Application of orthogonal functions to determination of nystatin in the presence of its degradation products

Direct spectrophotometric measurement of nystatin at its absorption maxima is unsuitable for stability studies because of interference by absorbing degradation products. Although the colorimetric method of Chang, Honig & others (1963), in which nystatin is heated with sodium hydroxide solution, has been recommended for stability studies, a spectrophotometric method was sought that could easily be automated.

The compensation method (Schiafano, Loy & others, 1956; Tardif, 1961; Hiskey, 1961) was used to study the spectrum of the degradation products of nystatin (Fig. 1). It was found that the irrelevant absorption was nearly rectilinear from about 280 to 322 nm. This suggested that the method of orthogonal polynomial functions (Glenn, 1963) could be used to correct for irrelevant absorption. It was found that with an eight-point polynomial over the wavelength range 298 to 326 nm at 4 nm intervals p4, the coefficient of the quartic polynomial P4, was independent of the concentration of the degradation products of nystatin and proportional to the concentration of nystatin.

The value of p4 (1 %, 1 cm) for pure nystatin was 100·63.

Procedure. Dissolve an accurately weighed quantity of the sample, containing about 100 mg of nystatin, in 10 ml of dimethylformamide and dilute with methanol to 100 ml. Dilute 1 ml of this solution with methanol to 100 ml and measure the absorbance of a 1 cm layer over the wavelength range from 298 to 326 nm at intervals of 4 nm. Calculate the concentration of nystatin from the following equation:
$\%\ \text{nystatin} = (-7\ A_{298} + 13\ A_{302} + 3\ A_{306} - 9\ A_{310} - 9\ A_{314} + 3\ A_{318} + 13\ A_{322} - 7\ A_{326}) / (616 \times 100{\cdot}63 \times 1000)$, where the subscripts represent the wavelengths (nm) and 616 is the normalizing factor for the polynomial.

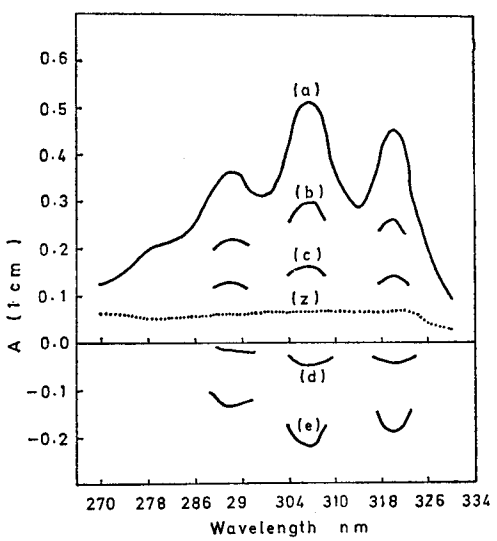

FIG. 1. Irrelevant absorption of nystatin. (a) Absorption curve of the sample [1 mg per 100 ml], As; (b) and (c) difference curves = As—Ar; (z) difference curve at the balance point = irrelevant absorption [Cr = 0·65 mg 100 ml]; (d) and (e) over compensation difference curves.

Table 1. *Determination of nystatin in degraded samples.*

Sample no.	Recovery %	
	Present method	Compensation method
1	65·00	65
2	65·63	66
3	53·00	50
4	42·35	42
5	41·90	42

Table 2. *Determination of nystatin in pharmaceutical preparations.*

Preparation*		% Recovery	
	Labelled content**	Present method	Compensation method
Mycostan capsules (Memphis Co)	100 mg per capsule	106·9	106·0
Mycostan vaginal Tablets (Memphis Co)	20 mg per tablet	110·55	105·0
Memcostan cream (Memphis Co)	20 mg per g	106·45	106
Mycostatin oral Suspension (Squibb)	20 mg per dose	97·85	98

* Pharmaceutical preparations collected at random from the local market of A.R.E.
** Assuming that each mg nystatin contains 5000 nystatin units.

Seven determinations of pure nystatin at concentrations of 0·2 to 1 mg of nystatin per 100 ml gave a mean recovery of 100·6% with a coefficient of variation of 0·6%. The mean recovery and the coefficient of variation by the compensation method were 99·3% and 0·5%.

The results of the application of both methods to degraded samples of nystatin and to pharmaceutical preparations obtained from the local market are summarized in Tables 1 and 2.

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