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# Application of orthogonal functions to spectropolarimetric analysis of some penicillins

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The orthogonal function method is extended to correct for interferences in spectropolarimetric analysis. The method has been applied to the determination of some penicillins in presence of the degradation product penicilloic acid. The results obtained suggest that the method can be widely applied for similar problems.

According to Glenns methods of orthogonal functions (Glenn, 1963, 1967; Perks & Glenn, 1971), the ORD curve, $\alpha(\lambda)$ of an optically active compound can be represented as follows:

$$\alpha(\lambda) = p_0P_0 + p_1P_1 + p_2P_2 + \cdots\cdots + p_nP_n$$

where $\alpha(\lambda)$ denotes the observed angles of rotation, $\alpha$, in degrees of the sample measured under specified conditions at a wavelength $\lambda$ that belongs to a set of $(n + 1)$ equally-spaced wavelengths; $P_j$ are the orthogonal polynomials (Fisher & Yates, 1953) and $p_j$ are their respective coefficients which are proportional to the concentration of the compound. Thus, $p_j = R_jc_a$ where $R_j$ is the coefficient of $P_j$ corresponding to the specific rotation $[\alpha]_\lambda^t$ of the pure compound, a, $c_a$ is the concentration and t is the temperature. In the presence of optically active interferences, each observed coefficient is the sum of two terms,

$$p_j = R_jc_a + p_j(z)$$

where z denotes the contribution from interference. By a proper choice of polynominal and range, the number of wavelengths and the mean wavelength, $p_j(z)$ can be arranged to be negligibly small relative to $R_jc_a$. In that case, the concentration $c_a$ is directly proportional to $p_j$. The procedures for the choice of these assay parameters are similar to those for orthogonal functions in spectrophotometry (Wahbi, 1967, 1971; Agwu & Glenn, 1967; Abdine, Wahbi & Korany, 1971; Wahbi & Ebel, 1974a). When the ORD curve changes by changing the solvent used, the $\Delta p_j$ method (Abdine, Wahbi & Korany, 1972) can be applied provided that the interference does not change. When it is difficult to find a set of wavelengths over which the coefficient of the optically active interference is negligibly small relative to the assay coefficient, the combined polynomial method can be used (Wahbi & Ebel, 1974b).

Glenn (quoted by Wahbi, 1967) derived a formula for choosing polynomials to give appropriate reproducibility in the application of orthogonal polynomials to spectrophotometry. That formula is not applicable to spectropolarimetry and it is advisable to test the reproducibility of the assay coefficient by calculating the relative standard deviation from repeated measurements (separate weighings) of the investigated compound.

The present work presents an application of the method to the spectropolarimetric

determination of sodium ampicillin, potassium benzylpenicillin and potassium phenoxymethylpenicillin in the presence of the corresponding degradation products, mainly penicilloic acid.

*Choice of assay conditions.* The ORD curves of the different penicillins in 0·1M phosphate buffer pH 7·0 were found to possess maximum angle of rotation at 247 nm (Fig. 1). The quadratic polynomial, $P_2$, was chosen according to general rules collated by Wahbi (1967) as it makes a large contribution to the entire curve; hence the coefficient, $p_2$, should afford precise estimates of concentration. The correspond-

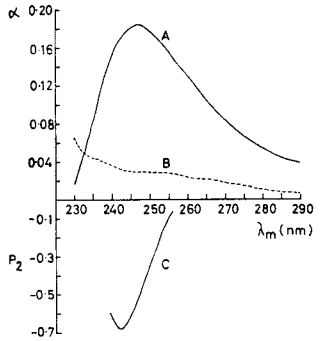


FIG. 1. The ORD curves of 12·5 mg per 500 ml buffer pH 7·0 of sodium ampicillin (A), the corresponding penicilloic acid (B) prepared by the sodium hydroxide method and the $p_2$ convoluted curve (C).

ing ORD curves of penicilloic acids prepared (i) by the sodium hydroxide method (B.P. 1973) followed by neutralization and (ii) by means of the specific enzyme, penicillinase (Rasmussen & Higuchi, 1971) were investigated in the same solvent. Differences between the two curves were negligible and were attributed to instrumental errors. The latter predominate whenever the measured angles of rotations are small. The quadratic component, $p_2P_2$ made a small contribution to both curves. The correction of such an interference can be carried out using the quadratic polynomial at a specified set of wavelengths.

In the ideal case, the analytical set of wavelengths is selected by plotting convoluted curves (Agwu & Glenn, 1967) using different points and intervals so that $|p_2|$ is maximal in the corresponding convoluted curve and negligibly small for the interference. Then the assay coefficient is tested for its reproducibility by calculating the relative standard deviation.

Several solutions of the different penicillins (10 to 20 mg per 500 ml buffer pH 7·0) were accurately prepared and measured without delay over the wavelength range 290 to 230 nm at 1 nm intervals using the proper polarimeter amplification at each wavelength whenever required. The data obtained were convoluted using the quadratic polynomial, $P_2$, for 8 to 24 points at different wavelength intervals and the relative standard deviation of the computed $R_2$ at each set of wavelengths was calculated. It was found that using 20 points over the wavelength range 259 to 240 nm at 1 nm intervals, the relative standard deviation of $R_2$ for the different penicillins was less than for all other possible cases including those sited on maxima in the corresponding convoluted curves (Table 1). These results were not surprising because the penicillins absorb strongly at wavelengths shorter than 255 nm (Rasmussen & Higuchi, 1971), and so the concentrations could not exceed 0·004% w/v, leading to relatively small angles of rotations (less than 0·3°) of low precision. In this connection, the

experimental ORD curve of this particular problem is considered to be the sum of two curves (i) a real or true curve and (ii) a non-negligible, unspecific error curve. The latter is reported to be of high frequency nature and would therefore contribute to higher components in equation 1 (Glenn, private communication). To correct for such an error curve, a relatively large number of points need to be used. With 20 point orthogonal polynomials, the calculation of $p_2$ will automatically reject all other components from $p_0P_0$ to $p_9P_9$ inclusive present in the ORD curves of penicillin and interference as well as the error curve. The $p_2$ coefficient was found to be negligibly small when calculated over the above mentioned wavelengths for penicilloic acid.

## METHODS

*Instrument.* A Perkin-Elmer polarimeter 141 fitted with a 10 cm quartz cell, 6·20 ml capacity which was thermostatted at 25°.

*Assay.* A solution of the penicillin salt was prepared by rapidly dissolving 10 to 20 mg, accurately weighed in 500 ml 0·1M phosphate buffer pH 7·0. Angles of rotation were measured without delay over the wavelength range 259 to 240 nm at 1 nm intervals. The coefficient $p_2$ was calculated by $\sum_{i=0}^{i=19} \alpha_i P_{2i}/N_2$ using 20 point orthogonal polynomials (Milne, 1949). The concentration was calculated from the corresponding $R_2$ by use of a reference sample or from a calibration curve (Table 1).

Table 1. *Reproducibility of coefficients.* ($P_2$, 20 points, $\lambda_i = 259$ nm, $\lambda_f = 240$ nm)

| Penicillin | Exp.* | $R_2$** | Relative s.d. % |
|---|---|---|---|
| Ampicillin Na .. .. .. | 6 | —17·510 | 1·25 |
| Benzylpenicillin K .. .. .. | 7 | —15·539 | 0·73 |
| Phenoxymethylpenicillin K .. | 8 | —14·338 | 1·20 |

* Concentration ranges from 10 to 20 mg per 500 ml phosphate buffer pH 7·0.

** $R_2 = \sum_{i=10}^{i=19} \left[\alpha\right]_{\lambda i}^{25°C} \cdot P_{2i}/N_2$;

## RESULTS AND DISCUSSION

Mixtures of the penicillin salts and the corresponding penicilloic acid (instantaneously prepared by the sodium hydroxide method followed by neutralization) were assayed by the above method. The results obtained suggest that the method can be applied for routine analysis.

Errors in the present method can be attributed to (i) wavelength setting errors, (ii) the non-zero coefficient that may have been contributed by penicilloic acid to the assay coefficient, (iii) overall shifts in the wavelength calibration which affect coefficients sited on slopes in the corresponding convoluted curves (Fig. 1), (iv) degradation of penicillin in aqueous solutions during measurements, (v) mutarotation of penicilloic acid (Rasmussen & Higuchi, 1971) and (vi) precision of the instrument.

The effect of ±0·5 nm overall shift in the wavelength calibration on the coefficient calculated at the above mentioned wavelengths for a solution of 0·003% w/v sodium ampicillin gave +3·7% and −2·7% error, respectively. However, such an error can

be minimized by keeping the instrument in a constant temperature laboratory or by calibration by use of the emission lines of the built-in mercury lamp.

With regard to the problem of degradation, Rasmussen & Higuchi (1971) reported that losses of approximately 1% were observed in 170 min for benzylpenicillin and 90 min for ampicillin and phenoxymethylpenicillin. The degradation was found to follow a zero order reaction and was therefore independent of concentration. The present method takes about 45 min from the time of solution preparation. Moreover, the error due to degradation can be said to be compensated for, or at least kept to a minimum, when the concentration of the sample is calculated from a reference similarly prepared and measured.

The mutarotation of penicilloic acids during the course of measurements may have lead to a curve of high frequency nature similar to the instrumental error curve. Both curves would certainly be correct for using 20 point orthogonal polynomials as previously described. The results obtained from the determination of samples of ampicillin sodium, benzylpenicillin K and phenoxymethyl penicillin K containing penicilloic acid corresponding to 4 mg of the penicillin support this argument. For ampicillin sodium, 9 samples 11·00–20·33 mg gave a % recovery of 100·2 $\pm$ 1·94 for benzyl penicillin K, 8 samples, 9·64–18·75 mg, gave a % recovery of 100·5 $\pm$ 1·50; for phenoxymethylpenicillin K, 7 samples, 10·51–17·52 mg gave % recovery of 100·5 $\pm$ 1·59.

The same mixtures of sodium ampicillin, potassium benzylpenicillin and potassium phenoxymethylpenicillin in the presence of the degradation products were assayed by measurement of the angle of rotation at 247 nm. Mean percentage recoveries were found to be 106·5 $\pm$ 2·5, 107·3 $\pm$ 3·8 and 105·1 $\pm$ 2·9 respectively. The high results obtained by this method are attributed to the contribution of the interferences to the measurements.

As will have been gathered from the above discussion the correction of interferences in spectropolarimetric and circular dichroism methods of analysis can be successfully carried out using Glenn's method of orthogonal functions.

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