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

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A simple ultraviolet spectrophotometric method for the determination of phenytoin in pharmaceutical preparations has been developed. The mean was 100·9% (6 determinations) and the coefficient of variation was 1·2%. The method was applied successfully to the determination of phenytoin in capsules and suspensions.

Methods for the determination of phenytoin include non-aqueous titration (Sellés & Flores, 1955; Ruggeri, 1956; Vincent & Blake, 1958; Rink & Schuster, 1960), argentimetric (Kalinoswka, Podkowska & Mieszczakowska, 1963) and complexometric (Hentrich & Pfeifer, 1967) methods. These methods are non specific and to achieve better selectivity, preliminary purification using either ion-exchange resin (Vincent & Blake, 1958) or partition column chromatography on Celite 535 (Hentrich & Pfeifer, 1967) have been suggested. The B.P. 1973 still recommends a gravimetric method for its determination in tablets.

Although phenytoin has no specific absorption in acid or alkaline media, a hyperchromic effect is demonstrated over the whole spectrum (220–274 nm) on changing the medium from the acid to the alkaline side which allows for the possibility of applying the $\Delta A$ (Aulin-Erdtman, 1955) and $\Delta p_2$ (Abdine, Wahbi & Korany, 1972) methods (Fig. 1).

## MATERIALS AND METHODS

Reagents and chemicals: Diphenylhydantoin (phenytoin); borate buffer 0·05M, pH 10; 0·1 N hydrochloric acid; 0·1 N sodium hydroxide; diethyl ether, solvent grade. All are chemically pure and pass the B.P. 1973 requirements.

### A. *Determination of phenytoin in capsules*

Dissolve an accurate weight of the contents, containing about 100 mg of phenytoin, as completely as possible by stirring for a few minutes with 20 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 the borate buffer solution and make up to volume with the same buffer. Filter, if necessary, transfer two 2-ml aliquots of the solution into two 100 ml volumetric flasks and make up to volume using 0·1 N hydrochloric acid in one flask and the borate buffer of pH 10 in the second flask. Measure the absorbances of a 1 cm pathlength of both solutions in the range 222 to 252 nm with 6 nm intervals and also at 232 nm. Calculate the content of phenytoin (i) from a calibration curve representing $\Delta p_2$ against concentration, where $\Delta p_2$ is the coefficient difference of the acid solution from that at pH 10, and (ii) from a calibration curve of $\Delta A$ *vs* concentration where $\Delta A$ is the absorbance difference at 232 nm. The coefficient $p_2$ is calculated for each solution as follows:

$$p_2 = (5\,A_{222} - A_{228} - 4\,A_{234} - 4\,A_{240} - A_{246} + 5\,A_{252})/84$$

### B. *Determination of phenytoin in suspensions*

Transfer 5 ml of the suspension (equivalent to 30 mg of phenytoin) into a 100 ml separating funnel, add 30 ml of 0·1 N sodium hydroxide, mix well then dilute with 20 ml of distilled water. Pass a stream of carbon dioxide through the solution for about 10 min or until no more phenytoin is precipitated. Extract once with 50 ml of ether, then with further 3 × 20 ml of ether. Wash the combined ether extracts with 2 × 10 ml portions of water and reject the washings. Extract the washed ether layer with 3 × 10 ml of 0·1 N sodium hydroxide followed by 2 × 10 ml of distilled water and reject the ether. Pass a stream of carbon dioxide-free air through the combined aqueous extract until no more ethereal odour is detected. Transfer two 5 ml aliquots of the aqueous solution to two 100 ml

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volumetric flasks and make up to volume with 0·1 N hydrochloric acid in one flask and with the borate buffer (pH 10) in the second flask. Complete as under 'A', starting with the words, 'Measure the absorbances. . . .'.

## DISCUSSION

The results obtained in Table 1, were subjected to statistical analysis. Student's $t$-test showed a non-significant difference between the two mean percentage recoveries; the calculated value of $t$ was found to be 0·8148 (theoretical value 2·228 at $P = 0{\cdot}05$). However, the variance ratio, F, was 8·9528 (theoretical value 5·1 at $P = 0{\cdot}05$) indicating that there is a significant difference between the precision of the two methods. The ΔA method is considered to be less precise than the $\Delta p_2$ method. The coefficient of variation calculated for five separate determinations of $\Delta p_2$ (1%, 1 cm) at the chosen set of wavelengths was found to be 0·94%; the corresponding value for ΔA (1%, 1 cm) at 232 nm was 1·19%.

Table 1. *Application of the $\Delta p_2$ and $\Delta A$ methods to the determination of phenytoin.*

| Sample No. | Used mg/100 ml | Recovery % $\Delta p_2$ | ΔA |
|---|---|---|---|
| 1 | 1·0 | 98·96 | 98·55 |
| 2 | 1·2 | 101·50 | 96·30 |
| 3 | 1·4 | 102·46 | 97·81 |
| 4 | 1·6 | 101·39 | 106·29 |
| 5 | 1·8 | 100·46 | 100·78 |
| 6 | 2·0 | 100·57 | 98·12 |
| Mean and confidence limits ($P = 0{\cdot}05$) | | 100·89 ±1·25 | 99·64 ±3·74 |

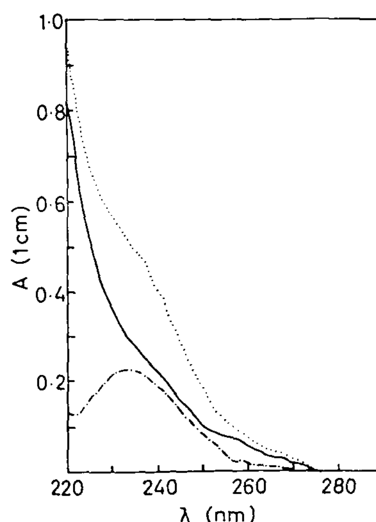


Fig. 1. Absorption curves for 2 mg/100 ml of phenytoin in 0·1 N hydrochloric acid (——), in borate buffer pH 10 (· · · · ·), and the ΔA curve derived from the acid and pH 10 solvents (—·—·—·).

The assumptions that, for a good ΔA method, the difference in absorbance at the analytical wavelength should be not less than 0·43 (Twyman & Lothian, 1933) and that the sum of the absorbances in both media should not exceed 1·0 (Junejo & Glenn, 1956) may explain the less satisfactory results of the ΔA method. In the present case, these two assumptions are not fulfilled as evidenced in Fig. 1.

Table 2. *Recovery experiments of added phenytoin to pharmaceutical preparations. ($\Delta p_2$ method).*

| Sample No. | Preparation and stated concn (mg/capsule or per 5 ml suspension) | Found (mg) | Standard addition (mg) | Recovery % |
|---|---|---|---|---|
| 1 | Capsules (PD) (50 mg) | 53·0 | — | — |
| | | | 60 | 101·50 |
| | | | 100 | 99·12 |
| 2 | Capsules (PD) (100 mg) | 97·0 | — | — |
| | | | 60 | 98·32 |
| | | | 100 | 100·48 |
| 3 | Capsules (Nile Co.) (100 mg) | 102·0 | — | — |
| | | | 80 | 100·86 |
| | | | 100 | 99·97 |
| 4 | Susp. (PD) (30 mg) | 31·64 | — | — |
| | | | 11 | 98·67 |
| | | | 20 | 98·80 |
| 5 | Susp. (PD) (30 mg) | 29·20 | — | — |
| | | | 12 | 100·92 |
| | | | 20 | 99·45 |

PD—Parke Davis & Co.

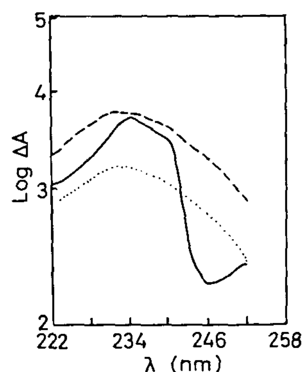


Fig. 2. ΔA curves for phenytoin (2 mg/100 ml) (– – –), phenytoin suspension directly diluted (1·2 mg/100 ml) (——), and phenytoin suspension after ether extraction (1·2 mg/100 ml) (· · · ·) [0·1 N hydrochloric acid and buffer solution of pH 10 solvents].

The method has been applied to the analysis of phenytoin in the presence of lactose; a mean percentage recovery of 101·68 ± 0·95 ($P = 0·05$) was obtained (5 experiments). This indicates that lactose, which is the main potential source of interference in tablets and hard capsules, does not interfere (Table 2).

When the proposed $\Delta p_2$ method for the determination of phenytoin was applied directly to phenytoin suspension without prior purification, erroneous results are obtained. To detect the source of interference, the graph of log $|\Delta A|$ *vs* wavelength of the phenytoin suspension derived from absorbances of solutions in alkaline and acid media was compared with a similar graph of pure phenytoin over the wavelength range 222 to 252 nm. The two graphs were not superimposable indicating that there was interference due to some pH-sensitive absorbing impurities (Fig. 2), but the impurities were removed by the procedure described in the experimental part of this paper (Table 2).

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