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INDIVIDUAL AND SIMULTANEOUS DETERMINATION OF EPHEDRINE AND PHENYLEPHRINE USING ZERO AND FIRST ORDER DERIVATIVE SPECTROPHOTOMETRY

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A simple and sensitive method for the individual and simultaneous determination of ephedrine (EP) and phenylephrine(PE) has been developed. The individual method is based on direct measuring the absorbance of the resulting reddish colour at 526 and 545 nm for EP and PE, respectively. The simultaneous determination is based on measuring the absorbance at isodeferential point of 545 and 526 nm in the first order derivative spectra for EP and PE. The calibration graphs in the direct measurements were found to be linear over the range of 5 - 40 and 2 - 50 μg/ml for EP and PE, with lower detection limit of 1.5 and 0.5 ppm, respectively. The molar absorptivity was calculated to be 0.2×10^4 and 0.6×10^4 l. mol^{-1} cm^{-1} with Sandell's sensitivity being 0.098 and 0.0276 μg/cm^2, respectively. While the calibration graphs in the first order derivative (simultaneous) were found to be linear in the range of 10 - 40 and 5 - 50 μg/ ml, respectively. The relative standard deviation for five replicate analyses of 10 μg/ml EP and PE were 1.3 and 1.15% respectively. The proposed method has been successfully applied for the determination of EP and PE in pharmaceutical formulation and in synthetic mixtures.

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1.Introduction

Ephedrine, [(1R, 2S)-(-)-α-(1-methylaminoethyl) benzylalcohol] is a sympathomimetic drug that stimulates both α- and β-adrenergic receptors [1]. Phenyl -ephrine, 1-m-hydroxy- α,α-[(methyl amino) methyl]benzyl alcohol hydrochloride, is a sympathomimetic agent which has a direct effect on α-adrenoceptor [1](Fig. 1).

Ephedrine has been determined in its pharmaceutical formulations by potentiometry using modified carbon paste electrode (CPE) [2, 3], PVC electrode [4], high performance liquid chromatography (HPLC) [5], capillary electrophoresis(CE) [6]and infrared (IR) [7]. Phenylepherine has also been determined in its pharmaceutical dosage by potentiometry using CPE[8], PVC electrode [9], voltammetry [10], HPLC [11], and CE [12]. However, most of these methods involve time-consuming procedures, derivatization and/or sophisticated instruments that are not available in most quality control laboratories.

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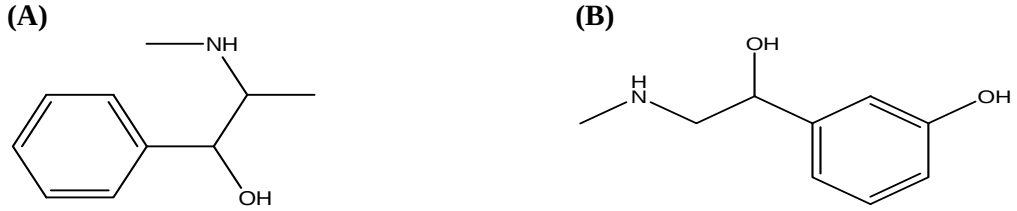

Fig. 1. Chemical structure of (A) ephedrine and (B) phenylephrine.

Spectrophotometry [13, 14] represents an attractive common technique adequate for solving many analytical problems, particularly when using capabilities of modern instruments available nowadays. Derivative spectrophotometric method has been utilized intensively for simultaneous determination in pharmaceutical analysis [15-17]. The resolution of overlapped spectra of binary mixture using derivative spectrophotometry is greatly simplified and their reliability increased by the determination of individual components without the need of chemical separation.

A quantitative determination of EP and PE in its formulations was published [18]. The method was based on the interaction of N-alkylvinylamine formed from the condensation of the free secondary amine group and acetaldehyde with chloranil to give a vinylamino-substituted quinone. The colored product for ephedrine and phenylephrine were exhibited at 320 and 680 nm, respectively. Beer`s law was obeyed over the concentration range of 1-25 μg/ml, for both drugs.

The present study describes a new approach for the individual and simultaneous determination of EP in ampoules and PE in nasal and eye drops as well as in synthetic mixtures. The method is based on the oxidation of the target analytes to the corresponding keton compounds. Ketons are react in alkaline media with AHMT, the reaction products are oxidized with potassium periodate to a reddish purple complex. The individual and simultaneous determinations are performed for the resulting reddish purple complex using zero order (direct measurement) and first order derivative spectra (simultaneous), respectively.

2.Experimental

Apparatus

A Shimadzu UV/VIS spectrophotometer, Model 2401 (Kyoto, Japan) was used for measuring the absorbance and recording the normal and derivative spectra. First derivative spectra were recorded using $\Delta\lambda = 16$ and scaling factor 20. An Orion model 330 pH meter was used for pH measurements.

Chemicals and reagents

All chemical used were of analytical grades and double distilled water was always used. Potassium periodate solution, 4×10^{-4}M KIO_4 was prepared in a 0.05M KOH, the solution was used within 2 days of preparation. 4-amino-3-hydrazino-5-mercapto-1, 2, 4-triazole solution, 0.5% m/V was prepared by dissolving 0.25 g amount of AHMT (Aldrich) in 50 ml of 0.5M hydrochloric acid, the solution was used within 2 days of preparation. Potassium hydroxide solution, 5M was prepared. Potassium periodate 1.5% m/V, 0.75 g, was dissolved in 50 ml of 0.2 M potassium hydroxide, the solution was used within 2 days of preparation. Standard stock solutions of 1000 μg/ml EP and PE (Aldrich) were prepared by dissolving 1.0g each in 1 liter of double distilled water. Working solutions containing 100 μg/ml of each were prepared freshly by accurate dilution of stock solution. Ephedrine ampoule, each ampoule (1ml) labeled to contain 30mg of EP, product of Chemical Industries Development -Giza-Egypt. Prefrin 10% solution eye drop, (Misr Co. Pharm. Egypt), and Vibrocil, nasal spray, Swiss Pharma were used.

Procedure

Transfer a known volume of the test solution, containing 125 - 1000 µg of EP or 50-1250 µg of PE, into a series of 25 ml calibrated flasks. Add 2 ml of 4×10^{-4}M KIO_4, 0.25 ml of 5M KOH, and 2 ml of 0.5% AHMT solutions for PE and EP, respectively. The flask is stoppered and allowed to stand for 20 min, and then the flask is vigorously shaken by hand until the iodine color in the mixed solution disappears. Dilute to the mark with doubly distilled water. The absorbance of the resulting color was measured at 526 and 545 nm for EP and PE, respectively against the corresponding reagent blank prepared in the same manner. The concentration of EP or PE was obtained from a preconstructed calibration graph under identical experimental conditions.

3. Results and discussion

A simple and sensitive method has been developed for determination of EP and PE. The method is based on oxidation of EP and PE to the corresponding ketones. Ketones react in an alkaline media with AHMT, the product is then oxidized with potassium periodate to a reddish purple color. The resulting color complex is measured individually for each analyte or simultaneously using first order derivative spectrophotometery. The method was reported [19] as specific method for determination of formaldehyde.

Absorption spectra

The Zero-order absorption spectra of EP and PE as well as a mixture of the two drugs, in solution containing KIO_4, KOH, and AHMT are shown in Fig.2. The wavelength of maximum absorbance (λ_{max}) was found to be 526 and 545 nm for EP and PE, respectively. At the same time spectra of EP and PE in the mixture overlap significantly, rendering the simultaneous determination of the two drugs using zero order spectra quite difficult (Fig.3). The different analytical parameters used for determination of EP and PE are summarized in Table (1).

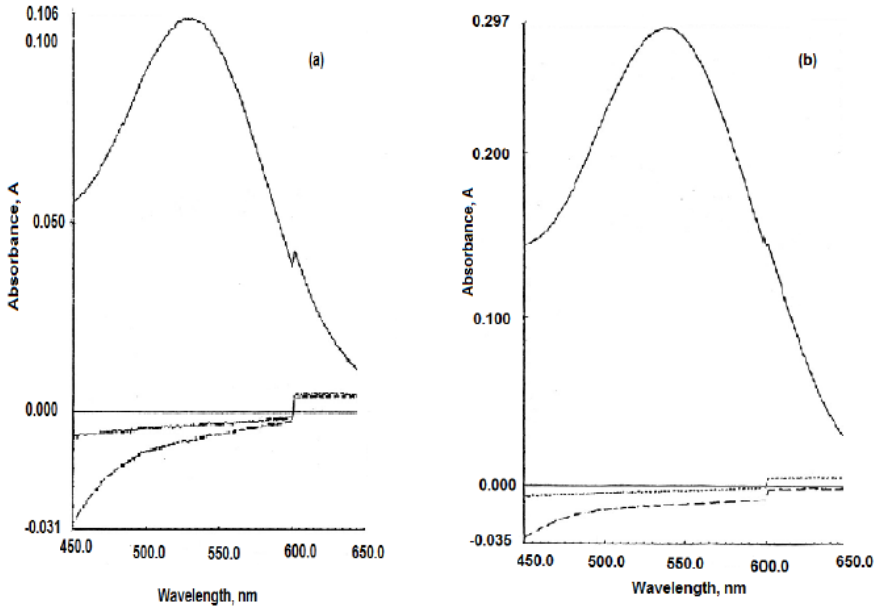

Fig. 2. The Zero-order absorption spectra of (a) 10 ppm of EP (curve —) , absorption spectra of AHMT without analyte (curve ----) and absorption spectra of analyte without KIO_4 (curve …..), (b) 10 ppm of PE (curve —), absorption spectra of AHMT without analyte (curve ----) and absorption spectra of analyte without KIO_4 (curve ……).

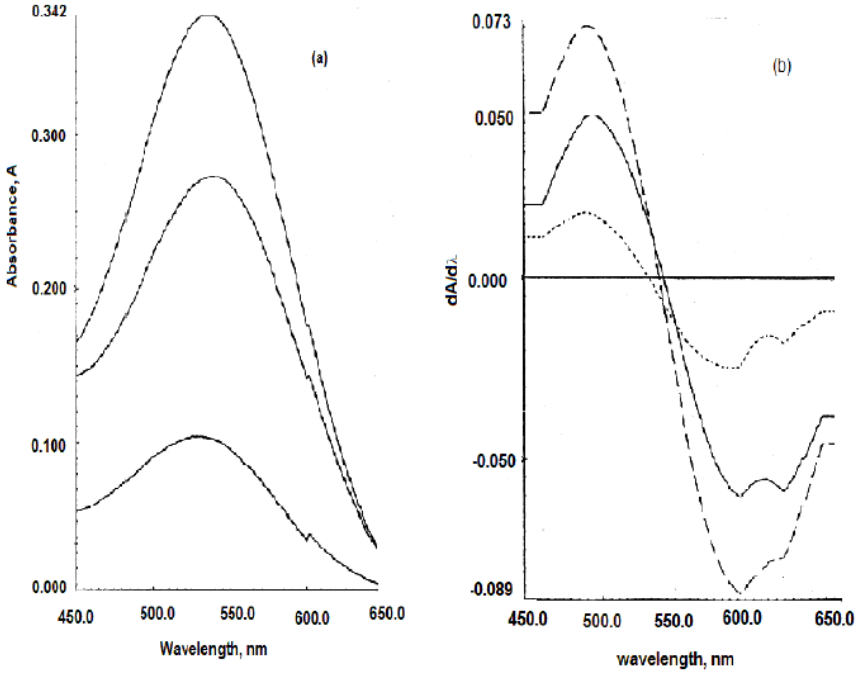

Fig. 3. The zero-order absorption spectra of (a) 10 μg/ml of EP, 10 μg/ml of PE , and mixture from down to up , respectively. (b) First order spectra of 10 μg/ml EP (Curve ….), 10 μg/ml PE (curve —), and mixture of both (curve ------).

Table 1. Analytical parameters of the proposed method.

Parameter	Ephedrine(EP)	Phenylephrine(PE)
Linear range(μg/ml)	5 - 40	2 – 50
LOQ, (μg/ml)	5.0	2.0
LOD, (μg/ml)	1.5	0.5
Molar absorpitivity, l mol^{-1} cm^{-1}	0.2×10^4	0.6×10^4
Correlation coefficient	0.9996	0.9998
Sandal sensitivity μg/Cm^2	0.098	0.0276

Derivative spectra

The first order spectra of EP and PE showed a zero-crossing point at 526 and 545 nm respectively, as shown in Fig. 3 (a and b) the zero-order spectra of EP and PE overlap significantly. Hence the simultaneous determination of that compound is difficult using the zero-order spectra. Therefore the first order derivative spectra satisfactorily resolved those overlaps.

In the first order spectra of the two series of solutions, one contains increasing amount of EP (10, 20, 30, and 40 μg/ml) and fixed amount PE (10 μg/ml) and the other contains increasing amount of PE (5, 10, 20, 30 and 40 μg/ml) and fixed amount of EP (20 μg/ml) were prepared. Each set contains 2 ml of 4×10^{-4} M KIO_4, 0.25 of 5M KOH, and 2 of 0.5% AHMT solutions respectively. The flask is stopped and allowed to stand for 20 min, before adding 1.25 ml of 1.5% potassium periodate solution and vigorously shaking the flask by hand until the iodine color in the mixed solution disappears. The first derivative spectra of the first series of solutions exhibit isodeferential point at 526 nm as shown in Fig. 4 (a and b). The height of peak (h_1) 526 nm (zero crossing of EP) varies with the concentration of PE. Similarly, for the second series of solutions isodeferential point was exhibited at 545 nm (zero crossing of PE), the concentration of EP varies with the trough (h_2) at 545 nm as shown in Fig. 4 (b). The concentration of EP and PE in their

binary mixture solutions were determined by measuring the distances (h_1) and (h_2) from the base line to the peak at 526 and from base line to the trough at 545 nm, in the first order derivative spectra. The concentrations were calculated by comparing the obtained values with the pre-constructed calibration curves for EP and PE respectively.

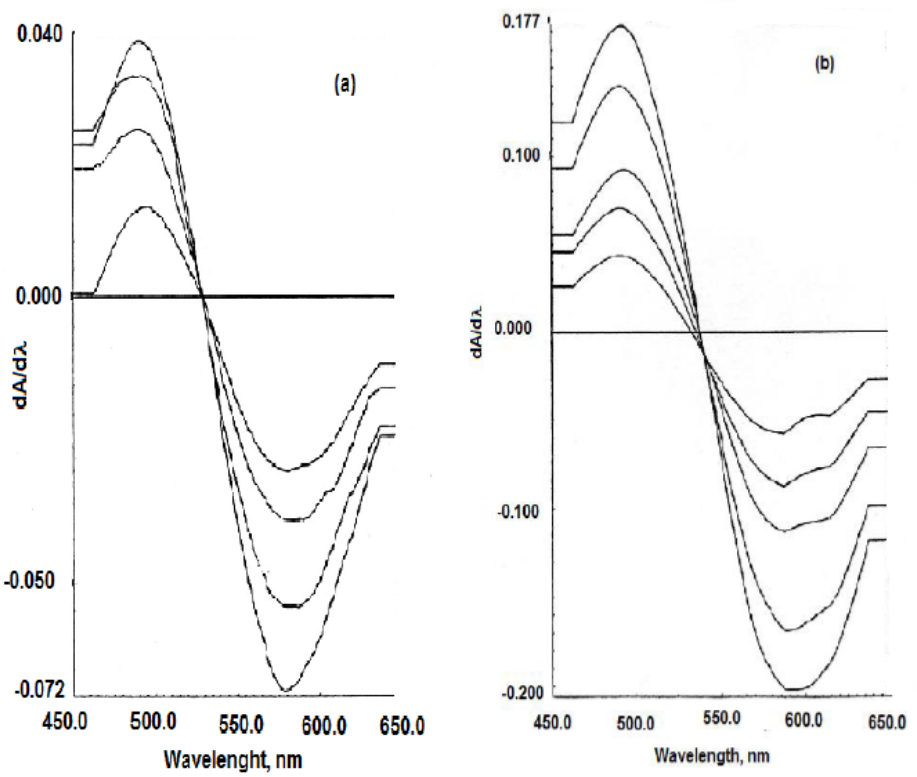

Fig. 4. The first order spectra of two sets of solutions. (a)Contains: 10 μg/ml of PE and different concentration of EP (10, 20, 30, and 40 μg/ml) (b)Contains: 20 μg/ml of EP and different concentration of PE (5, 10, 20, 30, and 40 μg/ml).

Effect of KIO_4 concentration

In order to find the most suitable concentration of KIO_4 for oxidized EP and PE, respectively to ketones, several experiments were conducted using 20 ppm EP and PE using 0.25 ml of 5 M KOH, 2 ml of 5% AHMT, and 1.25 ml of 1.5% KIO_4 respectively. Different concentrations of KIO_4 ranging from 4×10^{-6} - 6.4×10^{-5} M were used. The maximum absorbance of EP and PE was found to reach its optimum value at 2 ml of 4×10^{-4}M (3.2×10^{-5}M) in both instances. However, the absorption decreases at higher and lower concentrations of (2ml) 4×10^{-4}M. Therefore, for assured functioning concentration of KIO_4 reagent was fixed at 2 ml of 4×10^{-4} M throughout.

Effect of AHMT concentration

The maximum absorbance of 20 μg/ml EP and PE using, 0.25 ml of 5M KOH, 2 ml of 4×10^{-4} KIO_4, was tested as function of AHMT concentration. It was observed that the maximum absorbance for EP and PE was at 2 ml of 0.5% AHMT, respectively. Therefore 2ml of 0.5% AHMT was fixed throughout all the measurements for the determination of EP and PE.

Table 2. Determination and recovery of EP and PE using the proposed method.

Analyte	Added, (μg/ml)	Found (μg/ml)	Recover, %	RSD[a], %
EP	5	4.86	97.2	1.50
	10	9.8	98.0	1.40
	20	19.7	98.5	1.20
	40	38.9	97.25	1.15
PE	5	4.9	98.0	1.15
	10	9.8	98.0	0.90
	20	30.0	100	0.72
	40	50.75	101.5	0.63

[a] Average of five replicates

Table 3. Determination and recovery of binary mixture of EP and PE using the proposed method.

Added, (μg/ml)		Found, (μg/ml)		Recover, %		RSD[a], %	
EP	(PE)	EP	(PE)	EP	(PE)	EP	(PE)
10	(5)	9.6	(4.9)	96.0	(98.0)	0.83	(0.94)
20	(40)	19.5	(39.5)	96.0	(98.7)	0.93	(0.70)
40	(10)	39.0	(9.6)	97.5	(96.5)	0.63	(0.90)

[a] Average of five replicates

Table 4. Determination of EP and PE in some pharmaceutical preparations and their synthetic mixture using the proposed method.

Preparation	Certified value	Proposed method		British pharmacopoeia method	
		R %*	RSD, %	R %*	**RSD, %**
EP ampoule	30mg/ml	98.0	1.3	98.0	1.4
Vibocil (nasal drop)	1.2 mg/ml	97.5	1.30	97.0	1.4
PE 10% (eye drop)	10%	98.0	1.43	98.0	1.4
Mixture	(1:1)(2:1) (1:2) (EP: PE)	(96.75: 97.5)	(0.79:0.84)	(97.5/98.0)	1.4/1.3

[*] Average of five replicate analysis

Effect of KOH concentration

Since the color development is alkaline dependent, the variation of KOH concentration was investigated. It was found that the maximum absorbance were obtained, at 0.25ml of 5M KOH of EP and PE, respectively at the same reaction condition stated in the previous effect.

Effect of interferences

The influence of some selected cations e.g. Na^{+}, K^{+}, Fe^{2+}, Mg^{2+}, Ca^{2+}, Co^{2+}, Ni^{2+}, Al^{3+}, and some anions e.g. Carbonate, sulphate, acetate, nitrate and chloride present in 50 fold did not interfere except that of Cu^{2+}. The effect of several organic substances that commonly coexist with EP and PE were investigated. Antipyrine, polyvinylchloride, dimetindenum malice that is commonly present as excipients in perfrin eye drop were did not interfere in the concentration range present in eye preparation.

Precision and Accuracy of the method

The precision and the accuracy of the method was investigated by inter-day (repeatability) of the analysis of EP or PE or mixture of EP and PE, in five replicates. at the limit of quantification range. The precision and the accuracy of the method are expressed as RSD and % of deviation of the measured concentration. Also reproducibility (Day to Day or intraday) was investigated. The results obtained (Table 2 and 3) are within the acceptance range of less than 2.0 % (precision) and 2.3% (accuracy).

Ruggedness

The ruggedness of the spectrophotometric method was evaluated by carrying out the analysis using two different analyst (operator) and different instruments on different days. The RSD of less than 2.0% were observed for repetitive measurements in three different day time periods using two different instruments and two operators. The results of the ruggedness indicated that the method is capable of producing results with high precision.

Robustness

The robustness of the method is demonstrated by the versatility of the experimental factors that is affecting the spectrophotometric determination. Preliminary inspection of the results under various conditions suggest that the method is fairly robust, at the concentration range for each reagent, and the calibration graphs were applied at the optimum condition for each analyte.

Calibration graphs

The calibration graph for Ephedrine was found to be linear over the range of 5- 40 μg/ml, with lower limit of detection 1.5 μg/ml. The calibration graph for phenylepherine was found to be linear over the range of 2-50 μg/ml with a detection limit of 0.5μg/ml. The calibration graphs furnished molar absorptivities of 0.2×10^4 and 0.6×10^4 l. mol^{-1}. Cm^{-1} for EP and PE, respectively. The Sandell's sensitivity was calculated to be 0.098 and 0.0276 μg/ml in the same order.

To investigate the versatility of the method for the simultaneous determinations, two series of experiments were done, the first set was five test solutions containing different known amount of EP and fixed amount of PE, and the second set was five test solutions containing different known amount PE and fixed amount of EP. Both set were analyzed by the proposed method. Epherine and PE can be determined simultaneously by first order derivative by measuring the distance (h_1) and (h_2) for EP and PE at isodifferential point of 545 and 526 nm. The calibration graphs in binary mixtures were found to be linear in the range 10 - 40 and 4 - 50 μg/ml for EP and PE respectively, with 3 σ (Lower detection limit) of 2.5 and 1.0μg/ml in the same order. Moreover, the high value of correlation coefficient (0.9996 and 0.9998 for EP and PE, respectively) and intercept on Y-axis (~zero) indicate the good linearity of all calibration graphs and the conformity to Beer's law of the first-derivative measurements. The precision of the method is determined for five samples containing 10μg/ml of each, the relative standard deviation is 1.3 and 1.15% for each EP and PE, respectively. The results are shown in Fig. 3 and Table 3, and suggest that a satisfactory resolution of two overlapping spectra is obtained by the first-derivative method.

Applications

In order to investigate the applicability of the proposed method, EP and PE solutions at the concentration ranges of 5- 40 and 2- 50 μg/ml, respectively, and their binary mixtures were

prepared and analyzed by the proposed method. The absorbance recorded in these solutions was compared with the calibration graphs. The results obtained (Table 2 and 3) for each sample in five replicates, showed an average recovery of 98.5 and 98.0% and relative standard deviation of 1.2, and 1.15% at concentration of 20 μg/ml for EP and PE, respectively.

The results in Table 4 show an average recovery of 98% of the nominal values and a mean standard deviation of 1.3% for EP in ampoules. Phenylepherine was determined in some pharmaceutical preparations e.g. Vibrocil (nasal drops) and phenylephrine 10% solution by transfer of an equivalent amount of sample to 100 ml measuring flask, homogenized with water by shaking several times by hand and diluted with doubly distilled water to the mark. A portion of the sample was transferred into 25 ml measuring flask, and the reagent was added according to the recommended procedure. The absorbance was then measured and compared with a pre-constructed calibration graph. Results were obtained for the analysis of EP and PE in its formulation or in synthetic mixtures ((1: 1), (2:1), (1:2)) using both the proposed spectrophotometric and the standard British pharmacopeia method [19]. The recovery was 96.75 and 97.5% and relative standard deviation of 0.79 and 0.84% for PE and EP, respectively. Results are given in Table 4. These data suggest the proposed method can be carried out on real products with equal confidence and accuracy compared with official method.

Statistical analysis

Comparison of the experimental means for the two methods (developed and official methods) was carried out using the null hypothesis of $|t|_2$ for p=0.05and n = 5. It was found that $|t|_2$ =, 2.9 and 3.0 for PE and EP, respectively which is less than the tabulated value ($|t|_2$ =3.36)[20]. No significant difference was found between the two methods, which indicated that the proposed method is as accurate as the official method. Comparison of the precision of the proposed method with the official method to estimate the random errors of the two sets of data was also carried out using the two-tailed F-test [19]. Based on this results it is clear that the experimental $F_{4,4}$ values is 4.4, and 5.1 , for PE and EP, respectively . These values are obviously less than the tabulated value of $F_{4,4}$ for p=0.05 and n=5 (6.38) [21]. This proved that the results obtained by the two methods are not subject to random errors.

4. Conclusion

The individual method has been developed for the determination of EP and PE by measuring the absorbance of the reaction product at 526 and 545 nm, respectively. The simultaneous determination of EP and PE has been achieved using first-order derivative spectrophotometric method by measuring the absorbance at isodeferential points (545 and 526nm for PE and EP, respectively). The method has been successfully applied for the determination of EP and PE in nasal and eye drops and their synthetic mixtures.

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