# Resolution of quaternary mixtures of cadaverine, histamine, putrescine and tyramine by the square wave voltammetry and partial least squares method

W. Henao-Escobar, O. Domínguez-Renedo *, M.A. Alonso-Lomillo, M.J. Arcos-Martínez

Analytical Chemistry Department, Faculty of Sciences, University of Burgos, Pza. Misael Bañuelos s/n, 09001 Burgos, Spain

## ARTICLE INFO

## ABSTRACT

This work presents the simultaneous determination of cadaverine, histamine, putrescine and tyramine by square wave voltammetry using a boron-doped diamond electrode. A multivariate calibration method based on partial least square regressions has allowed the resolution of the very high overlapped voltammetric signals obtained for the analyzed biogenic amines. Prediction errors lower than 9% have been obtained when concentration of quaternary mixtures were calculated. The developed procedure has been applied in the analysis of ham samples, which results are in good agreement with those obtained using the standard HPLC method.

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## 1. Introduction

Biogenic amines (BAs) are organic, basic nitrogenous compounds of low molecular weight, usually formed by the removal of the alpha-carboxyl group from a proteinogenic amino acid as a result of normal metabolic activities in humans, animals, plants and microorganisms. They are frequently present in many foods and beverages including cheese, sausages, soy sauces, fish, fermented fish, wine, and beer, being histamine (His), tyramine (Tyr), putrescine (Put), cadaverine (Cad), spermine (Spm) and spermidine (Spd) the most important BAs occurring in foods [1,2]. The intake of foods or beverages containing a high concentration of BAs may cause food intoxication with different symptoms [3]. In this way, His is the causing agent of the food-borne chemical hazard called scombroid poisoning. Scombroid poisoning is usually a mild illness with a variety of symptoms, including rash, urticaria, nausea, vomiting, diarrhea, flushing, and tingling and itching of the skin [4]. Tyr is a potent vasoconstrictor, which may induce hypertension, migraines, brain hemorrhage and heart failure when high concentrations are present in an organism [5]. Put and Cad can react with nitrite to form carcinogenic nitrosamines [6].

Moreover, a high level of BAs indicates poor quality of raw materials, contamination with micro-organisms or improper processing, and storage in food industry [2]. Therefore, the development of sensitive methods for their analysis is of great importance.

Numerous authors have dealt with the detection of BAs content in different types of foodstuffs, under different conditions of treatment and storage, by means of HPLC methods, thin layer chromatography, gas chromatography, and capillary electrophoresis [7–10]. The above-cited techniques require in most cases tedious pre-treatment and derivatization steps of the sample, as well as expensive and complex instrumentation. Consequently, these techniques result not suitable for in situ analysis. Electrochemical biosensors have been often developed for the determination of BAs. These electrochemical systems are based on the use of commercial amine oxidases (AOs) enzymes as biological recognition elements for detecting BAs. In this way, plasma amine oxidase (PAO) [2,11], diamine oxidase (DAO) [11–17] and monoamine oxidase (MAO) [15,17–19] have been successfully applied in the determination of different BAs. The main problem of the described biosensors come from the poor enzyme selectivity towards the different BAs and/or the high working potentials needed for operation, leading to an increase in interferences [20]. In order to improve the selectivity of biosensors more specific enzymatic systems have been used in the construction of these electrochemical biosensors [21–25]. However, these systems present a higher cost associated with their manufacture, and limit the

* Corresponding author.
E-mail addresses: whenao@ubu.es (W. Henao-Escobar),
olgado@ubu.es (O. Domínguez-Renedo), malomillo@ubu.es (M.A. Alonso-Lomillo),
jarcos@ubu.es (M.J. Arcos-Martínez).

analysis to only one amine.

The determination of BAs by means of voltammetric techniques has been limited by the high oxidation potentials of these compounds. However, diamond thin-film and boron-doped diamond (BDD) electrodes have been successfully applied to the voltammetric determination of different polyamines [26]. The attractive features of conductive BDD include a wide electrochemical potential window in aqueous [27,28] and non-aqueous [29], very low capacitance [30], and extreme electrochemical stability media [31]. These properties have made BBD electrodes promising electrochemical systems for the electrochemical analysis of different compounds [26,32–35].

In this work a BDD electrode has been used for the simultaneous determination of His, Tyr, Put and Cad by square wave voltammetry (SWV). The analysis of these BAs in a same sample is complex due to their close oxidation potentials, which lead to high ovelapped signals. Thus, multivariate calibration techniques are then neccesary in order to achieve the simultaneous analysis of these compounds. In this way, a partial least squares (PLS) regression has been performed, which has been succesfuly proven in the resolution of overlapped signals in electrochemical techniques [36–38].

## 2. Experimental

### 2.1. Reagents

All reagents used were of analytical-reagent grade. Ultrapure water obtained from a Milli-Q water purifier (Millipore, Bedford, MA, USA) was used for the preparation of all solutions.

Put and Cad were obtained from Sigma-Aldrich (Steinheim, Germany). Tyr and His were acquired from Fluka (Steinheim, Germany).

The supporting electrolyte used for the electrochemical measurements was a 10 mM $Na_2CO_3$ and 100 mM $NaClO_4$ (Fluka, Steinheim, Germany) solution. The pH value was adjusted with a 5% $HClO_4$ solution (Panreac, Barcelona, Spain).

Ham samples were obtained from a local market. $HClO_4$ (Panreac, Barcelona, Spain), ethyl acetate, NaCl and butanol from Fluka (Steinheim, Germany) and, ammonia solution, acetone and $Na_2SO_4$ from Merck (Darmstadt, Germany) were used for the extraction of BAs from real samples.

### 2.2. Apparatus and measurements

Voltammetric measurements were taken using a μAutolab (Eco Chemie, Utrecht, The Netherlands). A conventional three-electrode configuration system was used for the determination of BAs, consisting of a Ag/AgCl (reference electrode), a platinum electrode (counter electrode) and a BBD electrode, used as working electrode.

All measurements were made at room temperature in a cell containing 15 mL of the supporting electrolyte solution (10 mM $Na_2CO_3$ and 100 mM $NaClO_4$) pH 10. Square wave voltammograms were acquired and processed by using the general purpose electrochemical system (GPES) software, making an anodic scan from + 250 mV (initial potential) to +1900 V (final potential). The experimental parameters were: scan rate 0.12 V/s, amplitude 20 mV, frequency 25 Hz. Data analysis and experimental designs were performed using Statgraphics statistical software package [39].

pH of buffer solutions was measured with a HI 221 pHmeter (HANNA Instruments, USA).

Chromatographic measurements of BAs were carried out using an HPLC system (Flexar) from PerkinElmer, Inc (Connecticut, U.S.A.) equipped with a binary LC pump and a solvent manager 3-CH

degasser. Separation was achieved using a reversed phase column (Col Kromophase C-18 125 × 4.0 mm (Scharlab, S.L.,Barcelona, Spain)). The detection was performed with an UV–vis system set at 240 nm.

### 2.3. Sample preparation

Extraction of BAs in ham samples was carried out using an ethyl acetate-acetone mixture, according to the procedure described by Yigit et al. [40]. In this way, 8 g of the previously homogenized ham and 10 mL of a 5% $HClO_4$ solution were mixed in a vortex mixer for 1 min. The mixture was placed in an ultrasonic bath for 10 min and then centrifuged for 10 min at 7000 rpm. The supernatant was recovered and neutralized with a 3% ammonia solution. Then, it was saturated with NaCl and extracted with 10 mL of an ethyl acetate–acetone (2:1) solvent system in a vortex mixer for 1 min. Then the mixture was briefly centrifuged, and the organic phase was separated. Finally, the solvent was evaporated.

## 3. Results and discussion

### 3.1. Square wave voltammetric analysis of BAs

The electrochemical determination of Put, His, Tyr and Cad using a BDD electrode was possible due to the wide potential window of this kind of electrodes. In this way, the analysis of these compounds by BDD electrodes is suitable using the experimental conditions described by Koppang et al. [26]. Fig. 1 shows the square-wave voltammograms obtained for these compounds in a 10 mM $Na_2CO_3$ and 100 mM $NaClO_4$ solution (pH 10) using a BDD electrode. These voltammograms exhibit well-defined oxidation peaks for the different BAs in the potential range from +250 to +1900 mV.

### 3.2. Partial least squares regressions

The electrochemical determination by SWV of the described BAs in a same sample leads to the overlapped voltammograms shown in Fig. 2. Therefore, the simultaneous determination of these compounds by means of direct measuring of peak current is not possible. Thus, a multivariate calibration analysis by PLS was carried out, which has been demonstrated to be very useful in the resolution of overlapped electrochemical signals [36, 41–43].

The first step in this analysis by means of PLS regression involved a calibration step in which the relation between current signals and the concentration of the different analytes was established from a set of standard samples (calibration set). The calibration set for quantitative analysis of the four analytes in a mixture consisted of 81 samples, which concentrations correspond to a three-level factorial design with four factors. The range of concentration used for Put, His, Cad and Tyr varied from 22 to 66 μM. A calibration model was constructed for each BA with original variables using 307 potentials ranging from +400 to +1900 mV.

The predicted concentrations with the different models were compared with the known concentrations and the prediction error sum of squares (PRESS) was calculated. The number of latent variables was then chosen in order to minimize PRESS by cross-validation [44].

The PLS models constructed for each analyte with the entire set of 81 samples led to poor analytical results. Thus, different models were constructed using different samples of the whole set. The best results were obtained for PLS models constructed in the concentration range from 22 to 65 μM for Cad, His and Put, and