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Short communication

Standard additions-dilution method for absolute quantification in voltammetry of microparticles. Application for determining psychoactive 1,4-benzodiazepine and antidepressants drugs as adulterants in phytotherapeutic formulations

Antonio Doménech-Carbó[a,*], Mariele Martini[b], Leandro Machado de Carvalho[b], Carine Viana[b], María Teresa Doménech-Carbó[c], Miguel Silva[c]

[a] Departament de Química Analítica, Universitat de València, Dr. Moliner, 50, 46100 Burjassot, València, Spain
[b] Universidade Federal de Santa Maria – UFSM, Campus Universitário, Caixa Postal 5051, CEP 97105-970 Santa Maria, RS, Brazil
[c] Institut de Restauració del Patrimoni/Departament de Conservació i Restauració de Bens Culturals, Universitat Politècnica de Valencia, Camí de Vera 14, 46022 València, Spain

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ABSTRACT

A standard additions-dilution solid-state electrochemical method for the determination of psychoactive 1,4-benzodiazepine and antidepressants drugs used as adulterants in commercial slimming herbal formulations is described and compared with conventional standard addition method. The proposed method, based on the voltammetry of microparticles approach, permits quantify, via standard additions methodology, 1,4-benzodiazepine and antidepressants drugs in phytotherapeutic formulations with no need of sample dissolution using dilution with a reference electroactive compound. The method was used to measure 1,4-benzobenzodiazepines (clonazepam, flurazepam, alprazolam, midazolam, bromazepam, chlordiazepoxide, lorazepam and diazepam) and antidepressants (bupropion, sertraline, paroxetine and fluoxetine) in slimming formulations that have been commercialized in Brazil.

1. Introduction

The use of 'natural' herbal formulations for phytotherapeutic purposes has experienced a considerable growth in developing countries. It has been detected, however, the presence of non-declared synthetic pharmaceuticals as adulterants in such formulations, including antidepressants and others [1]. The identification and detection of such adulterants is an obvious analytical target for reasons of public health. Disposable techniques include high performance liquid chromatography (HPLC), capillary electrophoresis (CE) [2], micellar electrokinetic capillary chromatography (MECC) [3] and electroanalytical techniques [4–7]. These last are based on the adsorption of the analytes on mercury electrodes, thus requiring the prior dissolution of the sample.

In a previous report [8], we have proposed a solid-state electrochemistry method for screening different families of adulterants based on the voltammetry of microparticles (VMP) methodology. This is a solid-state electrochemical technique developed by Scholz et al. [9] which has been applied for identification [10,11] and quantification of natural products [12], and estimation of antioxidant properties of vegetables [13,14]. Here, we report the application of VMP for the quantitative determination of adulterants in herbal formulations using solid-state standard additions-dilution method on the basis of the characteristic voltammetric signatures of such compounds, where the sample containing the analyte is spiked with a known amount of the same and diluted with known amounts of a reference compound. Ferrocene, Prussian blue and indigo are tested as reference compounds and the analytical performance of the additions-dilution method is compared with that of the standard addition method, previously applied to quantification, via VMP, of selected components in ceramic materials [15]. Sample dilution with sepiolite, a widely used phyllosilicate clay, was used in order to facilitate the homogeneization of the specimens.

Quantification of 1,4-benzodiazepine anxiolitics (clonazepam, flurazepam, alprazolam, lorazepam, midazolam, bromazepam, chlordiazepoxide, lorazepam, diazepam) and antidepressants (bupropion, paroxetine, sertraline, fluoxetine) in herbal formulations was performed. Atomic force microscopy (AFM) coupled with electrochemical runs was used to assess the genuine solid-state nature of the involved electrochemical processes, complementing prior reported data [8,16,17].

* Corresponding author.
E-mail address: antonio.domenech@uv.es (A. Doménech-Carbó).

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2. Experimental

Clonazepam 99.9%, bupropion 100.0% (Genix Pharmaceutical Industry, Pakistan); bromazepam 99.8%, alprazolam 100.18%, lorazepam 99.8%, chlordiazepoxide 99.57%, fluoxetine 100.5%, paroxetine 99.94%, sertraline 99.7% (Option Fênix Distribution of Inputs, Brazil); midazolam 100.06, flurazepam 99.56%, dizepam 98.99% (Deg Importation of Chemicals, India) were used as received. 0.25 M acetic acid (99.0%) plus sodium acetate (99.5%, both from Panreac, Spain) aqueous buffer (pH 4.75) was used as electrolyte, sepiolite, indigo, ferrocene (98.0%, Fluka, USA) and Prussian blue (Fluka, USA). The herbal formulations ($n = 15$) analyzed in this work were acquired from pharmacies in different regions of Brazil and were received by mail. The nominal composition of the samples is summarized in Table 1. Voltammetric measurements and in situ AFM-monitored electrochemical experiments were performed at sample-modified paraffin-impregnated graphite electrodes using equipment and methods previously described [8].

Table 1
List of samples in this study, all corresponding to commercial herbal formulations.

Sample	Composition
1	Slendesta™ 250 mg
2	*Centella asiatica* 100 mg, *Cynara scolymus* 100 mg, carboxymethylcellulose 200 mg, *Rhamnus purshiana* 100 mg, *Spirulina maxima* 200 mg
3	*Spirulina maxima*, *Amorphophallus konjak*, collagen, gelatin

3. Results and discussion

3.1. General voltammetric pattern

The electrochemical response of 1,4-benzodiazepines in solution is dominated by the proton-assisted reduction of the C=N motif [18,19]. Prior voltammetric data [8] for microparticulate deposits of the drugs on graphite electrodes immersed into aqueous acetate buffer indicated that intense proton-assisted reduction ca. −1.0 V vs. AgCl/Ag occurs (see Fig. 1). This voltammetry can be considered as consistent with theoretical modeling for ion-insertion solids [9,20], as previously reported [8]. Fig. 2 shows in situ atomic force microscopy (AFM) images from the upper face and sides of a deposit of acicular crystals of lorazepam (a) before and (b) after application of a reductive step at a constant potential of −1.05 V during 5 min. As shown in SWVs in Fig. 2c and d, significant changes occur in the voltammetric response. Consistently with theoretical modeling [9,20], and previous observations [8], the profile of the region of the crystal in contact with the base electrode/electrolyte boundary becomes modified with no significant dissolution of the crystals.

3.2. Standard additions method

Let us consider a sample of mass m of a solid material containing unknown amount m_X of an electroactive compound, X. Now, let us assume that the sample is mixed with a solid reference compound, R, which displays an electrochemical response independent from that of X. It is assumed that weighted amounts of sample (m) and the reference compound (m_R) are accurately powdered and mixed so that the mass ratio between the reference compound and the mass of the X-containing material, r_R ($= m_R/m$), is known. Assuming that X and R compounds display well-separated, independent signals in a suitable electrolyte, voltammograms of the mixture must provide peaks corresponding to their respective redox processes. In solid-state voltammetry, in which separate microparticles of each one of the electroactive compounds are mechanically transferred to the surface of an inert electrode, independent electrochemical processes should occur. Accordingly, peak currents, $i_p(X)$, $i_p(R)$ (or

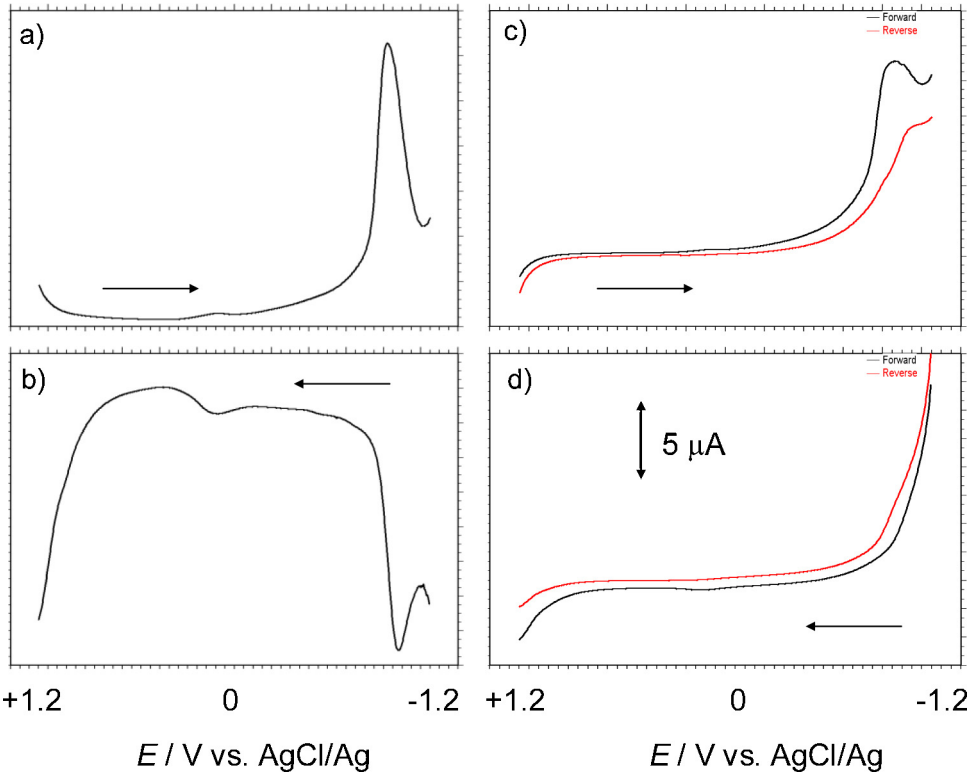

Fig. 1. SWV for diazepam-modified PIGE immersed into 0.25 M aqueous sodium acetate buffer at pH 4.75. Potential scan initiated at (a) +1.05 V in the negative direction, (b) −1.05 V in the positive direction (scan direction marked by arrows). Potential step increment 4 mV, square wave amplitude 25 mV, frequency 5 Hz. Forward and backward components are depicted.