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The rapid quantitative analysis of phenprobamate and acetaminophen by RP-LC and compensation technique

M. Levent Altun [a], Nevin Erk [b,*]

[a] *Department of Pharmacognosy, Faculty of Pharmacy, University of Ankara, 06100 Ankara, Turkey*
[b] *Department of Analytical Chemistry, Faculty of Pharmacy, University of Ankara, 06100 Ankara, Turkey*

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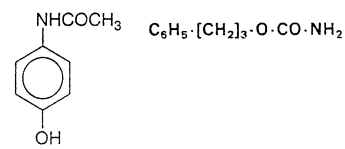
Abstract

For the analysis of phenprobamate and acetaminophen in combination, the main analytical methods used were spectrophotometric compensation technique and Vierordt's method with high performance liquid chromatography, used as an analytical reference method. The first procedure for the simultaneous quantitative determination phenprobamate and acetaminophen by high performance liquid chromatographic (HPLC) method was proposed. The method was standardized using a LiChrosorb® RP18-5 column, methanol–water–formic acid (120:80:1 v/v), apparent pH 4.25 with triethylamine, as mobil phase and UV detection at 254 nm. The peak area response versus concentration was linear in a concentration range from 4 to 28 μg ml^{-1} of phenprobamate and from 4 to 30 μg ml^{-1} for acetaminophen. The correlation coefficients were 0.9999 for phenprobamate and 0.9987 for acetaminophen. The second procedure, based on the compensation technique, is presented for the derivative spectrophotometric determination of binary mixtures with overlapping spectra. The proposed methods, which give thoroughly comparable data, are simple and rapid and allow precise and accurate results.

Keywords: Phenprobamate; Acetaminophen; Simultaneous determination; High performance liquid chromatography; Compensation technique

1. Introduction

Acetaminophen **(1)**, *N*-(4-hydroxyphenyl)acetamide, is the active metabolite of phenacetin, a so-called coaltar analgesic. Phenprobamate **(2)**, 1-carbamoyloxy-3-phenylpropane, is an anxiolytic agent. A combination dosage form of acetaminophen and phenprobamate is indicated in analgesic and myorelaxan preparations.



(1) **(2)**

* Corresponding author. Tel.: +90-312-2305000; fax: +90-312-231081.
E-mail address: erk@pharmacy.ankara.edu.tr (N. Erk).

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Different reported methods for the quantitative determination of these compounds, alone or in combination with other drugs, have been used. These methods use high performance liquid chromatography (HPLC) [1–6], spectrophotometric methods [7–10], capiller electrophotometry [11], polarography [12] and gas chromatography [13] in pharmaceutical preparations, either separately or in combination with other drugs so far. Phenprobamate and acetaminophen in compound preparations cannot be directly determined by conventional spectrophotometric methods because of the significant overlapping in the spectra.

A chromatographic method for the simultaneous determination of acetaminophen and phenprobamate in pharmaceutical preparations dosage forms, which presented a very good resolution in the chromatographic separation of both drugs, was developed in this work.

The compensation method [14,15] is a non-mathematical method for the detection and elimination of unwanted absorption during spectrophotometric analysis. In binary mixture analysis, the compensation method involves a comparison of several difference spectra (mixture–reference) using different concentrations of a reference solution in the reference cell. Hence, if A_m and A_r refer to the absorbances of the relevant cells against air at the same wavelength λ, then $\Delta A_\lambda = A_{m\lambda} - A_{r\lambda}$, where $A_m = A_a + A_b$ at a given wavelength λ, a and b refer to components a and b, respectively and A_r refers to A_a or A_b. If C_r for compound a is introduced into the reference cell, the absorption characteristics of the mixture gradually approach that of compound b as c_a increases and finally coincides with the absorption curve of compound b at the end-point, for which $c_r = c_a$ and by analogy c_b can be found by repeating the same steps using c_r for compound b in the reference cell. The accuracy of the method depends on the evaluation of the balance point [16,17]. In the Vierordt's method [18,19], A_1^1 (1%, 1 cm) values of acetaminophen and phenprobamate were determined at 249.2 and 259.2 nm in the zero-order spectra. The utility of the developed methods to determine the contents of pharmaceutical formulation is demonstrated.

2. Experimental

2.1. Apparatus

The HPLC instrument was equipped with a JASCO model PU-980 pump with a 7725 Rheodyne value injector 20 μl fixed loop and a JASCO UV-975 UV/VIS detector. The detector was set at 254 nm (0.02 absorbance units full scale (aufs)) and peak areas were integrated automatically by computer, using the Borwin software programme.

A double beam, Shimadzu 1601 spectrophotometer model with a fixed slit width (2 nm) connected to an IBM-PC computer loaded with a Lexmark printer was used for all the absorbance signals and treatment of data.

Other apparatus used included a Radiometer NEL pH 890 pH meter digital equipped with a combined glass–calamol electrode and ultrasound generator.

2.2. Reference substances, reagents and solutions

Authentic samples of phenprobamate and acetaminophen were kindly donated by Abdi Ibrahim Pharm. Ind., Turkey. The solvents used in the mobile phase were of HPLC grade. All other chemicals were of analytical-reagent grade.

2.3. Chromatographic conditions

The mobil phase used was methanol–water–formic acid solution in the ratio of 120:80:1 v/v, adjusted to pH 4.25 with triethylamine. All determinations were performed at ambient temperature (25°C) using C_{18}, 200 × 4.6 mm i.d., LiChrosorb® RP18-5 column. The column effluent was monitored at 254 nm, which represents the wavelength of maximum absorbancy of phenprobamate, acetaminophen and the sensitivity was set at 0.02 aufs. The injection volume was 20 μl, with a flow rate of 1 ml min^{-1}.

2.4. Pharmaceutical preparation

A commercial pharmaceutical preparation, KUIFLEX® sugar-coated tablets (produced by Abdi Ibrahim Pharm. Ind., batch no: BD1578,

containing 200 mg of phenprobamate, 200 mg of acetaminophen per sugar-coated tablet) was assayed.

2.5. Standards solutions and calibration graphs for chromatographic procedure

Standard solutions of phenprobamate, acetaminophen containing concentration ranges of 4.0–28.0 and 4.0–30.0 μg ml^{-1}, respectively, were prepared in the mobil phase. Triplicate 20-μl injections were made for each solution and the peak area ratio of each drug was plotted against the corresponding concentration to obtain the calibration graph.

2.6. Sample preparation for HPLC

Ten sugar-coated tablets were accurately weighed and powdered in a mortar. A portion of the powder equivalent to about one sugar-coated tablet was weighed accurately, transferred to a 100-ml calibrated flask and either suspended in the mobile phase. The flasks were completed to volume with the same solvent. The samples were filtered through a 0.45 μm membrane filter, then further diluted to suit the calibration graphs for HPLC.

2.7. Determination of standard ratios for compensation method

The first derivative spectra for each set of reference solutions using the appropriate solution were recorded. The first derivative maxima ($^1D_{\lambda 1}/^1D_{\lambda 2}$), where appropriate at the specified wavelengths (λ_1 and λ_2) as indicated in parentheses in Table 3.

2.8. Sample preparation for HPLC

Ten sugar-coated tablets were accurately weighed and powdered in a mortar. A portion of the powder equivalent to about one sugar-coated tablet was weighed accurately, transferred to a 100-ml calibrated flask and either suspended in the methanol. After 30 min of mechanically shaking, the solution was filtered through Whatman No. 42 filter paper. The flasks were completed to volume with the same solvent. The samples were used in the preparation of calibration graphs for compensation and Vierordt's methods.

2.9. Spectrophotometric measurements

2.9.1. Compensation technique

A series of solutions containing different concentrations of pure drugs, phenprobamate above and below, that presented in the binary mixture solution was prepared and placed in succession in the reference cell. The solution of the mixture (containing compounds phenprobamate and acetaminophen) was placed in the sample cell. The first (^1D) absorption spectra of the solutions prepared were recorded and calculated to the corresponding ratio (Table 3) in each instance and then the calculated ratio for pure compound acetaminophen was followed.

The exact balance point (the ratio of the sample is equal to that of pure compound acetamino-

Table 1
Analytical data of the calibration graphs for the determination of phenprobamate and acetaminophen by high-performance liquid chromatography

Compound	Linearity range (μg ml^{-1})	Regression equation ($D = a + bC$)[a]	r[b]	$S_{y/x}$[c]	S_a[d]	S_b[e]
Phenprobamate	4.0–28.0	$D = 0.04 + 8.89C$	0.9999	0.587	0.593	0.056
Acetaminophen	4.0–30.0	$D = 0.59 + 3.25C$	0.9987	0.459	0.111	0.089

[a] Peak area versus concentration C of each drug in μg ml^{-1}; standard specimens: $n = 5$.
[b] Correlation coefficient
[c] $S_{y/x}$, relative S.D.
[d] S_a, S.D. of intercept of regression line.
[e] S_b, S.D. of slope of regression line.

Table 2
Assay results of phenprobamate and acetaminophen in laboratory-made mixtures and in commercial sugar-coated tablets[a]

Sample	Recovery (mean ± S.D.)%[b]					
	Phenprobamate			Acetaminophen		
	HPLC	Compensation method	Vierordt's method	HPLC	Compensation method	Vierordt's method
Synthetic mixtures	99.16 ± 0.78	99.30 ± 0.48	98.01 ± 0.39	99.91 ± 0.87	98.60 ± 0.55	99.00 ± 0.40
	$t = 0.098$	0.658		$t = 0.412$	0.987	
	$F = 1.897$	1.637		$F = 2.242$	1.987	
Commercial tablets[c]	99.15 ± 0.17	99.41 ± 0.56	99.00 ± 0.29	99.17 ± 0.25	99.57 ± 0.88	99.33 ± 0.34
	$t = 1.024$	0.874		$t = 0.131$	0.598	
	$F = 1.007$	1.002		$F = 1.047$	0.657	

[a] Values in parentheses are the theoretical values at $P = 0.95$. Theoretical values at 95% confidence limits $F = 3.18$; $t = 2.26$.
[b] Mean and relative S.D. for ten determinations; percentage recovery from the label claim amount.
[c] KUIFLEX® sugar-coated tablets were labeled to contain 200 mg acetaminophen, 200 mg phenprobamate per tablets, respectively.

phen) at which the concentration of compound phenprobamate in the sample solution is equal to that in the reference solution was determined. For the similar procedure, the same steps using solutions of pure compound acetaminophen in the reference cell in order to determine its concentration in the binary mixture at the balance point was followed.

2.9.2. Vierordt's method

Absorptivity A_1^1 (1%, 1 cm) values for each compound in the binary mixtures at zero-order spectra were calculated by using the absorbances measured at the appropriate wavelengths in methanol. Similarly, the absorbances of the mixed sample solutions were measured and then the concentration of each compound was calculated from the following simultaneous equations:

$$A_1 = \alpha_1 \cdot C_{\text{Phe}} + \beta_1 \cdot C_{\text{Ace}} \quad A_2 = \alpha_2 \cdot C_{\text{Phe}} + \beta_2 \cdot C_{\text{Ace}}$$

where C_{Phe} and C_{Ace} are the concentrations of two compounds, 'phen' and 'ace', in binary mixtures calculated as 100 g ml^{-1}. A denotes the absorbance of the mixture solution, α and β represents the values of A_1^1 (1%, 1 cm) for ingredients. The subscripts 1 and 2 refer to $\lambda_1 = \lambda_{\max} = 259.2$ nm of phenprobamate compound and $\lambda_2 = \lambda_{\max} = 249.2$ nm of acetaminophen compound, respectively.

2.10. Standards solutions and calibration graphs for spectrophotometric measurements

Stock solutions were prepared by dissolving phenprobamate and acetaminophen in methanol to obtain a concentration of 1 mg ml^{-1} for each compound. The standard solutions were prepared by dilution of the stock solutions in methanol to reach concentration ranges in the compensation method 6.0–22.0, 5.0–25.0 μg ml^{-1} and in the Vierordt's method, 10.0–30.0 and 5.0–25.0 μg ml^{-1} for phenprobamate and acetaminophen, respectively.

3. Results and discussion

3.1. High-performance liquid chromatography

The purpose of this paper was to develop a specific, fast and reproducible determination of both phenprobamate and acetaminophen in a single run. The mobile phase, containing methanol–water–formic acid (120:80:1 v/v) was adjusted to

Table 3
Experimental parameters calculated for the simultaneous determination of phenprobamate and acetaminophen in binary mixture by compensation method

Preparation	Linearity range (μg ml^{-1})	Ratio	Mean*	RSD (%)
Phenprobamate	6.0–22.0	^1D (236)/^1D(262)	0.704	1.691
Acetaminophen	5.0–25.0	^1D(266)/^1D(269)	0.481	0.855

* Mean of ten separate determinations.

pH 4.25 with triethylamine. As shown in Fig. 1, at a flow rate of 1 ml min^{-1}, the retention times were 2.35 min for acetaminophen and 4.23 min for phenprobamate in combined pharmaceutical dosage forms. The optimum wavelength of 254 nm was selected in order to permit the simultaneous determination of both active substances in the sugar-coated tablets. Under the experimental conditions described, standard calibration curves for phenprobamate and acetaminophen were constructed by plotting absorbance versus concentration. Conformity with Beer's law was evident in the concentration range from 4 to 28 μg ml^{-1} of phenprobamate and from 4 to 30 μg ml^{-1} of acetaminophen. The regression curve was calculated by the least-squares method. The correlation coefficients were 0.9999 for phenprobamate and 0.9987 for acetaminophen, indicating good linearity. Five replicate determinations at different concentration levels were carried out to test the precision of the methods. The relative S.D. were found to be $<0.587\%$, indicating reasonable repeatability of the selected method. The detection limits (LOD) [20] were 0.78 μg ml^{-1} for phenprobamate and 0.056 μg ml^{-1} for acetaminophen, while the quantification limits (LOQ) [21] were 0.100 μg ml^{-1} for phenprobamate and 0.156 μg ml^{-1} for acetaminophen. Results of HPLC analysis of laboratory-prepared mixtures with different proportions of sugar-coated tablets are given in Table 2.

3.2. Compensation technique

The first derivative spectra of phenprobamate and acetaminophen in the 230–280 nm wavelength region are shown in Fig. 3. The first derivative spectra were recorded for each reference solution of the analyte components and the ratios of the ^1D maxima and ^1D maxima were calculated.

Table 3 shows the mean values of the ratios calculated for ten different determinations of each standard solution. The ratios are constant, characteristic of the pure substance, independent of concentration and whether another absorbing component is present. The determination of phenprobamate concentrations in phenprobamate–acetaminophen mixtures, the sample cell was filled with the mixture solution and the reference cell was filled, in succession, with a series of reference phenprobamate solutions with different concentrations. The ratios of the mixture calculated from the recorded ^1D spectra was compared with those of acetaminophen. At the balance point, the ratio of the mixture corresponds to that of acetaminophen, where the concentration of phenprobamate

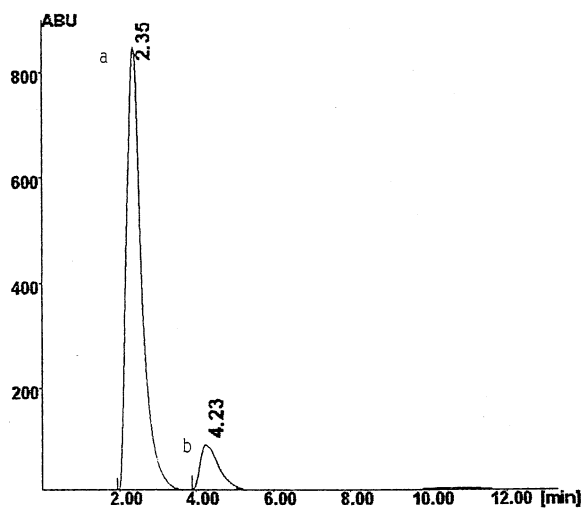

Fig. 1. A typical chromatogram of Kuiflex® sugar-coated tablet. (a) Acetaminophen; and (b) phenprobamate.

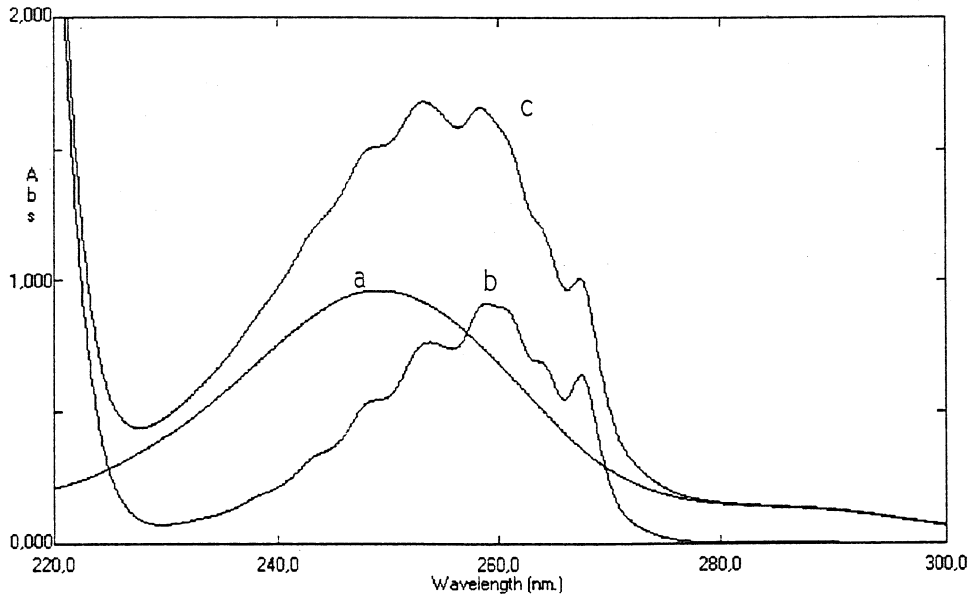

Fig. 2. Zero-order spectra of (a) 20 μg ml^{-1} acetaminophen; (b) 20 μg ml^{-1} phenprobamate; and (c) their mixture in methanol.

in the mixture in the sample cell is equal to that of the reference solution in the reference cell. For determining the other component, the same steps, using solutions of pure compound acetaminophen in the reference cell to determine its concentration in the mixture at the balance point, should be followed. Conformity with Beer's law was evident in the concentration range from 6 to 22 μg ml^{-1} of phenprobamate and from 5 to 25 μg ml^{-1} of acetaminophen. The detection limits (LOD) were 0.64 μg ml^{-1} for phenprobamate and 0.088 μg ml^{-1} for acetaminophen, while the quantification limits (LOQ) were 1.19 μg ml^{-1} for phenprobamate and 0.91 μg ml^{-1} for acetaminophen. Results of HPLC analysis of laboratory-prepared mixtures with different proportions of sugar-coated tablets are given in Table 2.

3.3. Vierordt's method

Fig. 2 show zero-order UV absorption spectra of phenprobamate and acetaminophen. It is evident that the two zero-order spectra of the compounds overlap greatly in the wavelength region 230–280 nm.

Table 4 shows the experimental parameters obtained by using the zero-order absorption spectra for the standard solutions of active substances. Conformity with Beer's law was evident in the concentration range from 10 to 30 μg ml^{-1} of phenprobamate and from 5 to 25 μg ml^{-1} of acetaminophen. The detection limits (LOD) were 0.99 μg ml^{-1} for phenprobamate and 0.159 μg ml^{-1} for acetaminophen, while the quantification limits (LOQ) were 1.99 μg ml^{-1} for phenprobamate and 0.89 μg ml^{-1} for acetaminophen. The application of the Vierordt's method to the mixed sample solutions prepared by adding known amounts of active substances yielded mean percent recoveries of 98.01 $\pm$ 0.39 and 99.00 $\pm$ 0.40 in phenprobamate–acetaminophen and its co-existing compounds, respectively (Table 2).

The HPLC method was chosen as the analytical reference method. Compensation and Vierordt's methods were compared with HPLC method. The results obtained were summarized in Table 2. No significant differences were found between the results obtained by the HPLC method, the compensation method and Vierordt's method, for the same batch at the 95% confidence level (Student's t-test and F-variance ratio test).

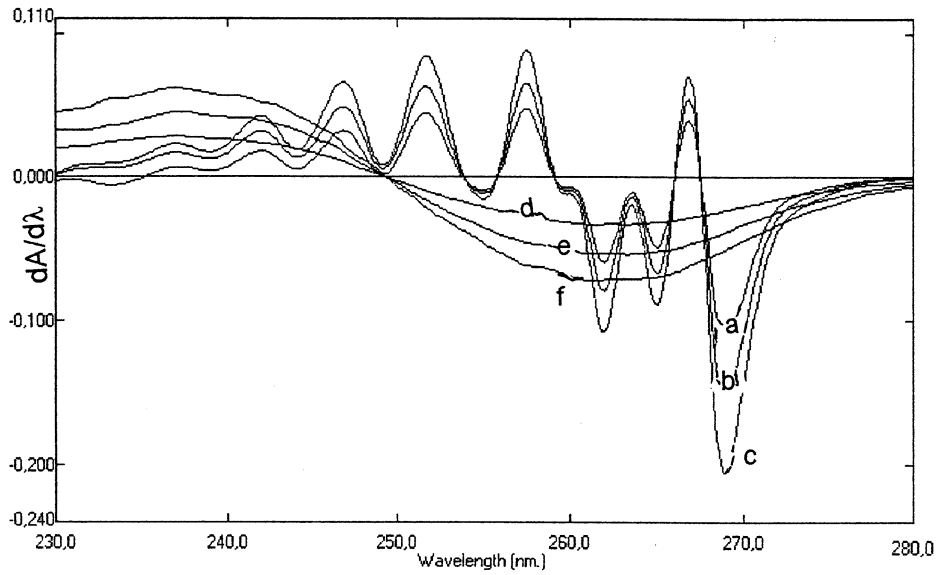

Fig. 3. First-derivative spectra of (a) 20 μg ml^{-1} acetaminophen; and (b) 20 μg ml^{-1} phenprobamate in methanol.

The stability of each component in its binary mixture is investigated by preparing a mixture containing phenprobamate and acetaminophen and calculating the contents of each component at different time intervals. The results indicate that these mixtures are stable for 1 h. The absorbance of the solutions were taken within 1 h of their preparation.

4. Conclusions

High-performance liquid chromatography and spectrophotometric methods (compensation technique, Vierordt's method) were found to be reproducible and accurate in the reliable analysis of combination of phenprobamate and acetaminophen, either in pure form or in sugar-coated tablets. There was no evidence of interference from excipients in the sugar-coated tablets analysed. All the proposed methods were linear with good reproducibility and sensitivity. The preferred method is the HPLC. The HPLC method gives a good resolution between phenprobamate and acetaminophen within a short analysis time. In general, all the proposed methods can be applied for either content uniformity or routine quality control.

Table 4
Experimental parameters calculated for the simultaneous determination of phenprobamate and acetaminophen in binary mixture by Vierordt's method

λ (nm)	Phenprobamate		Acetaminophen	
	α_2	α_2	β_1	β_2
λ_1: 259.2	1312	–	2242	–
λ_2: 249.2	–	428	–	2536
Linearity range (μg ml^{-1})	10.0–30.0		5.0–25.0	

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