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Determination of astemizole in pharmaceutical preparations using spectrophotometric methods

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Abstract

UV absorption and second derivative spectrophotometric methods were developed for the determination of astemizole in commercial pharmaceutical formulations containing this compound alone. Solutions of astemizole in 0.1 M HCl:methanol (1:3) were used in the methods and the linearity range was 4.6–45.8 μg ml^{-1} in both methods. The mean recoveries and relative standard deviations were calculated and the method was applied to two commercial preparations marketed in Turkey. Results were compared with the literature method, HPLC. Also, two new spectrophotometric methods are described for the simultaneous determination of astemizole and pseudoephedrine hydrochloride in their combination. In the first method, first derivative spectrophotometry, $dA/d\lambda$ values were read at selected wavelengths in zero-crossing points in the first derivative spectra of the mixture solution in 0.1 M HCl:methanol (1:3). In the second, ratio spectra derivative spectrophotometry, analytical signals were measured at the wavelengths corresponding to either maximums and minimums for both drugs in their solution in 0.1 M HCl:methanol (1:3) in the first derivative spectra of their ratio spectra. The procedures do not require any separation step. The mean recoveries were found satisfactory in the methods.

Keywords: Astemizole; Pseudoephedrine hydrochloride; Derivative spectrophotometry; Ratio spectra derivative spectrophotometry; Pharmaceutical preparation

1. Introduction

Astemizole (A) is frequently prescribed in medicine as an anthistaminic drug as alone or its combination with pseudoephedrine hydrochloride (PE) Various methods including spectrophotometry [1–12] and HPLC [13–16] have been used for the determination of A and PE in pharmaceutical preparations containing these drugs alone or in combination with other active ingredients.

We couldn't find any work about PE + A mixture in the literatures.

Salinas et al. [17], developed a new method for analysis of mixtures with overlapped spectra. Salinas's method is based on the use of the first derivative of the ratio spectra. In this method, the concentrations of active compounds were deter-

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mined by measuring the amplitudes of the minimum or maximum at points corresponding to the selected wavelengths. Berzas Nevado et al. [18–21] and Onur et al. [22–25] applied the same method to determine the active compounds in different mixtures.

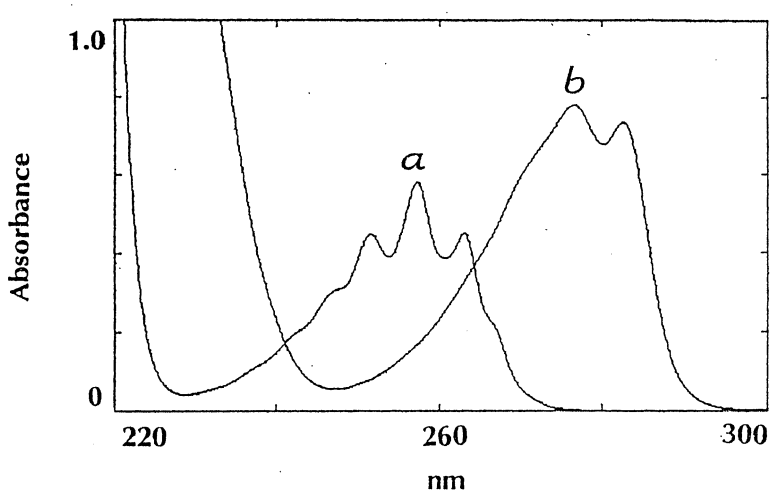

Fig. 1. Zero-order absorption spectra of: (a) 404 μg ml^{-1} solution of pseudoephedrine hydrochloride; (b) 22.9 μg ml^{-1} solution of Astemizole in 0.1 M HCl:methanol (1:3).

Table 1
Recovery results for PE and A in synthetic mixtures by UV absorption Spectrophotometry, first and second derivative spectrophotometry

Mixture no	PE		A						
	Added	recovery (%)	Added	recovery (%)					
	μg	247.2 nm $^{1}D^{a}$	μg	277.1 UV^{c}	283.2 UV^{c}	278.7 $^{1}D^{a}$	285.7 $^{1}D^{a}$	277.1 $^{2}D^{b}$	283.8 nm $^{2}D^{b}$
1	606	96.8	4.6	101.6	101.6	100.5	97.2	98.2	99.2
2	606	98.2	11.5	100.8	100.4	101.5	98.9	98.9	98.9
3	606	98.2	22.9	98.7	99.7	98.3	97.3	98.3	98.6
4	606	100.3	25.2	102.8	102.8	98.9	96.3	96.3	97.3
5	606	96.2	34.4	102.8	101.8	101.9	103.8	101.8	102.8
6	606	98.9	45.9	100.0	100.0	99.6	100.9	100.9	100.6
7	202	98.9	11.5	98.8	99.8	101.5	98.9	97.9	98.9
8	606	96.8	11.5	101.5	100.5	97.4	98.9	98.9	99.9
9	1010	101.9	11.5	100.8	100.8	97.8	100.5	100.1	100.2
10	1212	98.2	11.5	101.2	101.2	98.9	100.9	101.2	100.6
11	1414	98.3	11.5	98.0	98.0	101.5	100.8	100.8	100.8
12	1818	100.4	11.5	99.6	99.6	101.5	98.9	99.5	99.4
$n = 12$		$\bar{x} = 98.6$		100.6	100.5	99.9	99.4	99.4	99.8
		RSD = 1.67		1.55	1.24	1.65	2.07	1.61	1.39

[a] ^{1}D, derivative spectrophotometry.
[b] ^{2}D, second derivative spectrophotometry.
[c] UV, UV absorption spectrophotometry.

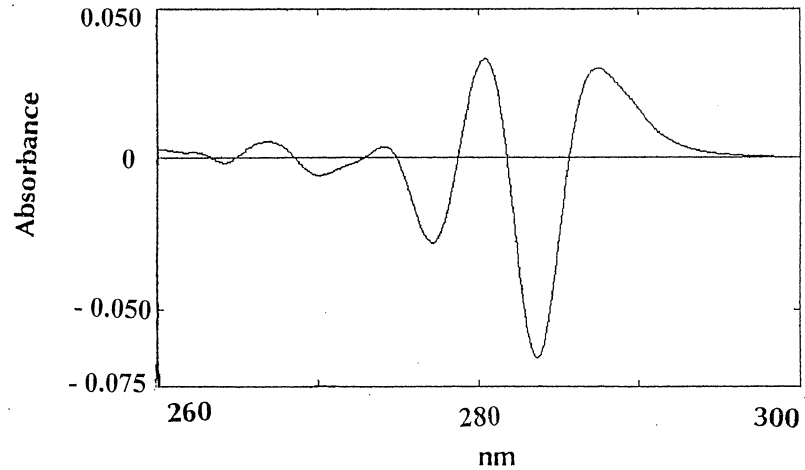

Fig. 2. Second derivative spectra of 20.6 μg ml^{-1} solution of Astemizole in in 0.1 M HCl:methanol (1:3).

In this study; UV absorption and second derivative spectrophotometry are proposed for the determination of A for its presence alone and, first derivative and ratio spectra derivative spectrophotometry are proposed for the simultaneous determination of A and PE in their mixtures.

2. Experimental

2.1. Apparatus

Shimadzu 1601 PC double beam spectrophotometer with a fixed slith width (2 nm) connected to a computer loaded with Shimadzu UVPC software was used for all the spectrophotometric measurements and treatment of data.

Zero-order absorption spectra were traced in 1 cm quartz cells over the ranges 250.0–300.0 nm.

First derivative curves of the zero-order spectra of references and test solutions were recorded in 1 cm quartz cells over the ranges 220.0–300.0 nm ($\Delta\lambda = 2$ nm). The ordinate maximum and minimum settings were (+ 1.0) and (− 2.0) for PE in PE + A mixture (scaling factor = 10).

Second derivative curves of the zero-order spectra of references and test solutions were recorded in 1 cm quartz cells over the ranges 250–300 nm.

In ratio spectra derivative spectrophotometry, range was selected as 220.0–300.0 nm ($\Delta\lambda = 2$ nm) for reading the analytical signals after smoothing with $\Delta\lambda = 2$ nm. The ordinate maximum and minimum settings were (+ 75)–(− 50) for PE in 220.0–280.0 nm range and (+ 400)–(− 500) in 220.0–300.0 nm range for A in their mixture.

2.2. Reagent and solutions

Pseudoephedrine hydrochloride and Astemizole were kindly donated by Eczacibaşi Pharm. Ind. Turkey and used without further purification.

All the solvents used in spectrophotometric analysis were of analytical reagent grade.

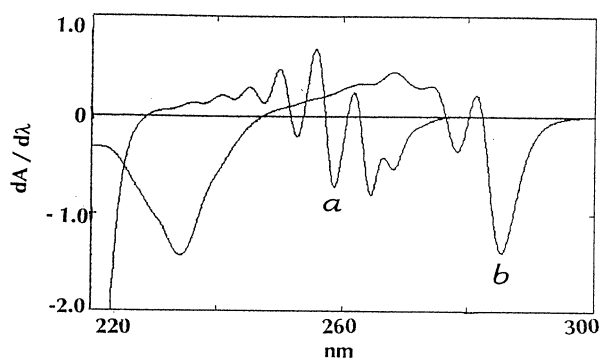

Fig. 3. First derivative spectra of: (a) 404 μg ml^{-1} solution of pseudoephedrine hydrochloride; (b) 22.9 μg ml^{-1} solution of Astemizole in 0.1 M HCl:methanol (1:3) ($\Delta\lambda = 2$ nm, scaling factor = 10).

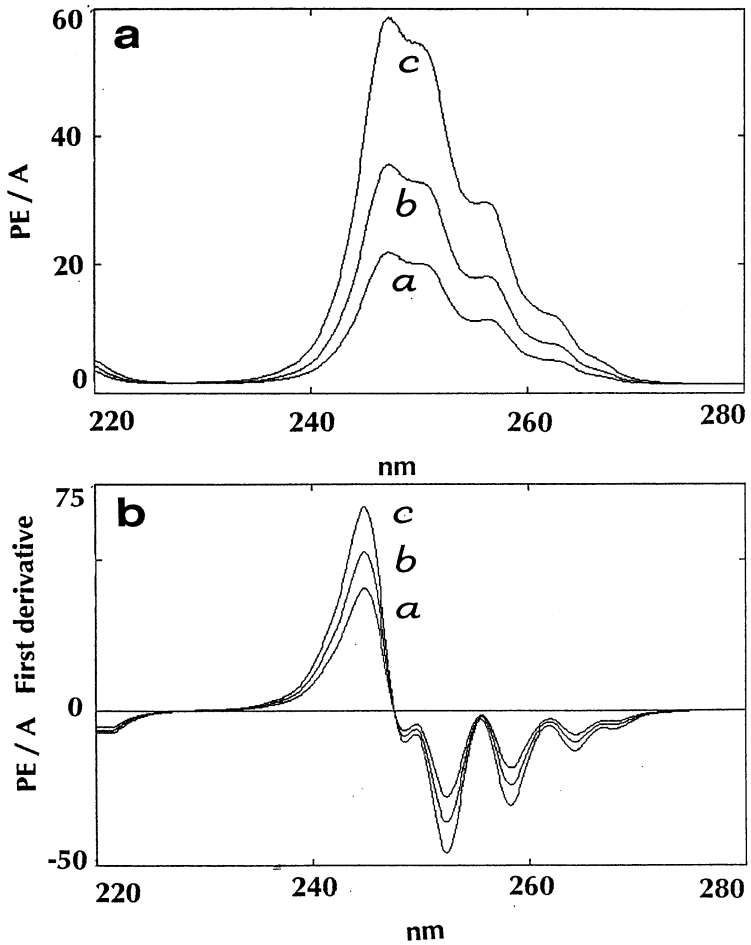

Fig. 4. Ratio spectra (a) and first derivative of the ratio spectra (b) of pseudoephedrine hydrochloride of: (a) 404 μg ml^{-1}; (b) 808 μg ml^{-1}; and (c) 1010 μg ml^{-1} solution in 0.1 M HCl: methanol (1:3) when 4.6 μg ml^{-1} solution of Astemizole in 0.1 M HCl:methanol (1:3) used as divisor ($\Delta\lambda = 2$ nm).

Solutions of 50 mg 100 ml^{-1} astemizole and 20 mg 100 ml^{-1} of pseudoephedrine hydrochloride were prepared, respectively, in 0.1 M HCl:methanol (1:3).

2.3. Procedure

Twenty tablets were accurately weighed and powdered in a mortar. An amount of the tablet mass equivalent to one tablet content was dissolved in 60 ml of 0.1 M HCl:methanol (1:3) mixture. After 30 min of mechanically shaking the solution was filtered in a 100 ml volumetric flask. The residue was washed three times with 10 ml solvent then the volume was completed to 100 ml with the same solvent (solution 1). Solution 1 was diluted 1:4 with the same solvent. All the spectrophotometric methods were applied to the latest diluted solution.

2.4. Commercial pharmaceutical preparations

Almizol® (10 mg astemizol/tablet) Nobel Pharm. Ind (batch no: 8D001) and Hismanal® (10 mg astemizol/tablet) Janssen-Cilag Pharm.Ind. (batch no: 905378) were assayed.

3. Results and discussion

3.1. For astemizole alone

3.1.1. UV spectrophotometry

UV absorption spectra of the solution of A in 0.1 M HCl:methanol (1:3) have two maxima, 277.1 and 283.2 nm. The determination of A can simply be made by reading absorbances at these wavelengths in its solution in 0.1 M HCl:methanol (1:3) (Fig. 1). In this procedure, mean recovery was found as 100.6% with the RSD (Relative Standard Deviation) = 1.55% at 277.1 nm and 100.5% with the RSD = 1.24% at 283.2 nm for A in synthetic mixtures (Table 1). Linearity range was 4.6–45.8 μg ml^{-1} for both wavelengths. The regression equations and correlation coefficients were $y = 3.2 \times 10^{-2}\ x - 4.0 \times 10^{-3}$ and $y = 3.0 \times 10^{-2}\ x - 3.0 \times 10^{-3}$ (where x is the concentration in μg ml^{-1}, y is the absorbance) and, 0.9994 and 0.9993 at 277.1 and 283.2 nm, respectively for A in the method (Table 3). This method was successfully applied to the pharmaceutical preparations by using absorbances read at 283.2 nm due to its lower RSD value and the results were illustrated in Table 4.

3.1.2. Second derivative spectrophotometry

In second derivative spectra (^{2}D) of the solution of A in 0.1 M HCl:methanol (1:3) in the range 250–300 nm there exist two minima corresponding to the maxima in zero-order spectra at 277.1 and 283.8 nm ($\Delta\lambda = 2$ nm) (Fig. 2). A linear relationship was observed between the $d^2A/d\lambda^2$ values and the concentration of A in the range 4.6–45.8 μg ml^{-1} for both wavelengths. So, A can be determined by measuring $d^2A/d\lambda^2$ values at these wavelengths. Mean recoveries, relative standard deviations, regression equations and correlation coefficients in the method were shown in Table 1 and Table 3.

This method was successfully applied to the pharmaceutical preparations selected and the results were illustrated in Table 4.

Table 2
Recovery results for PE and A in synthetic mixtures by ratio spectra first derivative spectrophotometry

Mixture no	PE			A					
	Added	recovery (%)		Added	recovery (%)				
	μg	244.8	258.4	μg	224.7	233.7	259.4	265.0	269.8 nm
1	202	100.0	99.2	11.5	99.0	103.4	100.8	100.0	102.9
2	404	99.8	100.6	11.5	99.0	101.1	100.8	100.7	102.9
3	606	101.3	99.1	11.5	97.6	101.0	100.4	101.0	102.4
4	808	100.0	102.6	11.5	99.0	100.3	101.2	101.0	102.9
5	1010	99.4	99.4	11.5	99.9	101.1	101.0	100.0	102.4
6	1212	101.0	99.9	11.5	99.0	103.4	101.0	100.4	99.1
7	1414	100.0	100.3	11.5	99.0	103.4	102.0	100.0	102.9
8	1616	99.5	101.7	11.5	99.2	101.1	100.8	101.9	100.0
9	1818	100.1	101.4	11.5	99.0	103.4	97.9	100.7	99.1
10	606	101.0	101.4	4.6	101.3	98.1	99.0	100.0	98.1
11	606	101.0	99.9	11.5	99.0	103.4	102.0	100.7	102.9
12	606	101.1	101.0	16.0	99.9	99.1	101.2	100.0	95.0
13	606	100.0	99.1	22.9	100.3	99.6	100.4	101.4	98.7
14	606	100.5	99.1	27.5	100.7	101.1	101.0	100.5	102.5
15	606	101.0	99.1	32.1	97.6	99.0	99.1	99.1	102.4
16	606	101.3	99.1	36.6	102.4	98.0	99.5	99.3	100.2
17	606	100.3	99.4	41.2	99.2	101.4	99.7	100.5	99.0
18	606	101.0	99.1	45.8	99.9	100.3	101.2	101.9	99.1
n = 18	$\bar{x}$	100.4	100.4		99.5	101.0	100.5	100.6	100.7
	RSD =	0.64	1.78		1.17	1.81	1.07	0.82	2.29

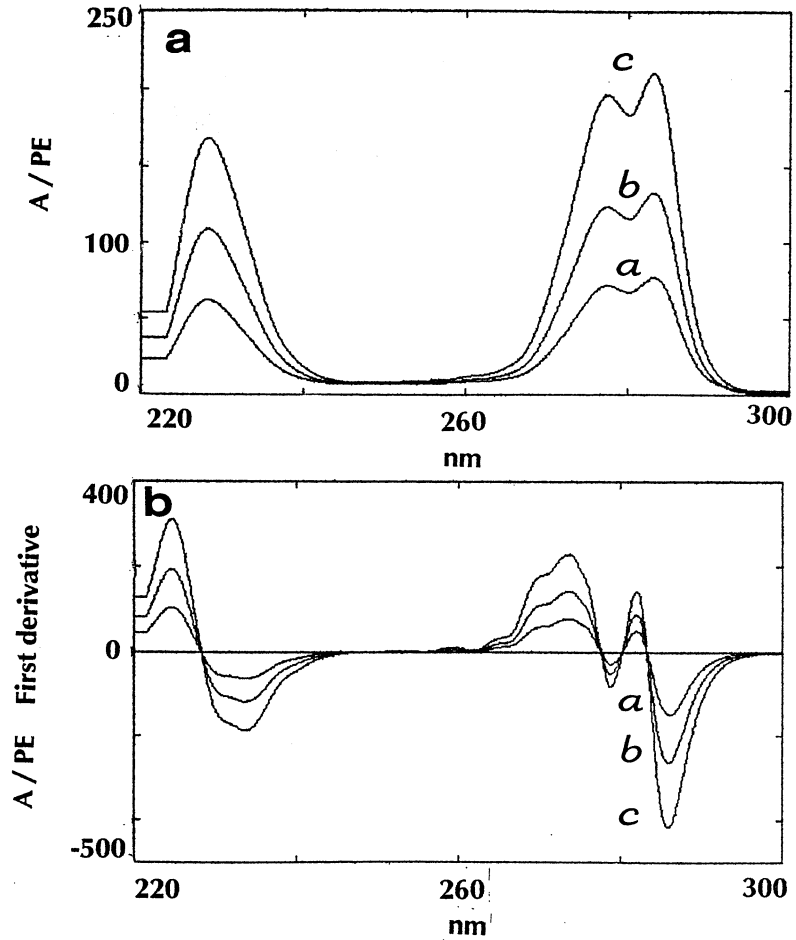

Fig. 5. Ratio spectra (a) and first derivative of the ratio spectra (b) of Astemizole of: (a) 9.2 μg ml^{-1}; (b) 20.6 μg ml^{-1}; and (c) 36.6 μg ml^{-1} solution in 0.1 M HCl: methanol (1:3) when 202 μg ml^{-1} solution of pseudoephedrine hydrochloride in 0.1 M HCl:methanol (1:3) used as divisor ($\Delta\lambda = 2$ nm).

We selected 283.8 nm for the assay of the pharmaceutical preparations by its lower RSD value in its recovery studies (Table 1).

Comparison of the zero- and second derivative spectra of A in standard and drug formulation solutions showed that the wavelenght of maximum absorbances did not changed. Also, standard addition technique was applied to one brand of preparations analysed by the calibration curve. The slopes of the standard calibration and standard addition curves of the methodes were found identical at the wavelenght selected in the methods. Therefore, It has been decided that excipients (Lactose, starch, avicel, povidon, sodium dodecylsulfate, aerosil, magnesium stearate in Almizol® and lactose, starch, avicel, povidon, sodium lauryl sulfate, magnesium stearate in Hismanal®) did not interfere the quantitation of A in those methods.

Summary of the assay results for commercial preparations were shown in Table 4. The results of two spectrophotometric methods and also HPLC method cited in the literature no.16 (we used this method as a reference method) for the

Table 3
Beer's law data and statistical analysis for the calibration graphs of PE and A using UV absorption spectrophotometry, derivative and ratio derivative spectrophotometric procedures[a]

Compounds	Method	λ (nm)	Regression equations		R	Concentration range (μg ml^{-1}))
			a (SE)	b (SE)		
PE	^{1}D	247.2	2.44×10^{-4} (1.25×10^{-5})	3.25×10^{-3} (2.25×10^{-3})	0.9991	202–1818
PE	^{1}DD	244.8	6.00×10^{-2} (2.29×10^{-3})	4.32×10^{-3} (8.05×10^{-3})	0.9999	202–1818
PE	^{1}DD	252.5	7.70×10^{-2} (6.65×10^{-2})	4.50×10^{-2} (4.55×10^{-3})	0.9995	202–1818
PE	^{1}DD	258.4	−8.166 (1.78)	2.50×10^{-2} (9.00×10^{-3})	0.9997	202–1818
A	UV	277.1	3.20×10^{-2} (7.66×10^{-2})	-4.00×10^{-3} (3.06×10^{-3})	0.9999	4.6–45.8
A	UV	283.2	3.00×10^{-2} (0.95×10^{-4})	-3.00×10^{-3} (1.01×10^{-4})	0.9998	4.6–45.8
A	^{1}D	278.7	-1.86×10^{-2} (7.25×10^{-3})	-6.88×10^{-3} (1.25×10^{-4})	0.9993	4.6–45.8
A	^{1}D	285.7	-6.00×10^{-2} (4.29×10^{-3})	-1.50×10^{-3} (4.22×10^{-4})	0.9991	4.6–45.8
A	^{2}D	277.1	1.26×10^{-3} (7.20×10^{-3})	1.55×10^{-4} (1.56×10^{-3})	0.9996	4.6–45.8
A	^{2}D	283.8	3.10×10^{-3} (1.25×10^{-2})	1.99×10^{-4} (1.38×10^{-5})	0.9996	4.6–45.8
A	^{1}DD	224.7	7.56 (1.01)	6.09 (3.03)	0.9995	4.6–45.8
A	^{1}DD	233.7	−5.13 (2.78)	1.13 (0.91)	0.9995	4.6–45.8
A	^{1}DD	259.4	3.9×10^{-1} (1.9×10^{-1})	$-2.4\ 10^{-1}$ (1.0×10^{-1})	0.9998	4.6–45.8
A	^{1}DD	265.0	8.9×10^{-1} (8.0×10^{-2})	$1.2\ 10^{-1}$ (2.0×10^{-2})	0.9995	4.6–45.8
A	^{1}DD	269.8	4.71 (2.78)	3.36 (1.56)	0.9992	4.6–45.8
A	^{1}DD	273.6	43.78 (11.45)	5.07 (2.08)	0.9991	4.6–45.8

[a] ^{1}D, derivative spectrophotometry; ^{1}DD, ratio spectra derivative spectrophotometry; UV, UV absorption spectrophotometry; a, slope; b, intercept; r, correlation coefficient; SE, standard error.

Table 4
Assay results of commercial preparations of Astemizol marketed in Turkey (mg/tablet)

Methods	Almizol®	(Label claim = 10 mg/tablet)	Hismanal®	(Label claim = 10 mg/tablet)
	Mean ± SD[a]	t values calculated ($P = 0.05$)	Mean ± SD[b]	t values calculated ($P = 0.05$)
Zero-order UV abs.spectr.	10.1 ± 1.3	UV − ^{2}D = 0.56	10.0 ± 1.2	UV − ^{2}D = 0.86
^{2}D	10.0 ± 0.9	UV − HPLC = 1.06	10.1 ± 0.9	UV − HPLC = 1.26
HPLC	10.0 ± 1.0	^{2}D − HPLC = 1.16	10.0 ± 1.1	^{2}D − HPLC = 1.08

[a] Mean of ten determination.
[b] Theoretical value for t at P: 0.05 level = 2.26. SD, standard deviation; abs. Spectr., absorption spectroscopy; ^{2}D, second derivative spectrophotometry.

Table 5
Assay results of synthetically prepared commercial preparation (240 mg PE + 10 mg A/tablet) (mg)

Methods	PE		A	
	Mean ± SD[a]	t values calculated ($P = 0.05$)	Mean ± SD[b]	t values calculated ($P = 0.05$)
Zero-order UV abs.spectr.			9.9 ± 0.2	UV − ^{1}D = 1.15
^{1}D	239.6 ± 0.7	^{1}D − ^{1}DD = 1.02	9.8 ± 0.4	UV − ^{1}DD = 0.89
^{2}D			9.9 ± 0.3	UV − ^{2}D = 0.66
^{1}DD	239.9 ± 0.2		10.0 ± 0.2	^{1}D − ^{1}DD = 1.56
				^{2}D − ^{1}DD = 0.76

[a] Mean of ten determination.
[b] Theoretical value for t at P: 0.05 level = 2.26; SD, standard deviation; abs. Spectr., absorption spectroscopy; ^{1}D, first derivative spectrophotometry; ^{1}DD, ratio spectra first derivative spectrophotometry.

same commercial formulation were compared by Student's t-test. The calculated (experimental) t-values did not exceed the tabulated (theoretical) values in the test, indicating that there was no significant difference between the methods compared (Table 4).

We observed that standard solution of A in 0.1 M HCl:methanol (1:3) was stable for 8 days. These two spectrophotometric methods can be used as a stability indicating procedure.

The limit of quantification (LOQ) for A was established by analysing ten different standard solutions containing the lowest concentration on the standard curves (4.6 μg ml^{-1} for both spectrophotometric methods). Relative standard deviations were 1.15% at 283.2 nm in zero-order UV spectrophotometry and 1.01% at 283.8 nm in second derivative spectrophotometry. The limit of detection (LOD) was considered as the concentration of A giving a signal-to-noise ratio greater than 3:1 and the signal-to-noise ratios were found to be 3.48 and 3.65 in zero-order and second derivative spectrophotometric procedures respectively in 4.6 μg ml^{-1} A solution.

3.2. In astemizole + pseudoephedrine hydrochloride mixture

3.2.1. UV absorption and second derivative spectrophotometry

The determination of A can simply be made by reading absorbances at 283.2 nm in zero-order UV spectra and by measuring $d^2A/d\lambda^2$ values in second derivative spectra of its solution in 0.1 M HCl:methanol (1:3) (Fig. 1 and Fig. 3) without interference of PE. In these procedures, same results were obtained as A exists alone as mentioned in Section 3.1.1. But the quantitation of PE by using UV spectrophotometry is impossible due to the overlapping spectra (Fig. 1).

3.2.2. First derivative spectrophotometry

In the first derivative spectra (^{1}D) traced with $\Delta\lambda = 2$ nm of the solution of these compounds in 0.1 M HCl:methanol (1:3) (scaling factor = 10) (Fig. 3), there exist five zero-crossing points; at 251.7, 253.7, 257.2, 260.8 and 262.7 nm for PE and, at 247.2 nm for A giving opportunity for their determination by reading $dA/d\lambda$ values at these wavelengths without interference from each other. In the method, the mean recoveries and relative standard deviations found for synthetic mixtures prepared in our laboratory are illustrated in Table 1 for PE at 247.2 nm. Also, Beer's law compliance for both compounds, the regression equations and correlation coefficients were shown in Table 3. But, although the determination of A seems possible in PE + A mixtures at the five wavelengths for PE mentioned above, no linear relationship was observed between the concentrations and $dA/d\lambda$ values in the mixtures.

By using same first derivative spectra (Fig. 3) in 220.0–300.0 nm range, the determination of A is also possible in the mixture by measuring of $dA/d\lambda$ values at 278.7 and 285.7 nm as free from interference from PE by the fact that PE has no derivative spectra in 220.0–300.0 nm interval.

Calibration graph was established at these wavelengths and used for the assay of synthetic formulation. Straight line was observed in the concentration range 4.6–45.8 μg ml^{-1} for A. Regression equations and relative standard deviations were found as follows:

$$y = -1.86 \times 10^{-2}\ x - 6.88 \times 10^{-3} \quad \text{and}$$

$$y = -6.00 \times 10^{-2}\ x + 1.5 \times 10^{-3},$$

(where y is $dA/d\lambda$ value with its sign, x is concentration in μg ml^{-1}), 0.9993 and 0.9991, respectively. The mean recovery and relative standard deviation was found 1.65 and 2.07%, respectively for synthetic mixtures in the method for A (Table 1). This method was successfully applied to a synthetically prepared mixture containing these active ingredients as similar as the pharmaceutical preparation (240 mg PE + 10 mg A/tablet) for the determination of A and PE and, the results were illustrated in Table 5.

3.2.3. Ratio spectra first derivative spectrophotometry

The ratio spectra of different PE standards at increasing concentrations in 0.1 M HCl:methanol (1:3) obtained by dividing each with the stored spectrum of the standard solution of A by computer aid are shown in Fig. 4a and the first derivative of these spectra (^{1}DD) traced with the interval of $\Delta\lambda = 2$ nm are illustrated in Fig. 4b. As seen in Fig. 4b, there exist more than one maxima and minima and we found that one maxima (244.8 nm) and four minima (252.5, 258.4, 264.3 and 268.0 nm) are suitable for the determination of PE in PE + A mixture. We selected 244.8 nm for the determination of this compound in the assay of synthetically prepared pharmaceutical preparation, tablet, due to its lower RSD value and more suitable mean recovery among the wavelengths mentioned (Table 2). The ratio and ratio derivative spectra of the solutions of A in different concentrations in 0.1 M HCl:methanol (1:3) traced with the interval of $\Delta\lambda = 2$ nm by using the standard spectrum of PE as divisor by computer aid was demonstrated in Fig. 5a and b, respectively. In these spectra, six maxima (224.7, 259.4, 265.0, 269.8, 273.6 and 281.7 nm) and two minima (233.7 and 273.6 nm) were found suitable for the quantification of A in PE + A mixture after smoothened with $\Delta\lambda = 2$ nm. Measured analytical signals at these wavelengths are proportional to the concentrations of the drugs. We selected 265.0 nm for the determination of this compound in the assay of synthetically prepared pharmaceutical preparation, tablet, due to its lower RSD value and suitable mean recovery among the wavelengths mentioned (Table 2).

Calibration graphs were established from analytical signals measured at 244.8, and 258.4 nm for standards containing 202–1818 μg ml^{-1} of PE and at 224.7, 233.7, 259.4, 265.0 and 269.8 nm for standards containing 4.6–45.8 μg ml^{-1} A corresponding to maxima and minima in the absence of each other. At other wavelengths mentioned above we didn't observe a linear relationship between the signals measured and concentrations.

In the method, the mean recoveries and relative standard deviations calculated for synthetic mixtures prepared in our laboratory are illustrated in Table 2. Also, Beer's law compliance for both compounds, the regression equations and correlation coefficients were summarized in Table 3. Mean recoveries and relative standard deviations of the method were found satisfactory.

Divisor concentration is main instrumental parameter. The standard spectra of 4.6 μg ml^{-1} of A and 202.0 μg ml^{-1} of PE was considered as suitable for the determination of PE and A, respectively as divisor. The $\Delta\lambda$ found as optimum for the first derivative of their ratio spectra was 2 nm.

A critical evaluation of all the proposed methods was performed by statistical analysis of the data, where slopes, intercepts including their standard errors and correlation coefficients were shown in Table 3.

Solution of PE in 0.1 M HCl:methanol (1:3) is stable for 4 days. For this reason, if it is necessary, these procedures should be completed before 4 days.

A pharmaceutical preparation containing A + PE is absent in Turkish drug market. So, we applied these methods only for the synthetically prepared mixtures as similar as the pharmaceutical formulation for its active ingredients. We suppose that these methods can easily be applied to its real

commercial preparations. Because, there exist more than one wavelengths suitable for the determination of these active ingredients.

All results obtained by using the methods described above were compared with each other and no significant difference was observed between the amounts of drugs found as theoretical values for t at $P = 0.05$ level (Table 5).

Common excipients such as lactose, starch, avicel, povidon, sodium dodecylsulfate, aerosil, magnesium stearate, sodium lauryl sulfate did not interfere these two spectrophotometric methods.

4. Conclusion

Two new spectrophotometric methods, UV absorption and second derivative spectrophotometry, were proposed in this study for the quantitation of A. UV absorption spectrophotometry was recently used for the determination of A [1]. But we used 0.1 M HCl:methanol (1:3) as solvent in our method and this made the our method more easy to use than that of shown in literature [1] in which chloroform was used for the same purpose. In addition linearity range is larger than the method cited in literature [1]. Second derivative spectrophotometry is an advantageous method by the elimination of possible interferences from the other materials placed in the commercial formulations. As seen in the Table 4, in assay results standard deviations of the second derivative method were smaller than those obtained by using direct UV absorption method. Also, these methods more easy than the extractive spectrophotometric methods explained in literatures ([1,5]) due to not need to any separation and extraction steps Table 5.

The assay results obtained using these methods for commercial preparations were also compared with HPLC method proposed in literature [16] (we used this HPLC method as a reference method due to absence of an official method for A) and good coincides was observed. HPLC method for analysis of A in pharmaceuticals needs expensive equipments and materials such as columns and HPLC grade solvents. The linearity range of the method 0.1–0.25 mg ml^{-1}. Consequently, the proposed methods in this text seemed to be more sensitive than the HPLC method. In addition, the described methods are direct methods for analysis of A, and do not need any expensive equipment. The methods can be easily applied in routine practices made in any laboratory possessing a spectrophotometer with a derivative accessory.

First derivative spectrophotometry and ratio derivative spectrophotometry, could be applied with great success for the simultaneous determination of PE and A in mixtures and tablets containing its mixture without interference of each other (except that the determination of A is possible by zero-order UV spectrophotometry without interference of PE easily). Easy mesurements on the separate peaks, higher values of analytical signals and no need to work only at zero-crossing points (sometimes co-existing compounds have no maximum or minimum at these wavelengths) is an advantage for ratio spectra derivative spectrophotometry in comparison with the derivative spectrophotometry. As we explained in the text, although there exist only one zero-crossing point for the determination of PE in the first derivative spectra, more than one wavelengths were appeared in the derivative spectra of ratio spectra. This gives an opportunity for the determination of PE in presence of many other active ingredients. By the fact that there was no official method for the analysis of A + PE mixture these two spectrophotometric methods were compared with each other. These two methods were found suitable for simple and precise routine analysis of the synthetically prepared pharmaceutical preparation selected. Good agreement was seen in the assay results of synthetically prepared pharmaceutical preparation for all the methods proposed.

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