

Application of orthogonal functions to the spectrophotometric determination of phenytoin and phenobarbitone in pharmaceutical preparations

M. M. AMER, A. K. S. AHMAD AND S. M. HASSAN*

Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Cairo, and the *Pharmaceutical Chemistry Department, Faculty of Pharmacy, Mansura University, Mansura, Egypt

Orthogonal polynomial functions have been used in the simultaneous determination of mixtures of phenytoin and phenobarbitone in pharmaceutical preparations. The mean recovery (9 determinations) and coefficient of variation were $100.0 \pm 1.4\%$ for phenobarbitone and $100.0 \pm 1.3\%$ for phenytoin. Neither the ΔA method of Pannderden & Fontein nor a ΔA modification of Vierordt's method of two-component analysis gave results of the same accuracy and precision as the proposed method.

Mixtures of phenytoin and phenobarbitone are frequently prescribed for the control of epilepsy. Such mixtures have been determined by titrimetry (Garrido, 1947), gas chromatography (Dill & Glazko, 1974) and spectrophotometry (Pannderden & Fontein, 1958). In the present paper, orthogonal polynomials (Glenn, 1963) have been used in the simultaneous spectrophotometric determination of this binary mixture.

By the proper selection of the polynomial and wavelength range (Wahbi, 1967) it is possible to determine phenobarbitone from the quadratic polynomial for 6 points over the wavelength range from 226 to 256 nm at 6 nm intervals without interference from phenytoin (Figs 1, 2). The gross absorbance for both

$$p_2 = (5 A_{226} - A_{232} - 4 A_{238} - 4 A_{244} - A_{250} + 5 A_{256})/84 \quad (1)$$

components is measured at 240 nm, i.e. at λ max for phenobarbitone. The contribution of phenobarbitone to the gross absorbance at 240 nm is calculated according to the following equation:

$$A_{ph} = p_{2ph} \cdot \frac{A_r}{p_{2r}} \quad (2)$$

where A_{ph} is the absorbance of phenobarbitone at 240 nm, p_{2ph} is the calculated quadratic coefficient and A_r/p_{2r} is the mean ratio of the absorbance at 240 nm to the calculated p_2 for several different concentrations of pure reference samples of phenobarbitone. The mean ratio as calculated for 6 separate determinations of phenobarbitone was found to be 20.41 (c.v. = 0.86%). By subtraction

of A_{ph} from the gross absorbance at 240 nm, the contribution of phenytoin at 240 nm is calculated, provided that there is no third substance that absorbs appreciably at this wavelength.

To emphasize the latter requirement in pharmaceutical preparations which are mainly hard capsules, four samples of synthetic mixtures of phenobarbitone and phenytoin, each containing as much as 5 times the usually added amounts of lactose in phenytoin (Parke Davis & Co., containing phenytoin sodium, 100 mg and phenobarbitone 50 mg in each capsule) with phenobarbitone capsules, were analysed by the method given below. The mean percentage recoveries and confidence limits were found to be 101.0 ± 2.8 and 100.5 ± 1.52 for phenytoin and phenobarbitone, respectively ($P = 0.05$).

MATERIALS AND METHODS

Reagents and chemicals: phenobarbitone, phenytoin, borate buffer pH 10 0.05 M, 0.1 N hydrochloric acid, 0.1 N and 0.5 N sodium hydroxide, all chemically pure met B.P. 1968 requirements.

Procedure. Dissolve an accurately weighed quantity of the mixture (or capsules) equivalent to 100 mg of phenytoin as completely as possible in 50 ml of 0.1 N sodium hydroxide in a small beaker. Transfer the solution quantitatively to a 100 ml volumetric flask with the aid of a few ml of borate buffer of pH 10 and make up to volume with the buffer solution. Transfer a 1 ml aliquot to a 100 ml volumetric flask and make up to volume with the buffer solution. Measure the absorbance of the resulting solution in a 1 cm pathlength cell at 226 to 256 nm at 6 nm intervals and also at 240 nm.

* Correspondence.

RESULTS AND DISCUSSION

The simultaneous determination of authentic mixtures of phenytoin and phenobarbitone by the proposed method, in which phenytoin concentrations were varied from 0.6 to 1.6 mg/100 ml and phenobarbitone from 0.8–1.6 mg/100 ml gave mean recoveries of $100.02 \pm 1.03\%$ for phenytoin and

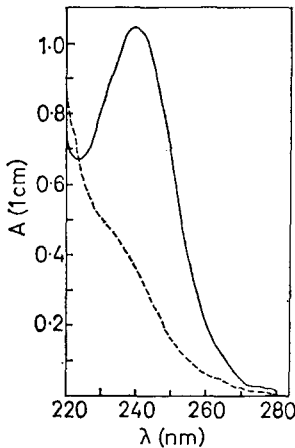


FIG. 1. Absorption curves for 2 mg/100 ml of phenobarbitone (—), and 2 mg/100 ml of phenytoin (---) in buffer solution of pH 10.

100.04 ± 1.06 for phenobarbitone. The results are compared statistically with the method of Pannerden & Fontein (1958) and a proposed two-component ΔA method in Table 1.

The method of Pannerden & Fontein (1958) depends upon measurement of a suitable concentration of the mixture of phenytoin and phenobarbitone in 0.5 N sodium hydroxide and in borate buffer (pH 10) at 240 nm, where the absorbance difference is

Table 1. *Statistical comparison of the results obtained using the orthogonal function method with those of two other possible methods.*

	Phenytoin			Phenobarbitone		
	Pannerden & Fontein method	Δp_2 method	ΔA method	Pannerden & Fontein method	Δp_2 method	ΔA method
Mean and confidence limits (%)	99.85 ± 5.1	100.02 ± 10.3	92.43 ± 6.03	101.15 ± 5.26	100.04 ± 1.06	98.51 ± 1.08
No. of experiments	4	9	5	4	9	5
Tabulated <i>t</i>	2.20	2.18		2.20	2.18	
Actual <i>t</i>	0.14	4.52		0.89	2.24	
Tabulated <i>F</i>	4.1	6.2		4.1	6.2	
Actual <i>F</i>	5.8	13.2		5.8	2.5	

$P = 0.05$ for confidence limits, *t* and *F* tests.

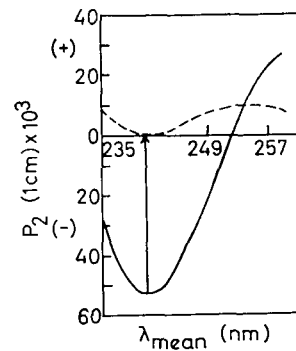


FIG. 2. p_2 -Convolved absorption curves for phenobarbitone (—) and phenytoin (---) as derived from Fig. 1.

taken as a measure of the phenobarbitone content. Phenytoin concentration is calculated by subtracting the absorbance due to phenobarbitone from the gross absorbance at 240 nm.

The other method depends upon measurement of the absorbance difference (ΔA) of the mixture in 0.1 N hydrochloric acid and in 0.1 N sodium hydroxide at 232 and 254 nm, i.e. at the λ_{max} of the ΔA curves for phenytoin and phenobarbitone, respectively, in these solvents (Fig. 3). The concentrations of both components are then calculated by the solution of a pair of simultaneous equations:

$$\Delta A_1 = C_A \Delta \alpha_1 + C_B \Delta \beta_1 \quad \dots \quad (3)$$

$$\Delta A_2 = C_A \Delta \alpha_2 + C_B \Delta \beta_2 \quad \dots \quad (4)$$

where the subscripts 1 and 2 refer to wavelengths, C_A and C_B are the concentrations of the two components A and B, and α and β are their respective absorptivities. This principle has been adopted for the analysis of morphine and phenol (Wahbi & Farghaly, 1970).

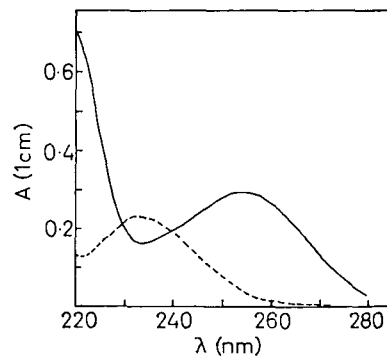


FIG. 3. ΔA Curves for 1 mg/100 ml of phenobarbitone (—), and 2 mg/100 ml of phenytoin (---) 0.1 N sodium hydroxide and 0.1 N hydrochloric acid solvents.

It is evident from Fig. 4 that the assumptions for a good ΔA method, i.e. the difference in absorbance at the analytical wavelength is about 0.43 (Twyman & Lothian, 1933) and that the sum of the absorbances in both media should not exceed 1.0 (Junejo & Glenn, 1956) are not fulfilled. This in addition to the fact that the intensity of absorption of phenobarbitone in strong alkaline solution such as 0.5 N sodium hydroxide falls with time (Sunshine & Gerber, 1963) would explain the inferior results of Pannerden & Fontein's method relative to the orthogonal function method.

However, the two component ΔA method being a modification of Vierordt's method (Stern & Timmons, 1970) suffers from its general drawback,

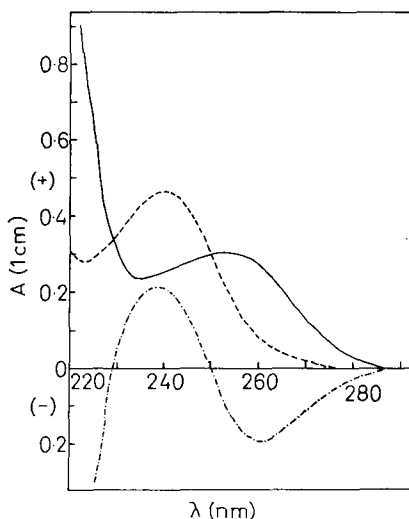


FIG. 4. Absorption curves for 1 mg/100 ml of phenobarbitone in 0.5 N sodium hydroxide (—) and borate buffer of pH 10 (---) and the ΔA curve (— · —) derived therefrom.

namely that it is more sensitive to wavelength errors than is the spectrophotometric determination at a single wavelength. In addition, for this specific case, the unsatisfactory results obtained for phenytoin may be attributed to: (a) The overlapping of the phenobarbitone ΔA curve at the analytical wavelength for phenytoin, and (b) The low contribution of phenytoin to the mixture's total absorption curve.

Table 2. Recovery experiments of added phenytoin and phenobarbitone to phenytoin with phenobarbitone capsules.

Present (mg capsule ⁻¹)		Added (mg capsule ⁻¹)		Recovery %	
Pheno- barbitone	Pheny- toin sodium	Pheno- barbitone	Pheny- toin	Pheno- barbitone	Pheny- toin
51.3	92.5	—	50	—	101.5
		—	100	—	101.0
		50	—	101.64	—
		100	—	100.05	—
53	104	—	50	—	98.3
		—	100	—	100.9
		50	—	101.2	—
		100	—	99.5	—

Moreover, the error in a result of an assay based on a procedure involving four absorbances measured at two wavelengths for the two substances and their mixture would be greater than that in a result based upon two absorbances measured at a single wavelength in the one component ΔA method. In the former case, it is almost certain that some of the absorbance measurements will have to be made on the slopes of the absorption curves.

The results in Table 2 indicate that the proposed orthogonal function method can be applied in routine analysis.

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