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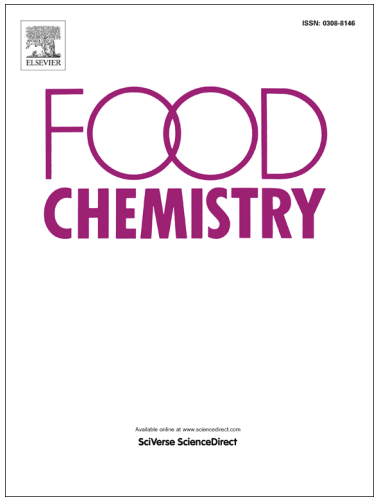

Analytical methods

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

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 x 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.

Keywords: Core-shell particle column; Biogenic amines; Dansyl chloride; Wine; Fruit nectar; HPLC.

Chemical compounds studied in this article

Serotonin (PubChem CID: 5202); ethylamine (PubChem CID: 6341); methylamine (PubChem CID: 6329); histamine (PubChem CID: 774); spermine (PubChem CID: 1103); spermidine (PubChem CID: 1102); agmatine (PubChem CID: 199); putrescine (PubChem CID: 1045); cadaverine (PubChem CID: 273); tyramine (PubChem CID: 560).

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 decarboxilation 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 2007).

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 interest not only due to their toxicity, but because they can also be used as good indicators of spoilage (Önal, Evrim, Tekkeli, & Önal, 2013; Vinci & Antonelli, 2002) 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 utraviolet, fluorescence and mass spectrometric detection), gas

chromatography (Flame ionization and mass spectrometric detection), biochemical assays and capillary electrophoresis have so far been described (Önal, Evrim, Tekkeli, & Önal, 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, benzoylchloride, fluorescamine, and dabsyl chloride (Önal, Evrim, Tekkeli, & Önal, 2013), but most frequently used is probably dansyl chloride with pre-column derivatization (Soufleros, Bouloumpasi, Zotou, & Loukou, 2007; Proestos, Loukatos, & Komaitis, 2008;). 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 (Nguyen, Guillarme, Rudaz, & Veuthey, 2006; Gritti & Guiochon, 2012). 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-2μm totally porous particles while maintain low back pressure thus could be used on conventional HPLC instrument (Gritti, Leonardis, Abia, & Guiochon, 2010; Guiochon & Gritti, 2011; Fekete, Olah, & Fekete, 2012). This is especially true for the sub-3μm Core-shell particles because they offer much improved reduced plate height and lower backpressure compared to the sub-2μm 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 (Kaufmann & Widmer, 2013; Chocholou, Vackova, Sramkova, Satınsky, & Solich, 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-2μm particle size column coupled with UV or FL detectors (Latorre-Moratalla et al. 2009; Dadakova, Krizek, &. Pelikanova, 2009; Mayer, Fiechter, & Fischer, 2010; Fiechter, Sivec, & Mayer, 2013) others involving mass detection (Jia, Kang,. Park, Lee, S.W &. Kwon, 2011; Jia, Kang,. Park, Lee, S.W &. Kwon, 2012). A pre-column derivatization method with dansyl-chloride with the use of sub-2μm particle size column with HPLC/UV for the analysis of biogenic amines in seafood has been proposed by Simad and Dalgaard in 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 $HClO_4$ 10.3M to reach a final acid concentration of 0.2M and then dansylated (Vinci, Restuccia, & Antiochia, 2011). For the recovery experiments at two concentration levels, 0.3 and 1.5 mL of standard solution were added to 25 ml of acidified wine as to obtain, after the derivatization step, a final concentration in wine of 0.384 and 1.92 mg/L for each amine, respectively.

Nine fruit nectars made from apricot, pear and peach, as defined in Annex IV of European Council Directive 2001/112/EC (European Union, 2002), were analyzed.

Since biogenic amines content in this matrix has never been studied before, the extraction method has been tested for different acid concentration and volume and the final optimized conditions are hereby reported.

2.5 mL of fruit nectar previously added with IS (0.5 mL) were extracted with 7.5 mL of $HClO_4$ 0.4M, centrifuged at 2000 rpm for 5 minutes. Supernatant was filtered through a 0.20 µm membrane Millipore filter and sediment was added with 1.5 mL of $HClO_4$ 0.4M and centrifuged again for 3 min. The second extract was then filtered and added to the first. The final volume was adjusted to 10 mL with $HClO_4$ 0.4M. An aliquot of 1 mL of the final extract was then used for analysis after derivatization while the remaining volume was stored at 4°C for no more than one week.

The derivatization reaction was carried out following the procedure described in Chiacchierini et al. (Chiacchierini, Restuccia, & Vinci, 2006) modified for temperature and time of reaction. 1.0 mL of

acid standard solution or sample extract was added with 200 µL of NaOH 2M (to adjust the pH to 11), 300 µL of saturated $NaHCO_3$ solution, and 2 mL of dansyl chloride solution (10 mg/mL in acetone). Fresh dansyl chloride solutions were prepared each time just before use. After shaking, samples were left in the dark at 45°C for 60 min (Henríquez-Aedo, Vega, Prieto-Rodríguez, & Aranda, 2012), and the excess of dansyl-chloride was removed by adding 100 µL of NH_4OH 25% (v/v). The final volume was adjusted to 5 mL by adding acetonitrile. The dansylated amine solution obtained was filtered 0.22 µm (Polypro Acrodisc, Pall Gelmann Laboratory) and injected into the chromatograph.

For the recovery experiments at two concentration levels, 1 and 2 mL of standard solution were added to 25 mL of peach nectar as to obtain, after the derivatization step, a final concentration in fruit nectar of 0.32 and 0.48 mg/L for each amine, respectively. For agmatine, UV detection recovery were considered only at the higher level.

2.3. Chromatographic conditions

HPLC chromatographic separations were developed in a system consisting in a LC-10 ATVP binary HPLC pump with a SP-10 AVP UV-Vis detector and a RF-10AXL fluorescence detector (Shimadzu, Kyoto, Japan) in series. The injector was fitted with a 20 µl loop. The chromatographic data were collected and processed using Class-VP software (Shimadzu).

The analytical column was a 100 x 4.6mm I.D. Kinetex C18 column (particle size 2.6µm), with KrudKatcher Ultra, an integrated 0.5 µm 316 stainless steel depth filter that removes microparticulates from the flow stream with minimal contributions to system dead volume (0.2 µL), (all supplied by Phenomenex, Torrance, CA, USA). Analytical column temperature was kept at T=50°C by a CT 10AS oven. The maximum operating pressure on the system was 400 bar.

The statistical analysis of data was performed with STATGRAPHICS Centurion XV 15.2.06 (Statpoint Technologies, Inc., Warrenton, VA, USA) and Matlab 7.6.0.324 (Math Works, Natick, MA, USA).

The final optimized conditions were as follows: a linear gradient was from 65% acetonitrile to 75% acetonitrile in 3.5 min and then increased to 100% acetonitrile in 9 min, and then kept for 2 min for a total run time of 11 min at a constant flow rate of 0.6 mL/min. Two minutes were necessary to for column equilibration at the initial condition, for a total elution time of 13 min. The UV detection wavelength was set at 254 nm. Fluorescence detection at 320 nm for excitation and 523 nm for emission was applied. The fastest sampling (10 Hz) and minimum response time rate (0.1 s) of FLD and UV and were applied to all runs to assure the narrowest peak was recorded completely.

The matrix effect was evaluated by the slope comparison method as described in Jia et al. (Jia, Kang, Park, Lee, & Kwon, 2012) and Taverniers et al. (Taverniers, De Loose, & Van Bockstaele, 2004). Standard additions to each matrix were performed as in 2.4 section at the same concentration levels of the pure aqueous standards, to construct standard addition calibration curves. Then the slopes of the two calibration curves were compared for each biogenic amines studied.

3. Results and Discussion

3.1 Chromatographic method development

The chromatographic conditions were optimized in order to achieve the separation of the 11 components in a single chromatographic run on a 13 min elution program, keeping good resolution and sensitivity in the three food matrices considered (Fig.1 for FLD). The retention times in the UV chromatograms are delayed of 0.05 min due to the volume of the connection tube between the two detectors.

The new conditions reduced at least 3-fold the elution time and the solvent consumption reported by others previous HPLC methods for the determination of a similar number of biogenic amines in wine and or meat products, with comparable results of methods involving the use of sub-2µm particles columns (Table 1). As a result of the time reduction, the spent of solvents is drastically reduced making the present HPLC method of lower reagent cost and less environmental impact.

This paper is also the first on the determination of biogenic amines in fruit nectars, more extensive results on the samples analyzed will be reported in a future article.

The high efficiency of the non-porous core column for small molecules is due to the combination of a reduced longitudinal diffusion parameter and Eddy diffusion coefficient of the Van Deemter equation. The trans-particle mass transfer resistance coefficient for these chromatography columns for compounds of molecular weight below 1000 Da is small or negligible (Gritti &. Guiochon, 2013), this characteristic makes these columns optimal for the separation of biogenic amines.

However, as high-performance liquid chromatographs were not initially designed for the recently developed high-efficiency packed columns, some adjustments of the HPLC apparatus were needed. First of all the replacement of the conventional connecting tubes and the cell of detectors with similar parts having a smaller volume to improve column performance and bring it to a level comparable to the one observed for the same column, operated with the new ultra-high pressure instruments (Gritti &. Guiochon, 2013). Also some method modifications were necessary, above all in order to maintain the instrument backpressure under 300 bar.

The mobile phase was acetonitrile (A)/water (B). The initial gradient step was (A)= 65 %, this was indispensable to achieve a good separation of the first two amines eluted, methylamine and ethylamine, and of histamine and cadaverine, for which was also necessary an intermediate gradient step of 75% (A) at 3.5 min. The flow rate was kept constant at 0.6 ml/min, which is lower the limit of the flow range recommended by the column producer (from 0.7 to 3.0 ml/min). With such a flow rate an analysis time of 13 min was achieved. The column temperature kept at 50°C gave an important aid to maintain the pressure under the required limits and to shorten the retention time, without the loss of separation efficiency. In fact, the temperature employed influences the chromatographic efficiency since it affects the mobile-phase viscosity, the analyte retention factor, and the diffusion coefficient (Alexander, Waeghe, Himes, Tomasella, & Hooker, 2011).

3.2 Optimization of the extraction procedure in fruit nectar

For biogenic amines extraction from wine we referred to the method optimized by our group (Vinci, Restuccia, & Antiochia, 2011). Being fruit nectar an unexplored matrix, we started our optimization of the extraction procedure following the work by Chiacchierini et al. (Chiacchierini, Restuccia, & Vinci, 2006), that considered tomato products. As previously demonstrated, perchloric acid was chosen for the sample treatment and has been tested at two different concentrations (0.2 and 0.4 M). The recovery tests were carried out in triplicate on peach nectar following the procedure described in section 2.4. As shown in Figure 2a, acid concentration of 0.4 M resulted in significant higher recoveries than those obtained with acid at 0.2 M ($p < 0.05$). As the recoveries at 0.4 M were not significant different from 100%, no further tests with different acid concentrations were carried out. In order to improve the sensitivity of the method, especially for UV detection, an acid final volume of 5 mL (0.4 M) was tested. As can be seen from Figure 2b, a final acid volume of 10 ml appeared to be more suitable for a complete extraction of all amines.

3.3. Analytical method validation

The matrix effect was assessed by the slope comparison method as performed by Jia group (Jia, Kang, Park, Lee, & Kwon, 2011). Here, for each analyte, the slope of calibration curves prepared with aqueous standards was compared with those obtained with the sample matrix extract and the slope ratio not significant different from 1.0 ($p > 0.05$) indicated no matrix effect, both in red wine and fruit nectar (Supplementary Table S1).

The same results has been reported by Jia et al. in the determination of amino acids and biogenic amines in fermented food by liquid chromatography/quadrupole time-of-flight mass spectrometry. Unlike UV and FL detectors, the different matrices in mass detection could cause dramatic signal changes; and therefore the matrix effect evaluation is of big concern. The explanation that the authors gave in their article can be fitted to our work. In summary, the matrix effect was effectively solved by better chromatographic separation, using an high resolution column and the application of a structural analogue internal standard.

This result has allowed a direct injection of the derivatized acid extract with the advantage of quantitative recoveries of all the researched amines. This simple and rapid procedure has been already reported for food matrices with relatively low free amino acids content such as meat, fish and vegetables. In fact, the presence of an high free amino acids content can cause interference problems for the first eluted amine peaks (Innocente, Biasutti, Padovese, & Moret, 2007).

Parameters of the chromatographic method are reported in Table 2. Linearity was tested at six different concentrations between 0.01-8 mg/L for FLD, and between 0.06-8 mg/L for UV detection, performing three measurements at each level. The data linearity was assessed by analysis of the variance of the regression. Least–squares analysis resulted in a correlation coefficient of $r \geq 0.999$ for all the biogenic amines studied in both detectors ($p \leq 0.001$). The standard curves for each BAs had a coefficient of determination (R^2) above 0.997 for FL detection and above 0.995 for UV detection.

The chromatographic limit of detection (LOD) and the limit of quantification (LOQ) for each analyte were obtained by a serial dilution of stock solution until the peak heights were respectively 3σ and 10σ (σ being three times and ten times the standard deviation of the blank signal calculated over ten injections of the blank). The derivatized perchloric acid (0.2 and 0.4 M) was used as blank.

LOQ for each biogenic amine (Table 3) in the different matrices were determined on spiked samples and by taking into account the dilution factor of extraction so that LOQs were expressed in mg/L.

LOD values for FLD were between 0.002 (PUT) and 0.023 mg/L (AGM). The corresponding limit of quantification (LOQ) values were 0.006 and 0.077 mg/L. For UV detection LOD and LOQ ranged between 0.013-0.112 mg/L, and 0.043-0.373 mg/L, respectively. The intraday and interday instrumental precisions were determined by analyzing a standard solution of biogenic amines at 1 mg/L level, five times in one day and five times over five consecutive days, respectively. The RSD values were calculated for the eleven studied biogenic amine and for the internal standard. RSD values obtained using FLD are lower than the obtained when UV detection is employed. However,

both intraday and interday repeatability values of UV and FL were always lower 4%, which guarantees a satisfactory level of precision of the proposed method. The intraday precisions were in the range of 0.94-2.12 %, and the interday precisions ranged from 1.32-3.03% for FLD, were 1.16-3.07% and 1.67- 3.72 % for UV.

To assess the accuracy and the precision of the method, a standard biogenic amines solution of 160 mg/l was added to each food samples as described in the previous section. Five determinations were carried out at each addition level. The mean recoveries were above 95 % which was not statistically different from the theoretical value of 100% ($p>0.05$ according to Student's t test), for all the biogenic amines in all the three matrices considered (RSD lower than 5.0%) both for fluorescence and UV detection (Table 3). To assess precision acceptability the Horwitz equation for intra-laboratory study has been applied (Horwitz, 1982). The experimental RSD values for instrumental precision and method precision were all under two-thirds the upper limit of the acceptable range for RSD calculated by the formula (from 5.7 to 7.0), which indicates that the method can be carried out with a satisfactory level of precision.

The assumption of homogeneity of variances between the two spiking levels was tested using Cochran's C test. All the experimental values remained under the Cochran's test tabled value [Cochran C test maximum limit (4, 2, 0.05) =0.9392], confirming that the variance of recovery values was not dependent on the amine content.

Less resolved peaks were those from methylamine and ethylamine with resolution of 1 ppm standards and samples close to one, allowing for reliable quantitation of these compounds. (Maurer et al., 2014). The results showed that the present method was reliable for the analysis of biogenic amines in food samples.

3.4 Biogenic amines determination in real samples

Our method will be employed to evaluate the BAs profiles of a large set of Italian red wines and fruit nectars. In Table 4 are presented the identified BAs and their concentrations for FL detection

of a preliminary subset of those results, that will be fully reported in future articles. It is noticeable from the data reported that lower detection limits achieved by FL allow the identification of a wider number of BAs in beverages. This is especially true in case of semi-solid such as fruit nectar where an extraction procedure with subsequent dilution of the extracts is required. When only UV detection is available the proposed extraction methods should be optimized by the inclusion of one or more concentration steps as to obtain appropriate dilution of the extracts prior to analysis.

The concentration values obtained in this study are in the order of the concentration values reported in literature for red wine (Proestos, Loukatos, & Komaitis, 2008; Dugo, Vilasi, La Torre, & Pellicano, 2006; García-Marino, Trigueros, & Escribano-Bailón, 2010), taking into account that the biogenic amines content in food is influenced by different factors, such as the environmental conditions or the technological aspects of production. For fruit nectars, we have only references of studies on orange juice (Vieira, Theodoro, & Gloria, 2007; Basheer et al. 2011) but this is the first work dealing with BAs in fruit nectars from other fruits, and therefore the detailed results will be reported in a future article.

Wine was the matrix with the richest number of biogenic amines, with putrescine, ethylamine and cadaverine the most abundant amines in the wine samples analysed. The relative concentrations of biogenic amines (mg/L) approximated the following order: putrescine > ethylamine > cadaverine > tyramine > methylamine > agmatine > β-phenylethylamine > histamine > spermidine > spermine. In wine samples ethanol was also derivatized and therefore the correspondent peak has been detected (Figure 1b).

In fruit nectars the biogenic amines detected were only five, spermidine, spermine, putrescine and cadaverine, with cadaverine the most abundant. Ethylamine was found in only one sample of apricot nectar.

4. Conclusion

The present study describes the application of new technology core-shell column to the analysis of 11 biogenic amines in three food matrices of different complexity.

The use of a core-shell particles column permits to obtain highly reproducible and resolved chromatograms in a short analytical run (13 min), using a traditional HPLC pump.

In comparison to previously reported analytical methods this HPLC method allows a reduction of the run time by 60% and of the solvent use by 75%. These results are comparable to those achieved by the use of sub-2µm particles column. The detection has been carried out using the UV and FL detector, with higher sensitivity as the FL detector main advantage. However, since FL detector is not always available, it has been shown that also UV can be a useful detection mode for dansylated biogenic amines in food when a sample extraction with an high enrichment factor is designed.

The method demonstrated to be reliable, rapid and green and therefore suitable for laboratories involved in the analysis of a large volume of samples, for example those who work in quality or authentication assessment because it allows a great saving in time and solvent, with affordable instruments and reagents.

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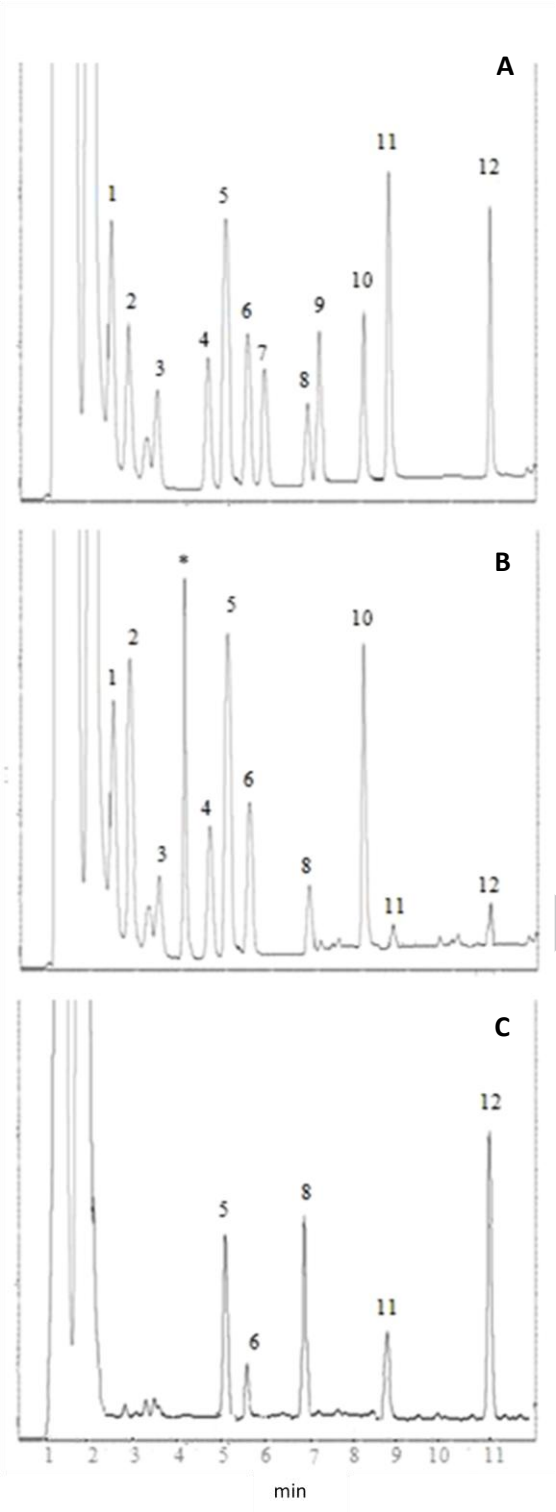
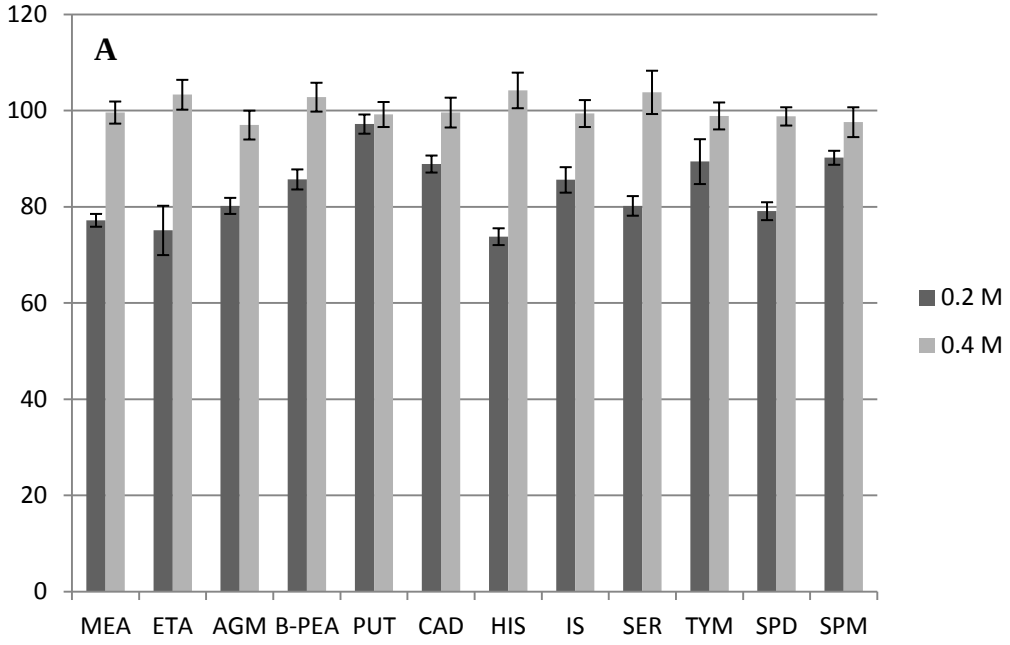
Fig. 1. HPLC/FLD chromatograms of a biogenic amine solution of 1 mg/L (A), wine (B) and of a apricot nectar (C) extracts. Peaks: (1) methylamine, (2) ethylamine, (3) agmatine, (4) β-phenylethylamine, (5) putrescine, (6) cadaverine, (7) histamine, (8) internal standard, (9) serotonin, (10) tyramine; (11) spermidine, (12) spermine.
***dansylated ethanol peak.**

Fig. 2 Influence of $HClO_4$ concentration (0.2 M or 0.4 M) (A), and of its final volume (B) on biogenic amines percentage of recovery in fruit nectar. Data are the mean value ± SD of three independent analysis on a peach nectar spiked as described in section 2.4.





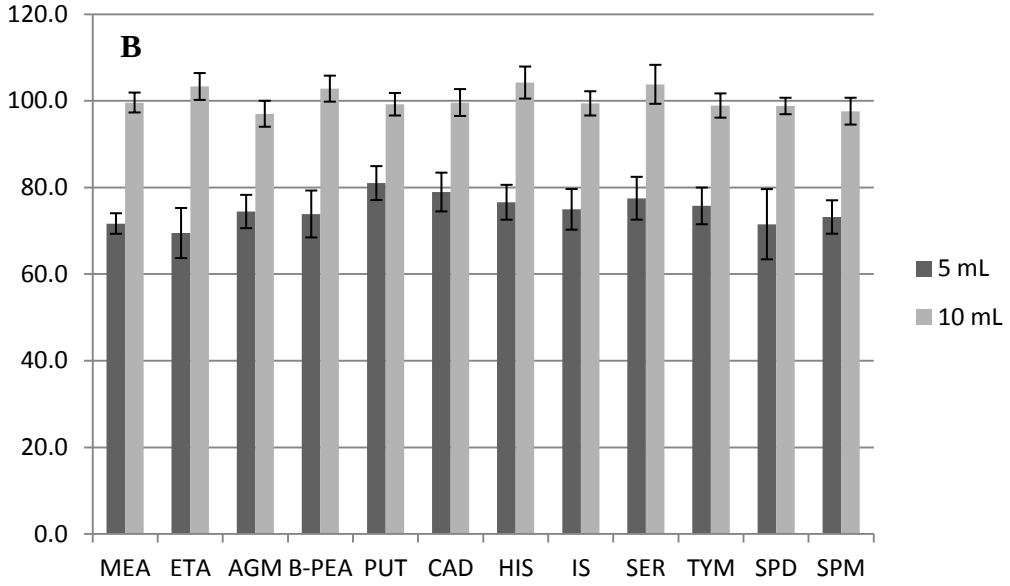


Table 1. Literature comparison to the proposed method for the determination of biogenic amines.

Apparatus Derivatization agent	Biogenic amines	Analytical column Flow	Run Time (min)	Time per analyte (min)	Solvent consume (mL)	Matrix	Ref.
HPLC-RF Dansyl-Cl	MEA, ETA, AGM, β-PEA, PUT, CAD, HIS, IS, SER, TYM, SPD, SPM.	Kinetex C18 100 x 4.6mm 2.6µm Φ = 0.6 ml/min	**13**	1.1	**7.8**	Wine, fruit nectar	**This study**
HPLC-UV Dansyl-Cl	AGM, β-PEA, PUT, CAD, HIS, IS, TRYP, TYM, SPD, SPM.	Chrompack Hypersil C18 250 x 3.0 mm 5µm Φ = 1 ml/min	29	2.9	29	Fish	Simad, & Dalgaard 2011
HPLC-UV Dansyl-Cl	CAD, PUT, HIS, TYR, TRYP, PHE, IS, SPD, SPM, MEA, ETA.	C18 Supelco Discovery 150 x 2.1 mm 5µm Φ = 0.4 ml/min	30	2.7	12	Wine	Soufleros et al. 2007
HPLC-RF Dansyl-Cl	CAD, PUT, HIS, TYR, TRYP, AGM, ISO, MEA, ETA.	C18. Luna 5 lm 250 x 4.6 mm 5µm Φ = 1 ml/min	35	3.9	35	Wine	Proestos et al., 2008
Ion pair HPLC OPA	SPD, SPM, PUT, AGM, SYN, TYM, OCT, HIS, SER, CAD, TRYP, β-PEA.	Bondapak C18 300 x 3.9 mm 10 µm Φ =0.7 ml/min	71	5.9	50	Orange juice	Vieira et al., 2007
HPLC-UV Dansyl-Cl	AGM, β-PEA, PUT, CAD, HIS, IS, TRYP, TYM, SPD, SPM.	Zorbax Eclipse C18 50 x 4.6 mm 1.8 µm Φ = 1 ml/min	12	1.2	12	Fish	Simad, & Dalgaard 2011
UHPLC-UV Dansyl-Cl	TRYP, β-PEA, PUT, CAD, HIS, IS, TYM, SPD, SPM.	Zorbax Eclipse C18 50 x 4.6 mm 1.8 µm Φ = 1 ml/min	12	1.3	12	Meat, Cheese mush-rooms	Dadakova et al., 2009
UHPLC RF OPA	OC, DO, TYR, PUT, SER, CAD, HIS, AGM, β-PEA, SPD, TRYP, SPM.	Acquity UPLC BEH 50 x 4.6 mm 1.7 µm Φ = 0.8 ml/min	7	0.6	5.6	Wine, fish, cheese, dry sausages	Latorre-Moratalla et al., 2009

OPA, o-phthalaldehyde; SPD, spermidine; SPM, spermine; PUT, putrescine; AGM, agmatine; SYN, synephrine; TYM, tyramine; OCT, octopamine; CAD, cadaverine; HIS, histamine; SER, serotonin; TRYP, tryptammine; ETA, ethylamine; MEA, methylamine; HIS, histamine; β-PEA, β-phenylethylamine; DO, dopamine; ISO, isopropylamine, IS, internal standard.

Table 2. Method performances for UV and FL detection for standard solution of dansylated biogenic amines

	FLD						UV					
BAs	**Linear range**	**R^2**	**LOD**	**LOQ**	**Intra day**	**Inter day**	**Linear range**	**R^2**	**LOD**	**LOQ**	**Intra day**	**Inter day**
MEA	0.03-6	0.999	0.008	0.027	2.12	3.03	0.20-6	0.997	0.050	0.167	3.07	3.72
ETA	0.02-8	1	0.006	0.020	1.64	2.24	0.14-8	0.997	0.038	0.127	1.75	2.77
AGM	0.09-6	0.999	0.023	0.077	1.75	2.83	0.40-6	0.996	0.112	0.373	2.64	3.70
B-PEA	0.03-6	0.999	0.007	0.023	1.67	2.58	0.15-6	0.999	0.041	0.137	2.32	3.02
PUT	0.01-8	1	0.002	0.006	1.05	1.32	0.06-8	1	0.013	0.043	1.44	1.73
CAD	0.02-8	1	0.005	0.017	1.22	2.04	0.15-8	0.999	0.033	0.110	1.36	1.97
HIS	0.06-6	0.998	0.012	0.040	1.07	2.10	0.50-6	0.998	0.057	0.190	1.91	2.62
IS	0.04-4	1	0.007	0.024	0.79	1.01	0.30-4	0.999	0.051	0.170	0.90	1.14
SER	0.08-4	0.997	0.022	0.074	2.04	2.41	0.40-4	0.995	0.095	0.317	3.05	3.28
TYM	0.06-6	0.999	0.016	0.053	1.08	2.19	0.40-6	1	0.089	0.297	1.68	2.05
SPD	0.02-8	0.999	0.006	0.020	1.28	2.28	0.15-8	0.999	0.038	0.128	1.59	1.94
SPM	0.03-8	0.999	0.007	0.023	0.94	1.32	0.20-8	0.999	0.050	0.166	1.16	1.67

R^2, square regression coefficient.

Intra-day and inter–day precisions are expressed as RSD (%): relative standard deviation for five determinations in one day and five times over five consecutive days, respectively

LOD: limit of detection, Signal/Noise ratio=3; LOQ, limit of quantification Signal/Noise =10; and linear range are in mg/L.

Table 3. Recovery percentages and LOQs of biogenic amines in wine and fruit nectar spiked at the higher level as described in section 2.4.

	WINE				FRUIT NECTAR			
Amines	**Rec[A]**	**Rec[B]**	**LOQ[A] mg/L**	**LOQ[B] mg/L**	**Rec[A]**	**Rec[B]**	**LOQ[A] mg/L**	**LOQ[B] mg/L**
MEA	97.5 (3.4)	95.8 (3.6)	0.12	0.84	99.6 (2.3)	99.2 (3.1)	0.52	3.42
ETA	101.6 (2.9)	99.8 (3.7)	0.10	0.65	103.3 (3.1)	99.8 (3.4)	0.39	2.64
AGM	104.3 (3.7)	99.9 (3.9)	0.40	1.88	97.0 (3.0)	96.3 (2.7)	1.65	7.56
B-PEA	100.5 (3.1)	103.4 (3.3)	0.10	0.69	102.8 (3.0)	104.1 (3.5)	0.42	2.84
PUT	99.5 (2.2)	99.6 (2.5)	0.03	0.21	99.2 (2.6)	100.2 (2.8)	0.11	0.92
CAD	98.8 (3.6)	98.4 (3.3)	0.07	0.61	99.6 (3.1)	100.8 (3.2)	0.32	2.44
HIS	98.7 (3.1)	97.9 (3.6)	0.17	0.98	104.2 (3.7)	101.7 (4.0)	0.79	4.02
IS	99.8 (2.8)	100.8 (3.3)	0.11	0.90	99.4 (2.8)	99.8 (3.1)	0.46	3.52
SER	99.2 (3.7)	95.9 (4.6)	0.33	1.60	103.8 (4.5)	102.9 (4.8)	1.42	6.46
TYM	98.2 (2.1)	98.2 (3.0)	0.28	1.53	98.9 (2.8)	98.8 (3.0)	1.12	6.09
SPD	97.4 (2.7)	96.3 (3.7)	0.10	0.68	98.8 (1.9)	99.4 (2.6)	0.36	2.70
SPM	96.2 (3.2)	95.5 (3.7)	0.11	0.85	97.6 (3.1)	98.0 (2.7)	0.42	3.48

Rec: mean recovery percentages and standard deviation in parentheses.
A: fluorimetric detection; B: UV detection

Table 4. Biogenic amines contents in Italian red wine (Wn) and in fruit nectars samples determined by fluorescence detection.

	Biogenic amines mg/L (SD)										
	HIS	**β-PEA**	**AGM**	**MEA**	**ETA**	**PUT**	**CAD**	**TYM**	**SPD**	**SPM**	**SER**
W1	ND	0.22 (0.01)	0.52 (0.02)	ND	1.58 (0.03)	5.97 (0.04)	1.11 (0.03)	1.07 (0.03)	0.44 (0.02)	1.07 (0.02)	ND
W2	NQ	0.85 (0.03)	NQ	0.36 (0.01)	1.87 (0.03)	4.86 (0.06)	0.65 (0.03)	0.67 (0.02)	0.61 (0.03)	NQ	ND
W3	0.36 (0.01)	0.97 (0.03)	0.58 (0.03)	0.74 (0.02)	0.79 (0.04)	8.94 (0.05)	0.49 (0.03)	1.38 (0.04)	NQ	ND	ND
W4	0.52 (0.01)	0.79 (0.02)	ND	1.41 (0.04)	1.22 (0.03)	3.84 (0.03)	0.94 (0.03)	0.95 (0.03)	0.67 (0.02)	0.46 (0.02)	ND
W5	NQ	0.68 (0.03)	0.56 (0.02)	NQ	1.04 (0.02)	11.13 (0.09)	1.58 (0.04)	1.97 (0.04)	0.25 (0.01)	0.38 (0.03)	ND
W6	0.76 (0.03)	0.88 (0.03)	NQ	NQ	1.11 (0.02)	3.94 (0.09)	1.08 (0.03)	0.88 (0.03)	0.23 (0.02)	0.62 (0.03)	ND
W7	ND	1.03 (0.04)	0.86 (0.03)	0.95 (0.03)	0.67 (0.02)	4.81 (0.07)	1.14 (0.04)	1.13 (0.03)	0.38 (0.03)	0.48 (0.02)	ND
W8	0.95 (0.01)	1.07 (0.03)	NQ	NQ	1.05 (0.03)	8.64 (0.05)	0.86 (0.03)	0.97 (0.03)	NQ	1.01 (0.04)	ND
W9	NQ	0.59 (0.02)	1.08 (0.04)	0.97 (0.04)	1.32 (0.04)	5.68 (0.04)	0.96 (0.02)	1.09 (0.03)	0.48 (0.03)	0.51 (0.03)	ND
W10	1.02 (0.04)	0.67 (0.03)	0.53 (0.03)	0.87 (0.03)	0.86 (0.03)	3.76 (0.04)	0.75 (0.02)	1.24 (0.03)	NQ	NQ	ND
Apricot 1	ND	ND	ND	ND	2.45 (0.07)	2.57 (0.04)	6.81 (0.10)	ND	2.95 (0.09)	2.50 (0.07)	ND
Apricot 2	ND	ND	ND	ND	NQ	3.25 (0.03)	1.96 (0.05)	ND	1.32 (0.06)	2.74 (0.04)	ND
Apricot 3	ND	ND	ND	ND	ND	1.10 (0.05)	11.25 (0.20)	ND	2.83 (0.10)	2.21 (0.07)	ND
Peach 1	ND	ND	ND	ND	ND	7.22 (0.04)	13.03 (0.15)	ND	1.71 (0.05)	1.91 (0.09)	ND
Peach 2	ND	ND	ND	ND	ND	1.88 (0.03)	7.86 (0.09)	ND	1.34 (0.03)	1.58 (0.05)	ND
Peach 3	ND	ND	ND	ND	ND	2.45 (0.04)	6.51 (0.07)	ND	1.96 (0.11)	3.58 (0.08)	ND
Pear 1	ND	ND	ND	ND	ND	1.23 (0.05)	17.20 (0.13)	ND	1.70 (0.06)	2.19 (0.07)	ND
Pear 2	ND	ND	ND	ND	ND	3.10 (0.03)	5.88 (0.07)	ND	2.17 (0.08)	1.81 (0.09)	ND
Pear 3	ND	ND	ND	ND	ND	1.65 (0.02)	8.47 (0.09)	ND	2.13 (0.09)	1.47 (0.04)	ND

ND, not detected

Highlights:

1. A fast HPLC determination of 11 biogenic amines in food has been described.
2. A new technology Core Shell particle column has been used with UV and FLD detection.
3. This is the first article reporting the analysis of biogenic amines in fruit nectar.
4. The developed method resulted in a significant saving of analysis time and solvents.