenermine and 20 mg etilefrine per capsule both bound to an ion exchangeresin were purchased from local pharmacies
- Water usedthroughouttheprocedureswas freshlydouble distilled.

Standard Solution

A stock solution of Etilefrine (100μg/ml) was prepared by dissolving 10 mg of Etilefrine in 50 ml of methanol and complete to 100 ml with methanolandstock solution of Chlorophenermine(100 μg/ml) was prepared by dissolving 10 mg of CPM in 50 ml of methanol and complete to 100 ml with methanol and was further diluted with the same solvent as appropriate

Procedure

Construction of the calibration curves (general procedures):

Method A(Ratio derivative method)

Aliquots equivalent to(0.1 – 1mg) ETFand (0.1– 1mg) CPMwere accurately transferred from their standard working solution (100 µg ml⁻¹) into two separate series of 10 ml volumetric flasks then completed to volume with methanol. The spectra of the prepared standard solutions were scanned from 200 - 400 nm and stored in the computer. For the determination of ETF in presence of its interferent product, the stored spectra of ETFwere divided by the spectrum of 60 µg ml⁻¹CPM, smoothed with $\Delta\lambda = 2$ nm and scaling factor= 1, then the first derivative of the ratio spectra (¹DD) with $\Delta\lambda = 2$ nm was obtained. The amplitude of the first derivative trough of (ETF /CPM) was measured at 279 nm. A calibration graph relating the trough amplitude at 279 nm to the corresponding concentrations in µg ml⁻¹of ETFwas constructed alternatively, the regression equation was derived.

Method B (Ratio difference method)

Aliquots equivalent to (0.1 – 1 mg) were accurately transferred from ETF standard stock solution (100 µg ml⁻¹) into a series of 10 - ml volumetric flasks then completed to volume with methanol. The spectra of the prepared standard solutions were scanned from 200 - 400 nm and stored in the computer, For the determination of ETFin presence of CPM, the stored spectra of ETFwere divided by the spectrum of (60 µg ml⁻¹) of CPM. The amplitude difference at 260 and 281 nm (dP260– 281) was plotted against the corresponding ETF concentration in µg ml⁻¹ and the regression equation was computed.

Method C (Mean centering method)

Aliquots equivalent to (0.1 – 1 mg) of ETF working standard solution were accurately transferred into a series of 10 - ml volumetric flasks then completed to volume with methanol. The spectra of the prepared standard solutions were scanned from 200 - 400 nm using methanol as a blank and stored in the computer. The absorption spectra of ETFwere divided by the spectrum of (60 µg ml⁻¹) of CPM. The amplitude of the mean centered peak of (ETF / CPM) was measured at 283 nm. A calibration graph relating the peak amplitude to the corresponding concentrations in µg ml⁻¹ of ETF was constructed.

MethodD(Dual wavelength method)

Aliquots of standard ETF solution in methanol (100 µg ml⁻¹) containing (0.1 – 1 mg) of the drug were added to a series of 10 -ml volumetric flasks

and then diluted to the mark with methanol. The utility of dual wavelength data processing programis to calculate the unknown concentration of acomponent of interest present in a mixture containingETF andCPM interfering component by the mechanism of theabsorbance difference between two points on themixture spectra. This is directly proportional to theconcentration of the component of interest, independentof the interfering components. From the overlay of two drugsfor estimation of ETF, two wavelengths selected (244 nm and 269nm)wherethe CPM shows same absorbance. Six working standard solutions havingconcentration 10, 20, 30, 50, 90 and 100the drugs were prepared separately in methanol and the absorbance at 244 nm and 269 nm were measured and absorptive coefficients were calculated usingcalibration curve.

Method E(Area under curve method)

Aliquots of standard ETFsolution in methanol (100 µg ml^{-1}) containing (0.1 – 1) mg of the drug were added to a series of 10 -ml volumetric flasks and then diluted to the mark with methanol The solution of drugs were scanned in the range of 200-400 nm. For Area under Curve method, the sampling wavelength ranges selected for estimation of ETF in presence of CPM are 237-247 nm (λ_1-λ_2) and 265-275 nm (λ_3-λ_4). Mixed standards were prepared and their Area under the Curve were measured at the selected wavelength ranges. Concentration of two drugs in mixed standard and the sample solution were calculated using equation (1) and (2).

A1 =$\alpha_1 C_{ET}$ +$\beta_1 C_{CH}$…………... (1) at 237-347 nm.

A2 = $\alpha_2 C_{ET}$ + $\beta_2 C_{CH}$………..... (2) at 265-275 nm.

Where, $\alpha 1$ And$\alpha_2$are absortivities of ETF at (λ_1-λ_2) and (λ_3-λ_4) respectively. β_1 and $\beta_2$areabsortivities of CPM at (λ_1-λ_2) and (λ_3-λ_4) respectively.

A_1 and A_2 are area under curve of mixed standard at (λ_1-λ_2) and (λ_3-λ_4) respectively. C_{ETF} and C_{CPM} are the concentrations in g/100ml.

Analysis of pharmaceutical preparation

Procedure

The following procedure was followed in the dark(using aluminum foil to cover all flasks). To determine the content of ETF in commercial Balkis®capsules(each capsule labeled to contain20 mg ETF and6mg CPM)The following procedures were carried out as a modification to the manufacturer method of analysis obtained by personal communication[6] [25]., The contents of 12 capsules were carefully emptied and weighed. A portion of the contents equivalent to the average weight of two capsules and half was accurately weighed and transferred toa250-mlconicalflask. This portion was washed three times with 100 ml of warm double distilled water using amagneticstirrer for10 minutes each

Extraction was carried out using the following procedure

i. Extracting solution was prepared as follows: dissolve 5 g of sodiumchloride dissolved in 220 mlof distilledwater, and then mixed with 20 ml of 25% hydrochloricacid and 760 ml methanol.

ii. The extraction was repeated using 100 ml of the extracting solution but the extraction was continued overnight (12hours).

iii. The extracting solution was collected, in a1000-ml evaporating flask, by decantation, keeping the resinin the extracting vessel.

iv. The extracting solution was trans ferred by the same method to the evaporating flask.

v. The pH was adjusted to7 using 20 % W/V sodium hydroxide solution. Methanol was evaporatedusing arotaryevaporator (Rotavap). Then extracted the remaining by using double distilled water,and filtered through0.5µm What manfilterpaper. From the above prepared solution, further dilutions were prepared in the linearity range using methanol

Calculation optimization

After extraction, Etilefrine will be available in the final extraction solution as etilefrineHClbut (ETF)in the capsule is present as etilefrineresinate.. Standard solutions were calculated according to the authentic etilefrineHCl (ETF). So, to convert the etilefrineHCl

(ETF) found percentage to etilefrine found percentage; it is multiplied by the conversion factor of

0.8323.

Etilefrine conversion factor = (Mol. Weight of etilefrine/Mol. Weight of etilefrinHCl= 181.2/217.7 = 0.8323

RESULTS AND DISCUSSION

Spectral characteristics

The zero order (D_0) absorption spectra of ETF (60 μg ml^{-1}) and CPM (60 μg ml^{-1}) were recorded against methanol as blank over the rangeof 200 – 400

For method A

Salinas et al.[14] designed a spectrophotometric method, which is based on the derivation of the ratio-spectra for resolving binary mixtures. 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)[15-17]. 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 interfere the assay. In this method the absorption spectrum of the mixture (absorbance at each wavelength) is divided by the absorption spectrum of a standard solution of one of the components, and the first derivative of the ratio spectrum is obtained. The concentration of the other component is then determined from a calibration graph.
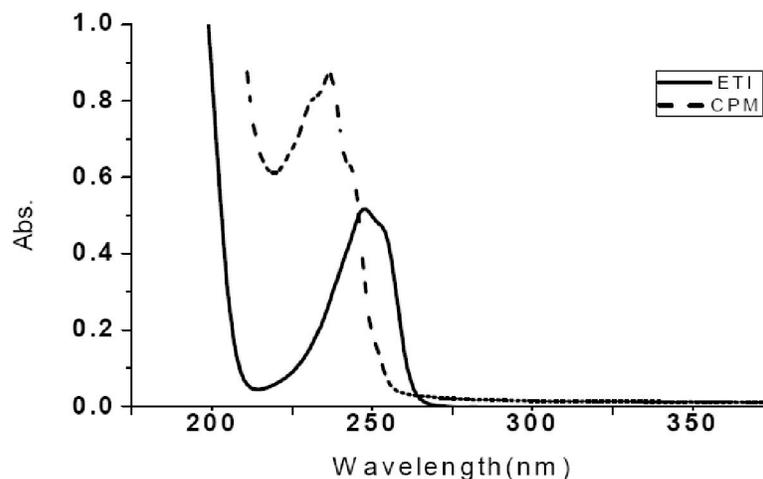

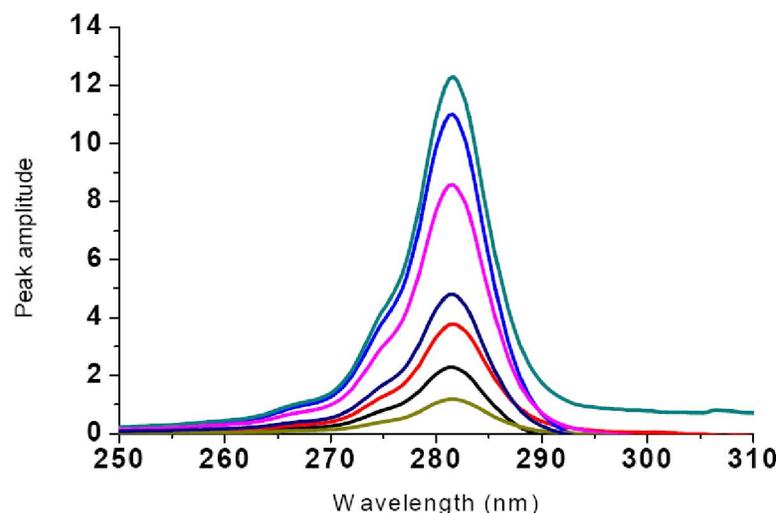**Figure 2 : Zero-order absorption spectra of ETF (60μg ml^{-1}) (—) and CPM (60 μg ml^{-1}) (…..) in methanol**

Figure 3 : Ratio spectra of ETF (10 -100μgml^{-1}) using (60 μgml^{-1}) CPM as divisorand methanol as blank

The main parameters that affect the shape of the ratio spectra were wavelength, scanning speed, the concentration of the standard solution used as a divisor; the wavelength increment over which the derivative was obtained ($\Delta\lambda$) and the smoothing function was carefully tested. The ratio spectra presented in Figure(3)and the first derivative of the ratio spectra presented in Figure (4)may provide a good proof for this understanding. The effect of wavelength scanning speed is studied. It was found that at high speed noisy spectra were obtained while at low scanning speed, the noise was decreased but a longer time was needed for the measurements, so medium scanning speed was chosen to perform measurements. Effect of divisor concentration was also tested, different concentrations of divisor were used (10,30 and60 µg ml⁻¹) of CPM and the divisor of concentration 60 µg ml⁻¹of CPM was found the best regarding average recovery percent when it was used for the prediction of ETFconcentrations in bulk powder as well as in laboratory prepared mixtures.

The absorption spectra of ETF were divided by the absorption spectrum of 60 µg ml⁻¹CPM an Figure(3) for determination of ETF in the presence of CPM. These gave the best compromise in terms of sensitivity, repeatability and signal to noise ratio. The choice of wavelength for the measurement was carefully studied. The trough amplitude at 274.0 and peak amplitude at 279 nm of the first derivative of ratio spectra are then recorded respectively. Good linearity was observed but the recovery percent at 279.0 nm was better, which may be attributed to its higher signal to noise ratio.

For method B

Ratio difference[18] is a new simple, rapid and selective method for the simultaneous determination of components having overlapping spectra in binary mixtures, having the advantages of minimal data processing and wider range of application. The binary mixture of ETF and CPM was chosen as an example for the application of the new ratio difference method.

The absorption spectra of ETF and CPM show a degree of interference as shown in figure (2), that the application of direct spectrophotometry failed to determine ETF in the presence of CPM. Several approaches have been developed to remove the overlapping constant in the ratio spectrum, either using certain order derivativeor through a sophisticated subtraction followed by multiplication procedure[19]; the latter was capable of determining only the component with the less extended spectrum in the mixture. The ratio difference method is a simple innovative method was capable of determining ETF in presence of CPM with minimal data processing, high selectivity regardless which component has more extended spectrum.

The method comprises two critical steps, the first is the choice of the divisor, and the selected divisor should compromise between minimal noise and maximum sensitivity. Different concentrations of divisors are used (10, 30 and 60µg ml⁻¹) of CPM and the divisor concentration 60 µg ml⁻¹ was found the best regardingaverage recovery percentwhen it was
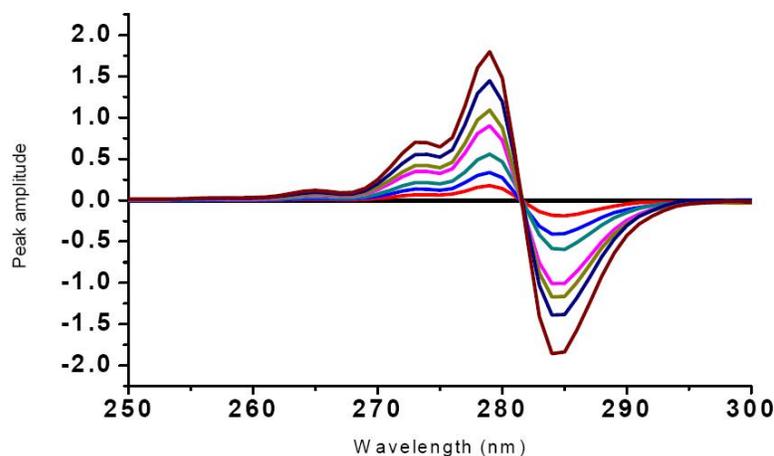
Figure 4 : First derivative of ratio spectra of ETF (10-100µgml⁻¹)using 60 µgml⁻¹OfCPM as a divisor and methanol as blank

Full Paper

used for the prediction of ETF concentration in bulk powder as well as in laboratory prepared mixtures.

The second critical step is the choice of the wavelengths at which measurements are recorded. Any two wavelengths can be chosen provided that they exhibit different amplitudes in the ratio spectrum and a good linearity is present at each wavelength individually. Linear correlation was obtained between the differences in amplitudes at 260.0 and 281.0nm, against the corresponding concentration of ETF. Good linearity is obtained in the concentration range of 10 - 100 µg ml^{-1}ETF.

For method C

In this method[20], the absorption spectra of the drug were divided by a suitable absorption spectrum of the interfering drug (divisor) to get the ratio spectra Figure (4). The best divisor concentration was 60 µg/ml of CPM. The obtained ratio spectra were mean centered using MATLAB and the concentration of ETF was determined by measuring the amplitude at 283 nm Figure (5)

For method D

The utility of dual wavelength[21] method is to calculate the unknown concentration of a component of interest present in a mixture containing both the component of interest and an unwanted interfering component by the mechanism of the absorbance difference between two points on the mixture spectra. The calibration curves were prepared at absorbance difference of two wavelengths (269 nm – 244 nm). The response for the ETF was found to be linear in the concentration range 10 to 100 µg/ml and at ab-

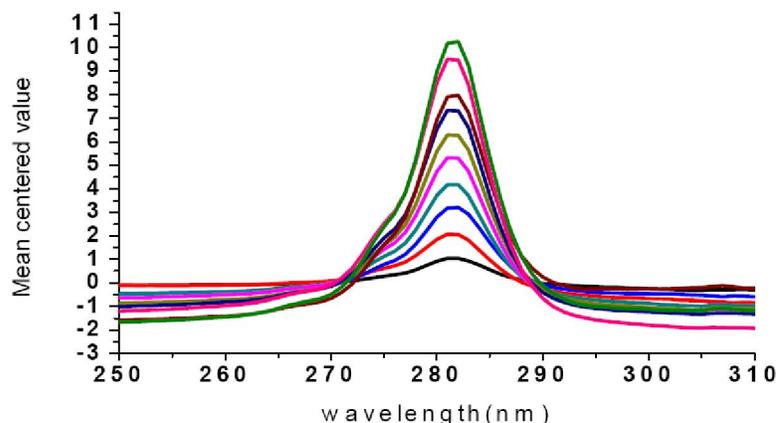**Figure 5 : Mean centered ratio spectra of ETF (10 – 100 µg ml^{-1}) using (60 µg ml^{-1})ofCPM as a divisor and methanol as blank**
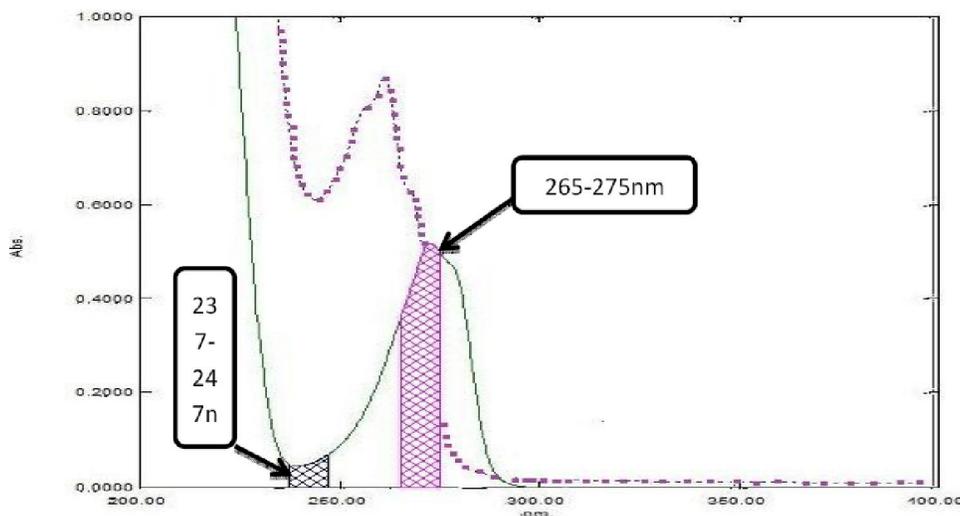

Figure 6 : Zero order absorption spectra of (60 µgml-1) ETF (—) and(50 µgml-1) CPM (…) showing wavelength ranges for area under curve method using methanol as blank

sorbance difference of two wavelengths (244 nm – 269 nm), the response was found to be linear in the concentration range 10 to 100 µg/ml. The linearity of the calibration curve was validated by the high values of correlation coefficient, Figure (2).

For method E

Selection of the wavelength region to construct AUC method has a great effect on the analytical parameters such as slope, intercept and correlation coefûcient. Different wavelength regions are tested where the wavelength ranges 237–247 nm and 265–275 nm are selected which show good selectivity and percentage recovery, (Figure 6).

Area under curve of the absorption spectra in the wavelength ranges 237–247 nm (λ_1-λ_2) and 265–275 nm (λ_3-λ_4) of Etilefrine in the concentration range of 10–100 µg ml^{-1}werecalculated. For chlorophenerminearea under curveof the absorption spectra in the wavelength ranges 237–247 nm(λ_1-λ_2) and 265–275 nm (λ_3-λ_4) in the concentration range of 10–100 µg ml^{-1}werealso calculated. The absorptivity 'Y' values of Etilefrine and Chlorophenermine were calculated at each wavelength range. The concentrationsof Etilefrinecan be obtained by applying Cramer's rule and matrices in Eqs. (1) and (2). Concentration of two the drugs in mixed standard and the sample solution are calculated according to the following equations[22-23]

A1 =89C$_{ET}$ +834C$_{CH}$...............(1) at 237-347 nm.

A2 = 781C$_{ET}$ + 882C$_{CH}$.............(2) at 265-275 nm.

Where C$_{ET}$ and C$_{CH}$ are the concentrations of Etilefrine and Chlorophenermineing/100ml, respectively. 89 and 781 are the absorptivity value of Etilefrine at (λ_1-λ_2) and (λ_3-λ_4) respectively. 834and 882are absorptivity valueof chlorophenermine at (λ_1-λ_2) and (λ_3-λ_4) respectively. A1 and A2 are the area under curve of sample solutions at the wave length range (λ_1-λ_2) and (λ_3-λ_4) respectively.

VALIDATION OF THE METHODS

Linearity

Method A

Under the described experimental conditions, the calibration graph for the method was constructed by plotting troughheight versus concentration in µg/ml. The regression plot was found to be linear over the range of 10-100 µg/ml. The linear regression equation for the graph is:

P$_{279\ nm}$= 0.0181 C - 0.0079 r = 0.9997

Where C is the concentration of ETF in µg ml^{-1}, P is the trough height of the first derivative of the ratio spectrum curve at 279 nm and ris the correlation coefficient.

Method B

Linear correlation was obtained between the differences in amplitudes at 260.0 and 281.0nm, against the corresponding concentration of ETF. Goodlinearity is obtained in the concentration range of 10-100 µg ml^{-1}. The corresponding regression equation was computed to be:

ΔP$_{260.0 - 281.0}$ = 0.117 C - 0.0536 (r = 0.9998)

Where ΔP is the amplitude difference at the selected wavelengths, C is the concentration in µg ml^{-1} and r= the correlation coefficient.

Method C

Linear correlation was obtained between the mean centered values at 283 nm, against the corresponding concentration of ETF. Good linearity is obtained in the concentration range of (10 - 100µg ml^{-1}) ETF The corresponding regression equation was computed to be:

MCN$_{283}$ = 0.0996 C - 0.0452 (r = 0.9997)

Where MCN is the peak amplitude of the mean centered ratio spectrum curve, C is the concentration in µg ml^{-1} and r = the correlation coefficient, as shown in TABLE 1.

Method D

The calibration curves were plotted over a concentration range of 10-100 µg/ml for ETF.

ΔP$_{244.0 - 269.0}$ = 0.0069 C - 0.0025 (r = 0.9997)

Where ΔP is the amplitude difference at the selected wavelengths, C is the concentration in µg ml^{-1} and r= the correlation coefficient.

Method E

Under the described experimental conditions, the

Full Paper

TABLE 1 : Spectral data for determination of etilefrine by the proposed methods

Parameters	Ratio derivative	Ratio difference	Mean centering	Dual wavelength	Area Under curve
λ_{max} (nm)	279	260-281	283	244-269	237-247 265-275
Linearity range (μgml^{-1})	10-100	10-100	10-100	10-100	10-100
LOD (μgml^{-1})	0.294	1.452	1.005	0.919	0.372
LOQ (μgml^{-1})	0.893	4.40	3.04	2.785	1.128
Regression equation*					
Slope (*b*)	0.0181	0.117	0.0996	0.0069	0.0792
Intercept (*a*)	0.0079	0.0536	0.0452	0.0025	0.0889
Correlation Coefficient (*r*)	0.9997	0.9998	0.9997	0.9997	0.9996

TABLE 2 : Intra-day and inter-days accuracy and precision for the determination of etilefrine by the proposed methods

	Conc. μg.ml^{-1}	Intra-day		Inter-days	
		Accuracy (R %)	Precision (RSD %)	Accuracy (R %)	Precision (RSD %)
Method A	50	100.39	1.222	100.17	0.939
	60	99.83	1.014	100.14	1.650
	100	99.97	1.882	99.29	0.620
Method B	50	100.30	1.156	100.22	0.147
	60	99.28	1.216	100.10	1.354
	100	100.10	1.551	99.03	0.829
Method C	50	100.46	1.393	100.48	0.368
	60	99.12	1.162	100.03	1.531
	100	99.84	0.779	99.13	0.658
Method D	10	100.24	.836	99.75	0.147
	50	100.53	0.729	100.33	1.531
	100	99.25	1.716	101.23	0.144
Method E	50	101.61	0.415	101.05	0.174
	80	99.05	0.858	98.60	0.450
	100	98.93	0.223	99.00	0.162

calibration graph for the method was constructed by plotting area under curve versus concentration in μg/ml. The regression plot was found to be linear over the range of 10-100 μg/ml. The linear regression equation for the graph is:

$P_{AUC} = 0.0792C - 0.0889$ (r = 0.9996)

WhereP_{AUC} is area under curve at the selected wavelengths, C is the concentration in μg ml^{-1} and r= the correlation coefficient.

Sensitivity

The limit of detection (LOD) and the limit of quantitation (LOQ) werecalculated according to ICH Q$_2$ Recommendation[26]from the following equations:

LOD = 3.3 S$_a$ / slope

LOQ = 10 S$_a$ / slope

Where S$_a$ is the standard deviation of the intercept

of regression line. LOD was found to be 0.294, 1.452, 1.45, 0.919 and 0.372μg/ml, while LOQ was found to be 0.893,.4.40, 3.04, 2.785 and 1.128μg/ml for methodA,B,C,D and E respectively. The small values of LOD and LOQ

Accuracy and precision

Three replicate determinations of three different concentrations of ETFin pure form within linearity range were performed in the same day (intraday) and in three successive days (inter-day). Accuracy as recovery percent (R%) and precision as percentage relative standard deviation (RSD%) were calculated and results are listed in TABLE 2. The small values of RSD% indicate high precision of the method. Morever, the good R% confirms excellent accuracy.

Full Paper

TABLE 3 : Determination of ETF in Mixtures withCPM by the proposed methods

Intact (µg ml⁻¹)	Degradate (µg ml⁻¹)	Degradate %	Ratio derivative	Recovery% of Intact		
				Ratio difference	Mean centering	Dual wavelength
30	9	23	100.90	101.67	101.24	101.08
40	12	23	99.43	98.30	98.97	98.79
50	15	23	100.87	99.60	100.20	98.73
70	21	23	100.39	99.71	99.30	100.43
90	27	23	101.59	99.63	99.68	100.36
Mean ± SD			100.64±0.797	99.78±1.205	99.88±0.891	99.88+1.060

Intact (µg ml⁻¹)	Degradate (µg ml⁻¹)	Degradate %	Recovery% of Intact , Area under curve			
20	6	23	100.31			
30	9	23	101.03			
40	12	23	99.81			
70	21	23	99.68			
80	24	23	101.14			
Mean ± SD			100.39 ±0.674			
20	6	23	100.31			
30	9	23	101.03			
40	12	23	99.81			
70	21	23	99.68			
80	24	23	101.14			

TABLE 4 : Determination of ETF in BALKIS® Tablets by the proposed and reported methods

Parameters	First derivative ratio	Ratio Difference		Proposed Methods Mean Dual Area centering wave length under curve		Reported*** method[3]
N*	5	5	5	5	5	5
$X^{?}$	100.26	99.73	99.15	99.63	99.41	98.87
SD	0.624	0.582	0.665	0.859	1.218	1.23
RSD%	0.622	0.583	0.670	0.862	1.225	1.34
t**	0.562 (2.306)	0.484 (2.306)	0.141 (2.306)	0.466 (2.306)	0.339 (2.306)	____
F**	3.39 (6.38)	3.89 (6.38)	2.98 (6.38)	1.78 (6.38)	2.11 (6.38)	____

* No. of experimental; ** The values in the parenthesis are tabulated values of t and F at (p= 0.05); ***The reported method (MBTH) method

Specificity

The specificity of the proposed methods was assured by applying the laboratory prepared mixtures of the Etilefrine together with chlorophenermine maleate. The proposed methods were adopted for the specific determination of ETF in presence of up to23 % of CPM as the same ratio in the pharmaceutical preparation (Balkis®capsule). The percentage recovery±SD% was 100.64 ±0.797,99.78±1.205, 99.88±0.891, 99.88±1.060 and100.39±0.674for method A, B, C, D and E respectively, as shown in TABLE 3.

Pharmaceutical applications

The proposed methods were applied to the determination of the studied drug in its Tablet preparation. The results were validated by comparison previously reported method[3]. No significant difference was found by applying t-test and F-test at 95% confidence level, indicating good accuracy and precision of the proposed method for the analysis of the studied drug in its pharmaceutical dosage form,as seen in TABLE 4.

CONCLUSION

The proposed methods are simple, rapid and inexpensive. So, it is good alternative to the other few

Full Paper

reported methods and to the high cost HPLC methods.

ACKNOWLEDGMENT

The authors thank Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, and Al-Azhar University for supporting this work.

REFERENCES

[1] The merck index, An encyclopedia of chemicals, Drugs, and Biologicals. 14th Edition, Merck & Co., Inc., Whitehouse station, NJ, USA, **(2006)**.

[2] N.W.Beyene, J.F.Van Staden, R.I.Stefan; IlFarmaco., **59**, 1005-1010 **(2004)**.

[3] G.H.Ragab, M.S.Elmasry, A.A.Aboul-Kheir; Egypt J.Pharm.Sci., **45,** 21-32 **(2004)**.

[4] R.S.Bakry, A.F.El Walily, S.F.Belal; Anal.Lett., **28**, 2503-2519 **(1995)**.

[5] M.N.El Bolkiny, S.Hana, M.M.El-Henawee, M.M.Ayad; Egyptian J.Pharm.Sci., **33**, 31-42 **(1992)**.

[6] A.M.Mohsen, A.M.Badawey, M.A.Shehata, S.Z.Elkhateeb; Int.J.Pharm.Sci., **6**, 595-603 **(2014)**.

[7] R.S.Bakry, A.F.M.ElWalily, S.F.Belal; Mikrochim Acta, **127**, 89-93 **(1997)**.

[8] A.E.El-Gendy; Anal.Lett., **33**, 2927-2938 **(2000)**.

[9] F.A.Aly, S.A.Tamimi, A.A.Alwarthan; j.Aoac.Int., **83**, 1299–1305 **(2000)**.

[10] K.Kojima, M.Yamanaka, Y.Nakanishi, S.Arakawa; J.Chromatogr.Biomed.Sci.and App., **525**, 210-217 **(1990)**.

[11] B.D.Ahrens, B.Starcevic, A.W.Butch; Methods Mol.Biol., **902**, 115–128 **(2012)**.

[12] A.S.Christophersen, E.Hovland, K.E.Rasmussen; J.Chromatogr A., **234**, 107-20 **(1982)**.

[13] A.F.Shoukry, Y.M.Issa, M.S.R.M Rizk El-Nashar; Anal.Lett, **29**, 1463-1475 **(1996)**.

[14] F.Salinas, J.Nevado, A.Mansilla; Talanta, **37**, 347–351 **(1990)**.

[15] A.El-Gindy, A.Ashour, L.Abdel-Fattah, M.Shabana; J.Pharm.Biomed.Anal., **24**, 527–534 **(2001)**.

[16] J.Lemus, P.Arroyo; J.Anal.Chim.Acta., **437**, 247-257 **(2001)**.

[17] R.C.Tena, Delgado, M.J.Sanchez, F.G.Montelongo; Talanta, **44**, 673-683 **(1997)**.

[18] E.S.Elzanfaly, A.S.Saad, A.E.Abd-Elaleem; Saudi.Pharm.J., **20**, 249–253 **(2012)**.

[19] M.G.El-Bardicy, H.M.Lotfy, M.A.El-Sayed, M.F.El-Tarras; J.AOAC Int., **91**, 299–310 **(2008)**.

[20] H.W.Darwish, S.A.Hassan, M.Y.Salem, B.A.El-Zeiny; Spectrochim.Acta Part A, **83**, 140–148 **(2011)**.

[21] G.Bharat; Chaudhari 1, J.Heena.Patel, Inte.J.Pharm.anal., **2**, 148-154 **(2014)**.

[22] S.L.Prabu, Shirwaikar, C.D.Kumar, A.Joseph, R.Kumar; Indian j.pharm sci., **70**, 128-131 **(2008)**.

[23] S.S.Chitlange, A.L.Mulla, G.R.Pawbake, S.B.wankhede; Int.J.Pharm.Quality assurance, **2**, 31-34 **(2010)**.

[24] The british pharmacopoeia, Her majesty's stationary office, London, UK, **(2009)**.

[25] Egyptian international pharmaceutical industries Co.(EIPICO), Personal Communication, **(2012)**.

[26] ICH Q2 (R1); Validation of analytical procedure, Text and methodology, Geneva, International Conference on Harmonization, **(2005)**.