International Journal of Pharmaceutics, 92 (1993) 15–21

IJP 02991

Research Papers

Application of orthogonal functions to spectrophotometric analysis. Determination of dissociation constants

A.M. Wahbi, F.A. El-Yazbi, M.H. Barary and S.M. Sabri

Faculty of Pharmacy, University of Alexandria, Alexandria (Egypt)

(Received 16 March 1992)
(Modified version received 21 July 1992)
(Accepted 30 July 1992)

Key words: Spectrophotometry; Orthogonal function; Dissociation constants; Graphical method

Summary

Glenn's method of orthogonal functions (*J. Pharm. Pharmacol.*, 15 (1963) 123T–130T) is extended to the spectrophotometric determination of dissociation constants of some weakly acidic and basic drugs. The calculated comparative coefficients, Q_j, of the dissociated, undissociated and partially dissociated forms of the drug have been used to calculate the pK_a values. A graphical technique based upon plotting orthogonal function titration curves is also presented. The results obtained are in good agreement with the reported values.

Introduction

Changes of pH may affect the absorption spectra of organic compounds especially those containing acidic or basic groups that exhibit bathochromic or hypsochromic shifts. In this case, application of the ΔA method may help in the analysis of these compounds (Wahbi et al., 1970). However, the peaks of certain compounds may split into subsidiary peaks without any appreciable change in intensity or the ΔA value may be small. The behaviour of these compounds restricted the application of the ΔA method. In these circumstances, application of the orthogonal function (Δp_j) method offered a solution for the analysis of such compounds (Abdine et al., 1972).

Most medicinal substances are either weak acids or weak bases and, in aqueous solutions, are only partially dissociated. In order to obtain a quantitative measure of the extent of dissociation at a certain temperature, the dissociation constant is determined. When the absorption spectra of the different species in solution overlap, the use of the A_{max} method may give erroneous results, since it will not fulfill the requirements. Therefore, the application of the orthogonal function (Δp_j) method helps in the resolution of the spectral overlapping which facilitate the determination of the ionised species at any pH value.

Correspondence to: A.M. Wahbi, Faculty of Pharmacy, University of Alexandria, Alexandria, Egypt.

In the present work, a new application of the orthogonal function method is introduced for the determination of the dissociation constants of some acidic and basic drugs by using Eqns 1 and 2, respectively:

$$pK_a = pH + \log\frac{(p_{jd} - p_{jb})}{(p_{jb} - p_{ju})} \quad (1)$$

$$pK_a = pH + \log\frac{(p_{jb} - p_{ju})}{(p_{id} - p_{jb})} \quad (2)$$

where p_{jb}, p_{jd} and p_{ju} are the coefficients of the polynomial, p_j, of the buffered, dissociated and undissociated drug solution, respectively. The proposed method is applied for the determination of the pK_a value(s) of drugs with single, double and triple pK_a values.

Experimental

Apparatus

A Perkin-Elmer model 550S UV-Vis spectrophotometer with 1-cm quartz cuvettes and a spectral slit width of 2 nm, and a Hitachi Model 561 recorder were used. The pH measurements were carried out by means of a Schott Gerate pH Meter CG 710 at 20°C.

Materials and reagents

All materials and solvents used were of analytical grade. Double-strength phosphate-citrate buffer solutions of different pH values were used (Lenter, 1984).

Reference solutions

500 ml of aqueous solutions containing 100 mg of benzoic acid, 10 mg of paracetamol, 5 mg of methyl paraben, 21.5 mg of salicylamide, 20 mg of desipramine, 25 mg of nikethamide, 100 mg of phenytion sodium, 20 mg of phenobarbitone, 20 mg of chloroquine phosphate or 15 mg of tetracycline hydrochloride were prepared. In the case of desipramine, it was first dissolved in 20 ml of 0.5 N hydrochloric acid followed by dilution with water to 500 ml. Phenobarbitone was first dissolved in approx. 20 ml of methanol followed by dilution with water to 500 ml.

Procedure

Into a set of 50-ml volumetric flasks, 25-ml aliquots of the reference drug solution were transferred to each flask and the volume was

TABLE 1

Assay parameters for the determination of dissociation constants for different drugs

Drug	Buffer pH values			Q_j	No. of points	Wavelength range (nm)	Interval (nm)	λ_m (nm)	$\Delta Q_j(\times 10^3)$ [a]
Benzoic acid	3.85	4.20	4.70	Q_2	6	260–290	6	275	367
Paracetamol	9.00	9.45	9.65	Q_2	8	238–280	6	259	203
Methyl paraben	7.90	8.20	8.60	Q_2	6	282–312	6	297	331
Salicylamide	8.20	8.60	8.90	Q_2	12	306–350	4	328	1764
Desipramine	9.90	10.1	10.6	Q_2	12	244–310	6	277	231
Nikethamide	2.85	3.45	4.40	Q_2	12	228–294	6	261	936
Phenytoin sodium	8.10	8.40	8.60	Q_2	12	242–264	2	253	455
Phenobarbitone	7.05	7.60	7.70	Q_2	8	246–274	4	260	245
	11.1	11.7	12.1	Q_2	8	236–278	6	257	597
Chloroquine PO_4	8.00	8.30	8.60	Q_4	8	320–348	4	334	155
	10.6	11.0	11.4	Q_2	8	332–360	4	346	30
Tetracycline HCl	2.90	3.30	3.60	Q_2	8	246–288	6	267	190
	6.80	7.10	7.50	Q_2	12	276–320	4	298	52
	9.50	9.60	9.75	Q_2	12	256–330	4	278	62

[a] ΔQ_j: difference in Q_j for two subsequent dissociation steps.

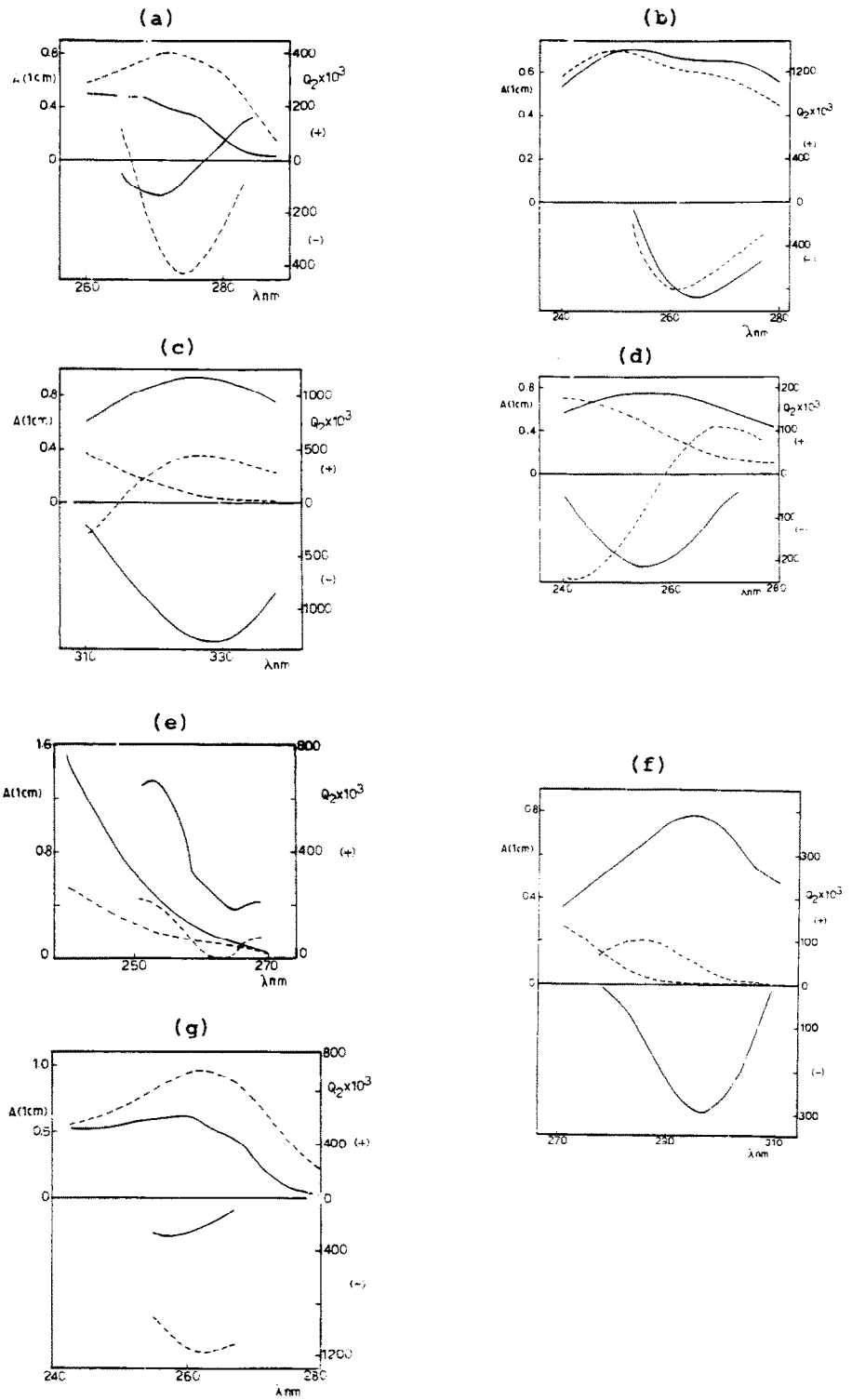
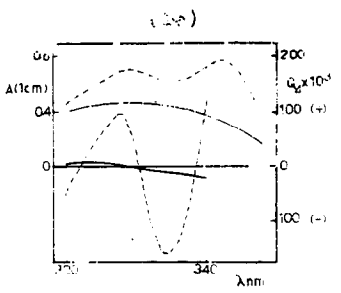
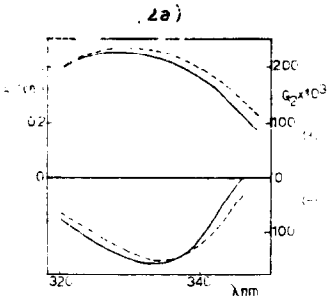
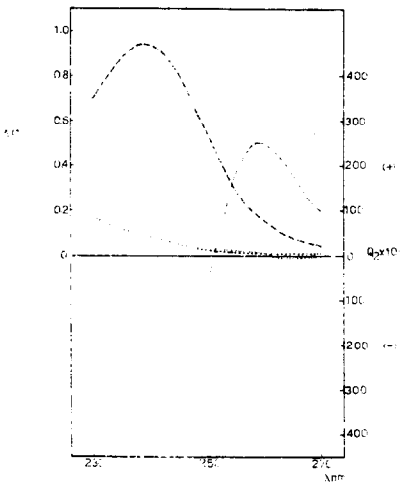


Fig. 1. Absorption spectra of (a) 0.01% w/v benzoic acid, (b) 0.002% w/v desipramine, (c) 0.00215% w/v salicylamide (d) 0.001% w/v paracetamol, (e) 0.01% w/v phenytoin, (f) 0.0005% w/v methyl paraben and (g) 0.0025% w% nikethamide in 0.1 N hydrochloric acid (- - - - -) and in 0.1 N NaOH (———) and their corresponding convolution curves.

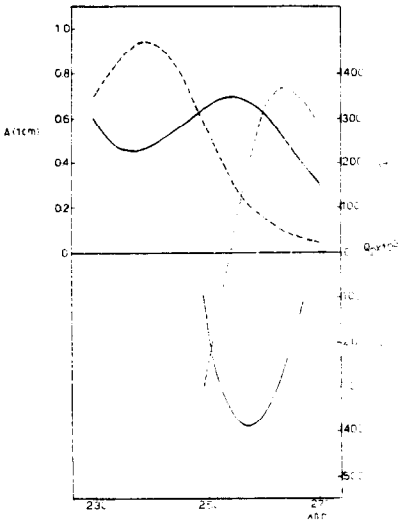


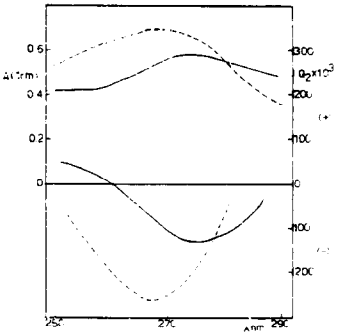


(1b)

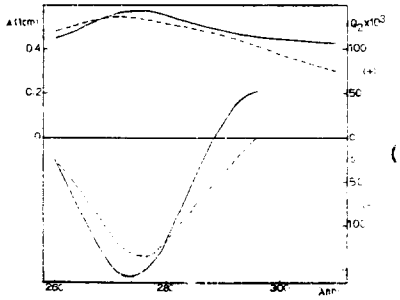


(2b)





(1c)



(2c)

(3c)

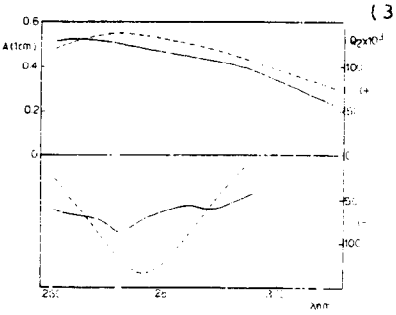


TABLE 2

Assay results for the determination of the dissociation constants for different drugs

Drug	pK_a			
	Reported [a]	Graphical method	Orthogonal function	
			Mean [b]	Relative S.D. [c]
Benzoic acid	4.2	4.15	4.13	0.11
Paracetamol	9.5	9.65	9.75	0.06
Methyl paraben	8.4	8.15	8.15	0.05
Salicylamide	8.2	8.5	8.47	0.13
Desipramine	10.2	10.2	10.21	–
Nikethamide	3.5	3.4	3.46	0.72
Phenytoin sodium	8.3	8.2	8.21	0.14
Phenobarbitone	7.3	7.3	7.31	0.16
	11.8	11.9	11.99	0.16
Chloroquine phosphate	8.4	8.3	8.34	0.12
	10.8	10.75	10.75	–
Tetracycline	3.3	3.3	3.31	0.14
hydrochloride	7.7	7.15	7.13	0.21
	9.7	9.65	9.64	–

[a] For reported pK_a values refer to Moffat (1986).
[b] Grand mean of 15 values (5 replicates at each of 3 different pH values given in Table 1).
[c] Overall relative standard deviation.

completed with: (i) 0.2 N hydrochloric acid solution; (ii) 0.2 N sodium hydroxide solution; (iii) double-strength phosphate-citrate buffer solution of different pH values (Table 1).

The accurate pH value of each solution was measured.

The absorbances at the selected wavelengths (Table 1) were measured against a blank prepared similarly but using distilled water instead of drug solution.

In the case of phenobarbitone and tetracycline hydrochloride, 1 N sodium hydroxide solution and buffer solution of pH 12.5 were used, respectively, to achieve full dissociation instead of 0.2 N sodium hydroxide solution.

Results and Discussion

The determination of dissociation constants of weak acidic or basic drugs is based upon recording the absorption spectra in strong acid, strong alkali and in a series of buffer solutions of different pH values. Because the conjugate acid and base forms of compounds are in equilibrium, the relative concentrations of either component will be determined by the pH of the solution. Accordingly, the pH can be adjusted so that there will be a very great preponderance of one form in the solution and the observed spectrum will be that of the predominant form. At pH values intermediate between the extremes, both forms are pre-

Fig. 2. (1a) Absorption spectra of 0.002% w/v phenobarbitone in 0.1 N hydrochloric acid (...), in buffer solution pH 10 (- - -) and their corresponding convolution curves. (2a) Absorption spectra of 0.002% w/v phenobarbitone in buffer solution pH 10 (— — —), in 0.5 N sodium hydroxide (———) and their corresponding convolution curves. (1b) Absorption spectra of 0.002% w/v chloroquine phosphate in 0.1 N hydrochloric acid (— — —), in buffer solution pH 9.4 (———) and their corresponding convolution curves. (2b) Absorption spectra of 0.002% w/v chloroquine phosphate in buffer solution pH 9.4 (———), in sodium hydroxide (- - -) and their corresponding convolution curves. (1c) Absorption spectra of 0.0015% w/v tetracycline hydrochloride in 0.1 N hydrochloric acid (- - -), in buffer solution pH 5.5 (———) and their corresponding convolution curves. (2c) Absorption spectra of 0.0015% w/v tetracycline hydrochloride in buffer solution pH 5.5 (———), 8.5 (— — —) and their corresponding convolution curves. (3c) Absorption spectra of 0.0015% w/v tetracyclin hydrochloride in buffer solution pH 8.5 (— — —), 12.5 (———) and their corresponding convolution curves.

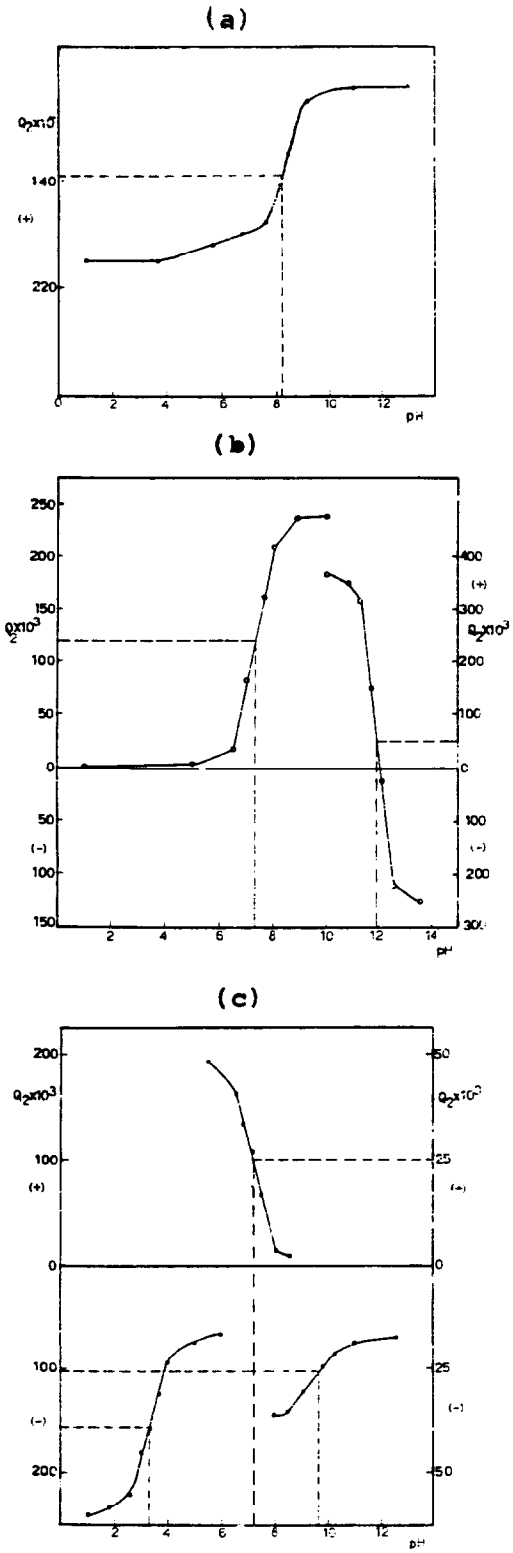


Fig. 3. Graphical representation of the spectrophotometric orthogonal function titration curves of (a) phenytoin sodium, (b) phenobarbitone, and (c) tetracycline hydrochloride.

sent and the observed spectrum is a linear combination of both components providing that Beer's law is followed. If the compounds do not show appreciable change in their spectra over a suitable pH range, the Δp_j method is applied.

To obtain precise values of Δp_j the comparative coefficients, ΔQ_j, have been applied where $\Delta Q_j = \Delta p_j N_j^{1/2}$ and N_j is the normalizing factor. For the relative standard deviation to be less than unity, ΔQ_j should exceed 140×10^{-3} for the set of absorbances used in the calculation.

Choice of assay conditions

According to general rules (Wahbi, 1967), the order of the polynomial, p_j, is chosen such that it makes a large contribution to a certain segment in the absorption curves of the compound at a given pH value and a small contribution over the same segment at another pH value. Hence, the coefficient difference, Δp_j, will correspond with the maximum in the convoluted curve. The assay parameters for the determination of the pK_a of the tested compounds are summarised in Table 1.

Fig. 1 shows the absorption spectra and the respective convoluted curves of compounds with one pK_a value in 0.1 N hydrochloric acid and 0.1 N sodium hydroxide. The quadratic coefficient, Q_2, was chosen for the calculation of pK_a since it makes a large contribution to the absorption curves in its dissociated form over the optimum wavelength range (Table 1) and a small contribution over the same segment in the undissociated form.

Fig. 2 shows the absorption spectra and the respective convoluted curves of chloroquine phosphate in 0.1 N hydrochloric acid, 0.1 N sodium hydroxide and phosphate-citrate buffer pH 9.4. It has two pK_a values (Table 2). In the buffer solution pH 9.4, the predominant form is the monodissociated one. For the calculation of the first pK_a value, the quartic coefficient, Q_4, was chosen while for the calculation of the second pK_a value, the quadratic coefficient, Q_2, was used at the selected wavelengths (Table 1).

Fig. 2 also shows the absorption spectra of phenobarbitone in 0.1 N hydrochloric acid, 0.5 N sodium hydroxide and phosphate-citrate buffer pH 10, with their respective convoluted curves. It has two pK_a values. The monodissociated form is

the predominant form in the buffer solution pH 10. The quadratic coefficient, Q_2, was chosen for the calculation of both pK_a values at the selected sets of wavelengths (Table 1).

Fig. 2 shows the absorption spectra of tetracycline hydrochloride in 0.1 N hydrochloric acid, phosphate-citrate buffer pH 5.5, buffer pH 8.5 and buffer pH 12.5. Sodium hydroxide was not used in this case due to the decomposition of tetracycline. It has three dissociation constants. The most predominant form in buffer solution pH 5.5 is the monodissociated form, while in the buffer solution pH 8.5, the predominant form is the didissociated form. In the buffer solution pH 12.5, the predominant form is the totally dissociated form. The quadratic coefficient, Q_2, was chosen for the calculation of the three values of the pK_a at the selected sets of wavelengths (Table 1).

To facilitate the study of the optimum assay conditions for the application of orthogonal function method and the calculation of the comparative coefficient, Q_j, for each case, a computer program written in BASIC is used in these calculations (Wahbi et al., 1991). By calculation of the comparative coefficient, Q_j, Eqns 1 and 2 can be rewritten as follows:

$$pK_a = pH + \log\frac{(Q_{jd} - Q_{jb})}{(Q_{jb} - Q_{ju})} \quad (3)$$

$$pK_a = pH + \log\frac{(Q_{jb} - Q_{ju})}{(Q_{jd} - Q_{jb})} \quad (4)$$

where Q_{jb}, Q_{jd} and Q_{ju} are the comparative coefficient of the buffered, dissociated and un dissociated drug solution, respectively.

Graphical technique

An alternative method is the graphical technique wherein the dissociation constant is determined by obtaining the value of pH when the concentrations of the dissociated and undissociated forms of the compound are equal. Thus, plots of Q_j calculated under the optimum conditions vs pH lead to the spectrophotometric orthogonal function titration curves. $Q_j^{1/2}$ (at half-neutralization) can be calculated using $(Q_{ja} + Q_{jb})/2$, where a and b denote fully acidic and fully basic solutions for singly dissociated compounds. The pH which corresponds to $Q_j^{1/2}$ is taken as the pK_a of the compound.

Fig. 3 shows the titration curves of singly, doubly and triply dissociated compounds, respectively. In the singly dissociated compounds, the titration curve shows only one break. In the case of compounds that have two or three stages of dissociation, the titration curves are plotted at narrow intervals of pH. When the measured parameter (Q_j) shows an abrupt change with pH, this indicates that the concentration of the different dissociation forms changes rapidly. When the change in the concentration reaches a steady value, this can be taken to be the end of the dissociation of a stage and the begining of the second stage.

However, it should be taken into consideration that the dissociation steps overlap and the calculated values of pK_a for the doubly and triply dissociated compounds are only approximate.

The results obtained for the tested compounds are shown in Table 2. The pK_a values determined by the application of the orthogonal function method and the graphical technique are in closel correspondence with the reported pK_a values (Moffat, 1986).

References

Abdine, H., Wahbi, A.M. and Korany, M.A., Application of orthogonal functions to spectrophotometric analysis: The Δp_j method. *J. Pharm. Pharmacol.*, 24 (1972) 518–521.

Glenn, A.L., The use of orthogonal functions to correct for irrelevant absorption in two component spectrophotometric analysis. *J. Pharm. Pharmacol.*, 15 (1963) 123T–130T.

Lenter, C., *Geigy Scientific Tables*, 1984, p. 58.

Moffat, A.C., *Clark's Isolation and Identification of Drugs*, Pharmaceutical Press, London, 1986, p. 384, 455, 516, 762, 813, 849, 883, 897, 964, 1005.

Wahbi, A.M., Application of orthogonal functions to spectrophotometric analysis, Ph.D Thesis, University of London (1967).

Wahbi, A.M. and Farghaly, A.M., Application of the ΔA method to the determination of morphine. *J. Pharm. Pharmacol.*, 22 (1970) 848–850.

Wahbi, A.M., El-Yazbi, F.A., Barary, M.H. and Sabry, S.M., Two BASIC programs for the calculations of orthogonal function coefficients and their comparative coefficients for the first six terms. *Alex. J. Pharm. Sci.*, 5 (1991) 220–224.