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Journal of Pharmaceutical and Biomedical Analysis
13 (1995) 1437-1440

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Quantitation of mixtures from two-dimensional data sets using orthonormal functions

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Received for review 26 April 1995

Abstract

The use of orthogonal polynomials in mixture analysis has been extended to two-dimensional data sets. Orthonormal two-dimensional functions, constructed from Legendre polynomials, are fitted to the standard and test data sets. Weighted combinations of coefficients are used to correct for interferences from other components whilst quantifying the analyte of interest.

The general procedure can be extended to data sets of higher dimensionality or to different orthogonal basis sets.

Keywords: Orthogonal polynomials; Orthonormal functions; Two-dimensional data; Acyclovir; Guanine

1. Introduction

The use of orthogonal polynomials to correct for interferences in spectroscopy was introduced by Glenn [1]. It was one of the first methods suitable for applying to digitised data. Many applications have been published, mainly involving UV analysis of pharmaceutical mixtures [2,3]. Modern advances in technology have made it easy to collect multi-dimensional data sets from a variety of analytical techniques. The smoothing of multi-dimensional data sets using orthogonal polynomials has already been reported [4]. This paper presents the extension of the orthogonal polynomial method for quantitative analysis in the presence of other absorbing species to two-dimensional data sets. Typical examples include fluorescence excitation–emission plots or UV data over a range of solvents.

Two-dimensional orthonormal arrays are generated by cross-multiplying normalised orthogonal polynomials and can be used to quantify the composition of mixtures if the spectra of the pure components are known.

2. Theory

A series of vectors $V_{0x}, V_{1x}, \ldots, V_{nx}$ is defined in the x direction. Each vector represents a normalised Legendre polynomial. A similar series $V_{0y}, V_{1y}, \ldots, V_{my}$ may be defined in the y direction. A series of two-dimensional orthonormal arrays can be defined by tensor multiplication:

$$A_{00} = V_{0x} \times V_{0y}, \qquad A_{01} = V_{0x} \times V_{1y} \qquad \text{etc.}$$

The number of points along each axis may be varied according to the size of the data array which is to be fitted. The data set S, or a selected subset, may be represented as a sum of orthonormal arrays:

$$S = C_{00}A_{00} + C_{01}A_{01} + \cdots \tag{1}$$

We define the inner product of two arrays (A_{ij}, A_{kl}) by multiplying the corresponding terms together and summing the results.

SSDI 0731-7085(95)01587-6

From Eq. (1), the coefficients are determined using this inner product as follows:

$$(A_{00}, S) = C_{00}(A_{00}, A_{00}) + C_{01}(A_{00}, A_{01}) + \cdots \quad (2)$$

Now, by definition, $(A_{ij}, A_{kl}) = 0$ unless $i = j$ and $k = l$.

Eq. (2) reduces to

$$C_{ij} = (A_{ij}, S)$$

In this work, the constituent orthogonal polynomials used to construct A_{ij} were varied from zero to fifth order, giving a total of 36 orthonormal arrays. Quantitation was carried out following one of the procedures defined by Glenn [1] selecting a weighted combination of two coefficients which gave a suitably large value for the analyte of interest, with minimal interference from the other analyte. The procedure was as follows.

(1) Select a wavelength range where all the spectra are on scale and of a reasonable magnitude.

(2) Calculate the coefficients of the orthonormal functions, and select those coefficients which have a reasonable magnitude and are additive within 2% when comparing the separate analytes with the mixture.

(3) Determine a weighted sum of coefficients $W = C_{ab} + nC_{cd}$ which gives zero response for the interfering analyte and a recovery within 2% of theory for the analyte of interest in the mixture.

3. Experimental

Two-dimensional data sets were generated by measuring UV spectra using a range of pH values. The test system selected was a mixture of acyclovir (9-(2-hydroxyethoxymethyl)-guanine) and guanine, which is a precursor and potential degradation product. A total of six different pH values were used. Since the measurements were labour intensive, the experimental work was limited to a feasibility study without subsequent method validation.

3.1. Materials

Acyclovir complied with the requirements of the European Pharmacopoeia. Guanine was of 99.2% purity. 0.1 M hydrochloric acid and 0.1 M sodium hydroxide were prepared from analytical-reagent grade materials. Phosphate buffer pH 3.0, phosphate buffer pH 6.5 mixed, phosphate buffer pH 8.0, 0.02 M and carbonate buffer pH 9.7 were prepared according to the British Pharmacopoeia.

3.2. Spectrometry

UV spectra were measured using a Perkin Elmer Lambda 7 UV-visible spectrometer controlled by an IBM PS/2 computer.

Measurements were made over the range 200–350 nm using a path length of 10 mm and a 1 nm bandwidth, maintaining the cell at 25°C.

3.3. Calculations

Calculations were carried out off-line using an IBM PS/2 computer. Spectra were saved in JCAMP-DX format and the header information deleted using the Microsoft Windows utility Notepad. Spectra were then read into a worksheet developed using Mathcad 4.0 for calculation of the coefficients from two-dimensional arrays [5]. Following determination of the coefficients, subsequent calculations were carried out with a Microsoft EXCEL spreadsheet.

3.4. Procedure

The following solvents were used for spectrometric measurements: 0.1 M hydrochloric acid, phosphate buffer pH 3.0, 6.5, 8.0, carbonate buffer pH 9.7, 0.1 M sodium hydroxide. Three solutions were prepared in each solvent, containing 2 mg per 100 ml of acyclovir, 2 mg per 100 ml of guanine or 2 mg per 100 ml of both compounds. Measurements were made against the appropriate solvent 'blank. The files were stored in JCAMP-DX format for further processing as described above.

The wavelength range of the array was varied, and those coefficients which gave values of a reasonable magnitude and where the coefficients were additive within 2% were selected for further manipulation. A weighted sum of two coefficients which gave a recovery within 2% of theory for each analyte was determined as described earlier.

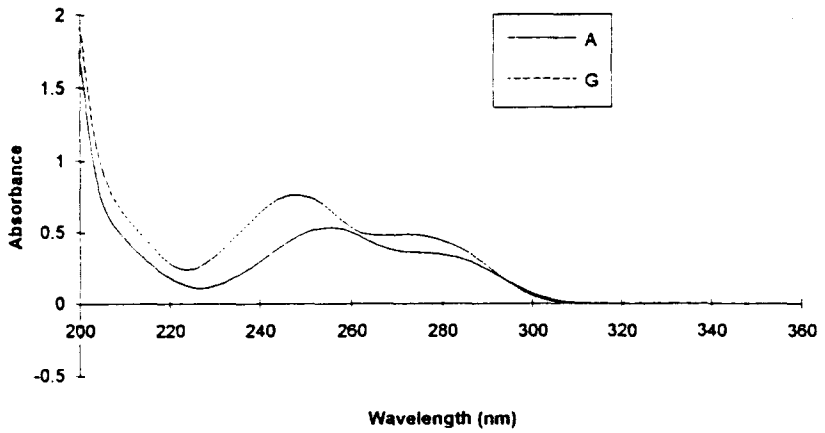

Fig. 1. Spectra of acyclovir (A) and guanine (G) in 0.1 M hydrochloric acid.

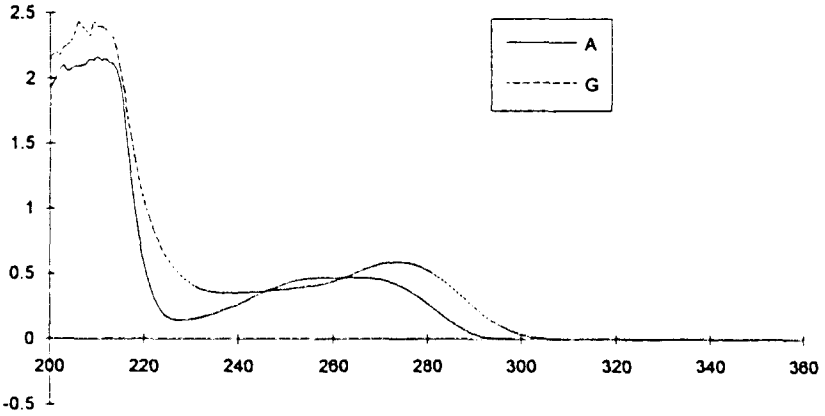

Fig. 2. Spectra of acyclovir (A) and guanine (G) in 0.1 M sodium hydroxide.

4. Results and discussion

Two of the pK_a values of acyclovir (2.3, 9.3) and guanine (3.3, 9.2, 12.3) are fairly close. Although the solvents chosen will to some extent exploit the differences in pK_a, in practice the spectra are somewhat similar in acidic solution but differ more at alkaline pH (Figs. 1 and 2). In the calculation, the spectra were assembled into the matrix in order of increasing pH. For each compound the spectra, in 0.1 M acid and 0.1 M base, were compared using the correlation coefficient. Over the range 239.6–294 nm, $r = 0.87$ for acyclovir and 0.18 for guanine, indicating that guanine exhibits the larger change in UV spectrum.

The orthonormal functions generated are recognisable in that slices taken parallel to an axis are proportional to one of the constituent polynomials (Fig. 3). In every case, the coefficient C_{00} which represents a zero offset was larger than the higher coefficients. It was usually used in quantitation together with higher coefficients, for which the ratio of responses from the two analytes differed markedly from the ratio of C_{00} values. Typical results are shown in Table 1 for different wavelength ranges.

It was initially thought that a larger wavelength range would increase the contribution of higher coefficients in the wavelength direction. There is some evidence that this is the case for the range 239.6–294 nm.

Legendre polynomials were selected because they had been used previously for UV spectra [1–3]. Although other orthogonal functions could be used, there is no theoretical basis for supposing that they would better represent the lineshape for electronic transitions. It is possible to use different orthogonal basis sets in the x and y directions if it proves appropriate for a particular problem.

The data in Table 1 are sufficient to show the feasibility of this approach. However, full method validation was not carried out owing to the difficulty of collecting a large number of data sets over a range of solvents.

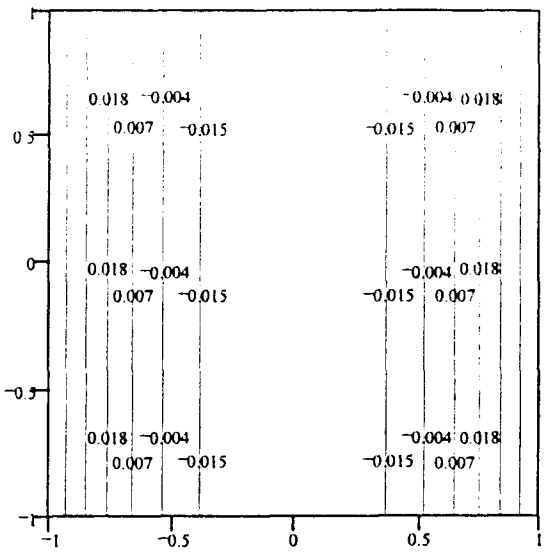

Fig. 3. Orthonormal functions A_{20} and A_{31}. The absolute values of the contour lines are low owing to an increase in the number of points to produce smooth curves.

5. Conclusions

The fitting of orthonormal arrays has been shown to be potentially useful for quantitative analysis with two-dimensional data arrays. The theory can easily be extended to three or more dimensions if required.

Table 1
Quantitation of acyclovir and guanine

Wavelength range	Analyte	Weighted sum of coefficients	% Recovery for analyte
247.6–271.6 nm	Acyclovir	$C_{00} - 17.10C_{20}$	100.4
	Acyclovir	$C_{20} + 4.018C_{31}$	98.0
	Guanine	$C_{20} - 2.639C_{31}$	98.1
	Guanine	$C_{00} + 36.65C_{30}$	99.1
239.6–279.6 nm	Acyclovir	$C_{00} - 36.69C_{20}$	100.6
	Acyclovir	$C_{20} + 0.7142C_{31}$	101.4
	Guanine	$C_{00} + 18.56C_{20}$	101.8
	Guanine	$C_{20} - 3.587C_{31}$	101.3
239.6–294 nm	Acyclovir	$C_{00} - 15.13C_{50}$	99.3
	Acyclovir	$C_{00} + 19.62C_{50}$	100.5
	Guanine	$C_{00} + 26.70C_{30}$	99.9
	Guanine	$C_{00} - 57.07C_{50}$	101.2

Acknowledgements

I am grateful to John Appleby for measurement of the UV spectra and to John Wood of the Wellcome Research Laboratories for expert statistical advice.

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