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Determination of biogenic amines in chocolate by ion chromatographic separation and pulsed integrated amperometric detection with implemented wave-form at Au disposable electrode

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Abstract

A rapid and selective cation exchange chromatographic method coupled to integrated pulsed amperometric detection (PAD) has been developed to quantify biogenic amines in chocolate. The method is based on gradient elution of aqueous methanesulfonic acid with post column addition of strong base to obtain suitable conditions for amperometric detection. A potential waveform able to keep long time performance of the Au disposable electrode was set up. Total analysis time is less than 20 min. Concentration levels of dopamine, serotonin, tyramine, histamine and 2-phenylethylamine were measured, after extraction with perchloric acid from 2 g samples previously defatted twice with petroleum ether. The method was used to determine the analytes in chocolate real matrices and their quantification was made with standard addition method. Only dopamine, histamine and serotonin were found in the analysed real samples. Repeatabilities of their signals, computed on their amounts in the real samples, were 5% for all of them. Repeatabilities of tyramine and phenethylamine were relative to standard additions to real samples (close to 1 mg/l in the extract) and were 7 and 3%, respectively. Detection limits were computed with the 3 s of the baseline noise combined with the calibration plot regression parameters. They were satisfactorily low for all amines: 3 mg/kg for dopamine, 2 mg/kg for tyramine, 1 mg/kg for histamine, 2 mg/kg for serotonin, 3 mg/kg for 2-phenylethylamine.

Keywords: Biogenic amines; Integrated pulsed amperometric detection; Au disposable electrode; Chocolate; Ion chromatography

1. Introduction

It is well known that biogenic amines (BAs) are a group of naturally occurring amines derived from enzymatic decarboxylation of some natural amino acids and proteins. Many of them have powerful physiological effects (e.g., histamine, serotonin, dopamine, tyramine) and have an important biological activity [1]. For these reasons, it is important to monitor biogenic amine levels in foodstuffs [2,3], beverages [4,5] and plants [6,7], in view of their importance for human health and food safety as they are members of pressor amine group and tend to cause hyper or hypotension [8]. Some of them, in particular aliphatic diamines, were found in tumour cells and their detection in organ transplant recipients was the basis for monitoring the extent of tissue rejection [9]. Moreover, secondary amines such as putrescine and cadaverine play an important role in food poisoning as they can potentiate the toxicity of histamine [10]. All of them can also react with nitrites to form nitrosamines, which are carcinogenic compounds. The detection of aromatic BAs has become particularly important also in chocolate [11] as they are found at significant concentration levels. Tyramine, serotonin, 2-phenylethyl amine and dopamine content can be modified during technological processes from cocoa to the final product and is influenced by the prevailing hygienic conditions.

Determination of BAs is mainly based on gas or liquid chromatographic separation followed by different detection approaches including FID [12], UV [2,11,13], fluorescence

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[5,14]. The main drawbacks of these methods are related to the process of pre- or post-column derivatisation leading to long analysis times, low reproducibility, interferences and problems connected to the stability of derivatisation products. Other methods [3,15,16] do not involve derivatisation and use conductometric detection after ion chromatographic separation with chemical suppression of the eluent or integrated pulsed amperometric detection (PAD) in various operative conditions [17,18]. In particular, integrated square wave detection (ISWD) was used for standard mixtures [19], and applied to determine BAs in soybean seeds [20]. Pulsed amperometric detection was used in milk samples [21] and electrochemical detection at fixed potential at carbon electrodes [22] was used for serum matrices. Moreover, carbon electrodes modified with gold nanoparticles [23] were applied to extracted sardine samples. The drawback of conductometric detection is the large presence of alkaline and alkaline-earth cations detected together with amines usually much less concentrated. Amperometric detection ensures good sensitivity and does not require derivatisation, however, the reported methods usually lack in repeatability above all when applied to real matrices owing to electrode surface poisoning. In the present work an improved method of detection of non derivatised dopamine (DOP), serotonin (SER), tyramine (TYR), histamine (HIS) and 2-phenylethylamine (PHE) in chocolate has been developed by using a suitable ion chromatographic separation coupled with an integrated pulsed amperometric detection at gold disposable electrodes able to improve signal repeatability. This detection technique resulted more selective than the potentially usable UV one.

2. Experimental

2.1. Reagents

All reagents were of analytical grade and were used as purchased: dopamine 99% (3-hydroxytyramine hydrochloride, Janssen, Geel, Belgium), serotonin hydrochloride 99% (Lancaster, Morecambe, England), tyramine >99% (Fluka, Milan, Italy), histamine base >97% (Fluka, Milan, Italy), 2-phenylethylamine >99% (Fluka, Milan, Italy), $HClO_4$ 70% RP Normapur (Prolabo, France), NaOH (Riedel de Haën-Fluka, Milan, Italy), methanesulfonic acid 99% (Sigma-Aldrich, Milan, Italy), petroleum ether 35–60 °C (Prolabo, France), CH_3CN HPLC grade 99.8% (Baker, Deventer, Holland). The solutions were prepared with milliQ water (Millipore Plus System, Milan, Italy, resistivity 18.2 Mohm·cm). Standard solutions of BAs were prepared in $HClO_4$ 0.1 M at 100 mg/l and stored at 5 °C. For the evaluation of recovery from chocolate, a more concentrated solution was used (about 4000 mg/l), to avoid the addition of large amount of solution.

2.2. Instrumentation

A Dionex GP50 gradient pump equipped with an EG40 electrochemical detector and a LC25 oven was used as chromatographic system. All devices were controlled by the Chromeleon software, version 6.20. The column used was a Dionex CS17 (2 mm × 250 mm, 5 μm) with a pre-column CG17 (2 mm × 50 mm, 5 μm) thermostated at 40 °C. The eluent was methanesulfonic acid (MSA) 6 mM for 3.5 min, then linearly to 27 mM in 7.5 min. This last concentration was maintained constant for 7 min. The chromatographic run was preceded by 7 min of equilibration at 6 mM methanesulfonic acid. The flow rate was 0.35 ml/min. A post column addition of 0.1 M NaOH was made by using a Biorad 1350T pump at 0.35 ml/min. The injected volume was 10 μl. Extracted samples were centrifuged with a Serafini centrifuge, mod. 322, and sonicated with a Branson, mod. 2200, ultrasonic bath. The flow-through electrochemical cell (Dionex) consisted of a 1.0 mm diameter Au disposable working electrode, a titanium counter electrode and a pH–Ag/AgCl combination reference electrode. The adopted waveform and the corresponding integration interval (charge is measured) is reported in Fig. 1.

2.3. Procedures

2.3.1. Extraction of amines from chocolate

Chocolate was grated and fats were twice extracted from 2 g of sample with two aliquots of 10 ml petroleum ether. Extraction were made by shaking for 10 min and then centrifuging for 10 min at 3000 rpm. Then, the petroleum ether solution was discarded and the residual ether eliminated with a nitrogen flow. Amines were then extracted with 10 ml of 0.1 M $HClO_4$ in an ultrasonic bath for 20 min and centrifuged at 4500 rpm for 45 min. Solution was filtered with a 0.2 μm PTFE filter and diluted 1:1 prior injection. A second extraction with 5 ml of $HClO_4$ was made to verify the completion of extraction. Ether used for fats extraction was tested for the presence of amines.

2.3.2. Standard additions calibration and recovery tests

Three standard additions were made to calibrate the analytical method and to evaluate the recovery of the analytes in the extraction procedure. For this purpose 2 g of grated plain chocolate were added of 10, 20, 30 μl of a standard solution containing DOP (4000 mg/l), TYR (2000 mg/l), HIS (900 mg/l), SER (4000 mg/l) and PHE (3000 mg/l). The added solution was

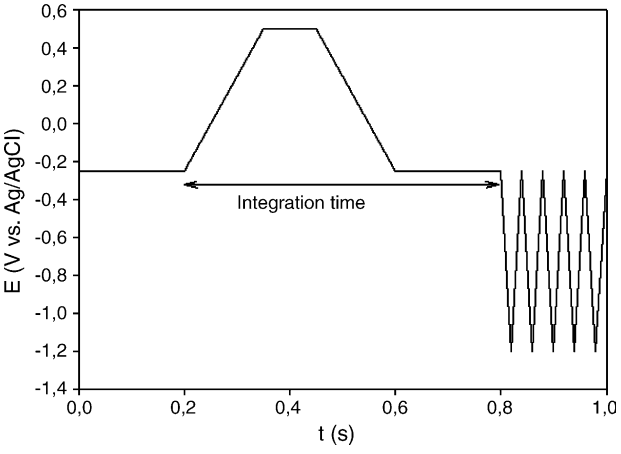

Fig. 1. Detection waveform.