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Determination of biogenic amines in fish tissues by ion-exchange chromatography with conductivity detection

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Abstract

Some biogenic amines, such as putrescine, cadaverine, spermidine and histamine, have been found to be useful as quality indices for the decomposition of fish, so research on the simultaneous analysis of various biogenic amines in food is of interest and importance. The intake of histamine may cause an allergic intoxication known as "scombroid poisoning" while secondary biogenic amines can potentiate the toxicity of histamine and in addition can react with nitrite to form carcinogenic nitrosamines. A new method for the simultaneous determination of underivatized biogenic amines based on ion-exchange chromatography with conductivity detector has been developed. The proposed method offers a number of advantages over previous pulsed amperometric detection and integrated pulsed amperometric detection methods such as simpler extraction procedure and sharp peaks. Separations were performed on a new low hydrophobic weak cation-exchange analytical column. This technique is simple, rapid and useful for routine checks in repetitive analyses.

Keywords: Fish; Food analysis; Biogenic amines

1. Introduction

Biogenic amines are low-molecular-mass aliphatic, alicyclic and heterocyclic organic compounds displaying biological activity. They may be found in a wide range of food products of both animal and plant origin, as well as in fermented foods. They are produced mainly by the microbial decarboxylation of amino acids, in particular of the amino acid histidine.

It is important to monitor biogenic amine levels in foodstuffs and beverages in view of their importance for human health and food safety. Biogenic amines occasionally cause food poisoning in man. The most frequent kind of poisoning due to the histamine is known as "scombroid fish poisoning", linked to consumption of fish like tuna and sardines, which contain high levels of histamine.

Also secondary amines such as putrescine and cadaverine play an important role in food poisoning as they can potentiate the toxicity of histamine [1–5] and react with nitrites to form nitrosamines, which are carcinogenic compounds. The detection of biogenic amines has become particularly important in recent years as an indicator of food quality. The biogenic amine content of foodstuffs can be modified during technological food processing and is influenced by the prevailing hygienic conditions.

Various methods are described in the literature for the determination of biogenic amines. The main drawbacks of these methods are related to the process of pre- or post-column derivatization leading to long analysis times, low reproducibility, interference and problems of derivatization stability.

Other methods [6–9] do not involve derivatization but use pulsed amperometric detection (PAD) and/or integrated pulsed amperometric detection (IPAD).

Amperometric detection ensures good sensitivity and does not require derivatization. However, the methods reported to date all require alkalinization of the eluent phase prior to detection. Furthermore, amperometric cell management proves unwieldy and requires special maintenance.

In the present work a new method has been developed using ionic chromatographic separation with conductimetric detection after chemical suppression and automatic regeneration.

It is possible to operate in conditions of conductivity also on real matrixes thanks to the use of a new low hydrophobic

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and moderate capacity weak ionic exchange polymeric chromatographic phase.

This method can be used to detect four biogenic amines: putrescine, cadaverine, histamine and spermidine in fish tissues using simple aqueous extraction.

2. Experimental

2.1. Reagents

The reference standards histamine dihydrochloride, cadaverine dihydrochloride, putrescine dihydrochloride and spermidine trihydrochloride (99% pure) and methanesulfonic acid (MSA; >99% pure) were supplied by Sigma–Aldrich (Poole, UK).

The water used was obtained using a Milli-Q purification system from Millipore (Milan, Italy).

2.2. Preparation of standard solutions

The stock solutions of biogenic amines, each containing 1000 mg/l, were prepared by dissolving respectively 165.6 mg of histamine dihydrocholoride, 171.4 mg of cadaverine dihydrochloride, 183.0 mg diputrescine dihydrochloride and 175.4 mg of spermidine trihydrochloride in 100 ml of an aqueous solution of 3 mmol/l MSA.

The stock solutions were stable for 6 months at +4 °C.

The working solutions at concentrations of 2, 5, 10, 15 and 30 mg/l were obtained by suitably diluting the respective stock solutions in an aqueous solution of 3 mmol/l MSA.

The working solutions must be prepared fresh each day.

2.3. Equipment and analytical conditions

A model DX-500 equipped with a GP 50 gradient pump, an electrochemical detector ED 40 in the conductivity mode and an autosampler AS 50 (Dionex, Sunnyvale, CA, USA) were used for the analyses with the Software PeakNet 6.4 (Dionex).

The suppressor used was CRSR Ultra (Dionex) with the suppressor current set at 100 mA.

The chromatographic column used was a weak ion-exchange IonPac CS17 column, 250 mm × 4 mm, particle size 7 μm (Dionex).

The method used involved an MSA gradient of from 3 to 18 mmol/l in 10 min, 4 min at 18 mmol/l and a second gradient from 18 to 25 mmol/l in 4.5 min. The system was then re-equilibrated for 10 min.

The flow rate was 1 ml/min and the injection loop 25 μl.

2.4. Sample preparation

Five grams of previously homogenized tuna fish were weighed out into a glass centrifuge tube and 20 ml of MSA 0.1 mol/l added; the mixture was vortex stirred for 1 min and then placed in an ultrasound bath for 15 min; it was then centrifuged at +4 °C for 15 min at 4000 rpm, the supernatant removed and filtered through a Whatman filter into a 50 ml round-bottomed flask. The procedure was repeated a second time; the extracts were then gathered and made up to volume with water. Before injecting into the HPLC system it was filtered through a 0.45 μm PTFE.

3. Results and discussion

A simple, rapid and specific method has been developed for the determination of four biogenic amines: cadaverine, putrescine, histamine and spermidine in tuna fish samples preserved in olive oil.

Several different tests were run on the samples to extract the analytes.

Several different extracts were used: HCl, $HClO_4$, phosphate buffer (pH 2.5) and MSA at different concentrations: 0.05, 0.1 and 1 mol/l as shown in Table 1.

As extraction solvent 0.1 mol/l MSA was chosen as it provided the best recovery (from 92 to 102% for each amine). Furthermore, MSA was also the eluent used in subsequent chromatographic analysis and so by means of injection in the homogeneous phase it was possible to obtain also a good symmetry and resolution of chromatographic peaks.

The method applicability has been verified even for different fish matrices (mackerels, anchovies and crabs) and the results can be compared to the tuna samples. One of the problems normally encountered in liquid phase chromatography of amines is the relatively poor peak symmetry. The strong cation-exchange polymer columns were effectively used to separate aliphatic amines [7,8] and also the more recent weak cation exchangers [6,9] display excellent selectivity. However, the problem remains of the strong hydrophobic interaction which makes it necessary to add organic solvent in the eluent phase. In spite of this, many amines, such as histamine, always display very broad and comparatively non symmetric bands, thus worsening separation and above all sensitivity. This led researchers to use particularly sensitive detection techniques, such as amperometry. This valid detection technique, in particular in its pulsed potential version, has given good results but requires normally acid eluents with high ionic strength and a post-column derivatization with hydroxide. The use of hydroxide is necessary to switch the eluent pH from acid to alkaline, otherwise oxidation of the amine group on the gold electrode would not be possible. Furthermore, the eluents prove very aggressive to the amperometric cell, obliging the operator to perform frequent maintenance on it to avoid corrosion and damage to the reference electrode as well as to remove dirt accumulating on the working electrode.

The recent introduction of a weak ion-exchange column, the IonPac CS17 (Dionex), offers new detection potential and simplicity of use. It consists of an ethylvinylbenzene–divinylbenzene polymer phase covered

Table 1

Extracting solution	Recovery (%) ($n = 18$)			
	Putrescine	Cadaverine	Histamine	Spermidine
HCl, 0.05 mol/l	62 ± 8	58 ± 9	62 ± 6	68 ± 5
HCl, 0.1 mol/l	82 ± 7	83 ± 6	84 ± 4	82 ± 7
HCl, 1 mol/l	91 ± 5	96 ± 8	95 ± 3	98 ± 6
$HClO_4$, 0.05 mol/l	59 ± 4	56 ± 8	55 ± 11	61 ± 10
$HClO_4$, 0.1 mol/l	84 ± 6	83 ± 5	80 ± 8	85 ± 5
$HClO_4$, 1 mol/l	96 ± 5	94 ± 5	92 ± 3	100 ± 5
Tampone fosfato (pH 2.5), 0.05 mol/l	55 ± 4	61 ± 10	59 ± 9	61 ± 11
Tampone fosfato (pH 2.5), 0.1 mol/l	75 ± 6	74 ± 7	71 ± 8	78 ± 8
Tampone fosfato (pH 2.5), 1 mol/l	90 ± 7	91 ± 10	92 ± 9	95 ± 7
MSA, 0.05 mol/l	63 ± 5	61 ± 8	±7	78 ± 6
MSA, 0.1 mol/l	95 ± 3	95 ± 2	98 ± 4	100 ± 2
MSA, 1 mol/l	95 ± 4	96 ± 5	97 ± 4	99 ± 4

with a hydrophilic polymer layer that restricts the hydrophobic interaction of the amine and short polymer chains supporting the carboxylic sites where the exchange takes place are grafted on to it.

The result is a moderate capacity weak cation exchange and above all a low hydrophobic chromatographic phase. The chromatographic advantage offered is to be able to elute using low acid concentrations with simple fast gradients and obtaining narrow, sensitive chromatographic bands without the need for organic solvents. This enhanced chromatographic yield allows us to use simple conductimetric detection after chemical suppression of the eluent using continuous automatic regeneration suppressor which had hitherto been rejected as being too insensitive to the above amines.

Fig. 1 displays a chromatogram for a standard mixture of biogenic amines.

Under the conditions adopted, the HPLC analyses were completed in 23.00 min with symmetrical peaks: the order of elution was putrescine (retention time, $t_r = 11.35$), cadaverine ($t_r = 11.78$), histamine ($t_r = 12.59$) and spermidine ($t_r = 17.95$).

The retention times for each analyte corresponded to that of the calibration standard within a tolerance of ±2.5%

To control the linear range of HPLC method relations between the area responses of injected standard solutions to the corresponding amine concentrations were measured. For each amine, we used five calibration standards dissolved in 3 mmol/l MSA ranging from 2 to 30 mg/l to generate external calibration curves.

The correlation coefficient exceeded 0.999 for the histamine and the spermidine and 0.998 for the cadaverine and putrescine, respectively.

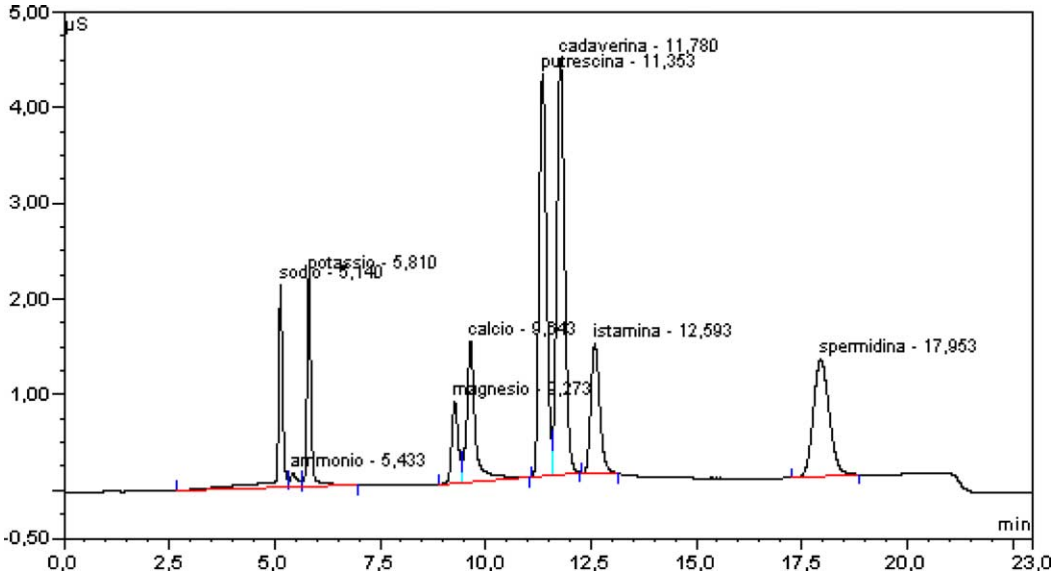

Fig. 1. Chromatogram for a standard mixture of biogenic amines (putrescine, cadaverine, histamine and spermidine).

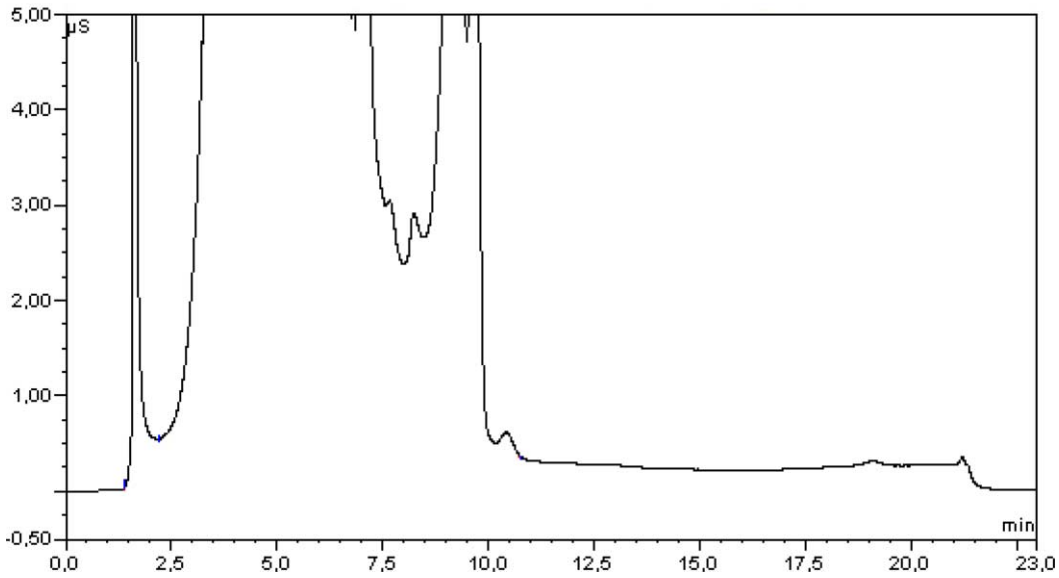

Fig. 2. Chromatogram of a blank tuna fish sample.

In order to verify the specificity of the method, 20 blank tuna fish samples from different origins were analysed. No interference was observed in the region of interest where the analytes were eluted as is shown in the blank sample chromatogram (Fig. 2).

In view of the absence of interferents, it was decided to skip the purification step.

The limits of detection (LODs), calculated as the lowest concentration from which it is possible to deduce the presence of the analyte with reasonable statistical certainty (signal/noise, 3:1) were 0.15 mg/kg for cadaverine, 0.15 mg/kg for putrescine, 0.45 mg/kg for histamine and 0.50 mg/kg for spermidine.

The limits of qualifications (LOQs), calculated as the smallest measured content of the identified analyte in a sample that may be quantified with a specified degree of accuracy ($n = 6$) and within-laboratory reproducibility, were 0.5 mg/kg for cadaverine, 0.5 mg/kg for putrescine, 1 mg/kg for histamine and 1 mg/kg for spermidine.

In order to evaluate the precision, accuracy and recoveries of the analytical method, the tuna fish samples were spiked at 100 mg/kg for each biogenic amine and then analysed (Fig. 3). Inter-day precision and accuracy data on three different days are shown in Table 2. The precision of the method was determined by calculating the relative standard deviation (R.S.D., %) for the repeated measurements. The

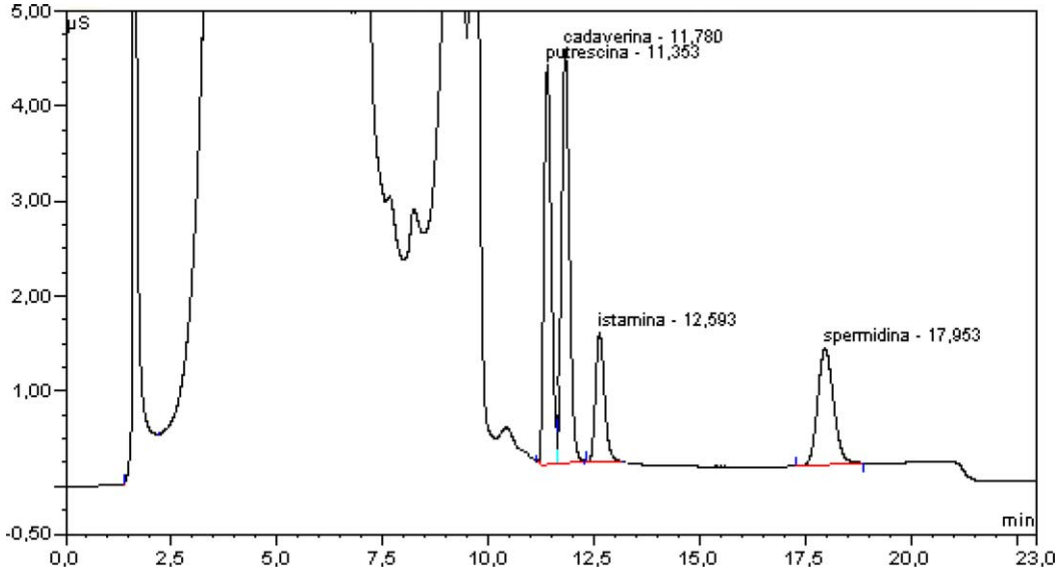

Fig. 3. Chromatogram of a spiked tuna fish sample.

Table 2
Inter-day precision and accuracy data in tuna fish samples

	Spiked sample level (100 mg/kg)			
	Cadaverine	Putrescine	Histamine	Spermidine
Average (mg/kg)	94.63	94.74	97.71	99.79
S.D. (mg/kg)	2.77	2.42	3.60	2.44
Precision (R.S.D., %)	2.93	2.56	3.69	2.44
Trueness (%)	−5.37	−5.26	−2.29	−0.21
n	18	18	18	18

accuracy of the method (trueness, %) was determined by assessing the agreement between the measured and nominal concentrations of analysed samples.

4. Conclusions

The method described herein may be considered one of the simplest and most complete for determining the main biogenic amines. It is complete, in so far as a single chromatographic run is sufficient to obtain the principle parameters; simple, as the use of reagents has been reduced to methanesulphonic acid alone. Considering that ionic chromatographs with suppressed conductimetric detection are now available that are capable of autogenerating also MSA, the analysis can be managed completely by means of an automated system thus eliminating operator errors. This makes available a robust method that can provide greater homogeneity among the data supplied by different operators and different laboratories to the benefit of data quality and in harmony with modern quality concepts.

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