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# Two and three way spectrophotometric-assisted multivariate determination of linezolid in the presence of its alkaline and oxidative degradation products and application to pharmaceutical formulation

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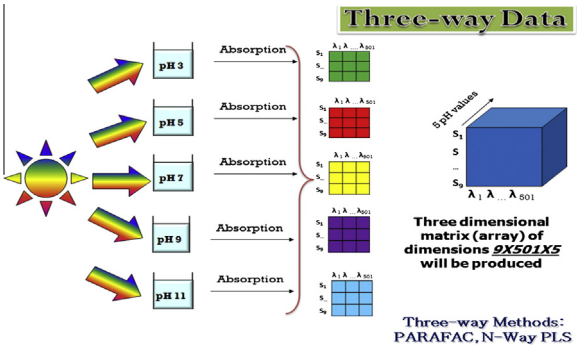
## HIGHLIGHTS

- Most of reported methods of LIN are based on chromatographic technique.
- Chromatographic methods are relatively of high cost and consuming time.
- Multivariate calibration, based on UV spectrophotometric data was used.
- It is suitable method for an accurate, rapid and less expensive determination.
- Several methods were simultaneously determine LIN and its degradation products.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Linezolid (LIN) is determined in the presence of its alkaline (ALK) and oxidative (OXD) degradation products without preliminary separation based on ultraviolet spectrophotometry using two-way chemometric methods; principal component regression (PCR) and partial least-squares (PLS), and three-way chemometric methods; parallel factor analysis (PARAFAC) and multi-way partial least squares (N-PLS). A training set of mixtures containing LIN, ALK and OXD; was prepared in the concentration ranges of 12–18, 2.4–3.6 and 1.2–1.8 μg $mL^{-1}$, respectively according to a multilevel multifactor experimental design. The multivariate calibrations were obtained by measuring the zero-order absorbance from 220 to 320 nm using the training set. The validation of the multivariate methods was realized by analyzing their synthetic mixtures. The capabilities of the chemometric analysis methods for the analysis of real samples were evaluated by determination of LIN in its pharmaceutical preparation with satisfactory results. The accuracy of the methods, evaluated through the root mean square error of prediction (RMSEP), was 0.058, 0.026, 0.101 and 0.026 for LIN using PCR, PLS, PARAFAC and N-PLS, respectively. Protolytic equilibria of LIN and its degradation products were evaluated using the corresponding absorption spectra-pH data obtained with PARAFAC. The obtained p$K_a$ values of LIN, ALK and OXD are 5.70, 8.90 and 6.15, respectively. The results obtained were statistically compared to that of a reported HPLC method,

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and there was no significant difference between the proposed methods and the reported method regarding both accuracy and precision.



## Introduction

Linezolid (LIN) or (S)-N-[3-3-fluoro-4-(4-moropholinyl)phenyl]-2-oxo-5-oxazolidinyl]methyl] acetamide, is considered as the first available oxazolidinone antibacterial agent. LIN is currently the only antibacterial agent which can be orally administered. It may be particularly useful as an alternative to vancomycin in patients with renal function impairment, poor or lack of intravenous access and those who require outpatient therapy, or who do not tolerate glycopeptides [1,2].

Literature survey reveals some LC methods for the determination of LIN in tablets and in biological fluids [3–12]. Chiral LC method was also reported [13]. Three methods determined LIN in the presence of its alkaline-induced degradation products using TLC densitometry, first derivate and first derivate of ratio spectra methods [14]. Studies using capillary electrophoresis to determine LIN were also reported [15,16]. Its electrochemical characterization was also described [17]. LIN was recently determined by HPLC in the presence of both alkaline and oxidative degradation products [18].

With the intent of improving the quality of the active pharmaceutical ingredient (API) and its formulation, there is an increasing need of separation, identification, quantification, and characterization of the most probable and possible degradation products generated under the various ICH guidelines for forced degradation [19]. These guidelines require the stress testing to be conducted to elucidate the inherent stability characteristics of the active substance. An ideal stability-indicating method is one that quantifies the drug and also resolves its degradation products.

Most of the reported methods for the determination of LIN in pharmaceutical preparations are based on chromatographic techniques, presenting relative high cost and time consumption. Multivariate calibration utilizing spectrophotometric data can be considered a suitable method for an accurate, rapid and less expensive determination. The unique feature and the novelty of the proposed spectrophotometric-assisted multivariate calibration methods is that it is the first time that several chemometric methods were applied for the simultaneous determination of LIN in presence of both its alkaline (ALK) and oxidative (OXD) degradation products.

The aim of the present work is to develop simple, sensitive, accurate, and specific multivariate calibration methods which can be performed easily with adequate software support and thus providing a clear example of the their efficient resolving power. Also, to use multi-way models, such as PARAFAC and N-way PLS to improve the predictions and to use the three-way PARAFAC model to study the equilibria of LIN and its degradation products; ALK and OXD and calculating their physical constants and trying to extract their respective spectra. Furthermore, to test for the capability of the adopted chemometric methods for the analysis of real samples through the determination of LIN in its pharmaceutical preparation.

## Theory

### *Two way factor based methods*

Factor based methods combine principal component analysis (PCA) with an inverse least square (ILS) regression to create a quantitative model for complex samples. Among these methods are principal component regression [20,21] (PCR) and partial least squares (PLS).

PLS is a quantitative spectral decomposition technique that is closely related to PCR. However, the decomposition is performed in a slightly different fashion. In PCR method only the information in the matrix is used during data decomposition, but in the PLS method, the concentration data matrix is also used in this step. So PLS not only has the advantage of PCR, but also it produces more robust model as it removes noise from both absorbance and concentration data [22–24].

### *Parallel factor analysis (PARAFAC)*

PARAFAC, one of several decomposition methods for N-way data, is a generalization of PCR [25] to higher orders. It is less flexible, uses fewer degrees of freedom and provides unique solution independent of rotation. This last feature is a great advantage to the modeling of spectroscopic data. The true underlying spectra (or whatever constitutes the variables) will be found if the data is indeed tri-linear, the right number of components is used and the signal-to-noise ratio is appropriated [26].

A PARAFAC model of a three-way array is given by three loading matrices, A, B and C, with elements $a_{if}$, $b_{jf}$ and $c_{kf}$ (Eq. (1)), respectively ($f = 1 - F$ principal components). The tri-linear model is found to minimize the sum of squares of the residues, $e_{ijk}$ in the model [26], which is represented as follows:

$$X_{ijk} = \sum a_{if} b_{jf} c_{kf} + e_{ijk} \quad (1)$$

where $a_f$, $b_f$ and $c_f$ are the $f$th columns of the loading matrices A, B and C, respectively.

An important difference between the two-way PCR and the multi-way PARAFAC is that the PARAFAC model is not nested. This fact means that the parameters of an $F + 1$ component model are not equal to the parameters of an $F$ component model plus one additional component. The reason for this is that the components are not required to be orthogonal, hence independent. Therefore, every model has to be calculated specifically with all its components.

The algorithm used to solve the PARAFAC model is alternating least squares (ALS) [27]. ALS successively assumes the loadings in two modes and then estimates the unknown set of parameters of the last mode. The algorithm converges iteratively until the relative change in fit between two iterations is below a certain value (the default is $1 \times 10^{-6}$). It is initialized by either random values or values calculated by a direct tri-linear decomposition based on the generalized eigenvalue problem [26]. Constraining the PARAFAC solution can sometimes be helpful in terms of the interpretability or the stability of the model. The fit of a constrained model will always be lower than the fit of an unconstrained model, but if the constrained one is more interpretable and realistic, this may justify the decrease in fit. The used constraints are unimodality, orthogonality and non-negativity. The resolution of spectra used to require the non-negativity constraint since negative spectral parameters do not make sense.

A new tool that helps for determining the correct number of components is the core consistency diagnostic (Corcondia) [28] which can be given by the following equation:

$$Corcondia = 100 \times \left(1 - \frac{\sum_{d=1}^{F}\sum_{e=1}^{F}\sum_{f=1}^{F}(g_{def} - t_{def})^2}{\sum_{d=1}^{F}\sum_{e=1}^{F}\sum_{f=1}^{F} t_{def}^2}\right) \quad (2)$$