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SIMULTANEOUS DETERMINATION OF ATROPINE SULFATE AND MORPHINE HYDROCHLORIDE IN THEIR BINARY MIXTURE USING SPECTROPHOTOMERIC METHODS

İKİLİ KARIŞIMINDA ATROPİN SULFAT VE MORFİN HİDROKLORÜRÜN SPEKTROFOTOMETRİK YÖNTEMLER KULLANILARAK AYNI ANDA MİKTAR TAYİNLERİ

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ABSTRACT

In this study, four spectrophotometric methods are used for the simultaneous determination of atropine sulfate and morphine hydrochloride in their binary mixture. In the first and second methods, Vierordt's and modified Vierordt's methods, quantitation of atropine sulfate and morphine hydrochloride were realized by using A (% I, I cm) values determined at 257.3 nm and 284.4 nm in their solution in distilled water. In the third method, derivative spectrophotometry, dA/dλ values were read at 260.8 nm for atropine sulfate and at 244.7 nm for morphine hydrochloride in the first derivative spectra of both compounds in their solution in distilled water. In the fourth method, ratio spectra derivative spectrophotometry, analytical signals were measured at 255.8 nm for atropine sulfate and 273.6 nm for morphine hydrochloride in the first derivative of ratio spectra obtained by using their spetra as divisor in their solution in distilled water. The procedures do not require any separation step. Mean recoveries and relative standard deviations of the methods were calculated in synthetic mixtures.

Key words: *Atropine sulfate, morphine hydrochloride, simultaneous determination, Vierordt's method, derivative spectrophotometry.*

ÖZET

Bu çalışmada, atropin sulfat ve morfin hidroklorürün ikili karışımında aynı anda miktar tayinleri için dört spektrofotometrik yöntem kullanılmıştır. Birinci ve ikinci yöntemlerde, Vierordt ve modifiye Vierordt yöntemi, atropin sulfat ve morfin hidroklorürün miktar tayinleri bunların distile su içerisindeki çözeltilerinde 257.3 nm ve 284.4 nm lerde hesaplanmış olan A (% 1, 1 cm)

değerlerinden yararlanılarak gerçekleştirilmiştir. Üçüncü yöntemde, türev spektrofotometri, dA/dλ değerleri her iki maddenin distile su içerisindeki çözeltilerinin birinci türev spektrumlarında 260.8 nm de atropin sulfat ve 244.7 nm de morfin hidroklorür için okunmuştur. Dördüncü yöntemde ise , spektrum oranları türev spektrofotometri, analitik sinyaller bu maddelerin distile su içerisindeki çözeltilerinde kendilerinin spektrumları bölücü olarak kullanılarak elde edilen bölüm spektrumlarının birinci türevinde 255.8 nm de atropin sulfat için ve 273.6 nm de morfin hidroklorür için ölçülmüştür. Yöntemlerde hiçbir ayırma işlemi gerekmemektedir. Yöntemlerin ortalama geri kazanımları ve bağıl standard sapmaları hazırlanan sentetik karışımlarda hesaplanmıştır.

Anahtar kelimeler: *atropin sulfat, morfin hidroklorür, aynı anda miktar tayini, Vierordt yöntemi, türev spektrofotometri.*

INTRODUCTION

Combinaton of atropine sulfate (A) and morphine hydrochloride (M) is prescribed as narcotic analgesic for severe pains. Only one work was found in the literatures for the simultaneous determination of A and M in their mixture (1). Various methods including spectrophotometry (2-8), spectrofluorimetry (9), high pressure liquid chromatography (10-13), refractometry (14) , NMR (15) and gas chromatography (16) have been used for the determination of A and M in pharmaceutical preparations containing these drugs in combination with other active ingredients.

Many researcher have used Vierordt's and modified Vierordt's methods (17-19) , derivative spectrophotometry (20-23) and ratio spectra derivative spectrophotometry (24-28) for the analysis of pharmaceutical preparations containing drug mixtures. Also, we used these methods for the analysis of multicomponent formulations (29-40).

In this study; Vierordt's and modified Vierordt's methods, first derivative spectrophotometry and ratio spectra derivative spectrophotometry are proposed for the simultaneous determination of A and M in their mixtures.

EXPERIMENTAL

Apparatus

Shımadzu 1601 PC double beam spectrophotometer with a fixed slit width (2 nm) connected to a computer loaded with Shımadzu 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 240.0 - 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 240.0 - 300.0 nm ($\Delta\lambda$ =1 nm). The ordinate maximum and minimum settings were (+ 0.250) and (- 0.500) for M in M + A mixture .

In ratio spectra derivative spectrophotometry, range was selected as 240.0 - 300.0 nm ($\Delta\lambda$ = 2 nm) for reading the analytical signals. The ordinate maximum and minimum settings were (+ 2.0) - (- 2.0) for M and A in their mixture.

Reagent and solutions

Morphine hydrochloride was supplied by Turkish Monopoly Adm. and atropine sulfate was kindly donated by Eczacıbaşı Pharm.Ind. Turkey and used without further purification.

Solutions of 400 mg / 100 mL morphine hydrochloride and 600 mg / 100 mL of atropine sulfate were prepared respectively, in distilled water and used as stock solutions.

RESULTS AND DISCUSSION

i) Vierordt's method: This method is based on the solving of equations with two unknown using A^{1}_{1} (absorbance value of the % 1 solution in a 1-cm cell) values calculated from the absorbance measurements at a pair of suitable wavelengths in which two compounds in the mixture have an absorption minimum and maximum, inversely. The concentrations of the ingredients in the mixture is then calculated from a pair of simultaneous equations as follows;

$$A_1 = \alpha_1 . C_1 + \beta_1 . C_2$$

$$A_2 = \alpha_2 C_1 + \beta_2 . C_2$$

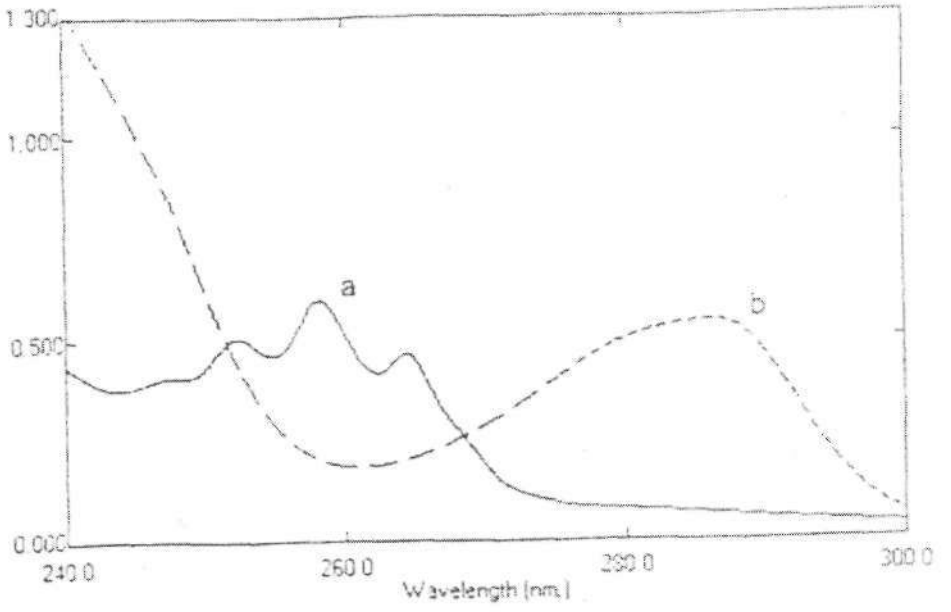

Figure 1: Zero-order absorption spectra of a) 120 μg / ml solution of morphine hydrochloride, b) 600 μg / ml solution of atropine sulfate in distilled water.

where C_1 and C_2 are the concentrations of morphine hydrochloride and atropine sulfate respectively, in g/100 mL. A_1 and A_2 denotes the absorbances of the mixture solution measured and α and β represent the values of A (% 1, 1cm) calculated for atropine sulfate and morphine hydrochloride respectively. The subscript 1 and 2 refer to λ\(257.3 nm) and λ_2 (284.8 nm) respectively for these drugs.

In Figure 1, the absorption spectra of the solutions of A and M in distilled water are overlapped at the region 240.0 - 300.0 nm. By using the Vierordt's method, the determination of these two compounds is possible for direct absorbance measurements in zero-order absorption spectra. For this procedure, the absorbance values were measured at 257.3 nm (λ_{max} for A) and at 284.8 nm (λ_{max} for M). The determination of both compounds in Vierordt's method was realized by using the α and β values calculated at 257.3 and 284.8 nm in the zero-absorption spectra of the solution of A and M in distilled water (Table 1) and solving the equations explained above. For spectra the interval of $\Delta\lambda$=0.1 nm and 300 nm/min of scanning speed was selected in the spectrophotometer. Under these conditons, the obtained original spectra was stored in the computer. Mean recoveries and relative standard deviations of the

method were found as 100.0 % and 0.43 % for A and 99.9 % and 0.36 % for M respectively in the synthetic mixtures prepared by adding known amounts of A and M (Table 2).

Beer's law was valid in the concentration range 200.0 - 1200.0 μg/mL for A and 40.0 - 240.0 μg/mL for M.

ii) Modified Vierordt's method: In the method, using the same readings in (i) and using the following equations, determination of M and A were realized in their UV absorption spectra :

$$C_1 = \frac{A_1}{\alpha_1} \times \frac{b - m}{b - a}$$

$$C_2 = \frac{A_2}{\beta_2} \times \frac{b(m - a)}{m(b - a)}$$

$$m = \frac{A_2}{A_1}, \quad a = \frac{\alpha_2}{\alpha_1}, \quad b = \frac{\beta_2}{\beta_1}$$

other symbols are identical with those cited in (i).

TABLE 1. Experimental parameters for Vierordt's method used for the simultaneous determination of A and M.

	A		M	
	α_1	α_2	β_1	β_2
λ_1 = 257.3 nm	5.54		14.59	
λ_2 = 284.8 nm		5.53		40.65
linearity range μg/ml	200.0 - 1200.0		40.0 - 240.0	

In the same conditions as cited in Vierordt's method, but using the equations explained above (a and b were calculated as 0.0957 and 2.786 respectively), mean recoveries and relative standard deviations of the method were found as 100.0 % and 0.35 % for A and 100.4 % and 0.74 % for M respectively in the synthetic mixtures prepared by adding known amounts of A and M (Table 2).

Beer's law was valid in the concentration range 200.0 - 1200.0 μg/mL for A and 40.0 - 240.0 μg/mL for M.

iii) First derivative spectrophotometry; Figure 2 shows the first derivative spectra (D) of A and M obtained with $\Delta\lambda$ = 1 nm intervals from the stored absorption curves illustrated in Figure 1. In Figure 2, there are eight zero-crossing points for A and two zero-crossing points for M. This means that there is no interference from the co-existing substances at these wavelengths. Linear relationship between the concentration and the $dA/d\lambda$ values was observed at two wavelengths, 260.8 nm and 244.7 nm for A and M respectively. Simultaneous determination of A and M was realized by reading $dA/d\lambda$ values at 260.8 nm for A and at 244.7 nm for M in the first derivative spectra of their solution in distilled water.

The regression equations and correlation coefficients for both compounds at these wavelengths were found as follows:

$y = 3.18\ 10^{-2}x + 1.69\ 10^{-3}$ for A $(r = 0.9994)$

$y = 1.00\ 10^{-3}\ x - 7.50\ 10^{-4}$ for M $(r = 0.9998)$

where y is $dA/d\lambda$ values, x is the concentration in μg / mL .

TABLE 2. Results obtained in the determination of A and M in synthetic mixtures by using Vierordt's and modified Vierordt's methods

Mixture		Vierordt's method				modified Vierordt's method			
Added µg		Found µg		Recovery %		Found µg		Recovery %	
A	**M**	**A**	**M**	A	**M**	A	**M**	**A**	M
600	40	599.4	39.8	99.9	99.4	604.0	40.2	100.6	100.5
600	80	603.0	80.1	100.5	100.1	598.9	80.9	99.8	101.1
600	120	599.4	119.6	99.9	99.7	603.0	120.5	100.5	100.4
600	160	603.0	160.5	100.5	100.3	600.9	160.6	100.2	100.4
600	200	600.0	201.0	100.0	100.5	600.4	197.5	100.1	98.7
600	240	600.6	240.0	100.1	100.0	598.1	238.9	99.7	99.5
200	120	200.0	120.0	100.0	100.0	198.9	120.9	99.5	100.8
400	120	395.2	119.4	98.8	99.5	400.2	121.2	100.1	101.0
600	120	599.4	119.4	99.9	99.5	597.9	119.4	99.7	99.5
800	120	797.6	120.2	99.7	100.2	795.8	121.0	99.8	100.8
1000	120	1002.0	120.2	100.2	100.2	997.8	120.9	99.8	100.8
1200	120	1201.2	119.5	100.1	99.6	1195.4	121.0	99.6	100.8
n = 12			$\bar{x}$	100.0	99.9			100.0	100.4
			% RSD=	0.43	0.36			0.35	0.74

*RSD = Relative standard deviation

Mean recoveries and relative standard deviations of the method were found as 100.1 % and 0.59 % for A and 100.0 % and 0.24 % for M respectively in the synthetic mixtures prepared by adding known amounts of A and M (Table 3).

Beer's law was valid in the concentration range 200.0 - 1200.0 µg/mL for A and 40.0 - 200.0 µg/mL for M.

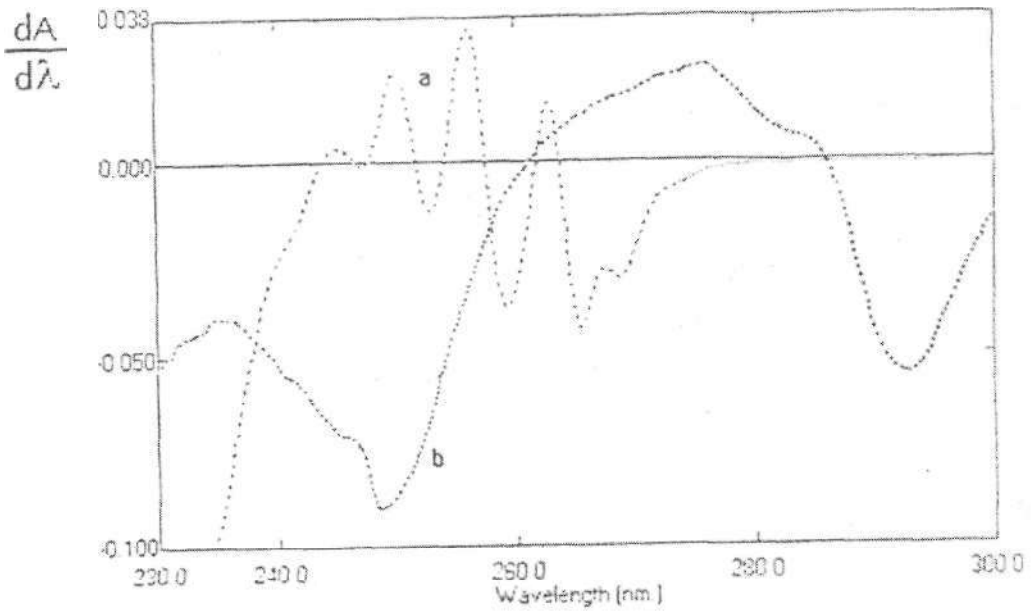

Figure 2: First derivative spectra of a) 120 μg / ml solution of morphine hydrochloride, b) 600 μg / ml solution of atropine sulfate in distilled water ($\Delta\lambda$= lnm).

The amounts of A and M in their mixture can therefore be determined without prior chemical separation and without any interference from each other.

In the method, in order to obtain sharp peaks and zero-crossing points the experimental conditions were optimized. The influence of $\Delta\lambda$ in obtaining the best derivative spectra was tested and $\Delta\lambda$ = 1 nm was considered suitable for the determination of both compounds.

iv) Ratio spectra derivative spectrophotometry: The ratio spectra of different M standards at increasing concentrations in distilled water obtained by dividing each with the stored spectrum of the standard solution of A by computer aid are shown in Figure 3a and the first derivative of these spectra ('DD) traced with the interval of $\Delta\lambda$ = 2 nm are illustrated in Figure 3b. As seen in Figure 3b, there exist more than one maxima and minima and we found that one maxima (255.9 nm) is suitable for the determination of M in M + A mixture. Therefore, we proposed 255.9 nm for the determination of this compound in the assay of synthetically prepared pharmaceutical preparation, tablet (Table 4). The ratio and ratio derivative spectra of the solutions of A in different concentrations in distilled water traced with the interval of $\Delta\lambda$ =2

nm by using the standard spectrum of M as divisor by computer aid was demonstrated in Figure 3a and 3b, respectively. In these spectra, one maxima (273.6 nm) was found suitable for the quantification of A in M + A mixture. Measured analytical signals at this wavelength are proportional to the concentrations of the drugs. We proposed 273.6 nm for the determination of this compound in the assay of synthetically prepared pharmaceutical preparation, tablet (Table 4).

Calibration graphs were established from analytical signals measured at 255.9 nm and 273.6 nm for standards containing 40 - 200 μg / ml of M and 200 - 1200 μg / mL of A corresponding to the maxima in the absence of each other. At other wavelengths seen in the spectra 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 3. Mean recoveries and relative standard deviations of the method were found satisfactory.

The regression equations and correlation coefficients for both compounds at the wavelengths selected were found as follows:

$y = 4.07\ 10^{-4}x + 8.50\ 10^{-5}$ for A $(r = 0.9999)$

$y = 9.1710^{-3}x - 2.50\ 10^{-4}$ for M $(r = 0.9998)$

where y is the value of analytical signal, x is the concentration in μg / mL .

Divisor concentration is main instrumental parameter in the method. The standard spectra of 600 μg / mL solution of A and 120 μg / mL

TABLE 3. Results obtained in the determination of A and M in synthetic mixtures by using first derivative and ratio spectra derivative spectrophotometry

Mixture		First derivative spectrophotometry				Ratio spectra derivative spectrophotometry			
Added µg		Found µg		Recovery %		Found µg		Recovery %	
A	M	A	M	A	M	A	M	A	M
600	40	600.6	40.0	100.1	100.0	596.0	41.0	99.3	102.5
600	80	600.0	79.7	100.0	99.6	595.8	80.3	99.3	100.4
600	120	599.4	120.4	99.9	100.3	609.9	120.1	101.7	100.1
600	160	594.6	160.5	99.1	100.3	600.7	161.0	100.1	100.6
600	200	600.0	200.6	100.0	100.3	599.5	198.0	99.9	99.0
600	240	598.8	239.3	99.8	99.7	603.0	240.1	100.5	100.0
200	120	198.4	119.8	99.2	99.8	195.3	120.0	97.7	100.0
400	120	402.8	120.2	100.7	100.2	394.3	119.5	98.6	99.6
600	120	600.0	120.1	100.0	100.1	598.3	120.9	99.7	100.8
800	120	799.2	119.9	100.2	99.9	804.7	120.2	100.6	100.2
1000	120	1003.0	120.2	100.3	100.2	1013.5	120.0	101.4	100.0
1200	120	1216.8	120.0	120.0	100.0	1205.2	119.5	100.4	99.6
n= 12			$\bar{x}$	100.1	100.3			99.9	100.2
			% RSD=	0.59	0.24			1.13	0.86

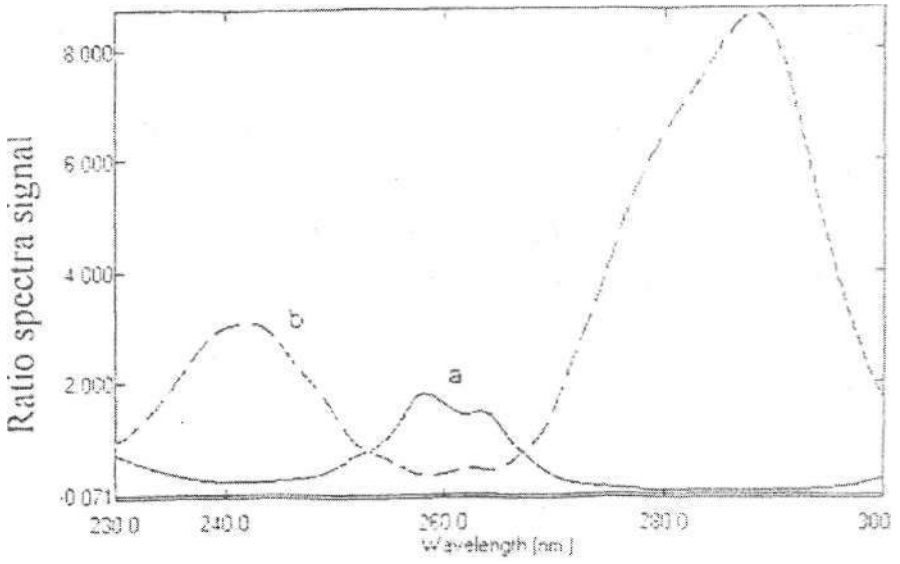

Figure 3. Ratio spectra of (a) 600 µg / ml solution of atropine sulfate (spectra of 120 µg / ml solution of morphine hydrochloride was used as divisor) and (b) 120 µg / ml solution of morphine hydrochloride(600 µg / ml solution of atropine sulfate was used as divisor) in. distilled water ($\Delta\lambda$ = 2 nm.

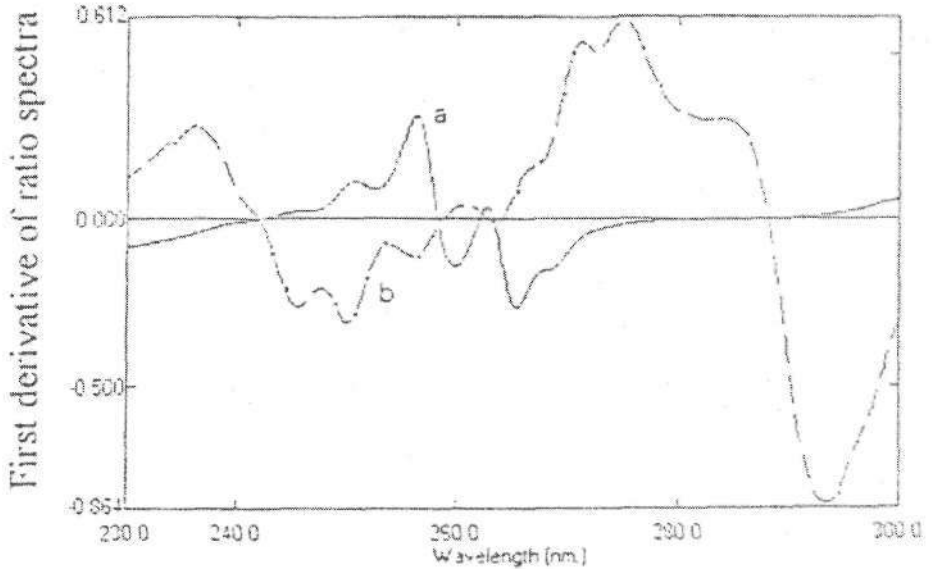

Figure 4. First derivative of the ratio spectra of (a) 600 µg / ml solution of atropine sulfate (spectra of 120 µg / ml solution of morphine hydrochloride was used as divisor) and (b) 120 µg/ml solution of morphine hydrochloride (600 µg / ml solution of atropine sulfate was used as divisor) in distilled water ($\Delta\lambda$ 2 nm).

solution of M in distilled water were considered as suitable for the determination of M and A, respectively as divisor. The $\Delta\lambda$ found as optimum for the first derivative of their ratio spectra was 2 nm.

A pharmaceutical preparation containing A + M mixture is absent in Turkish drug market but it exists in "Rote Liste", Germany. And, by the fact that A + M mixture is used as narcotic analgesic and need a special prescription we couldn't obtain its commercial preparation. So, we applied these methods only for the synthetically prepared mixtures as similar as the pharmaceutical formulation for its active ingredients and all the methods proposed in this text were applied only to this synthetic mixture (Table 4). All the 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 4).

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

CONCLUSION

The proposed methods, Vierordt's, modified Vierordt's and first derivative and ratio spectra derivative spectrophotometry could be applied with great success for the simultaneous determination of morphine hydrochloride and atropine sulfate in mixtures without interference from each other. Easy measurements 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). Derivative ratio method is also an advantageous method by not needing any additional mathematical calculations and not working in different mediums and different measurements in comparison with the methods explained in literatures such as ion-pair extraction spectrophotometry and chemometric methods. Relative standard deviations for morphine hydrochloride in ratio spectra derivative spectrophotometry and modified Vierordt's method and for atropine sulfate in ratio spectra derivative spectrophotometry were found

TABLE 4. Comparison of the results for synthetically prepared tablet mixture with respect to only their active ingredients (0.5 mg A + 10 mg M /tablet) (mg)

Methods	A mean ± SD*	*t* values calculated (P=0.05)	M mean ± SD*	t values calculated (P=0.05)
Vierordt's	0.50 ± 0.03	V - ^{1}D = 1.15	9.96 + 0.36	V - ^{1}D = 1.15
Modified Vierordt's	0.49 ± 0.04	V - ^{1}DD = 1.05	9.89 + 0.72	V - ^{1}DD = 1.05
^{1}D	0.48 ± 0.05	V - MV = 0.46	10.00 ± 0.24	V - MV = 0.46
^{1}DD	0.48 ± 0.09	^{1}D - ^{1}DD = 1.56	9.98 ± 0.64	^{1}D - ^{1}DD = 1.56
		^{1}D - MV = 0.76		^{1}D - MV = 0.76
		^{1}DD - MV = 1.15		^{1}DD - MV = 1.15

*Mean of ten determination,

** Theoretical value for t at P : 0.05 level = 2.26

SD : standard deviation, V : Vierordt's method, MV : modified Vierordt's method, ^{1}D : first derivative spectrophotometry, ^{1}*DD* : ratio spectra first derivative spectrophotometry

little higher than those obtained in other methods. Linearity range was found same in all the methods. These four methods were found suitable for simple and precise routine analysis of atropine sulfate + morphine hydrochloride mixture. Also, these methods are proposed for the analysis of pharmaceutical formulation mentioned above.

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