

Application of orthogonal functions to correct for quadratic irrelevant absorption in spectrophotometric analysis

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The seven-point correction method of Ashton and Tootill has been examined. The method is generalized for other groups of points from $n = 5$ to $n = 14$. Glenn's method of orthogonal functions proved to be suitable for assaying cyclandelate in the presence of interference with quadratic curvature.

The mathematical methods used for the correction of linear irrelevant absorption involve the measurements at three wavelengths and therefore stem from Wright's "base line" technique (1941)—a graphical method, which Banes & Eby (1946) transposed into algebra. The well known procedure of Morton & Stubbs (1946) is an algebraic version of the base-line method (Mulder, Spruit & Keuning, 1963).

The methods developed for the correction of non-linear irrelevant absorption are mainly based upon the use of orthogonal polynomials. Thus, Ashton & Tootill (1956) developed a seven-point method for the correction of quadratic irrelevant absorption during the determination of griseofulvin in fermentation samples. Glenn (1963) outlined general procedures for the correction of irrelevant absorption in two-component spectrophotometric analysis. Both methods depend upon the orthogonal polynomial expansion:

$$A = p_0P_0 + p_1P_1 + p_2P_2 + p_3P_3 + \dots + p_nP_n \quad \dots \quad (1)$$

where A represents an absorption curve, p_0, p_1, p_2 etc. are coefficients of the constant, linear, quadratic components respectively and P_j are the values which can be obtained from tables covering a wide range of points (Milne, 1949; Fisher & Yates, 1953). The coefficients, p_j , are linearly related to concentration (Glenn, 1963).

Ashton & Tootill developed a set of coefficients highly correlated to the absorption curve of griseofulvin but orthogonal to constant, linear and quadratic components. When these coefficients are multiplied by the seven absorbances of the sample, the concentration of griseofulvin can be obtained. In the absence of a full explanation by the authors of their mathematical procedure, the general formula for computing correction coefficients at each of the seven wavelengths and the principle of the evaluation procedure is simplified in the present work.

In view of the orthogonality of equation (1), any coefficient, p_j , can be obtained by forming the sum of products $\sum_{i=0}^{i=n} A_i P_{ji}$ then dividing by $\sum_{i=0}^{i=n} P_{2ji}$. The latter is known as the normalizing factor. Denoting the former product by p_j and the normalizing factor by N_j where $j = 0, 1, 2, \dots, n$ in equation (1) at λ_j ,

$$A_1 = \frac{p'_0 P_{01}}{N_0} + \frac{p'_1 P_{11}}{N_1} + \frac{p'_2 P_{21}}{N_2} + \frac{p'_3 P_{31}}{N_3} + \dots + \frac{p'_n P_{n1}}{N_n} \quad \dots \quad (2)$$

and if $n = 6$ (seven point orthogonal polynomials),

$$A_1 = \frac{p'_0}{7} + \frac{p'_1 P_{11}}{28} + \frac{p'_2 P_{21}}{84} + \frac{p'_3 P_{31}}{N_3} + \frac{p'_4 P_{41}}{N_4} + \frac{p'_5 P_{51}}{N_5} + \frac{p'_6 P_{61}}{N_6} \quad \dots \quad (3)$$

taking in consideration that $P_{01} = 1$ for all values of n . Multiplying equation (3) by 84 and transferring the constant, linear and quadratic terms to the left hand side (Wahbi, 1967),

$$84A_1 - 12p'_0 - 3p'_1 P_{11} - p'_2 P_{21} = 84 \left[\frac{p'_3 P_{31}}{N_3} + \frac{p'_4 P_{41}}{N_4} + \frac{p'_5 P_{51}}{N_5} + \frac{p'_6 P_{61}}{N_6} \right] \quad (4)$$

The left hand side of equation (4) is exactly the formula used by Ashton & Tootill (Note 1, p. 227) to compute the quadratic correction coefficients at λ_i , viz:

$$84\Sigma S - 12(\Sigma S) - 3 X_1(X_1\Sigma S) - X_2(X_2\Sigma S) \quad \dots \quad (5)$$

where ΣS is the sum of the absorbances at λ_i equivalent to A_1 in this paper, (ΣS) is the sum of the seven absorbances equivalent to p'_0 ; X_1 , X_2 , $(X_1\Sigma S)$ and $(X_2\Sigma S)$ are equivalent to P_{11} , P_{21} , p'_1 and p'_2 respectively. Given that:

$$T_1 = 84A_1 - 12p'_0 - 3 p'_1 P_{11} - p'_2 P_{21} \quad \dots \quad (6)$$

the divisor computed by Ashton & Tootill (Note 2, p. 227) is obtained by forming the product

$$\left[\sum_{i=0}^{i=6} A_i T_1 \right] / c = \left[84 \sum_{i=0}^{i=6} A_i^2 - 12(p'_0)^2 - 3(p'_1)^2 - (p'_2)^2 \right] / c \quad (7)$$

where c , is the concentration of griseofulvin.

According to Ashton & Tootill's method of calculation, the concentration c_x , of a sample of griseofulvin which is known to contain quadratic irrelevant absorption is

obtained by forming the product $\sum_{i=0}^{i=6} A'_i T_1$ [where A'_i is $A(c_x, 10 \text{ mm})$ at λ_i], then

dividing by equation (7).

The right hand side of equation (4) shows that the estimated concentration of griseofulvin depends upon cubic, quartic, quintic and sexic terms. Alternatively, the formula used to compute the quadratic correction coefficients (eqn 6) can be written as follows:

$$T_1 = 84 \left[\frac{p'_3 P_{31}}{N_3} + \frac{p'_4 P_{41}}{N_4} + \frac{p'_5 P_{51}}{N_5} + \frac{p'_6 P_{61}}{N_6} \right] \quad \dots \quad (8)$$

and the divisor

$$\left[\sum_{i=0}^{i=6} A_i T_1 \right] / c = 84(p'_3 \pi_3 + p'_4 \pi_4 + p'_5 \pi_5 + p'_6 \pi_6) \quad \dots \quad (9)$$

where c , is the concentration % w/v and π_j is the normalized coefficient, $p_j(1\%, 1\text{ cm}) = p_j/c$ %w/v

The concentration c_x , of the sample can be obtained from equation (8) which is orthogonal to constant, linear and quadratic components by forming the product:

$$\sum_{i=0}^{i=6} A'_i T_i = 84 c_x (p'_3 \pi_3 + p'_4 \pi_4 + p'_5 \pi_5 + p'_6 \pi_6) \dots \dots \quad (10)$$

then dividing by the left hand side of equation (9).

Ashton & Tootill's expressions (eqns 6 & 7) have been generalized for a number of points ranging from $n = 5$ to $n = 14$ (Table 1).

In an attempt to simplify the calculation of Ashton & Tootill, Daly (1961) derived a six-point method for eliminating quadratic irrelevant absorption. Thus,

$$c_x = \frac{(A_1 + A_2 + A_6) - (A_0 + A_4 + A_5)}{(\alpha_1 + \alpha_2 + \alpha_6) - (\alpha_0 + \alpha_4 + \alpha_5)} \dots \dots \quad (11)$$

where α denotes the $A(1\%, 1\text{ cm})$ of griseofulvin and the subscripts 0, 1, 2, . . . , 6 are the chosen set of wavelengths.

Despite the absence of any obvious dependence upon the principle of orthogonal functions, Daly's equation follows naturally from the method outlined by Glenn for the application of orthogonal functions to spectrophotometric analysis. Thus, for $n = 6$

$$p'_3 = (-1)A_0 + (+1)A_1 + (+1)A_2 + (0)A_3 + (-1)A_4 + (-1)A_5 + (+1)A_6 \quad (12)$$

where the numbers in parentheses are the tabulated values of P_3 . Alternatively,

$$p'_3 = (A_1 + A_2 + A_6) - (A_0 + A_4 + A_5) \dots \dots \dots \quad (13)$$

$$\text{and } \pi'_3 = (\alpha_1 + \alpha_2 + \alpha_6) - (\alpha_0 + \alpha_4 + \alpha_5) \dots \dots \dots \quad (14)$$

where π'_3 is the unnormalized coefficient, $p'_3(1\%, 1\text{ cm})$. Therefore, $c_x = p'_3/\pi'_3$ which is identical to equation (11).

Rekker (1965) applied Ashton & Tootill's method to the assay of cyclandelate. A set of seven correction coefficients was established for the purpose of the assay. The author dealt with this method and Glenn's method as if they were two completely different methods. However, both methods originate from the orthogonal polynomial expansion given by equation (1) and represent an attempt to separate the absorption curve of a pure compound from that of the irrelevant absorption present in the analytical sample. The only difference between the two methods is the assumptions made about irrelevant absorption. Ashton & Tootill assumed that the irrelevant absorption contributes only to the constant, linear and quadratic components and the concentration of griseofulvin was therefore evaluated from a suitable function of all coefficients other than p_0 , p_1 and p_2 . On the other hand, Glenn considers that assumptions about the irrelevant absorption curve should be kept to an absolute minimum. The concentration of a single substance in the presence of irrelevant absorption is calculated from just one coefficient.

The data published by Rekker were computed for determining the concentration of cyclandelate using P_3 ($n = 6$) over the chosen set of wavelengths (eqn. 12). The recovered quantity was found to be within 0.2% of the published result. This means

that Glenn's method can, in fact, give good results in the assay of cyclandelate (c.f. Rekker's statement).

Table 1. *The generalization of Ashton & Tootill's expressions for a number of points.*

General formulae:				
Correction coefficient at $\lambda_i = eA_i - fp'_0 - gp'_1P_{1i} - hp'_2P_{2i}$				
Divisor = $\left[e \sum_{i=0}^{i=n} A_i^2 - f(p'_0)^2 - g(p'_1)^2 - h(p'_2)^2 \right] / \text{concentration}$				
n*	e	f	g	h
5	420	70	6	5
6**	84	12	3	1
7	168	21	1	1
8	13860	1540	231	5
9	660	66	2	5
10	4290	390	39	5
11	12012	1001	21	1
12	2002	154	11	1
13	3640	260	4	5
14	185640	12376	663	5

* Number of points less 1.

** Ashton & Tootill's expressions.

In an attempt to generalize Ashton & Tootill's expressions for $(n + 1)$ -point orthogonal polynomials, Rekker stated that coefficients 84 and 12 (eqn 6) should be replaced by $3\Sigma(\xi')^2$ and $\frac{3\Sigma(\xi')^2}{n + 1}$ respectively, where $\Sigma(\xi')^2$ representing the sum of the squares of the reduced linear coefficients of an $(n + 1)$ -point orthogonal polynomial and this implies that the ratio between the first and second coefficients in Ashton & Tootill's expressions should always be equal to $(n + 1)$, for any number of points. This conclusion agrees with the data given in Table 1, i.e. $e/f = n + 1$. However, Rekker's generalization is not considered to be exact and is also incomplete because the coefficients of the other two terms should, of course, change with the number of points (g & h in Table 1).

As will have been gathered from the above discussion, Glenn's method is considered to be the most simple for the correction of irrelevant absorption. However, unless the principles of choosing the proper polynomial, number of points, wavelength range and intervals are well understood, the method may give unsatisfactory results.

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