Kinetic approach for the enzymatic determination of levodopa and carbidopa assisted by multivariate curve resolution-alternating least squares

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ABSTRACT

A combination of kinetic spectroscopic monitoring and multivariate curve resolution-alternating least squares (MCR-ALS) was proposed for the enzymatic determination of levodopa (LVD) and carbidopa (CBD) in pharmaceuticals. The enzymatic reaction process was carried out in a reverse stopped-flow injection system and monitored by UV–vis spectroscopy. The spectra (292–600 nm) were recorded throughout the reaction and were analyzed by multivariate curve resolution-alternating least squares. A small calibration matrix containing nine mixtures was used in the model construction. Additionally, to evaluate the prediction ability of the model, a set with six validation mixtures was used. The lack of fit obtained was 4.3%, the explained variance 99.8% and the overall prediction error 5.5%. Tablets of commercial samples were analyzed and the results were validated by pharmacopeia method (high performance liquid chromatography). No significant differences were found ($\alpha = 0.05$) between the reference values and the ones obtained with the proposed method. It is important to note that a unique chemometric model made it possible to determine both analytes simultaneously.

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1. Introduction

Levodopa [(S)-2 amino-3-(3,4-dihydroxyphenyl) propionic acid] and carbidopa [(S)-3-(3,4-dihydroxyphenyl)-2-hydrazino-2-methylpropionic acid] are drugs used in the treatment of Parkinson's disease. Levodopa (LVD) and carbidopa (CBD) combination is also used to treat tumors, spasms and poor muscle control caused by CO and manganese intoxication, as well as in ophthalmology [1,2]. In order to achieve better curative effect and lower toxicity, it is very important to control the content of these compounds in pharmaceutical tablets.

Several methods have been reported in the literature for the assay of LVD and CBD. These are commonly carried out by potentiometry [3], voltammetry [4,5], high performance liquid chromatography (HPLC) [6–8], capillary electrophoresis (CE) [9,10], NMR [1]H [11], fluorescence [12], synchronous fluorescence [13] and UV–vis spectrophotometry [14–17].

Spectrophotometry is within the most frequently used analytical techniques in pharmaceutical analysis, and has practical and significant economic advantages over other methods. Some reported spectroscopic methods also exploit the advantages provided by kinetic in the quantitative analysis [12,17]. The advantages of kinetic methods have been already mentioned elsewhere [18]. Normally, the kinetic modelling (hard-modelling) involves solving differential equations based on a well defined reaction mechanism. However, such models are frequently complex and need previous knowledge about orders of reaction and rate constants, which are often unavailable [19]. Another disadvantage is that hard-modelling methods do not work in the presence of unmodelled noise or spectral artifacts, such as baseline drift [20]. On the other hand, some of the problems of hard-modelling analysis can be overcome by using soft-modelling methods. In these resolution methods, which do not use an explicit physicochemical model, the analysis focuses on determining response curves, usually concentration and spectral profiles of the reacting species. Soft-modelling can be applied to obtain information from experimental data produced by both equilibrium and kinetic systems, and no assumptions about the kinetic model need to be made. Using soft-modelling methods makes it possible to avoid the errors caused by selecting an incorrect model. At the same time, the signals corresponding to species that do not take part in the reaction can be modelled. Kinetic monitoring has two main and different purposes: the first one is focused on obtaining qualitative information, identifying the species involved in a particular process throughout the time and finding out the underlying reaction model and the derived kinetic parameters; and the second one focused on getting quantitative information, taking advantage of the behaviour that the diverse species show in a kinetic process.

The literature is still poor in analytical procedures based on kinetics, especially for the determination of drugs in commercial dosage forms. Most of the studies are aided by chemometrics tools. Thus, some studies using parallel factor analysis (PARAFAC) [21,22],

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multivariate curve resolution-alternating least squares (MCR-ALS) [23,24], partial least squares (PLS) [16,25,26], three way partial least squares (N-PLS) [27,28] and artificial neural networks (ANN) [29,30] were reported.

Within the group of kinetic processes, enzymatic processes, particularly those using non-selective enzymes, have become a powerful tool for the resolution and quantifying of multicomponent mixtures [31,32]. On the other hand, the extraction of enzymes from natural products is inexpensive and enables to perform highly selective analyses [33,34]. Polyphenol oxidase (PPO; EC 1.14.18.1) is an enzyme widely distributed in the nature and several studies have been reported using crude extracts containing PPO [35,36]. This enzyme catalyses the ortho-hydroxylation of phenols and the oxidation of catechols (such as LVD and CBD) to ortho-quinones, which have a strong absorption in the UV–vis region [37].

In the current study, a kinetic determination of LVD and CBD in pharmaceutical preparations is proposed using PPO as a catalytic agent. The enzymatic reaction process was carried out in a reverse flow injection system and monitored by UV–vis spectroscopy. The spectra, recorded over time for each enzymatic process, were analyzed by MCR-ALS. The results obtained were in close agreement with the values obtained by the pharmacopeial reference method. To the best of our knowledge, it is the first time that MCR-ALS is applied to the kinetic determination of levodopa and carbidopa with PPO in pharmaceutical preparations. Among the advantages of the MCR-ALS could be mentioned the ability of dealing with data having certain lack of trilinearity, and that only one model is needed to determine both analytes. The proposed method results inexpensive, consumes low amounts of reagents and avoids separation techniques to achieve feasible quantitative results.

2. Experimental

2.1. Apparatus and software

Spectrophotometric measurements were carried out by using a Hewlett-Packard model 8452 A UV–vis diode array spectrophotometer with a Hellma 178-010-QS flow cell (inner volume of 18 μL).

A Gilson Minipuls 3 peristaltic pump and a Rheodyne 5041 injection valve were used.

The reaction coils, sampling loop and flow lines consist of PTFE tubing (0.5 mm id).

An Orion model 710 A pH Meter with an Orion-Ross® model 81-02 electrode was used to carry out the pH measurements.

Data treatment was performed using MATLAB® 7.0 (The MathWorks) and the MCR-ALS subroutines [38].

2.2. Reagents and solutions

All reagents were of analytical grade. All solutions were prepared with ultra pure water (18 MΩ, Barnstead).

Table 1
Concentration data corresponding to the calibration set.

Calibration mixture	LVD	CBD
1	1.05	0.66
2	5.67	0.66
3	3.16	0.16
4	3.16	0.94
5	1.70	0.37
6	5.02	0.37
7	1.70	0.98
8	5.02	0.98
9	3.16	0.57

The values are expressed in mg mL^{-1}.

A 0.1 mol L^{-1} phosphate buffer solution (pH 7.0) was used for preparation of LVD and CBD standard solutions.

Stock solutions of LVD and of CBD (both from Saporiti) 0.800 and 0.400 mg mL^{-1} in concentration, respectively, were freshly prepared in medium of phosphate buffer. All stock solutions were protected from light and stored at 4 °C. The working standard solutions were freshly prepared by adequate dilutions of the stock solutions in phosphate buffer.

PPO extracts were obtained from sweet potatoes roots (*Ipomoea batatas*), purchased in local supermarkets, as described in a previous paper [36].

Pharmaceutical preparations of Lebocar® (Pfizer) and Parkinel® (Bagó) were purchased in a local pharmacy. These preparations were presented in the form of tablets, with a nominal content of 250 mg of LVD and 25 mg of CBD (Lebocar® A and Parkinel® A) and 100 mg of LVD and 25 mg of CBD (Lebocar® B and Parkinel® B).

2.3. Methods

2.3.1. Preparation of the calibration and validation sets

A calibration set of nine mixtures of LVD and CBD was prepared as shown in Table 1. The concentration ranged from 1.05 to 5.67 mg mL^{-1} for LVD and from 0.16 to 0.94 mg mL^{-1} for CBD. The component ratios were selected considering the usual LVD/CBD relationship in the commercial pharmaceutical products, i.e., from 4:1 to 10:1. Also, a validation set of six mixtures (Table 2) was prepared in order to evaluate the overall error of prediction according to the following expression:

$$\text{error}(\%) = \frac{\sqrt{\sum_{i=1}^{\text{sample}}(C_{i\,\text{true}} - C_{i\,\text{calc}})^2}}{\sqrt{\sum_{i=1}^{\text{sample}}(C_{i\,\text{true}})^2}} \times 100 \qquad (1)$$

where $C_{i\,\text{true}}$ was the true concentration of the analyte in the synthetic mixture i and $C_{i\,\text{calc}}$ was the concentration calculated by the proposed method.

2.3.2. Sample preparation

Four samples were analyzed, corresponding to the commercial formulations mentioned in Section 2.2. Ten tablets of each phar-

Table 2
Model validation results.

Validation mixture	LVD			CBD		
	Nominal	Predicted	Recovery (%)	Nominal	Predicted	Recovery (%)
1	3.23	3.15	97.5	0.21	0.20	95.2
2	1.61	1.52	94.4	0.33	0.34	103.3
3	4.84	4.53	93.6	0.33	0.31	93.9
4	1.61	1.69	105.0	0.91	0.87	95.6
5	4.84	4.88	100.8	0.91	0.99	108.8
6	3.23	3.54	109.6	0.62	0.68	109.7

The nominal and predicted values are expressed in mg mL^{-1}.