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A comparative survey of two analytical methods for identification and quantification of biogenic amines

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Abstract

Recent trends in food security are promoting an increasing search for trace compounds that can affect human health such as biogenic amines. The present paper describes a comparative study between two modified quick and simple HPLC methods for evaluating biogenic amines. In both methods biogenic amines were separated by reversed-phase chromatography. In the method with pre-column derivatization with *o*-phthaldialdehyde after modifications we obtained excellent results to separate and to quantify both biogenic amines and amino acids in a single run. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Biogenic amines; RP-HPLC; Amino acids

1. Introduction

Biogenic amines are low molecular weight organic bases that possess biological activity. They can be formed and degraded as a result of normal metabolic activity in animals, plants and microorganisms, and are usually produced by the decarboxylation of amino acids (Halász, Baráth, Simon-Sarkadi, & Holzapfel, 1994).

Recent trends in food security are promoting an increasing search for trace compounds that can affect human health. Biogenic amines, the so-called natural amines with physiological significance, belong to this group of substances. Although they are present in fermented foods and beverages in low quantities, they exhibit interactions with normal human metabolism, (e.g. vasoactive or psychoactive properties) that justify the research based on their presence in foods and the possible related toxicological effects that they may cause (Cabanis, 1985; Lehtonen, 1996).

The most frequent food-borne intoxications caused by biogenic amines involve histamine. Another phenomenon is the ''cheese reaction'' caused by high levels of tyramine in cheese (Stratton, Hutkins, & Taylor, 1991). Putrescine, cadaverine and agmatine have been identified as potentiators that enhance the toxicity of histamine to humans by depressing histamine oxidation (Taylor, 1986).

The estimation of the biogenic amines histamine, tyramine, agmatine, putrescine and cadaverine is important not only from the point of view of their toxicity, but also because they can be used as indicators of the degree of freshness or spoilage of food. Until recently, the difficulty of the detection and reliable quantification of amines has provided insufficient information about their occurrence in the different types of foods and beverages. These problems are related to matrix interference (e.g. presence of free amino acids) and the low levels at which the amines are found.

Early techniques for the determination of biogenic amines in foods were based on thin-layer chromatography. More modern analytical techniques have since been developed that enable the acquisition of reliable quantitative data and better separation resolution of various amines. The quantitative determination of biogenic amines is generally accomplished by over pressure-layer chromatography, high-performance liquid chromato graphy and gas chromatography (Halász et al., 1994).

Marcé, Callull, Guasch, and Borrull (1989) reported that the use of reverse-phase column and pre-column derivatization was more efficient and faster than the conventional ion-exchange techniques.

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This study was conducted to evaluate two HPLC derivatization methods for quantitative determination of biogenic amines.

2. Materials and methods

2.1. Chemical

Biogenic amines standards agmatine, cadaverine, histamine, putrescine, tyramine and 1,7-diaminoheptane were from Sigma. All solvents in the derivatization process and in the chromatographic separation were HPLC quality.

2.2. Method 1

Biogenic amines were determined by modification of the method of Gonzalez de Llano, Polo, and Ramos (1991) for amino acids analysis.

2.3. HPLC equipment

A reverse-phase high performance chromatography (RP-HPLC) using an ISCO system (ISCO, Lincoln, NE) and a model 121 fluorimeter (340 nm excitation filter and 425 nm emission filter) was used. A Waters Novapack C18 column, 3.9 × 150 mm, 4 μm particle size, was used for the stationary phase with a flow of 1.5 ml/min.

2.4. Mobile phase

Solvents used for the separation, A: methanol, 10 mM sodium phosphate buffer, pH 7.3 and tetrahydrofuran (19:80:1) and B: methanol and 10 mM sodium phosphate buffer, pH 7.3 (80:20). Solvent gradient conditions were as follows: 8 min (20% B); 8 min (30% B); 12 min (40% B); 16 min (80% B); 6 min (100% B) and 12 min (20% B). The entire gradient cycle lasted 62 min, including the time necessary for the stabilization of the column after each injection. The analysis time was only 50 min.

2.5. Derivatization

The formation of *o*-phthaldialdehyde (OPA) derivatives was performed automatically with reactant solution 100–150 folds higher in concentration than the amino nitrogen. The determination of amino nitrogen was performed using the method described by Doi, Shibata, and Matoba (1981). The reaction solution consisted of 200 mg OPA in 9 ml methanol, 1 ml 0.4 M sodium borate, pH 10 and 160 μl 2-mercaptoethanol (MCE). OPA/MCE reagent was not used within 24 h of preparation.

The standard solution of biogenic amines was prepared by dissolving each amine into a 0.1 N HCl solution to reach a concentration of 2.5 μmol/ml. This solution was stored at -18°C. 50, 100, 200 and 500 μl of these solutions were adjusted to 25 ml with borate buffer 0.4 M pH 10 and were filtered through a 0.45 μm filter.

Standards were derivatized prior to column injection as follows: 50 μl of sample were reacted with 50 μl OPA/MCE reagent for exactly 1 min and 25 μl of this solution were immediately injected.

The pre-column derivatization and the column apparatus were at room temperature.

Quantification: External standard method was used.

3. Method 2

Biogenic amines were determined by liquid chromatographic method as described by Eerola, Hinkkanen, Lindfors, and Hurvi (1993); with some additional changes.

3.1. HPLC equipment

Liquid chromatography was performed in a Gilson system connected to a Gilson 118 UV detector at 254 nm. A reversed-phase Phenomenex ODS2 column 4.6 × 300 mm i.d., 4 μm particle size, was used for the stationary phase with a flow of 1.0 ml/min.

3.2. Mobile phase

The solvents used for the separation were solvent A: 0.1 M ammonium acetate and solvent B: acetonitrile. Solvent gradient conditions began with 50% B and ended with 90% B in 19 min. The total run time was 35 min including the washing time. Washing was essential to maintain column performance.

3.3. Derivatization

Standards were derivatized prior to column injection as follows: dansyl derivatization was performed with addition of 100 μl 2 N sodium hydroxide solution, 150 μl saturated sodium bicarbonate and dansyl chloride solution (5 mg dansyl chloride in 0.5 ml acetone). The reaction mixture was incubated at 40°C for 45 min. After incubation the residual dansyl chloride was removed by addition of 50 μl ammonia. After 20 min the sample was adjusted to 2.5 ml with acetonitrile, then was filtered with a 0.22 μm filter.

The pre-column derivatization and the column apparatus were at 40°C.

The standard curves were prepared with pure compounds at different concentrations.

Quantification: The method of internal standard (1,7-diaminoheptane) was used.

Statistical analysis: To validate the methods the MINITAB Student test was used. Five replicate determinations were carried out.

4. Results and discussion

In this survey the amines were determined by HPLC using two methods based on pre-column derivatization and a polarity gradient. Figs. 1 and 2 show the chromatograms of amine standards obtained using methods 1 and 2 (see Sections 2 and 3). In both methods we used reverse-phase column. Without derivatization the amines would elute as broad peaks shown by fluorescence detection.UV absorbance detection is only possible for the heterocyclic and aromatic amines, therefore derivatization is necessary for the detection of aliphatic amines and for increased sensitivity. Pre-column derivatization was chosen, since it allows secondary product separation in the column, avoiding loss during the analysis. The peaks of the biogenic amines were satisfactorily resolved and there were no interfering peaks, so simple observation of chromatograms suggest the presence of these compounds. The elution program was developed to provide chromatograms of high-resolution peaks. The programmed elution with a polarity gradient was necessary to obtain optimum separation and quantification of the corresponding amines due to the wide range of polarities of these molecules.

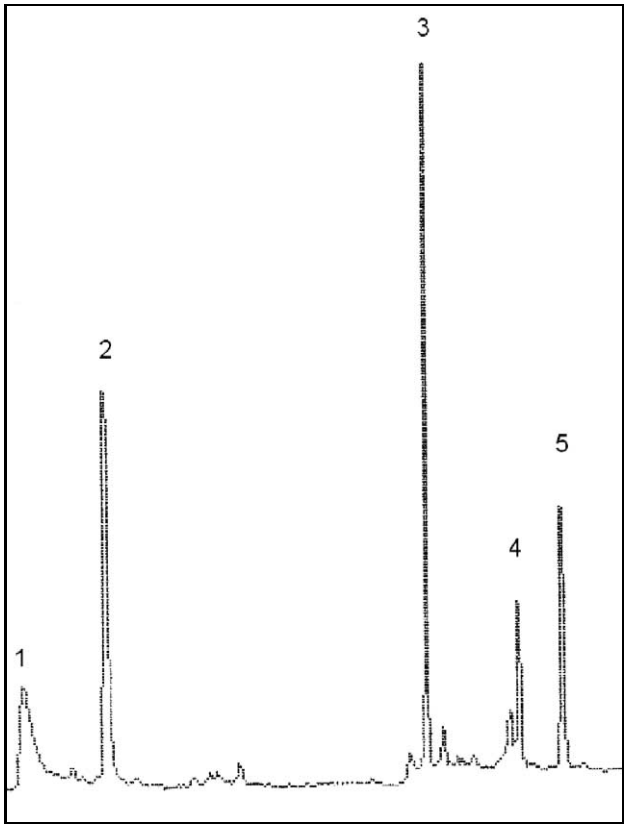

Fig. 1. HPLC profile of amines in standard solution (method 1): 1 – agmatine, 2 – histamine, 3 – tyramine, 4 – putrescine and 5 – cadaverine.

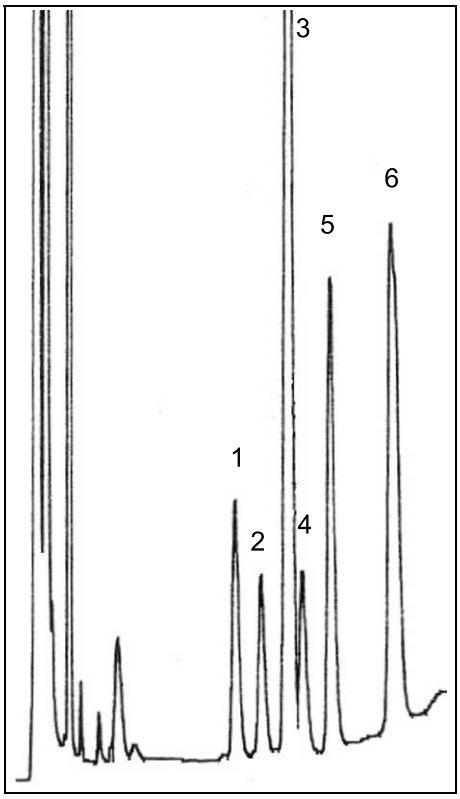

Fig. 2. HPLC profile of amines in standard solution (method 2): 1 – putrescine, 2 – cadaverine, 3 – agmatine, 4 – histamine, 5 – 1,7 diaminoheptane and 6 – tyramine.

The standard solutions employed for the identification were injected five times and analyzed under the chromatographic conditions described previously. A relative standard deviation (RSD) less than 6.00% for response factor was obtained by method 1. The repeatability of the method 2 was better than method 1, with RSD ranging from 1.30% to 3.50% for response factor (Table 1).

Agmatine and putrescine had an RSD for response factor greater than the average (2.99%) for method 1. For method 2 putrescine and cadaverine had an RSD greater than the average (2.30%). Hernández-Jover, Izquierdo-Pulido, Nogués, and Vidal-Carou (1996); using OPA derivatization obtained RSD less than 10%. Mafra, Herbert, Santos, Barros, and Alves (1999) reported that the aliphatic diamines, putrescine and cadaverine

Table 1
Study of the response factor and RSD of HPLC methods[a]

Biogenic amine	Method 1		Method 2	
	Response factor	RSD (%)	Response factor	RSD (%)
Agmatine	5.11	4.00	3.56	2.05
Cadaverine	8.14	1.81	27.10	3.50
Histamine	13.70	1.60	25.64	1.30
Putrescine	5.96	5.80	19.49	2.82
Tyramine	16.90	1.78	6.52	1.81

[a] Means of five replicates.

present the greatest RSD (more than 20%). In our study RSD for tyramine was approximately 1.80% for both methods tested. However Mafra et al. reported that tyramine has an RSD% greater than average (17.90%), probably due to difficulties in the derivatization procedure. In fact the hydroxyl group attached to the aromatic ring may confer acidic properties, which interfere in the derivatization reaction, whose yield is maximized in basic medium.

Our results point out the possibility to obtain good resolution for concentrated samples of biogenic amines, without the interference in the analysis of the other less concentrated biogenic amines, which are next to these compounds in the chromatogram.

Table 2 shows the retention time and its RSD for both methods. The elution time in method 1 was between 11.23 and 42.27 min. Agmatine was the first amine eluted followed by histamine, tyramine, putrescine and cadaverine. For method 2 the elution time lay within the range of 12.90–17.24 min. The elution order was putrescine, cadaverine, agmatine, histamine and tyramine. The internal standard 1,7 diaminoheptane eluted between histamine and tyramine. For method 1 RSD for retention time was less than 4.00% and for method 2 the values ranged from 2.08% to 5.30%.

The quantification by external standard method used in method 1 is based on the linearity of the detector response: a double concentration of product led to a peak of double area.

Table 2
Study of retention time and RSD of HPLC methods[a]

Biogenic amine	Method 1		Method 2	
	Retention time	RSD (%)	Retention time	RSD (%)
Agmatine	11.23	3.90	14.23	2.85
Cadaverine	42.27	0.80	13.61	2.87
Histamine	16.03	2.80	14.79	2.08
Putrescine	39.63	0.90	12.90	2.30
Tyramine	34.46	1.00	17.24	5.30

[a] Means of five replicates.

Table 3
Accuracy of the methods assayed expressed by the correlation factor[a]

Biogenic amine	Correlation coefficients	
	Method 1	Method 2
Agmatine	0.991	0.991
Cadaverine	0.998	0.980
Histamine	0.998	0.998
Putrescine	0.985	0.981
Tyramine	0.998	0.996

[a] Means of five replicates.

The fluorescence exhibited a linear correlation with the concentration in the range 1–10 mg/l. A good linear regression between peak area and concentration for each biogenic amine was obtained, with correlation coefficients ranging from 0.985 to 0.998 for method 1. This confirms the accuracy of this method for determining biogenic amines content (Table 3).

In method 2 the linearity was tested by dansylating the standard solutions at different concentrations. Dansylated amines showed linear responses in the concentration range 1–10 mg/ml, with correlation coefficients ranging from 0.980 to 0.998 (Table 3). The greatest variation in results was observed in the case of cadaverine.

In method 1, in order to achieve a better separation between amines and between amines and acidic and neutral amino acids, the time of analysis was sacrificed (50 min). The acidic and neutral amino acids those are present in higher levels than amines eluted first into the chromatogram. However the simple preparation of the sample and the rapid derivatization of the amines considerably reduce time and effort.

In addition using this method and beginning the gradient with 6 min (0% B) and 11 min (15% B), it was possible to separate and to quantify amines and amino acids from a sample in a single chromatogram (Fig. 3).

In method 2 internal standard was used. It involves a compound not present in the sample as an internal standard. Internal standardization compensates for variations in conditions during sampling and derivatization, as well as for variations in injection volumes and retention times during chromatographic run. Some modifications of this procedure were carried out: inclusion of agmatine, omission of centrifugation by filtration using a filter of 0.22 μm to avoid the microbial spoilage, increase of column length (higher plate number) and the detection was carried out with a UV detector.

In conclusion, in the method with pre-column derivatization with OPA after modifications we obtained excellent results to separate and to quantify both biogenic amines and amino acids in a single run.

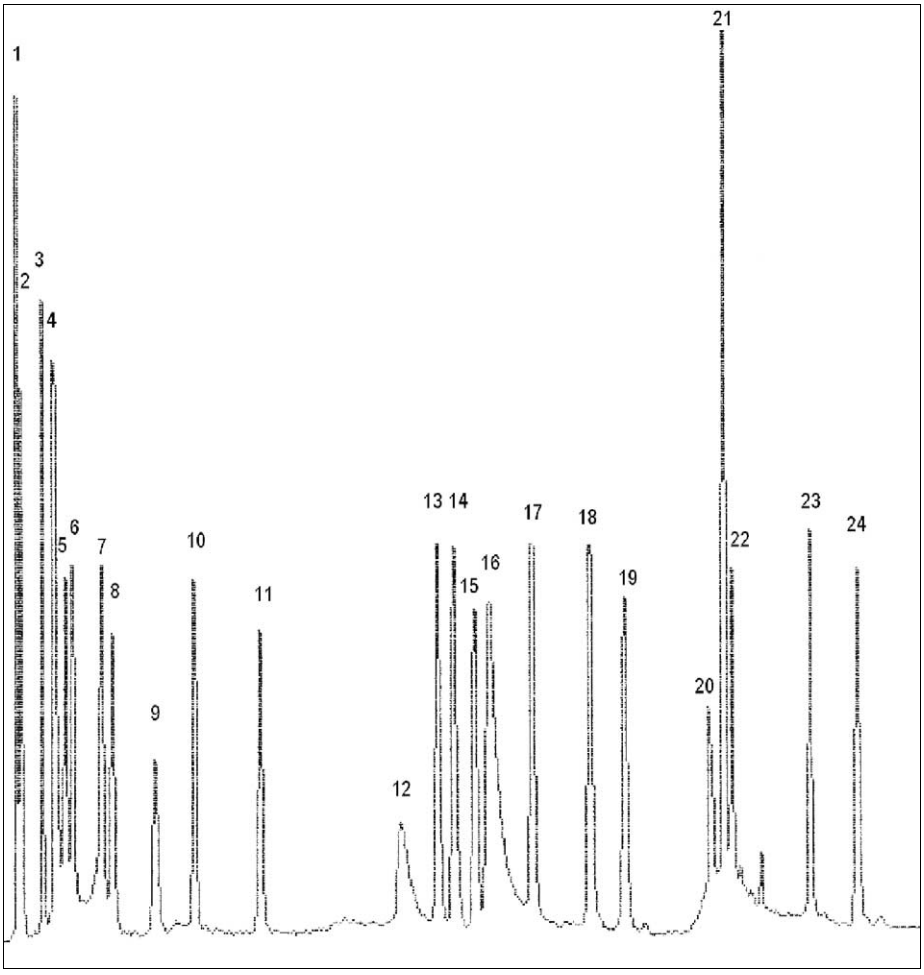

Fig. 3. HPLC profile of amines and amino acids (method 1): 1 – aspartic acid, 2 – glutamic acid, 3 – asparagine, 4 – serine, 5 – glutamine, 6 – histidine, 7 – glycine, 8 – threonine, 9 – arginine, 10 – alanine, 11 – tyrosine, 12 – agmatine, 13 – methionine, 14 – valine, 15 – tryptophan, 16 – histamine, 17 – phenylalanine, 18 – isoleucine, 19 – leucine, 20 – ornithine, 21 – tyramine, 22 – lysine, 23 – putrescine and 24 – cadaverine.

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