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Application of derivative spectrophotometry under orthogonal polynomial at unequal intervals: Determination of metronidazole and nystatin in their pharmaceutical mixture

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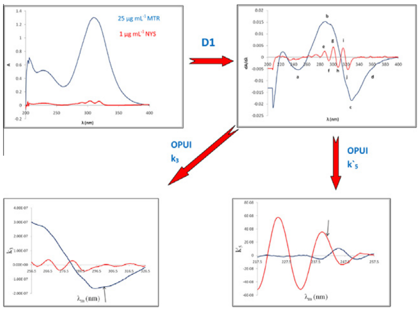
HIGHLIGHTS

- Derivative curves convolution using orthogonal polynomials for unequal intervals.
- Specific polynomial order for each drug was selected based on its derivative curve.
- Solving spectral interferences as spectral overlap and irrelevant absorption.
- Simultaneous determination of a binary mixture in its pharmaceutical matrix.
- Specific and selective determination of each drug in mixtures.

GRAPHICAL ABSTRACT



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ABSTRACT

This paper discusses a general method for the use of orthogonal polynomials for unequal intervals (OPUI) to eliminate interferences in two-component spectrophotometric analysis. In this paper, a new approach was developed by using first derivative D1 curve instead of absorbance curve to be convoluted using OPUI method for the determination of metronidazole (MTR) and nystatin (NYS) in their mixture. After applying derivative treatment of the absorption data many maxima and minima points appeared giving characteristic shape for each drug allowing the selection of different number of points for the OPUI method for each drug. This allows the specific and selective determination of each drug in presence of the other and in presence of any matrix interference. The method is particularly useful when the two absorption spectra have considerable overlap. The results obtained are encouraging and suggest that the method can be widely applied to similar problems.

Introduction

Background

UV–VIS absorption spectrophotometry is a rapid, inexpensive and reliable technique for simultaneous determination of several analytes without the need of prior separation. However, for multi-component analysis, the presence of spectral interference and/or spectral overlapping, would represent a major problem for spectrophotometric quantitation [1].

Different chemometric methods aiming at solving this problem have been applied. In this respect, using of multivariate analysis for spectrophotometric determination of multi-component mixtures has been extensively used. The most widely used of which are, principal component regression (PCR) [2–5], partial least squares (PLS) [2–5], classical least square (CLS) [3,5], principal component

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analysis (PCA) [4], Multiple Linear Regression (MLR) [4], inverse least squares (ILS) [3], rank annihilation factor analysis (RAFA) [6], radial basis function-artificial neural network (RBF-ANN) [5] and principal component-radial basis function-artificial neural network (PC-RBF-ANN) [5].

Other methods have also been successfully used, including derivative [7] and derivative ratio spectrum [8]. Vierordt's method has also been found beneficial [9]. Despite the simplicity and usefulness of these methods in solving certain problems, however, at certain conditions where the two analytes show high overlap and heavy spectral interferences, their applicability may be hindered. In this connection, a new approach was developed in the present work to the use of orthogonal polynomials [1].

The orthogonal functions method has been used to correct irrelevant absorption in multi-component spectrophotometric analysis depending on the use of orthogonal polynomials for the equally spaced intervals [10,11] and with the least-squares approach [10,11].

In the application of orthogonal polynomials for equal intervals to spectrophotometric analysis [10,11], a problem can be arisen where the polynomial integers (polynomial fundamental shapes) especially higher order polynomials may not exactly fit with the fine structure of the absorption curve. In this respect, Korany et. al. [1] introduced the use of orthogonal polynomials for unequal intervals (OPUI). The unequal interval wavelengths were selected according to the shape of a specified segment on the absorption curve. This method was applied for two component analysis where spectra of the analytes were highly overlapped [1]. The method was also extended to the multi-component [1] analysis and was considered to be highly advantageous over the use of orthogonal polynomials for equal intervals.

In this paper, a new hybrid technique was developed by using first derivative D1 curve instead of absorbance curve to be convoluted using OPUI method. In OPUI, the use of first derivative technique is advantageous over normal absorbance one as it eliminates any constant interference. Also, it gives different maxima and minima which facilitate choosing different polynomial orders for each drug to be solved in the presence of each other with good accuracy and precision.

This new approach is used for the determination of metronidazole (MTR) and nystatin (NYS) in their mixture and comparing the results with those obtained from first derivative method alone.

Microbiological and HPLC-based techniques are the common pharmacopeial methods for determination of nystatin and metronidazole, respectively but they are usually very time-consuming relative to spectroscopic techniques. Thus, a method capable of rapidly obtain the content of nystatin and metronidazole, in a reliable and simultaneous manner, is of great interest to pharmaceutical industries.

Different methods have been reported for the determination of MTR either in pharmaceutical dosage form [12–15] or in biological fluids [13,15]. Also for NYS, different methods have been reported either in pharmaceutical dosage form [16–18] or biological fluids [19,20].

To the best of our knowledge, only one method is reported for the analysis of NYS and MTR in their mixture. This method is based on diffuse reflectance NIR measurements and partial least squares regression in vaginal cream [21].

In general, NIR technique is characterized by producing spectra with low intensity, highly overlapping and broad bands. Thus, it is difficult to be used in measuring samples with low concentration. As a result, NIR is usually a chemometric based technique. Most of these chemometric methods, consume time as they need large number of training sets [22]. The proposed UV method lacks these disadvantages. Moreover, the chemometric method introduced in the study was specific and selective by fitting the fine structures of the derivative curves for each drug and it can be easily performed using Excel software.

Theory

Derivative technique (D method)

Application of derivative techniques to spectrophotometric data has become a well-established analytical method [23]. The elimination of interference by the use of derivative techniques depends on the fact that the first derivative of a constant function is zero. Consequently, a first derivative would eliminate constant interferences, thus:

$$\mathrm{D1} = \frac{\mathrm{d}A}{\mathrm{d}\lambda} \tag{1}$$

where, D1 is first derivative.

Derivative technique followed by orthogonal polynomials for unequal intervals D1/OPUI (the proposed method)

It is well known that the basis of harmonic analysis is that, a given function, for example D1 of absorbance curve, can be expanded in terms of an orthogonal function for unequal intervals. Thus,

$$\mathrm{D1} = k_0K_0 + k_1K_1 + k_2K_2 + k_3K_3 + k_4K_4 + k_5K_5 + \ldots + k_nK_n \tag{2}$$

where D1 is the first derivative of absorbance at a wavelength λ that belongs to a set of $n + 1$ unequally spaced wavelengths. K_0, K_1, K_2, K_3, K_4, K_5, . . ., K_n are the polynomials corresponding to constant, linear, quadratic, cubic, quartic, quintic etc. and $k_0, k_1, k_2, k_3, k_4, k_5, \ldots, k_n$ are their corresponding coefficients. In view of the orthogonality, the polynomials can be calculated according to the method of Grandage [24], based on the following

$$\sum_{i=0}^{n} K_{ij} = 0 \tag{3}$$

$$\sum_{i=0}^{n} K_{ij}K_{iu} = 0 \quad \text{where } j \neq u \tag{4}$$

where j, u represent different polynomial orders.Any coefficient, k_{ij}, can be calculated from a set of first derivative data by the equation

$$k_{ij} = \sum_{i=0}^{n} \mathrm{D1}K_{ij} \Big/ \sum_{i=0}^{n} K_{ij}^2 \tag{5}$$

The denominator of this equation is the sum of the squared individual values of K_{ij}.

After the construction of the convoluted polynomial curves of each analyte, the orthogonal polynomial coefficients, k_{ij}, at any λ are proportional to D1 and concentration, thus

$$k_{ij} = \alpha_j c_a \tag{6}$$

where $\alpha_j = k_{ij}$ (1%, 1 cm) is a constant analogous to absorptivity of the pure compound, a, and c_a is the concentration of the absorbing compound, a.

In binary mixture system (providing that each component obeys Beer's law and no interaction exists between the components), the orthogonal function for unequal interval could be applied for the analysis of binary component mixtures. Thus choosing the orthogonal polynomial order (quadratic, cubic, quartic, quintic etc.), number of points, wavelength intervals, the convoluted first derivative curve (orthogonal function coefficient, k, versus mean wavelength, λ_m) could be derived in order to select the optimum λ_m for a given component while the other component exhibits a zero coefficient at this wavelength and vice versa. The