J. Pharm. Pharmac., 1972, **24**, 518–521 *Received December* 13, 1971

Application of orthogonal functions to spectrophotometric analysis: The Δp_j method

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A general method is outlined for the use of orthogonal functions to eliminate interference in differential spectrophotometric analysis. The method is particularly useful when (i) the optimum conditions of the ΔA method are not fulfilled and (ii) the irrelevant absorption is linearly dependent upon the spectrum of the pure compound or its shape is completely unknown. The results obtained for the determination of clemizole in tablets are encouraging and suggest that the method warrants a careful study over a wide field of applications.

When properly applied, the ΔA method (Aulin-Erdtman, 1955) gives excellent results in the analysis of a single substance and a mixture of two substances (Wahbi & Farghaly, 1970) in the presence of absorbing impurities. The main limitation of the method centres around the assumption that the quantitative conversion of compound X to another form Y has no influence upon the absorbing impurities, Z, in which case the latter cancels as follows:

$$\Delta A = (A_X + Z) - (A_Y + Z) \quad .. \quad .. \quad .. \quad (1)$$

where A_X and A_Y are the absorbances of 1 cm layers of compounds X and Y at a given wavelength. The concentration c_X is given by

$$c_X = \Delta A/\Delta\epsilon \text{ where } \Delta\epsilon = \epsilon_X - \epsilon_Y \quad .. \quad .. \quad .. \quad (2)$$

and ϵ denotes absorptivity.

As with all spectrophotometric methods, substantial errors arise when absorbances lie outside a satisfactory range. Thus, providing that $(A_Y + Z) \approx 0$, the relative error in ΔA tends toward a minimum when (i) $(A_X + Z) + (A_Y + Z)$ is less than 1·0 (Junejo & Glenn, 1956; Glenn, 1965) and (ii) ΔA is about 0·43 (Twyman & Lothian, 1933).

Changes in pH have been more exploited than any other change of chemical condition in applying the ΔA method, especially for compounds showing bathochromic or hypsochromic shifts (or both). However, the peaks of certain compounds may split into subsidiary peaks without any appreciable change in intensity by changing the pH in a suitable interval. The behaviour of these compounds restricts the application of the ΔA method, since ΔA may not fulfil the above mentioned requirements. In these circumstances, the Δp_j method offers a solution for the analysis of such compounds in the presence of interference.

According to the orthogonal function method (Glenn, 1963), a single substance, X, can be estimated from a set of equally spaced absorbances by means of

$$p_{j1} = \alpha_{j1}\, c_X \quad .. \quad .. \quad .. \quad .. \quad (3)$$

where p_j is the coefficient of the polynomial, P_j; α_j is the coefficient for the A (1%, 1 cm) of the pure compound, X; c_X is the concentration and the subscript "i" denotes the wavelength range. In the presence of irrelevant absorption,

$$p_{ji} = \alpha_{ji}\, c_X + p_{ji}\,(Z) \quad .. \quad .. \quad .. \quad .. \quad (4)$$

where $p_{ji}\,(Z)$ denotes "contribution from irrelevant absorption." If the shape of the absorption curve changes significantly by changing the pH of the solvent from "a" to "b", the presence of a pH insensitive irrelevant absorption may be cancelled by means of

$$\Delta p_{ji} = [\alpha_{ji\mathrm{a}}\, c_X + p_{ji}(Z)] - [\alpha_{ji\mathrm{b}}\, c_X + p_{ji}(Z)] \quad .. \quad .. \quad (5)$$

and

$$c_X = \Delta p_{ji}/\Delta\alpha_{ji} \quad .. \quad .. \quad .. \quad .. \quad (6)$$

It may be possible to choose p_j and also the set of wavelengths "i" so that p_{ji} is optimum in one solvent and negligibly small in the other solvent. Accordingly, Δp_{ji} reaches the magnitude required to obtain precise and reproducible results.

Magnitude of Δp_j

To obtain precise estimates of concentration using the Δp_j method, the authors suggested the use of Glenn's theory of comparative coefficients (see Wahbi, 1967), that is, $|\Delta p_j|.N_j^{\frac{1}{2}} = |\Delta q_j|$ should exceed 140×10^{-3} if the coefficient of variation (Δp_j) is to be less than 1. Furthermore, Δp_j should correspond with a peak or a minimum in the polynomial's convoluted absorption curve (Agwu & Glenn, 1967).

The use of log $|\Delta p_j|$ *plots against* λ_m

By analogy with the use of graphs of log $|\Delta A|$ against wavelength, graphs of log $|\Delta p_j|$ ($\Delta p_j \neq 0$) against λ_m, the mean of the set of wavelengths, for sample and pure compound may be compared to detect changes in the irrelevant absorption. The two log $|\Delta p_j|$ graphs will only superimpose when the irrelevant absorption is unaffected by the change in pH.

Determination of clemizole in tablets

Clemizole exhibits a maximum absorption at 276 nm, [A (1%, 1 cm) = 300] in 0·1N sulphuric acid and four maxima at 253 nm, 267 nm, 276 nm [A (1%, 1 cm) = 190] and 282 nm in buffer pH 8 (Fig. 1). In these circumstances, the determination of clemizole in tablets constitutes an example for the application of the Δp_j method.

Application of the Δp_j *method*

Choice of assay conditions. According to general rules (Wahbi, 1967), the quadratic polynomial, P_2, was chosen as it makes a large contribution to segment MN and a small contribution over the same segment in the absorption curves of clemizole in buffer pH 8 and 0·1N sulphuric acid respectively (Fig. 1). Hence, the coefficient difference, Δp_2, should afford precise estimates of concentration.

Eight-point orthogonal polynomials were chosen as a compromise between the need for drawing maximal information from the continuous parts of the spectra and the laboriousness of the calculation.

The optimum wavelength range was obtained by plotting the p_2-convoluted absorption curves for both spectra at different wavelength intervals. λ_m was chosen

to correspond with a maximum or a minimum in the delta convoluted absorption curves (Table 1).

The wavelength range 238 to 266 nm at 4 nm intervals was finally chosen as the analytical set of wavelengths to make sure that $|\Delta q_2|$ is greater than 140×10^{-3} for solutions of concentration less than 0·003%. Moreover, the coefficient of variation calculated for seven separate determinations of Δp_2 (1%, 1 cm) at the finally chosen set of wavelengths was found to be 0·87%, the corresponding value for ΔA (1%, 1 cm) at 276 nm was 1·23%.

METHODS

Instrument. A Unicam SP500 photoelectric spectrophotometer.

Assay. Tablets were prepared, containing 20 mg of clemizole hydrochloride per 0·30 g tablet (Extra Pharmacopoeia). An accurately weighed quantity of the powder, representing 10–20 tablets, was quantitatively extracted with 0·1N sulphuric acid, filtered into a 100 ml volumetric flask and subsequently made up to volume. Two 1 ml portions of the filtrate were diluted to 100 ml using 0·1N sulphuric acid and a phosphate-borax buffer of pH 8, respectively. The absorbances of 1 cm path-lengths of both solutions were measured over the wavelength range 238 to 266 nm at 4 nm intervals and at 276 nm.

Table 1. *Optimum* Δp_2 *for* 0·0035% w/v *clemizole in buffer pH* 8 *and* 0·1N *sulphuric acid.*

Interval (nm)	λ_i (nm)	λ_f (nm)	λ_m (nm)	$\Delta p_2 \times 10^3$	$\lvert\Delta q_2\rvert \times 10^3$
2	246	260	253·0	— 7·196	93·3
3	242	263	252·5	—13·887	180·0
4	238	266	252·0	—19·446	252·0

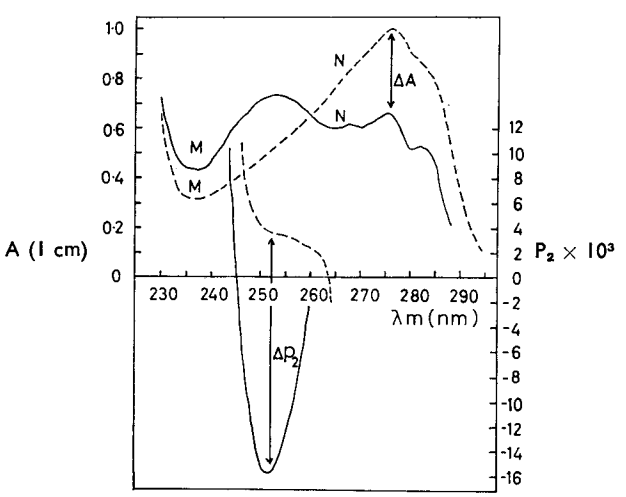

FIG. 1. The absorption curves of 0·003% w/v clemisole in buffer pH 8 or 0·1N sulphuric acid (the curves are superimposed) and the p_2 convoluted curves derived therefrom.

Table 2. *The determination of clemizole hydrochloride in tablets.*

Sample	mg added	ΔA % recovery	Δp_2 % recovery	p_2 % recovery
1	2·69	96·2	98·9	96·6
2	3·00	99·7	99·0	96·3
3	3·36	95·8	98·8	99·1
4	2·40	103·8	97·1	93·8
5	2·64	105·0	103·0	94·3
6	2·88	100·0	98·0	95·1
7	2·37	97·5	101·0	96·0
8	2·61	103·8	99·1	93·8
9	2·85	101·0	98·2	95·8
10	3·09	98·4	97·2	95·5
11	2·28	103·0	101·8	97·8
12	3·00	104·3	97·6	98·3

RESULTS AND DISCUSSION

When compared with the graph of log $|\Delta p_2|$ versus λ_m for clemizole hydrochloride, a similar graph for tablets was completely superimposable, thus showing the irrelevant absorption to be unchanged in the two solvents used. It was not, therefore, surprising that the above Δp_j procedure gave mean percentage recovery of $99{\cdot}1 \pm 1{\cdot}2\%$ ($P = 0{\cdot}05$) for the clemizole hydrochloride in twelve separate weighings of the powdered tablets. The mean percentage recovery obtained using the ΔA method was found to be $100{\cdot}7 \pm 2{\cdot}1\%$ ($P = 0{\cdot}05$) (Table 2). On the other hand, the mean percentage recovery calculated according to p_2 for the solutions prepared in buffer pH 8 was found to be $95{\cdot}2 \pm 1{\cdot}7\%$ ($P = 0{\cdot}05$). The poor results from the method using p_2 were due to irrelevant absorption in the tablets which was cancelled by the method using Δp_2. Furthermore, it must be noted that the irrelevant absorption contribution, $p_2(Z)$, was of opposite sign (eqn 4) to p_2, which leads to negative errors in all determinations.

Errors in the Δp_j method are mainly attributed to wavelength setting errors which affect absorbances measured on steep slopes in the absorption curves.

Although the Δp_j method gave marginally better results than the ΔA method, there must be other cases where the method proves to be superior over the ΔA method in cancelling irrelevant absorption contribution.

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