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Simultaneous voltammetric determination of levodopa, carbidopa and benserazide in pharmaceuticals using multivariate calibration

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ABSTRACT

An analytical methodology based on differential pulse voltammetry (DPV) on a glassy carbon electrode and the partial least-squares (PLS-1) algorithm for the simultaneous determination of levodopa, carbidopa and benserazide in pharmaceutical formulations was developed and validated. Some sources of bi-linearity deviation for electrochemical data are discussed and analyzed. The multivariate model was developed as a ternary calibration model and it was built and validated with an independent set of drug mixtures in presence of excipients, according with manufacturer specifications. The proposed method was applied to both the assay and the uniformity content of two commercial formulations containing mixtures of levodopa–carbidopa (10:1) and levodopa–benserazide (4:1). The results were satisfactory and statistically comparable to those obtained by applying the reference Pharmacopoeia method based on high performance liquid chromatography. In conclusion, the methodology proposed based on DPV data processed with the PLS-1 algorithm was able to quantify simultaneously levodopa, carbidopa and benserazide in its pharmaceuticals formulations using a ternary calibration model for these drugs in presence of excipients. Furthermore, the model appears to be successful even in the presence of slight potential shifts in the processed data, which have been taken into account by the flexible chemometric PLS-1 approach.

1. Introduction

The Parkinson's disease is related to a significant depletion of the dopamine neurotransmitter in the brain. Levodopa, a precursor of this neurotransmitter, is the principal drug used in the treatment of patients with Parkinson's disease. This catecholamine drug, in contrast to dopamine, is able to cross the blood–brain barrier and is metabolized into the central nervous system by dopadecarboxylase enzyme to dopamine. However, the enzymatic metabolization of levodopa does also occur in the peripheral system, producing different side effects associated to the increase of systemic dopamine. For this reason, levodopa is administered in pharmaceuticals in association with a peripheral dopadecarboxylase inhibitor, such as carbidopa or benserazide. The administration of these pharmaceuticals improves the efficiency of the treatment, because it makes possible a better control of dopamine levels, allowing to decrease the dose and the side effects [1]. The chemical structures of these compounds are shown in Fig. 1.

Different analytical methods have been employed for the determination of levodopa, carbidopa and benserazide in raw material, pharmaceutical formulations and biological fluids, mainly by high performance liquid chromatography [2–6], spectrophotometry [7–11], and capillary electrophoresis [12–15]. As with other catecholics and pyrogallics derivates, these drugs contain electroactive groups and can be electrochemically oxidized on carbon, platinum or gold electrodes. This has enabled the electrochemical characterization and determination of levodopa [16–21], carbidopa [22] and benserazide [23]. However, few electrochemical methodologies have been developed for simultaneous determination of these drugs, probably due to their similar structural patterns and electrochemical responses showing dramatic overlapping when using conventional electrodes. Recently, voltammetric methods have been reported for the simultaneous determination of these drugs using modified electrodes [24,25], but the treatment involved is time consuming and the associated cost is high. It is thus important to develop new methodologies for the simultaneous determinations of these drugs. An attractive possibility is the use of chemometric and multivariate calibration methods.

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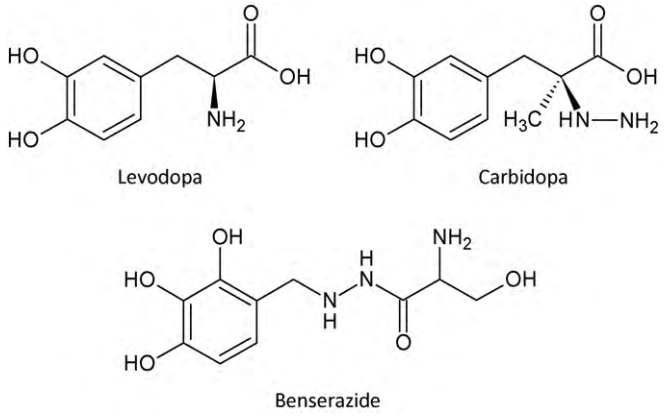

Fig. 1. Chemical structures of the drugs used in the treatment for Parkinson's disease.

In the recent years, multivariate calibration methods applied to absorptive spectral and electrochemical data are being increasingly used for the analysis of complex mixtures [26,27]. Several tools have been reported in the literature for processing these data [28], although the most popular are principal component regression (PCR) [29] and partial least-squares regression (PLS) [30]. All these techniques have the advantage of using the full spectral information and not only a characteristic peak value. Moreover, they allow a rapid determination of mixture components, often with no prior separation, and the calibration can be performed ignoring the concentrations of all components except the analyte of interest in complex samples. Recently, some spectrophotometric methods, assisted by multivariate calibration, have been described for the simultaneous determination of levodopa and carbidopa or levodopa and benserazide in pharmaceutical formulations [10,11]. Nevertheless, to the best of our knowledge, no reports exist in the literature about chemometric models applied to electrochemical data to resolve mixtures of these drugs.

In this work, we present the development of an electroanalytical methodology based on differential pulse voltammetry (DPV) on a glassy carbon electrode and the PLS-1 algorithm for the simultaneous determination of levodopa, carbidopa and benserazide.

2. Experimental

2.1. Reagents and standard solutions

Chile Laboratories (Santiago, Chile) supplied levodopa (99.3%) and carbidopa monohydrate (98.9%). Benserazide hydrochloride (98.5%) was supplied by Tecnofarma Laboratories (Santiago, Chile). Commercial tablets of Grifoparkin® (declared amount per tablet: 250 mg levodopa and 25 mg carbidopa, Chile Laboratories, Santiago, Chile) and Prolopa® (declared amount per tablet: 200 mg levodopa and 50 mg benserazide, Roche Laboratories, Santiago, Chile) were obtained commercially. All other reagents were of analytical grade unless indicated otherwise. Sodium hydrogen phosphate, phosphoric acid and acetonitrile HPLC grade were obtained from Merck. All solutions were prepared with ultrapure water (ρ = 18 MΩ) from Millipore-Milli-Q system.

Stock standard solutions of the drugs were prepared daily at a concentration of 1×10^{-2} mol L^{-1} in 0.1 mol L^{-1} perchloric acid solution and stored in amber glass material. Working solutions were prepared by diluting each stock standard solution before the measurements, using 0.1 mol L^{-1} Britton–Robinson buffer, 0.1 mol L^{-1} perchloric acid solution or 0.1 mol L^{-1} hydrochloric acid.

2.2. Apparatus

Differential pulse voltammetry measurements were performed with a BAS CV-50W electroanalyzer equipped with a 10-mL BAS cell. The components utilized in the three-electrode cell system were a glassy carbon (GC) (Ø = 3 mm, CHI) as working electrode, a platinum wire as auxiliary electrode and an Ag/AgCl (sat) as reference electrode.

High performance liquid chromatography (HPLC) measurements were carried out on a Waters assembly equipped with a model 600 Controller pump and a model 996 Photodiode Array Detector. The acquisition and treatment of data were made with the Millenium version 2.1 software. A Phenomenex C-8 column of 4.6 mm × 150 mm was used, and a C18 Bondapak (30 mm × 4.6 mm) was employed as column guard. The injector was a 20-μL Rheodyne valve. The column was kept at constant temperature using a Waters column heater cartridge model 600.

2.3. Calibration set for the PLS-1 model

For training the PLS-1 model, a calibration set of fourteen ternary mixtures was prepared using a central composite design with five concentration levels of each analyte: levodopa in the range 1.1×10^{-4} to 1.3×10^{-3} mol L^{-1}, carbidopa in the range 3.1×10^{-5} to 4.7×10^{-4} mol L^{-1} and benserazide 3.1×10^{-5} mol L^{-1} to 6.2×10^{-4} mol L^{-1}. A duplicated "center point" (level "0") solution was included in the calibration set, obtaining sixteen solutions for this set. The component ratios were selected considering the linear calibration ranges (previously established from univariate experiments for each drug) and the usual levodopa: carbidopa or levodopa: benserazide ratios present in commercial pharmaceutical formulations (4:1 to 10:1 for levodopa:carbidopa and 4:1 for levodopa:benserazide). All calibration samples were prepared by mixing appropriate volumes of levodopa, carbidopa and benserazide stock standard solutions and containing excipients according with manufacturer specifications. The excipients used for drug mixtures were cornstarch, microcrystalline cellulose, mannitol, polividone, ethylcellulose, titanium dioxide, talc, magnesium stearate and FD&C blue No. 2. Finally, the solutions were measured by triplicate and in random order.

2.4. Test samples for the PLS-1 model

An independent set of nine ternary mixtures was prepared by mixing appropriate volumes of each drug in the same concentration range used for calibration. The solutions were prepared containing the same excipients considered for calibration set. Each sample was measured by triplicate and in random order.

2.5. Electrochemical procedure

All DPV experiments were carried out at room temperature at the following operating conditions for the three drugs: sensitivity, 100 μA V^{-1}; potential range, −200 to 1400 mV at 4 mV intervals; sweep rate, 20 mV s^{-1}. Before each measurement, the working electrode surface was mechanically polished with 0.3 and 0.05 μm alumina slurries [31]. Working and sample solutions were analyzed in 0.1 mol L^{-1} perchloric acid.

2.6. Analysis of pharmaceutical forms

Assay and uniformity content of commercial samples were evaluated by both the DPV proposed method and HPLC official methods [32,33].