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Analytical Methods

Fast determination of biogenic amines in beverages by a core–shell particle column

Raffaella Preti [a,*], Marta Letizia Antonelli [b], Roberta Bernacchia [a], Giuliana Vinci [a]

[a] Laboratory of Commodity Science, Department of Management, Sapienza University of Rome, via del Castro Laurenziano 9, 00161 Rome, Italy
[b] Department of Chemistry, Sapienza University of Rome, Piazzale Aldo Moro 5, 00161 Rome, Italy

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ABSTRACT

A fast and reliable HPLC method for the determination of 11 biogenic amines in beverages has been performed. After pre-column derivatization with dansyl-chloride a Kinetex C18 core–shell particle column (100 mm × 4.6 mm, 2.6 μm particle size) has been employed and the biogenic amines were identified and quantified in a total run time of 13 min with ultraviolet (UV) or fluorescence detection (FLD). Chromatographic conditions such as column temperature (kept at 50 °C), gradient elution and flow rate have been optimized and the method has been tested on red wine and fruit nectar. The proposed method is enhanced in terms of reduced analysis time and eluent consumption with respect of classical HPLC method as to be comparable to UHPLC methods. Green and cost-effective, this method can be used as a quality-control tool for routine quantitative analysis of biogenic amines in beverages for the average laboratory.

1. Introduction

Bioactive amines (BAs) may be both essential and detrimental to health. When originating from metabolic pathway they are called "natural polyamines" and are indispensable compounds for cells either to grow or function in optimal manner. When they are formed by microbial decarboxylation of the corresponding amino acids, they are designated "biogenic" and are known to induce several negative physiological reactions. In fact, if present in high concentrations, they may induce headaches, heart palpitation, nausea, rash, hypertension and hypotension, and even anaphylactic shock syndrome and death (Rawles, Flick, & Martin, 1996).

Food poisoning may occur especially in conjunction with potentiating factors such as monoamine oxidase inhibiting (MAOI) drugs, alcohol, gastrointestinal diseases and other food amines (Rauscher-Gabernig, Grossgut, Bauer, & Paulsen, 2009).

Biogenic amines are organic bases occurring in different kinds of foods, such as wine, beer, cheese, fruit juices, fish and meat products (Önal, Evrim, Tekkeli, & Önal, 2013).

Fruit nectars consumption is increasing in recent years because they are recognized as good sources of vitamins, minerals, and other beneficial micronutrients, such as carotenoids, limonoids and lycopene as well as being enjoyable and easy to consume (AIJN, 2014). As fermentable beverages, fruit nectars present all the characteristic to be good contributors to BAs daily intake but this aspect have not been explored yet. Moreover, BAs profile and levels could be an important marker to assess the quality and safety of these beverages. In fact, BAs in food are of great

* Corresponding author. Tel.: +39 6 49766525; fax: +39 6 49766508.
E-mail address: raffaella.preti@uniroma1.it (R. Preti).

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interest not only due to their toxicity, but because they can also be used as good indicators of spoilage (Vinci & Antonelli, 2002; Önal et al., 2013) and therefore as a quality markers.

Furthermore, BAs have been recently used for authenticity assessment in combination with other parameters in wine (Galgano, Caruso, Perretti, & Favati, 2011; Saurina, 2010).

Several methods to analyze biogenic amines in food based on thin layer chromatography, liquid chromatography (with ultraviolet, fluorescence and mass spectrometric detection), gas chromatography (Flame ionization and mass spectrometric detection), biochemical assays and capillary electrophoresis have so far been described (Önal et al., 2013). Among these, HPLC is the most used due to its high resolution and sensitivity, especially when coupled with a fluorescence detector (FLD).

As BAs do not show satisfactory absorption in the visible and ultraviolet range nor do they show fluorescence, pre or post-column chemical derivatization is considered a necessary analytical step for this detection technology. The most common derivatization reagents are o-phthalaldehyde (OPA), fluorescein isothiocyanate, phenyl isothiocyanate, 9-fluorenyl methyl chloroformate, benzoyl chloride, fluorescamine, and dabsyl chloride (Önal et al., 2013), but most frequently used is probably dansyl chloride with pre-column derivatization (Proestos, Loukatos, & Komaitis, 2008; Soufleros, Bouloumpasi, Zotou, & Loukou, 2007). In fact, the pre-column derivatization technique is proven to provide a more sensitive detection than the post column technique and dansyl-chloride reacts with both primary and secondary amino groups and provides stable derivatives.

Recently, many ultra-high pressure (UHPLC) instruments coupled with columns packed with sub-2 μm fully porous particles have become commercially available. The main advantage of UHPLC methods is shorter analysis time, 5 to 10-fold faster separations than with conventional LC systems, which, together with the reduction of column length and diameter, makes the separation greener, with a significant decrease in solvent consumption, while maintaining or increasing resolution and reproducibility (Gritti & Guiochon, 2012; Nguyen, Guillarme, Rudaz, & Veuthey, 2006). The drawback of this instrument is the cost, prohibitive for the average laboratory, or difficult to switch from known procedures (Gritti & Guiochon, 2012; Mao, Lei, Yang, & Xiao, 2013).

In recent years, the rising interest in BAs content in food has led to the need of fast separations of these compounds with very high efficiency and adequate resolution to perform analysis within few minutes on more complex samples or on increasing numbers of samples.

Core–shell particles have a 1.7 μm solid core wrapped in a porous layer or shell of a 0.5 μm silica adsorbent, with a final particle size of 2.6 μm. This combination of materials provided columns with speed and efficiency similar to columns packed with sub-2 μm totally porous particles while maintain low back pressure thus could be used on conventional HPLC instrument (Fekete, Olah, & Fekete, 2012; Gritti, Leonardis, Abia, & Guiochon, 2010; Guiochon & Gritti, 2011). This is especially true for the sub-3 μm core–shell particles because they offer much improved reduced plate height and lower backpressure compared to the sub-2 μm totally porous particles (Wang, Barber, & Long, 2012). For these reasons, the new technology columns have been already successfully applied to the analysis of various compounds in several food (Chocholou, Vackova, Sramkova, Satinsky, & Solich, 2013; Kaufmann & Widmer, 2013) and environmental (Vinci, Antonelli, & Preti, 2013) matrices.

According to our knowledge, there is no previous published method that uses this new technology for biogenic amines determination. There are some recent articles that determine biogenic amines in food matrices using UHPLC, with sub-2 μm particle size column coupled with UV or FL detectors (Dadakova, Krizek, & Pelikanova, 2009; Fiechter, Sivec, & Mayer, 2013; Latorre-Moratalla et al., 2009; Mayer, Fiechter, & Fischer, 2010) others involving mass detection (Jia, Kang, Park, Lee, & Kwon, 2011, 2012). A pre-column derivatization method with dansyl-chloride with the use of sub-2 μm particle size column with HPLC/UV for the analysis of biogenic amines in seafood has been proposed by Simad and Dalgaard (2011). This method is able to determine nine biogenic amines in twelve minutes after dansylation, but it does not consider important biogenic amines as methylamine, serotonin and ethylamine.

The aim of this study was to develop a reliable and rapid method to quantify eleven biogenic amines in fermented (wine) and fermentable but unfermented (fruit nectar) beverages using a conventional HPLC system coupled with UV and FL detectors, by the use of a 4.6 mm ID Kinetex core–shell particles column. The developed method was validated in terms of linearity, sensitivity, precision, and recovery. To test the method, the analysis was carried out on red wine and different fruit nectar samples.

2. Materials and methods

2.1. Chemicals and reagents

Perchloric acid, petroleum ether, acetone (analytical-grade) and acetonitrile (HPLC-grade), as well as the other reagents, were purchased from Sigma–Aldrich (Milan – Italy). Ultrapure water (18.2 MΩ cm resistivity at 25 °C) was obtained by a Milli-Q (Millipore, Bedford, MA, USA). The eleven biogenic amines studied were: ethylamine (ETA), methylamine (MEA), histamine (HIS), serotonin (SER), spermine (SPM), spermidine (SPD), agmatine (AGM), putrescine (PUT), β-phenylethylamine (β-PEA), cadaverine (CAD), tyramine (TYM) all of which were supplied by Supelco, Bellefonte, PA, USA as well as the derivatizating agent dansyl chloride and the internal standard 1,7-diaminoheptane (IS).

Standard solutions of 2000 mg/L were prepared in purified water for each biogenic amine studied and for the internal standard. The standard solutions were protected from light and stored at 4 °C until use.

To perform calibration experiments, six standard solutions containing all the amines were obtained with different aliquots of each water solution, all diluted to 25 mL and added with $HClO_4$ 10.3 M to reach a final acid concentration of 0.2 M or 0.4 M, depending on the food sample to analyze.

After the derivatization procedure, the final dansylated amine concentration injected were in the range between 0.01 and 8.0 mg/L. The calibration curve was constructed by plotting the peak area ratios of analytes to internal standard against six analyte concentrations.

The standard solutions to perform the recovery experiments were prepared mixing aliquots of each individual water solution (amine and IS) and diluted to 25 mL (concentration 160 mg/L) with water acidified with $HClO_4$ in such a manner to obtain an acid concentration of 0.2 M or 0.4 M, depending on the beverage to analyze.

2.2. Samples and sample preparation

Samples of Italian red wine and fruit nectar were selected to check the recovery and the precision of the method. All samples were purchased from local markets. The procedures for the extraction of the different food matrices were as follows:

Ten Italian red wine samples were analyzed 25 mL of wine previously added with IS (0.5 mL), was acidified by $HClO_4$ 10.3 M to reach a final acid concentration of 0.2 M and then dansylated (Vinci, Restuccia, & Antiochia, 2011). For the recovery experiments at two concentration levels, 0.3 and 1.5 mL of standard solution