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Assay of ephedrine hydrochloride and theophylline in pharmaceutical formulations by differential-derivative spectroscopy

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Abstract

Rapid and accurate binary mixture resolution of ephedrine hydrochloride and theophylline was performed. Differential-derivative spectrophotometry with a zero-crossing measurement technique was used for the quantitative determination of ephedrine hydrochloride and theophylline in pharmaceuticals. Neither sample pretreatment nor separation were required. Linear calibration graphs of differential first derivative values (at 262.4 and 256.3 nm for theophylline and ephedrine hydrochloride, respectively) versus concentration (in the ranges 6.0–40.0 and 100.0–1000.0 μg ml^{-1} for theophylline and ephedrine hydrochloride, respectively) were obtained with negligible intercepts. Vierordt's method was also developed for a comparison method. Commercial tablet and laboratory-prepared mixtures containing both drugs were assayed using the developed methods. Both methods showed good linearity, precision and reproducibility.

Keywords: Theophylline; Ephedrine hydrochloride; Differential-derivative spectrophotometry; Pharmaceutical formulations

1. Introduction

Theophylline is a xanthine derivative that relaxes smooth muscles, relieves bronchospasm and has a stimulant effect on respiration. Ephedrine hydrochloride is reported to reduce the viscosity of tenacious sputum and is used as an expectorant. Theophylline and ephedrine hydrochloride in combination induce bronchodilation and assist the patient in coughing up viscid mucus, and have been used in the symptomatic treatment of bronchial asthma and other bronchospastic conditions.

A survey of the literature revealed that the analysis of theophylline and ephedrine hydrochloride either in single or multicomponent mixtures has been reported through high performance liquid chromatography [1–5], UV-densitometric [6], voltammetry [7,8], gas chromatography [9,10], micellar electrokinetic capillary chromatography [11,12] and spectrophotometry [13–20] in pharmaceutical preparations.

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Derivative spectrophotometry [21] is a useful technique for the suppression of additive interference, and it has been used extensively for the simultaneous determination of substances in mixture. Difference spectrophotometry has proved to be a powerful technique for determination of drugs [22–24] as well as detection and determination of decomposition products [25]. Derivative-difference spectrophotometry will offer further advantages in canceling heavy spectral interferences to drug analysis [26,27] when the irrelevant absorption is pH and solvent dependent.

The aim of this work was to study differential first derivative spectrophotometry and Vierordt's method assays of theophylline and ephedrine hydrochloride in binary mixtures without previous separation step. The utility of the developed methods to determine the contents of tablets was demonstrated.

2. Experimental

2.1. Instruments

All spectral measurements and treatment of data were carried out in 1-cm quartz cells using a Shimadzu 1601 double beam spectrophotometer with a fixed slit width (2 nm) connected to an IBM PC computer and a Lexmark printer. The wavelength range of 225.0–325.0 nm was selected for differential spectra and 235.7–305.5 nm for differential-derivative spectra, and ordinate maximum and minimum were adjusted to the magnitude of derivative values.

2.2. Reagents and samples

All experiments were performed with analytical grade chemicals and solvents. Authentic samples of ephedrine hydrochloride and theophylline were kindly donated by Carlo Erba, Turkey and were used without further purification. PiRASMiN® tablets (Carlo Erba, Turkey, batch no: ER 34) labeled to contain 125.0 mg ephedrine hydrochloride and 25.0 mg theophylline were obtained from the local market.

3. Procedures

3.1. Differential-derivative spectrophotometry

3.1.1. Calibration solutions

Standard solutions of ephedrine hydrochloride and theophylline were prepared, by dissolving ~ 50 mg each of the pure drugs in 50 ml of methanol. Appropriate volume aliquots of the

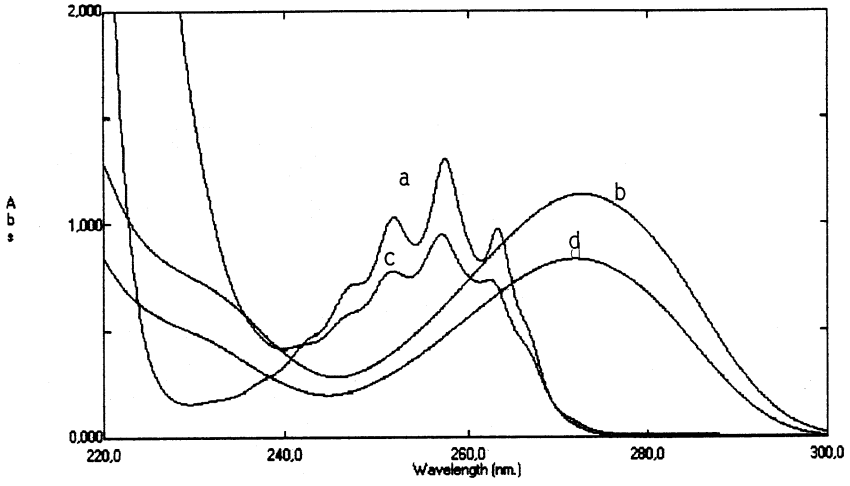

Fig. 1. Zero-order spectra of (a) 750.0 μg ml^{-1} ephedrine hydrochloride, (b) 30.0 μg ml^{-1} theophylline in methanol, (c) 750.0 μg ml^{-1} ephedrine hydrochloride, and (d) 30.0 μg ml^{-1} theophylline in 0.1 N NaOH.

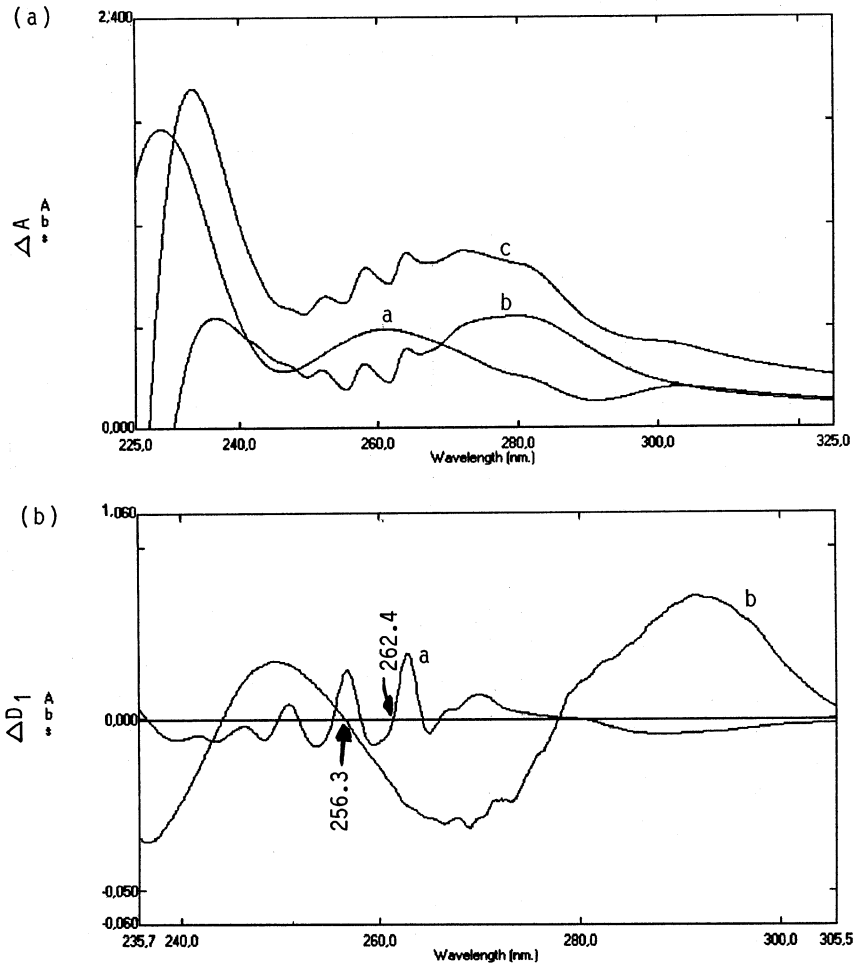

Fig. 2. (a) Differential spectra of a) 20.0 μg ml^{-1} ephedrine hydrochloride, b) 250.0 μg ml^{-1} theophylline and c) mixture of 250.0 μg ml^{-1} ephedrine hydrochloride and 30.0 μg ml^{-1} theophylline in methanol versus 0.1 N NaOH. (b) Differential-derivative spectra of a) 250.0 μg ml^{-1} ephedrine hydrochloride, and b) 30.0 μg ml^{-1} theophylline in methanol versus 0.1 N NaOH.

stock solution were transferred to 10-ml calibrated flasks in duplicate. Accurate volumes were transferred into two sets of 10-ml calibrated flasks. One set was diluted to volume with 0.1 N NaOH and the other set was diluted to volume with methanol. The first series contained a constant concentration of ephedrine hydrochloride (250.0 μg ml^{-1}) and a varying concentration of theophylline (6.0–40.0 μg ml^{-1}). The second contained a constant concentration of theophylline (30.0 μg ml^{-1}) and a varying concentration of ephedrine hydrochloride (100.0–1000.0 μg ml^{-1}). Calibration solutions ($n = 5$) were used to construct the calibration curves in the standardization of cited method.

3.1.2. Sample preparation

The proposed method was evaluated in the assay of commercial tablets. A total of ten replicate determinations were carried out. First ten tablets (PiRASMiN®), labeled to contain 25.0 mg of theophylline and 125.0 mg ephedrine hydrochloride and excipients were weighed and finely powdered, and samples equivalent to 25.0 mg of theophylline and 125.0 mg ephedrine hydrochloride (one tablet) were taken and dissolved

Table 1
Assay parameters for differential-derivative spectrophotometry and Vierordt's method determination of ephedrine hydrochloride and theophylline in binary mixture[a]

Parameters	Ephedrine hydrochloride		Theophylline	
	Diff. der. spec.	Vierordt's method	Diff. der. spec.	Vierordt's method
Range (μg ml^{-1})	100.0–1000.0	100.0–1000.0	6.0–40.0	6.0–40.0
Regression equation (Y)[b]				
Slope (b)	6.70×10^{-2}	2.10×10^{-3}	2.28×10^{-3}	9.32×10^{-2}
S.D. on slope (S_b)	1.41×10^{-4}	2.02×10^{-4}	4.45×10^{-5}	1.08×10^{-5}
Intercept (a)	3.31×10^{-2}	6.13×10^{-3}	8.74×10^{-3}	3.22×10^{-4}
S.D. on intercept (S_a)	4.52×10^{-3}	5.31×10^{-4}	4.87×10^{-3}	4.36×10^{-3}
S.E. of estimation (S_e)	0.57×10^{-4}	1.27×10^{-3}	4.11×10^{-3}	1.27×10^{-3}
Correlation coefficient (r)	0.9991	0.9993	0.9981	0.9998
RSD (%)[c]	0.95	2.07	0.75	2.19
% Range of error[c](95% confidence limit)	0.45	0.28	0.57	0.82

[a] Diff. der. spect., differential-derivative spectrophotometry.
[b] $Y=a+bC$ where C is concentration in μg ml^{-1} and Y is absorbance units.
[c] Five replicate samples ($n=5$).

in methanol in 100-ml calibrated flasks. After 20 min of mechanically shaking, the solution was filtered into a 100-ml calibrated flask through Whatman no. 42 filter paper. The residue was washed three times with 10 ml of solvent, then the volume was completed to 100 ml with methanol. The solution was diluted 1:10 with 0.1 N NaOH and methanol, separately. The difference spectra of the methanolic solution and equimolar 0.1-N NaOH solution of pure drugs and sample were recorded by placing the 0.1-N NaOH solutions in the reference compartment and the methanolic solution in the sample compartment. A first derivative spectrum of each of the differential curves was subsequently recorded. The solutions were measured at 256.3 and 262.4 nm for ephedrine hydrochloride and theophylline, respectively.

3.2. Vierordt's method

3.2.1. Calibration solutions

Samples were prepared in 50-ml calibrated flasks containing 100.0–1000.0 μg ml^{-1} of ephedrine hydrochloride and 6.0–40.0 μg ml^{-1} of theophylline in methanol. Absorption spectra were obtained in matched quartz cuvettes using the composite solvent as a reference. Then, the absorption spectra were recorded and the values of the absorbances were measured at suitably selected wavelengths (257.1 and 272.1 nm). Calibration solutions ($n=5$) were used to construct the calibration curves in the standardization of cited method.

3.2.2. Sample preparation

The proposed method was evaluated in the assay of commercial tablets. A total of ten replicate determinations were carried out. First ten tablets (PiRASMiN®), labeled to contain 25.0 mg of theophylline and 125.0 mg ephedrine hydrochloride and excipients were weighed and finely powdered, and samples equivalent to 25.0 mg of theophylline and 125.0 mg ephedrine hydrochloride (one tablet) were taken and dissolved

Table 2
Experimental parameters calculated for the simultaneous determination of ephedrine hydrochloride and theophylline in binary mixture by Vierordt's method

λ (nm)	α_1	α_2	β_1	β_2
	Ephedrine hydrochloride		Theophylline	
λ_1: 257.1	698.0	–	745.8	–
λ_2: 272.1	–	389.7	–	274.5

Table 3
Assay results of ephedrine hydrochloride and theophylline in laboratory-made mixtures and in commercial tablets

Preparation	Ephedrine hydrochloride recovery (mean ± S.D.)[a] (%)		Theophylline recovery (mean ± S.D.)[a] (%)	
	Diff. der. spect.[c]	Vierordt's method	Diff. der. spect.[c]	Vierordt's method
Laboratory-made mixture	99.00 ± 0.81 $t = 0.072$ $F = 1.137$	99.43 ± 1.63	99.09 ± 0.87 $t = 0.038$ (2.23)* $F = 2.870$ (5.19)*	98.72 ± 1.02
Commercial[b] tablets	99.85 ± 0.68 $t = 1.536$ $F = 1.877$	99.28 ± 1.07	99.91 ± 0.59 $t = 0.037$ $F = 2.980$	98.68 ± 0.92

[a] Mean of ten determinations ± S.D.
[b] PiRASMiN® tablets were labeled to contain 125.0 mg ephedrine hydrochloride and 25.0 mg theophylline per tablet.
[c] Diff. der. spect., differential-derivative spectrophotometry.
* Theoretical values for t and F at $P = 0.05$ level.

in methanol in 100-ml calibrated flasks. After 20 min of mechanically shaking, the solution was filtered into a 100-ml calibrated flask through Whatman no. 42 filter paper. The residue was washed three times with 10 ml of solvent, and the volume was completed to 100 ml with methanol. The solution was diluted 1:10 with methanol. The method described above was applied to the prepared solutions.

4. Results and discussion

4.1. Differential-derivative spectrophotometry

Fig. 1 shows the zero order absorption spectra of ephedrine hydrochloride (750.0 μg ml^{-1}) and theophylline (30.0 μg ml^{-1}) each in 0.1 N NaOH and in methanol. The differential absorption spectra of ephedrine hydrochloride, theophylline and a mixture of ephedrine hydrochloride and theophylline are shown in Fig. 2a. Fig. 2b shows the first derivative difference spectrum. For differential measurements, alkaline solutions were placed in the reference cell and methanolic solutions in sample cell. The delta absorbance ($\Delta A = A_{alk} - A_{acid}$) ΔD_1 curves were recorded. Attempts to analyze ephedrine hydrochloride and theophylline in two component mixtures by first differential-derivative spectrophotometry was successful, due to heavy contribution of ephedrine hydrochloride and theophylline spectra (Fig. 2b). The first derivative differential spectra of both the drugs Fig. 2b offered an advantage for their simultaneous determination by having zero crossing points. In particular absorbance at 256.3 nm for ephedrine hydrochloride and at 262.4 nm for theophylline were considered as the optimum working wavelengths for their determination. The first differential-derivative spectrum (Fig. 2b) of theophylline shows a well defined maximum at 262.4 nm while ephedrine hydrochloride has a zero ΔD_1 value at the same wavelength. Ephedrine hydrochloride has a ΔD_1 value at 256.3 nm at which theophylline exhibits no contribution. The derivative differential curves showed the best linear response to analyte concentrations used at these wavelengths. Least-squares regression analysis was carried out on the slope, the intercept and correlation coefficient (r) values. The relative standard deviation calculated for separate determinations of each drug was 0.75–0.95%, indicating good precision and reproducibility (Table 1). To prove the validity and applicability of the proposed methods, ten synthetic mixtures in the concentration range stated in Table 3 were assayed. The results obtained using the above methods were precise and accurate (Table 3). The detection limits (LOD) [28] were 0.92 μg ml^{-1} for ephedrine hydrochloride and 0.046 μg ml^{-1} for theophylline, while the quantification limits (LOQ) [29] were 2.89 μg

ml^{-1} for ephedrine hydrochloride and 0.128 μg ml^{-1} for theophylline. The selected method was successfully applied to the determination of these drugs in laboratory-prepared mixtures and commercial tablets. The results are summarized in Table 3. The results obtained show the high reliability and reproducibility of the method.

4.2. Vierordt's method

Differential-derivative spectrophotometric method also tested Vierordt's method for resolving the binary mixtures.

Absorptivity A_1^1 (1%, 1 cm) values for ephedrine hydrochloride and theophylline in the binary mixtures at zero-order spectra were calculated by using the absorbances measured at the appropriate wavelengths in methanol. Of these wavelengths 257.1 nm was selected as optimal for ephedrine hydrochloride and 272.1 nm for determination of theophylline in binary mixture. Similarly the absorbances of the mixed sample solutions were measured and then the concentration of each compound was calculated from the following simultaneous equations:

$$A_1 = \alpha_1 C_{\text{eph}} + \beta_1 C_{\text{the}} \qquad A_2 = \alpha_2 C_{\text{eph}} + \beta_2 C_{\text{the}}$$

where C_{eph} and C_{the} are the concentrations of two compounds, ephedrine hydrochloride and theophylline, in binary mixtures calculated as g 100 ml^{-1}. A denotes the absorbance of the mixture solution, and α and β represent the values of A_1^1 (1%, 1 cm) for ingredients. The subscripts 1 and 2 refer to $\lambda_1 = \lambda_{\max} = 257.1$ nm of ephedrine hydrochloride compound and $\lambda_2 = \lambda_{\max} = 272.1$ nm of theophylline compound, respectively. Tables 1 and 2 show the experimental parameters obtained by using the zero-order absorption spectra for the standard solutions of ephedrine hydrochloride and theophylline. The application of Vierordt's method of two-component analysis for the determination of these drugs in authentic mixtures and in tablets gave satisfactory results (Table 3). The detection limits (LOD) were 1.92 μg ml^{-1} for ephedrine hydrochloride and 0.051 μg ml^{-1} for theophylline, while the quantification limits (LOQ) were 3.13 μg ml^{-1} for ephedrine hydrochloride and 0.108 μg ml^{-1} for theophylline. The selected methods were successfully applied to the determination of these drugs in laboratory-prepared mixtures and commercial tablets. The results are summarized in Table 3. The results obtained show the high reliability and reproducibility of the method.

The proposed methods were statistically compared with those of Student's t-test and variance ratio F-test (Table 3). The calculated (experimental) t- and F-values did not exceed the tabulated (theoretical) values in either test, indicating that there was no significant difference with respect to accuracy and precision between the proposed methods. Commercially available tablets containing a mixture of ephedrine hydrochloride and theophylline were analyzed using the developed methods. The selected methods were successfully applied to the determination of these drugs in laboratory-prepared mixtures and commercial tablets.

5. Conclusions

The proposed methods are simple (as there is no need for solvent extraction), accurate and specific and can therefore be applied to the determination of the cited drugs in two-component pharmaceutical preparations.

References

[1] M.I. Saleh, C.S. Yoong, J. Phys. Sci. 4 (1993) 55.
[2] L. Oi-Wah, M. Chuen-Shing, J. Chromatogr. A 693 (1995) 45.
[3] C. Robing, W. Chaoyun, H. Qin, Zhongguo Yaoxue Zazhi 28 (1993) 91.
[4] Z. Zhiyao, Z. Zhenliang, Z. Shurun, L. Hongming, Yaowu Fenxi Zazhi 13 (1993) 162.
[5] H. Yuesheng, L. Zhisong, L. He, S. Yuqing, Yaowu Fenxi Zazhi 12 (1992) 28.
[6] R. Dusanka, Z. Ljiljana, A. Svetlana, G. Latinka, Arh. Farm. 40 (1990) 115.
[7] N.M.P. Alcada, L.F.C. Lima, M. Montenegro, B.S.M. Conceicao, J. Pharm. Biomed. Anal. 10 (1992) 757.
[8] P.R. Charmorro, R.C. Diaz, Talanta 40 (1993) 1461.
[9] O.W. Lau, Y.M. Cheung, Analyst 115 (1990) 1349.
[10] T.A. Zavrazhanaya, Farmatsiya (Moscow) 40 (1991) 31.
[11] D. Quanxun, Y. Lingxiao, S. Zengpei, L. Dakui, J. Chromatogr. 630 (1993) 363.

[12] Z. Chao-Xuan, T. Wolfgang, J. Capillary Electrophor. 1 (1994) 208.
[13] P. Campins Falco, A. Sevillano Cabeza, C. Molins Legua, Anal. Lett. 27 (1994) 531.
[14] G. Yang, P. Li, S. Bai, Shenyang Yaoxueyuan Xuebao 10 (1993) 199 (CA 120,307623r, 1994).
[15] W. Zhou, Fenxi Shiyanshi 12 (1993) 124 (CA 120,144293s, 1994).
[16] M.H. Abdel-Hay, M.S. El-Din, M.A. Abuirjeie, Analyst 117 (1992) 157.
[17] R. Marin Saez, M. Liobat Estelles, M.D. San Martin Ciges, A.R. Mauri Aucejo, Anal. Lett. 26 (1993) 641.
[18] K. Wang, C. Xu, W. Chen, B. Cao, Zhongguo Yiyuan Yaoxue Zazhi 13 (1993) 312 (CA 120,62442a, 1994).
[19] P. Parimoo, P. Umapathi, K.S. Srinivasan, Indian Drugs 29 (1992) 442.
[20] L. Qi, Y. Yumian, Q. Mingwei, C. Weixiang, Zhongguo Yaoke Daxue Xuebao 23 (1992) 124 (CA 117,157742a, 1992).
[21] J.E. Cahill, F.G. Padeka, Am. Lab. 12 (1980) 101.
[22] A.G. Davidson, Analyst 107 (1982) 422.
[23] A.G. Davidson, J. Pharm. Sci. 73 (1984) 1582.
[24] A.G. Davidson, J.B. Stenlake, Analyst 99 (1974) 476.
[25] A.G. Davidson, J. Pharm. Pharmacol. 30 (1978) 410.
[26] M.A. Korany, F.A. El-Yazbi, O. Abdel-Razak, M.A. Elsayed, Pharm. Weekbl. Sci. Ed. 7 (1985) 163.
[27] C.V.N. Prasad, A. Gautam, V. Bharadwaj, P. Parimoo, Talanta 44 (1997) 917.
[28] Nomenclature, symbols, units and their usage in spectrochemical analysis, II, Spectrochim. Acta Part B 33 (1978) 242.
[29] Guidelines for data acquisition and data quality evaluation in environmental chemistry, Anal. Chem. 52 (1980) 2242.