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Flow-batch technique for the simultaneous enzymatic determination of levodopa and carbidopa in pharmaceuticals using PLS and successive projections algorithm

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Abstract

An enzymatic flow-batch system with spectrophotometric detection was developed for simultaneous determination of levodopa [(*S*)-2 amino-3-(3,4-dihydroxyphenyl)propionic acid] and carbidopa [(*S*)-3-(3,4-dihydroxyphenyl)-2-hydrazino-2-methylpropionic acid] in pharmaceutical preparations. The data were analysed by univariate method, partial least squares (PLS) and a novel variable selection for multiple lineal regression (MLR), the successive projections algorithm (SPA). The enzyme polyphenol oxidase (PPO; EC 1.14.18.1) obtained from *Ipomoea batatas* (L.) Lam. was used to oxidize both analytes to their respective dopaquinones, which presented a strong absorption between 295 and 540 nm. The statistical parameters (RMSE and correlation coefficient) calculated after the PLS in the spectral region between 295 and 540 nm and MLR-SPA application were appropriate for levodopa and carbidopa. A comparative study of univariate, PLS, in different ranges, and MLR-SPA chemometrics models, was carried out by applying the elliptical joint confidence region (EJCR) test. The results were satisfactory for PLS in the spectral region between 295 and 540 nm and for MLR-SPA. Tablets of commercial samples were analysed and the results obtained are in close agreement with both, spectrophotometric and HPLC pharmacopeia methods. The sample throughput was 18 h^{-1}.
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1. Introduction

Levodopa [(*S*)-2 amino-3-(3,4-dihydroxyphenyl)propionic acid] is a precursor of the neurotransmitter dopamine used in the treatment of Parkinson's disease. It is a progressive neurological disorder that occurs when the brain fails to produce enough dopamine. This condition causes tremor, muscle stiffness or rigidity, slowness of movement and lost of balance.

Dopamine cannot be administered directly because it does not cross the blood brain barrier readily. Therefore, its precursor levodopa is given orally, which is easily absorbed through the bowel, there, the dopamine is formed by the action of the decarboxylase. High levels of dopamine also cause adverse reactions such as nausea, vomiting and cardiac arrhythmias. Usually, the peripheral decarboxylation of levodopa in extracerebral tissues is associated with an inhibitor of peripheral aromatic-L-amino acid decarboxilase, such as carbidopa [(*S*)-3-(3,4-dihydroxyphenyl)-2-hydrazino-2-methylpropionic acid]. Thus, the importance of the presence of carbidopa (CBD) together with levodopa (LVD) makes that the dopamine levels can be controlled properly. Also, it was observed that the side effects are reduced [1,2].

In order to achieve better curative effect and lower toxicity, it is very important to control the content of levodopa and carbidopa in pharmaceutical tablets. The most frequently analytical technique, used in quality control analyses of pharmaceutical products, is high-performance liquid chromatography (HPLC). However, this technique is expensive, labour-intensive task, time consuming and produces chemical waste.

The polyphenol oxidase (PPO; EC 1.14.18.1) is an enzyme widely distributed in the nature. This enzyme catalyses the oxidation of LVD and CBD to the corresponding dopaquinone

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which is converted to leucodopachrome by a rapid and spontaneous auto-oxidation. Then, the leucodopachrome is oxidized to its corresponding dopachrome. This kind of chemical reaction, where the dopachrome was formed, is produced with both analytes LVD and CBD and the products of both reactions present a strong absorption in the UV–vis spectra [3–5].

LVD and CBD determinations are commonly carried out by using high-performance liquid chromatography (HPLC) [6–8], capillary electrophoresis (CE) [9] and chemometrics-assisted spectrophotometric method [10]. Fatibello-Filho et al. [11] have published a paper on the FI spectrophotometric determination of LVD and CBD applying univariate calibration and using polyphenol oxidase. However, the mixture of both analytes shows a serious spectral overlapping, after the chemical reaction with the enzyme. Moreover, such flow manifold requires significant changes in their physical assemblies when it is necessary to analyse samples with a large variation of analyte concentration and/or physical–chemical properties.

Automated micro batch (AMBA) proposed by Sweileh and Dasgupta [12,13], and flow-batch analysers (FBA) proposed, developed and first named by Araújo et al. [14] constitute an excellent alternative to automate the quality control of pharmaceutical products because they are systems very flexible and versatile (multi-task characteristic). With AMBA or FBA, it is possible to work in wide analyte concentration range as well as to implement different analytical processes [14–25] without significant alterations on the physical configurations of the analyser. All these may be accomplished just by changing the operational parameters in their control software. These analysers have been used to implement several analytical procedures such as: liquid–liquid extraction [12], distillation of volatile analyte [13], kinetic approach [13], titrations [14,15], analyte addition [16,17], internal standard [18], screening analysis [19], exploitation of concentration gradients [20], on-line matching of pH [21] and salinity [22] and sample digestion [23].

FBA and AMBA combine favorable characteristics of both flow (FA) and batch analysers (BA). As in FA, the transportation of reagents, samples or other solutions are carried out in a flow mode, and, as in BA, the sample processing is carried out into a mixing chamber (MC). In AMBA, an injecting loop is used on the sampling stage (as in FA), while in FBA the sample amounts are added into the MC by controlling the ON switching time of one solenoid valve. As most of the FA, FBA and AMBA also present good precision and accuracy, high sample throughput and low contamination, consumption, manipulation of reagents and samples, cost per analysis and waste liberation for the environment, etc. Moreover, these analysers present high sensitivity because the physical and chemical equilibria inherent to the analytical processes may be attained and the dispersion and/or dilution of the samples may be negligible. In another hand, the analytical signal measurements can be performed in flow cells or directly inside MC and the multicommutation [24,25] may be used in order to manipulate the fluids in a simultaneous and/or in an intermittent way.

The application of multivariate calibration methods, such as multiple linear regression (MLR), principal component regression (PCR) and partial least squares (PLS), to the spectrometric data may require the use of variable selection for constructing well-fitted models. Several authors have presented theoretical and empirical evidence supporting the use of variable selection to improve the predictive ability of PCR, PLS [26–28] and, principally, MLR models. MLR yields models which are simpler and easier to interpret than PCR and PLS, since these calibration techniques perform regression on latent variables, which do not have physical meaning. In another hand, MLR calibration is more dependent on the spectral variables selection. To overcome this problem, Araújo et al. proposed a novel variable selection strategy for MLR calibration, which uses the “successive projections algorithm” (SPA) to minimize collinearity problems [29,30]. SPA is a forward selection method which operates on the instrumental response. The number of variables to be selected can be optimized in order to maximize model prediction capability [29].

The aim of the present work was to propose a chemometric-assisted flow-batch method for the simultaneous spectrophotometric determination of levodopa and carbidopa in medicaments. The method was based on the enzymatic oxidation of LVD and CBD with PPO obtained from a natural source (*Ipomoea batatas* (L.) Lam), in phosphate buffer medium (pH 7.0).

2. Experimental

2.1. Apparatus and software

Centrifugation of extracts was performed in a refrigerated-automatic Sigma centrifuge. The spectrometric measurements were carried out by using a Hewlett-Packard model 8453 UV-visible diode array spectrophotometer with a Hellma flow cell (inner volume of 18 μL). A model 713 Metrohm pHmeter was used to carry out the pH measurements.

SPA and MLR calculations and pre-selection were performed using programs developed in our own laboratory with the MATLAB®, Version 5.3 high-level programming language. The UNSCRAMBLER® chemometrics software (CAMO A/S), Version 9.5, was used for PLS calculations.

2.2. Reagents and solutions

All reagents were of analytical grade. To prepare all solutions ultra pure water (18 MΩ) was used.

A 0.1 mol L^{-1} phosphate buffer solution (pH 7.0) was prepared. 0.05 mol L^{-1} catechol stock solution used as substrate for enzymatic activity determination was prepared by dissolving 0.1375 g of catechol (Anedra) in 25 mL of the buffer solution.

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

Dowex 1×8 100–200 mesh (Fluka) strong basic, quaternary ammonium anion exchange resin was used as a protective and stabiliser agent in the sweet potato extract preparation. A 0.015%

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