ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
21 (1999) 429–437

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Application of derivative-differential UV spectrophotometry and ratio derivative spectrophotometric determination of mephenoxalone and acetaminophen in combined tablet preparation

Nevin Erk *

Department of Analytical Chemistry, Faculty of Pharmacy, University of Ankara, 06100 Ankara, Turkey

Received 30 December 1998; received in revised form 27 May 1999; accepted 8 June 1999

Abstract

A method is presented for the direct determination of mephenoxalone and acetaminophen in combined pharmaceutical dosage forms without prior separation. The first method, derivative-differential spectrophotometry, comprised of measurement of the difference absorptivities derivatized in the first order (ΔD_1) of a tablet extract in 0.1 N NaOH relative to that of an equimolar solution in methanol at wavelengths of 289.6 and 252.6 nm respectively. The second method is based on ratio derivative spectrophotometry. The amplitudes in the first derivative of the ratio spectra at 233.5 and 288.9 nm were selected to determine mephenoxalone and acetaminophen in the mixture. The proposed methods, which give thoroughly comparable data, are simple and rapid, and allow one to obtain precise and accurate results.

Keywords: Mephenoxalone; Acetaminophen; Pharmaceutical formulation; First derivative-differential spectrophotometry; Ratio derivative spectrophotometry

1. Introduction

A combination dosage form of acetaminophen and mephenoxalone is indicated in the analgesic and myorelaxan. Different reported methods for the quantitative determination of these compounds, alone or in their combination with other drugs have been used. These methods use high performance liquid chromatography[1–5] (HPLC) and although they are sensitive, they are laborious and expensive, spectrophotometric methods [6–12], have traditionally been the most widely employed, polarography [13] and gas chromatography [14] in pharmaceutical preparations.

Derivative spectrophotometry offers greater selectivity than does normal spectrophotometry in the simultaneous determination of two or more compounds without previous chemical separation

* Tel.: +90-312-23-05-000; fax: +90-312-21-31-081.
E-mail address: erk@pharmacy.ankara.edu.tr (N. Erk)

0731-7085/99/$ - see front matter
PII: S0731-7085(99)00157-0

[15–17]. Difference spectrophotometry based on pH changes has also been reported to be useful in the determination of binary mixtures [18]. There are few reports on utilization of the above two combined techniques for the estimation of individual drug substances [19] and for combined preparations [20,21].

Recently, a spectrophotometric method based on the use of the first derivative of the ratio spectra was developed by Salinas et al. [22,23], for resolving binary mixtures.

The purpose of this work was to develop a derivative-difference spectrophotometry and ratio derivative spectrophotometry for the simultaneous determination of mephenoxalone and acetaminophen in bulk and pharmaceutical formulations, the results obtained by these two approaches were compared.

2. Experimental

2.1. Apparatus

A Shimadzu 1601 double beam UV–Vis spectrophotometer, with 1 cm quartz cuvettes, a fixed slit width (2 nm) connected to an IBM-PC computer loaded with Shimadzu UVPC software was equipped with a Lexmark printer was used for all the absorbance measurements and treatment of data.

2.2. Chemicals used

Mephenoxalone and acetaminophen were kindly supplied by iLTAS Pharm. Ind.

Methanol and NaOH were of analytical reagent grade (Merck Chem. Ind.)

2.3. Pharmaceutical preparation

A commercial pharmaceutical preparation (DORSiLON® tablet iLTAS Pharm. Ind. Turkey, batch no: 123B1) was assayed. Its declared content was as follows: Mephenoxalone, 200.0 mg; Acetaminophen, 450.0 mg/tablet.

3. Application methods

3.1. Differential derivative spectrophotometry

The first derivative spectra of the mephenoxalone and acetaminophen solutions in 0.1 N NaOH were recorded against the corresponding methanolic solutions of the drugs as a blank. The ΔD_1 values of the first derivative difference spectrum of mephenoxalone at 289.6 nm (where the ΔD_1 value of acetaminophen is zero) and that of acetaminophen at 252.6 nm (where the ΔD_1 value of mephenoxalone is zero) have been used for the determination of two drugs. Thus at 289.6 nm the ΔD_1 value of the mixture will be due to the contribution of mephenoxalone alone and at 252.6 nm the contribution will be only that of acetaminophen.

3.2. Ratio spectra derivative spectrophotometry

The absorption spectra of the solutions prepared at different concentrations of mephenoxalone and of its binary mixtures with acetaminophen were recorded and divided by the spectrum of the standard solution of acetaminophen (25.0 μg ml^{-1} in methanol). The first derivatives of the ratio spectra were calculated with $\Delta\lambda = 8$ nm. In the binary mixtures the amount of mephenoxalone was determined by measuring the first derivative signals at 233.5 nm in the range of 214.0–250.0, for the mephenoxalone acetaminophen mixtures. A similar procedure was followed for the different concentrations of acetaminophen, when mephenoxalone was 12.5 μg ml^{-1}.

In the same way as describe above, the content of acetaminophen was determined by selecting the first derivative of the ratio spectra in the range 227.0–310.0 nm and measuring the signal at 288.9 nm.

4. Procedures

4.1. Differential derivative spectrophotometry

4.1.1. Calibration

Standard solutions of mephenoxalone and ac-

etaminophen were prepared, by dissolving approximately 25 mg, accurately weighed, in 250 ml methanol. Appropriate volume aliquots of the stock solution were transferred to 25-ml calibrated flasks. Accurate volumes were transferred into two sets of 25-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 mephenoxalone (20.0 μg ml^{-1}) and a varying concentration of acetaminophen (5.0–50.0 μg ml^{-1}). The second contained a constant concentration of acetaminophen (45.0 μg ml^{-1}) and a varying concentration of mephenoxalone (5.0–50.0 μg ml^{-1}). The solutions were protected from light throughout the study.

4.1.2. Sample preparation

A total of ten tablets (DORSiLON®) were weighed and finely powdered. Quantities of the powdered tablets equivalent to 450.0 mg of acetaminophen and 200.0 mg mephenoxalone (one tablet) were weighed accurately, taken and dissolved in methanol in 100 ml calibrated flasks. After 20 min of mechanically shaking, the solution was filtrated in 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 between the methanolic solution and equimolar 0.1 N NaOH solution of pure drugs and sample were recorded from 227.0–350.0 nm by placing the methanolic solution in the reference compartment and the 0.1 N NaOH solutions in the sample compartment. A first derivative spectrum of each of the differential curves was subsequently recorded. The solutions were measured at 289.6 and 252.6 nm for mephenoxalone and acetaminophen, respectively.

4.2. Ratio spectra derivative spectrophotometry

4.2.1. Calibration

Samples were prepared in 50-ml calibrated flasks containing 5.0–50.0 μg ml^{-1} of mephenoxalone and 5.0–50.0 μg ml^{-1} of acetaminophen in methanol. Absorption spectra were obtained in matched quartz cuvettes using the composite solvent as a reference. All spectra were stored in the IBM-PC. The stored spectra of the binary mixtures, mephenoxalone and acetaminophen, were divided by standard spectrum of acetaminophen (25.0 μg ml^{-1}) for determining mephenoxalone. Then, the first ratio spectra were recorded and the values of the derivatives were measured at suitably selected wavelengths. The solutions were protected from light throughout the study.

4.2.2. Sample preparation

A total of ten tablets (DORSiLON®) were weighed and finely powdered. Quantities of the powdered tablets equivalent to 450.0 mg of acetaminophen and 200.0 mg mephenoxalone (per tablet) were weighed accurately, taken and dissolved in methanol in 100 ml calibrated flasks. After 20 min of mechanically shaking, the solution was filtrated in 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 methanol. The method described above was applied to the prepared solutions.

5. Results and discussion

5.1. Differential derivative spectrophotometry

Fig. 1 shows the zero order spectrum of mephenoxalone (12.5 μg ml^{-1}) and acetaminophen (25.0 μg ml^{-1}) in 0.1 N NaOH and in methanol. The difference absorption spectra of mephenoxalone, acetaminophen and a mixture of mephenoxalone and acetaminophen shows in Fig. 2a. In the Fig. 2b shows that the first derivative difference spectrum. 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 289.6

nm for mephenoxalone and at 252.6 nm for acetaminophen were considered as the optimum working wavelengths for their determination. In the first differential derivative spectrum (Fig. 2b) of acetaminophen shows a well-defined maximum at 252.6 nm, while mephenoxalone has a zero ΔD_1 value at the same wavelength. Mephenoxalone has a ΔD_1 value at 289.6 nm at which acetaminophen exhibits no contribution.

Under the experimental conditions described, the graphs obtained by plotting ΔD_1 values versus concentration (in the range stated in Table 1) show linear relationships. Regression analysis using the method of least squares was made for the slope, intercept and correlation coefficient values. Separate determinations at different concentration levels were carried out for each drug to test reproducibility of the ΔD_1 values. The Relative Standard Deviations (RSDs) were found to be less than 1.05% Table 1. To prove the validity and applicability of the proposed methods, ten synthetic mixtures in the concentration range stated in Table 2 were assayed using procedures. The results obtained using the above methods were precise and accurate (Table 2). The present methods were applied for the simultaneous determination of the above-mentioned mixture in its commercial tablet form. The results obtained show the high reliability and reproducibility of the methods (Table 2).

5.2. Ratio spectra derivative spectrophotometry

In Fig. 3a are showed the ratio spectra of different mephenoxalone standards (spectra divided by the spectrum of a 25.0 μg ml^{-1} acetaminophen solution) in methanol. Then, the first derivatives of ratio-spectra were recorded in Fig. 3b and the values of the derivatives were measured at suitably selected wavelengths to the mephenoxalone concentration. As the noise levels were small a smoothing function was not needed. The influence of the $\Delta\lambda$ for obtaining the first derivative was tested and an $\Delta\lambda = 8$ nm was considered as suitable. The concentration of divisor (mephenoxalone in this case) can be modified, and different calibration graphs are then obtained. A concentration of 25.0 μg ml^{-1} of acetaminophen

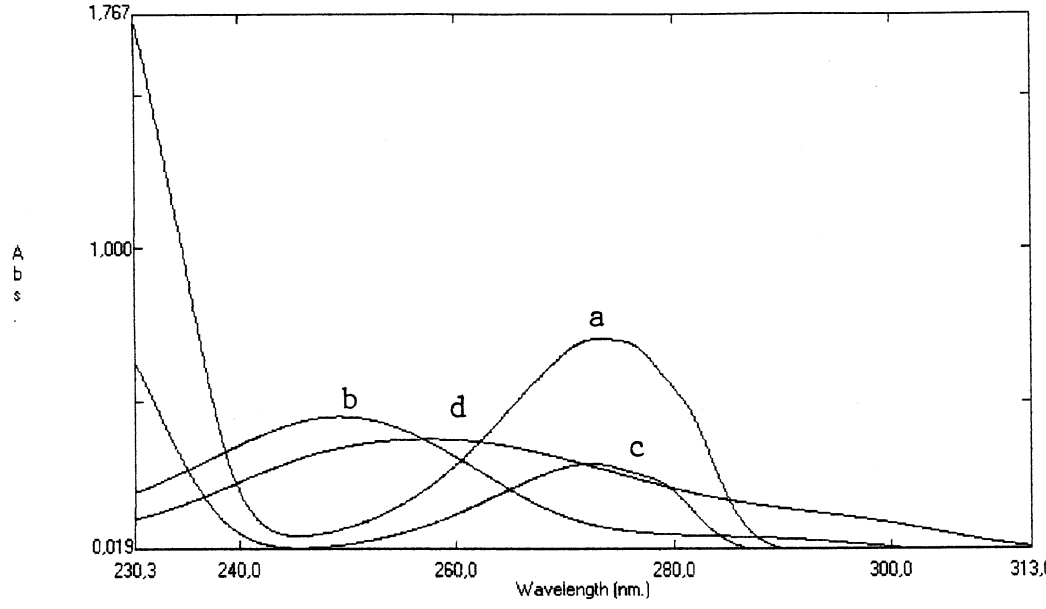

Fig. 1. Zero-order spectra of (a) 12.5 μg ml^{-1} mephenoxalone, (b) 25.0 μg ml^{-1} acetaminophen in methanol and, (c) 12.5 μg ml^{-1} mephenoxalone, (d) 25.0 μg ml^{-1} acetaminophen in 0.1 N NaOH.

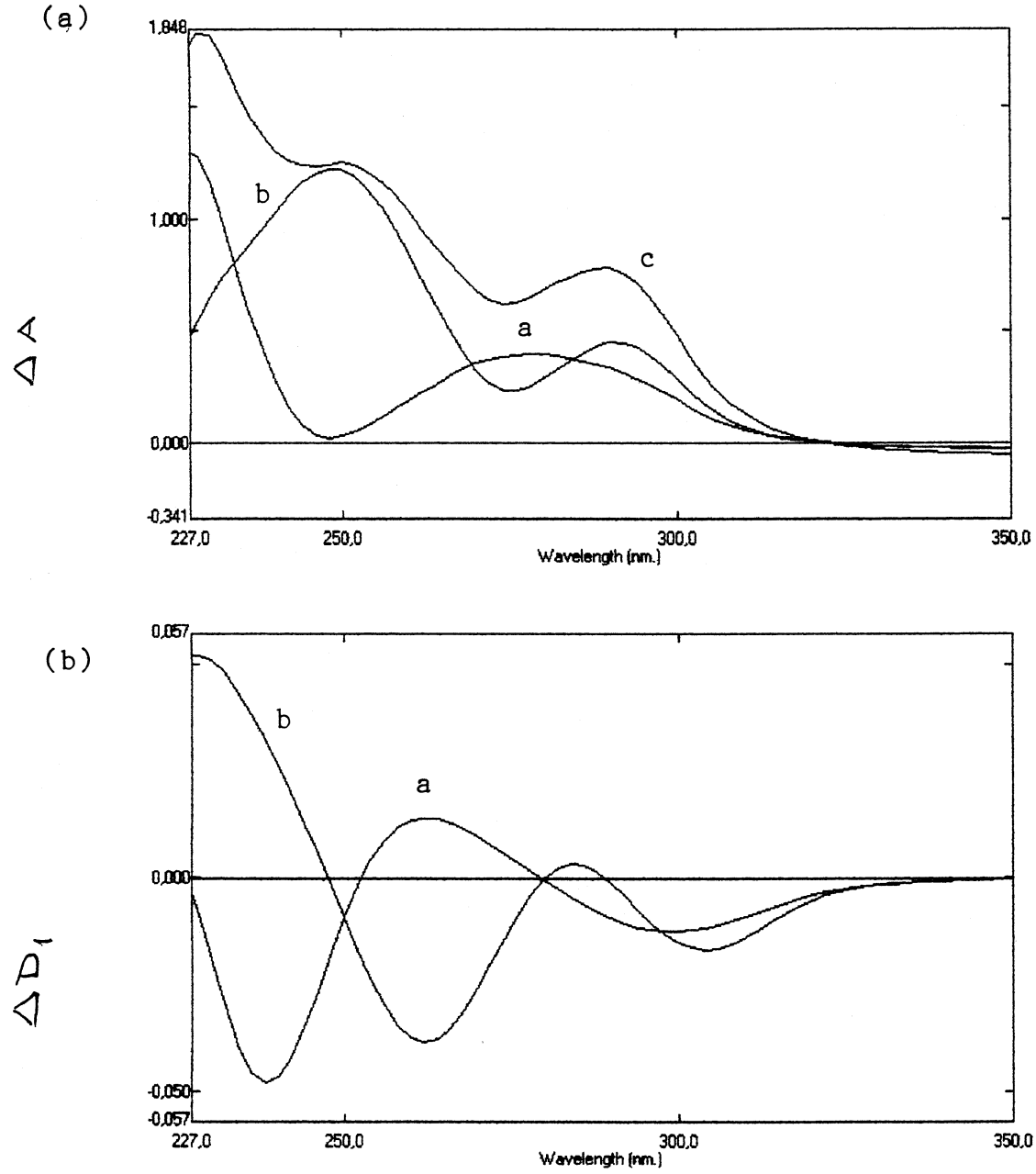

Fig. 2. (a) Differential spectra of (a) 12.5 μg ml^{-1} mephenoxalone, (b) 25.0 μg ml^{-1} acetaminophen and, (c) mixture of (12.5 μg ml^{-1} mephenoxalone and 25.0 μg ml^{-1} acetaminophen) in methanol versus 0.1 N NaOH. (b) Differential derivative spectra of (a) 12.5 μg ml^{-1} mephenoxalone, (b) 25.0 μg ml^{-1} acetaminophen in methanol versus 0.1 N NaOH.

was considered as suitable. The calibration graph was established by measuring at the amplitude at 233.5 nm corresponding to a minimum wavelength in Fig. 4b. Several mixture compositions of mephenoxalone and acetaminophen were prepared and tested between 5.0–50.0 μg ml^{-1} for

mephenoxalone in their binary mixtures shown in Table 1.

For determining the other component acetaminophen an analogous procedure was followed. I obtained the ratio spectra of different acetaminophen standards (spectra divided by the spectrum of a 12.5 μg ml^{-1} mephenoxalone solution) and their first derivatives were calculated. The calibration graph was obtained by measuring the amplitude at 288.9 nm corresponding to a maximum wavelength (Fig. 4b). The proposed were also applied for the determination of mephe-

Table 1
Optical characteristics, precision and accuracy

Parameters	Mephenoxalone		Acetaminophen	
	Differential derivative spectra	Ratio derivative spectra	Differential derivative spectra	Ratio derivative spectra
Range (μg ml^{-1})	5.0–50.0	5.0–50.0	5.0–50.0	5.0–50.0
Detection limits (μg ml^{-1})	2.0	2.0	2.0	2.0
Regression equation (Y)[a]				
Slope (b)	6.54×10^{-1}	1.24×10^{-1}	9.3×10^{-2}	8.42×10^{-1}
S.D. on slope (S_b)	7.80×10^{-4}	5.45×10^{-4}	2.58×10^{-5}	4.62×10^{-5}
Intercept (a)	9.87×10^{-1}	2.45×10^{-1}	7.11×10^{-1}	6.34×10^{-1}
S.D. on intercept (S_a)	7.39×10^{-3}	1.55×10^{-3}	1.37×10^{-3}	2.89×10^{-3}
S.E. of estimation (S_e)	4.58×10^{-3}	3.39×10^{-3}	1.30×10^{-3}	3.74×10^{-3}
Correlation coefficient (r)	0.9997	0.9986	0.9990	0.9989
R.S.D. (%)[b]	0.35	0.97	1.05	2.03
% Range of error[b] (% 95 confidence limit)	0.98	0.49	0.85	0.69

[a] $Y = a + bC$ where C is concentration in μg ml^{-1} and Y in absorbance units.
[b] Five replicate samples.

Table 2
Assay results for the determination of mephenoxalone and acetaminophen in laboratory synthetic mixture and commercial tablets[a]

Sample	Recovery (mean ± S.D.)%[a]			
	Mephenoxalone		Acetaminophen	
	Differential derivative spectra	Ratio derivative spectra	Differential derivative spectra	Ratio derivative spectra
Synthetic mixtures	99.7 ± 1.79	99.0 ± 0.79	99.0 ± 0.93	98.8 ± 0.29
t	0.198[b]		0.319[b]	
F	0.927[b]		0.040[b]	
Commercial tablets[c]	99.7 ± 1.88	99.6 ± 0.81	98.8 ± 1.66	98.8 ± 0.98
t	0.836		0.955	
F	0.576		0.9157	

[a] Mean and R.S.D. for ten determinations; percentage recovery from the label claim amount.
[b] Theoretical values at $P = 0.95$. Theoretical values at % 95 confidence limits $F = 3.18$; $t = 2.26$
[c] Dorsilon® tablet are the product of ilsan Pharm. Ind., Turkey; each one tablet was labelled to contain 200.0 and 450.0 mg of mephenoxalone and acetaminophen, respectively.

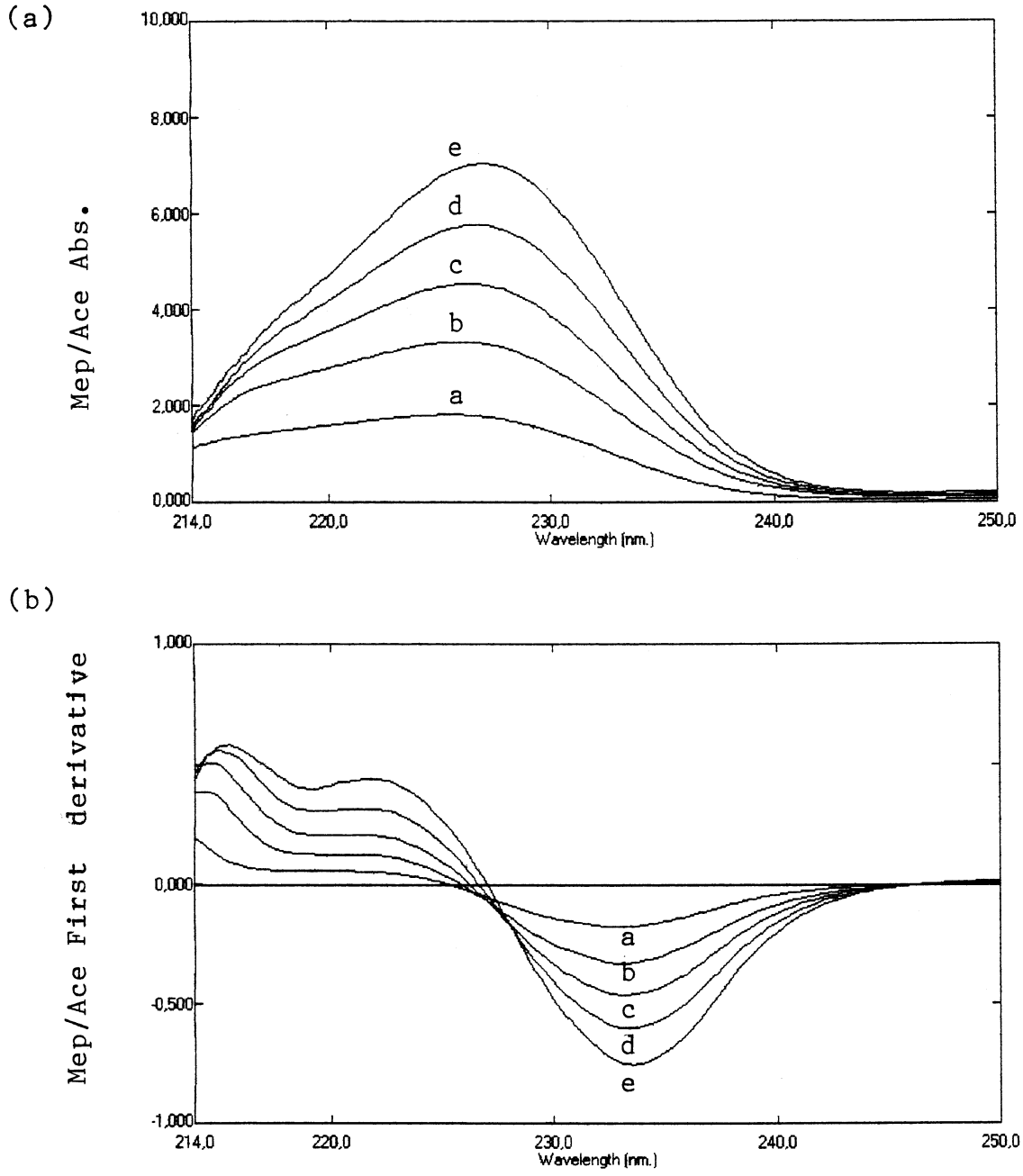

Fig. 3. (a) Ratio spectra of mephenoxalone of (a) 5.0 μg ml^{-1}, (b) 17.5 μg ml^{-1}, (c) 25.0 μg ml^{-1}, (d) 37.5 μg ml^{-1}, (e) 50.0 μg ml^{-1}, when 25.0 μg ml^{-1} acetaminophen used as divisor in methanol ($\Delta\lambda = 4$ nm). (b) First derivative of the ratio spectra of mephenoxalone of (a) 5.0 μg ml^{-1}, (b) 17.5 μg ml^{-1}, 25.0 μg ml^{-1}, (d) 37.5 μg ml^{-1}, (e) 50.0 μg ml^{-1}, when 25.0 μg ml^{-1} acetaminophen used as divisor in methanol ($\Delta\lambda = 4$ nm).

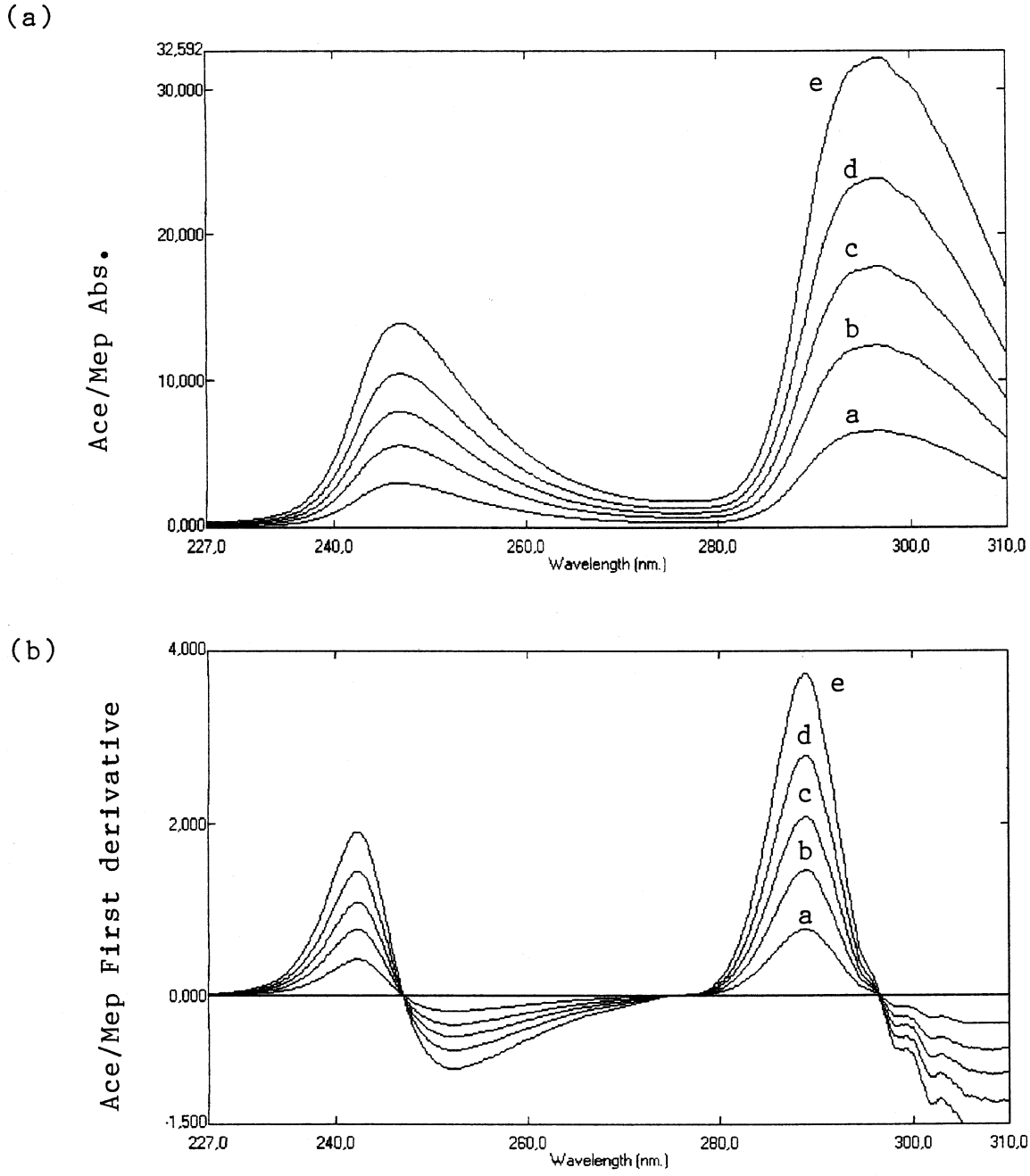

Fig. 4. (a) Ratio spectra of acetaminophen of (a) 5.0 μg ml^{-1}, (b) 17.5 μg ml^{-1}, (c) 25.0 μg ml^{-1}, (d) 37.5 μg ml^{-1}, (e) 50.0 μg ml^{-1}, when 12.5 μg ml^{-1}, mephenoxalone used as divisor in methanol ($\Delta\lambda = 4$ nm). (b) First derivative of the ratio spectra of acetaminophen of (a) 5.0 μg ml^{-1}, (b) 17.5 μg ml^{-1}, (c) 25.0 μg ml^{-1}, (d) 37.5 μg ml^{-1}, (e) 50.0 μg ml^{-1}, when 12.5 μg ml^{-1}, mephenoxalone used as divisor in methanol ($\Delta\lambda = 4$ nm).

noxalone and in pharmaceutical products. The results obtained show the high reliability and reproducibility of the methods.

In order to demonstrate the validity and applicability of the proposed methods (differential derivative spectrophotometry and ratio spectra derivative spectrophotometry), recovery studies were performed by analyzing synthetic mixtures that reproduced the composition of the commercial tablets. The results obtained (Table 2) were statistically compared using Student t- and the F-tests. As shown from the table, the calculated r- and F-values were less than the theoretical values, indicating no significant difference between the two methods. Commercially available tablets containing a mixture of mephenoxalone and acetaminophen were analyzed using the developed methods. The results are summarized in Table 2.

The optical characteristics and figures of merit are given in Table 1. The values obtained by the proposed methods for formulations are compared in Table 2, together with the results of recovery experiments.

6. Conclusions

Mephenoxalone and acetaminophen were simultaneously determined in pharmaceutical formulations using two different analytical techniques. The methods developed are simple, accurate, and specific. Differential derivative spectrophotometry and ratio derivative spectrophotometry may be recommended for routine and quality control analysis of the investigated drugs in two-component pharmaceutical preparations.

References

[1] R.A. Sodhi, J.L. Chawla, R. Sane, Indian Drugs 33 (6) (1996) 280–285.
[2] K.M. Thomas, D.A. Dabholkar, C.L. Jain, East Pharm. 36 (421) (1993) 177–178.
[3] G. Indrayanto, A. Sunarto, Y. Adrian, J. Pharm. Biomed. Anal. 13 (12) (1995) 1555–1559.
[4] W. Yong, G. Feng, G. Tang, H. Wu, Sepu 14 (4) (1996) 288–290.
[5] G. Liang, H.A. Qian, L. Zhu, Y. Wang, L. Sun, Zhongguo Yaoxue Zazhi 29 (8) (1994) 46–48.
[6] S.V. Erram, H.P. Tipnis, Indian Drugs 30 (3) (1993) 116–119.
[7] D. Mundhe, S.G. Kaskhedikar, East Pharm. 38 (452) (1995) 181–182.
[8] N.M. El Kousy, L.I. Ibrahim, J. Drug Res. 21 (1–2) (1994) 143–154.
[9] N. Erk, Sci. Pharm. 64 (1996) 173.
[10] N. Erk, F. Onur, Anal. Lett. 30 (6) (1997) 1201–1210.
[11] M.S. Bhatia, S.R. Dhaneshwar, Indian Drugs 32 (9) (1995) 446–450.
[12] M.A. Memon, M.A. Memon, M.U. Dahot, Sci. Int. 7 (1) (1995) 55–57.
[13] Y. Liang, J. Sun, Fenxi Huaxue 22 (4) (1994) 359–362.
[14] R.T. Sane, S.R. Surve, M.G. Gangrade, V.V. Bapat, N.L. Chonkar, Indian Drugs 30 (2) (1993) 66–72.
[15] B. Morelli, J. Pharm. Sci. 77 (1988) 615.
[16] B. Morelli, J. Pharm. Sci. 77 (1988) 1042.
[17] B. Morelli, J. Pharm. Sci. 84 (1995) 34.
[18] T.D. Doyle, F.R. Fazzari, J. Pharm. Sci. 63 (1974) 1921.
[19] A.M. Wahbi, M. Barary, H. Mahgab, M.A. El-Sayed, J. Assoc. Off. Anal. Chem. 68 (1985) 1045.
[20] A.G. Davidson, L.M.M. Mkoji, J. Pharm. Biomed. Anal. 6 (1988) 449.
[21] C.V.N. Prasad, A. Gautam, V. Bharadwaj, P. Parimoo, Talanta 44 (1997) 917–922.
[22] F. Salinas, J.J. Berzas Nevado, M.A. Espinosa, Talanta 37 (1990) 347–351.
[23] J.J. Berzas Nevado, C.C. Guiberteau, F. Salinas, Talanta 39 (1992) 547–553.