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ORIGINAL PAPER

A multiresidual method based on ion-exchange chromatography with conductivity detection for the determination of biogenic amines in food and beverages

Carmen Palermo · Marilena Muscarella · Donatella Nardiello · Marco Iammarino · Diego Centonze

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Abstract In the present work a sensitive and accurate method by ion chromatography and conductimetric detection has been developed for the determination of biogenic amines in food samples at microgram per kilogram levels. The optimized extraction procedure of trimethylamine, triethylamine, putrescine, cadaverine, histamine, agmatine, spermidine, and spermine from real samples, as well as the separation conditions based on a multilinear gradient elution with methanesulfonic acid and the use of a weak ionic exchange column, have provided excellent results in terms of resolution and separation efficiency. Extended calibration curves (up to 200 mg/kg, r>0.9995) were obtained for all the analyzed compounds. The method gave detection limits in the range 23–65 μg/kg and quantification limits in spiked blank real samples in the range 65–198 μg/kg. Recovery values ranged from 82 to 103 %, and for all amines, a good repeatability was obtained with precision levels in the range 0.03–0.32 % (n=4). The feasibility and potential of the method were tested by the analysis of real samples, such as tinned tuna fish, anchovies, cheese, wine, olives, and salami.

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C. Palermo (✉) · D. Nardiello · D. Centonze
Dipartimento di Scienze Agrarie, degli Alimenti e dell'Ambiente,
Università degli studi di Foggia,
via Napoli, 25,
71122 Foggia, Italy
e-mail: c.palermo@unifg.it

M. Muscarella · M. Iammarino
Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Università degli studi di Foggia,
Via Manfredonia 20,
71122 Foggia, Italy

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Introduction

The importance of biogenic amine levels in food and beverages is related to their impact on human health and food quality: the most frequent kind of poisoning due to histamine (HIS) is known as "scombroid fish poisoning," and the legal limit in fish has been fixed at 100 mg/kg [1]. Also, putrescine (PUT), cadaverine (CAD), spermine (SPM), spermidine (SPMD), and agmatine (AGM) and trimethylamine (TMA) are very important freshness indicators [2–10] since they can potentiate the toxic effects of tyramine and histamine by inhibiting the detoxifying enzymes [11] present in the human body. A quite recent review on dietary polyamines [12] summarizes the current knowledge on the biological implications of dietary polyamines for human health and collects the data on their formation and contents in manifold foods. This review underlines that extensive research is required to extend the current limited database and the need of new limits for such toxic compounds.

The analytical determination of biogenic amines (BAs) and polyamines is not a simple task owing to their structure and because they are usually present at low levels in complex matrices. Furthermore, biogenic amines and polyamines do not exhibit a satisfactory absorption and then strong signals at visible, ultraviolet, and fluorescence wavelengths. Therefore, a chemical derivatization is usually applied for their analysis, and several methods have been developed for assaying amines as derivatives. In 2007 Önal

reviewed [13] the analytical methods developed for the determination and quantification of biogenic amines in foods, based on chromatographic separations, and among them, reverse-phase liquid chromatography (RPLC) resulted as the most-used technique. The main drawbacks of these methods are related to the process of pre- or post-column derivatization [14, 15] that usually show an overall long analysis time and low reproducibility due to the instability of both derivatization reagents and derivatized compounds. Also, the use of a RPLC-ESI-tandem mass spectrometry-based methods showed a limited application and required an extensive sample cleanup step [16]. An alternative approach is represented by ion-exchange chromatography (IEC) coupled with pulsed amperometric detection (PAD) that is widely used [17] for the determination of ionic and ionizable compounds, such as inorganic cations and anions, amino acids, organic acids, amines, peptides, and proteins [18–20]. Nevertheless, acidic mobile phases, which are very important for an efficient separation of the analytes, are not useful to an effective PAD at gold working electrodes that require a post-column alkalinization of the eluent. Furthermore, amperometric cell management proves unwieldy and requires special maintenance.

A few applications based on IEC have been reported for the determination of biogenic amines. This is at least partially due to the strong hydrophobic interactions between the protonated amines and typical cation-exchange stationary phases resulting in long retention times and poor peak shapes. In addition, eluents required to separate the amines are often not compatible with suppressed conductivity detection, which can provide one of the simplest approaches for detecting some of the major biogenic amines [21].

In the last decade, a weak carboxylic acid-functionalized cation-exchange column (CS17 supplied by Dionex) has been proposed for the separation and analysis of common inorganic cations and a variety of amines and diamines, as well as biogenic amines, without requiring the use of any organic solvent in the mobile phase [22]. The use of both an eluent generator and a cation trap column to eliminate impurities in the deionized water is recommended, in order to obtain very clean eluents that provide flat baselines with either eluent step changes or eluent gradients. In particular, cation trap column can be installed to remove cationic contaminants from the eluent that would increase the background noise by lowering the sensitivity of the method.

Moreover, an acidic gradient at a controlled temperature (i.e., above 30 °C) should be used in order to provide both good peak efficiencies and peak symmetries. In addition, the presence of high concentrations of inorganic cations (e.g., sodium and calcium) in real samples, as well as the pH of the extraction solution, can strongly affect the ionization of relatively weak carboxylic acid cation-exchange sites and then the separation efficiency and method sensitivity.

As a consequence, a few applications of this new column for the analysis of biogenic amines in tuna fish and processed meat have been reported [23–26]. These methods are based on the extraction of biogenic amines by methanesulfonic acid (MSA) and their analysis by IEC coupled with conductivity detection (CD) [23, 24], integrated pulsed amperometric detection (IPAD) [25], and mass spectrometry detection (MS) [26]. The main problem of these analytical methods is represented by the inefficient resolution due to gradients and/or concentrations of MSA used in the separation and extraction of BAs. Moreover, in the IEC-MS-based methods, the mass detector was operated in SIM mode to overcome resolution problems, as for instance those relevant to putrescine and cadaverine. Furthermore, separation and detection of SPM was not reported, although this polyamine is very important for the determination of meat freshness [9, 10]. Also, the IEC-CD [23] multiresidual method suffers of resolution problems that do not allow, in particular in the presence of real sample interferents (e.g., metal cations), the detection of TMA and SPMD; for instance, accuracy and limit of detection of the method are strongly affected.

An attempt to overcome these problems has been recently carried out by the use of a new weak carboxylic acid-functionalized cation-exchange column (CS18 from Dionex) that was applied to the determination of biogenic amines in beer and wine samples [21]. This column, designed for the determination of polar amines, is aimed at the improvement of separation of closely eluting peak pairs, such putrescine and cadaverine, but it requires an eluent generator and a column thermostatation at 40 °C. Moreover, a post-column addition of NaOH, to increase the effluent pH, is needed for the detection by IPAD at a gold working electrode.

In the present work a sensitive and accurate method has been developed for the IEC separation and conductimetric determination of biogenic amines (putrescine, cadaverine, spermine, spermidine, triethylamine, agmatine, and trimethylamine). The use of a weak cation-exchange column (CS17 supplied by Dionex) operating at room temperature and without eluent generator has been proposed. The concentration of MSA for the extraction procedure and separation conditions have been carefully optimized to ensure an efficient recovery and accurate determination of biogenic amines in real samples of animal and vegetal origin. The proposed method has been submitted to an extensive validation procedure, to assess accuracy, sensitivity, reproducibility, and limits of detection and quantitation. The potential of the validated method has been tested by the analysis of biogenic amines in tinned tuna fish, anchovies, cheese, wine, olives, and salami.

Experimental

Chemicals and standard solutions

Methanesulfonic acid (> 99 %) of HPLC grade, HIS (dihydrochloride), PUT (dihydrochloride), CAD, SPM, SPMD (trihydrochloride), triethylamine (TEA), AGM, and TMA of analytical-reagent grade were purchased from Sigma-Aldrich (Poole, UK). Solutions and mobile phases were prepared with water purified (18 MΩcm^{-1}) by a Pure-Lab Prima and PureLab Classic-UV ELGA systems (Lab-Water, Buckingham Shire, UK). Stock solutions of biogenic amines (1,000 mg/L each) were prepared in water and stored in the dark at −20 °C. Working solutions were prepared freshly just before use by dilution in mobile phase.

Equipment and analytical conditions

A Dionex (Sunnyvale, CA, USA) DX-500 apparatus equipped with a GP 50 quaternary gradient pump, an electrochemical detector ED 50 in conductivity mode, and a CRSR Ultra suppressor (Dionex) with a current value set at 100 mA was used as ion chromatographic system. Data acquisition and processing were carried out by the software PeakNet 6.3 (Dionex). A Dionex IonPac CS17 column (250×4 mm I.D.; particle size, 7 μm; pore size, 150 Å; 55 % cross-linked polyethylvinylbenzene–divinylbenzene, grafted with carboxylated functional groups) coupled with an IonPac CG17 (50×4 mm I.D.) guard column was used for the chromatographic analyses. The experimental separation conditions involved a multilinear gradient operating at room temperature and consisting of a 6-min isocratic step with MSA at 6 mM, followed by a linear gradient from 6 to 11 mM in 16 min, then from 11 to 40 mM in 4 min, and finally, at 40 mM in 4 min. The system was then re-equilibrated for 10 min. The flow rate was 1 mL/min, and the injection volume was 25 μL.

Preparation of real samples

Two grams of a previously homogenized real sample (tuna fish, anchovies, wine, olives, salami, and cheeses) were weighed out into a glass centrifuge tube, and 3×5 mL of 20 mM MSA were added. After mixing by a vortex for 1 min, the mixture was placed in an ultrasound bath for 10 min. After centrifugation at 3,000 rpm for 10 min at +4 °C, the gathered extracts were made up to 20 mL volume in a conical flask with 20 mM MSA.

Wine (after a dilution 1:2 in water) and olive samples (after the extraction step) required an additional purification by a solid-phase extraction on a polyvynilpyrrolidone cartridge OnGuard II P® (Dionex, Sunnyvale, CA, USA) in order to eliminate the polyphenolic compounds. Sample solutions were filtered through a 0.45-μm PTFE membrane before the chromatographic analysis.

Results and discussion

Cation-exchange chromatographic separation of biogenic amines

Analysis of biogenic amines in complex real samples (e.g., tinned tuna fish, anchovies, cheese, etc.), obtained by moderate-capacity weak cation-exchange columns, can present crucial problems due to the presence, in particular, of high concentrations of mono- and bivalent metal cations and amino acids. Depending on the chromatographic conditions, similar retention times of metal ions and amines can lead to coelution or poor separation efficiency. On the contrary, amino acids, even if positively charged, do not interfere since they are not detected in suppressed conductivity. In order to minimize these analytical problems, proper step change and/or gradient elutions should be developed for accurate analyses of biogenic amines in real samples.

Preliminary experiments carried out on standard solutions of each biogenic amine evidenced that TMA, TEA, PUT, and CAD require low MSA concentrations to be eluted, while HIS, AGM, SPM, and SPMD need higher concentrations. Standard mix of biogenic amines and spiked real samples were also tested with different elution programs in order to optimize the separation of analytes.

Figure 1 displays a chromatogram obtained by the proposed method for a standard mixture of biogenic amines. TMA and TEA are eluted by using an isocratic step at low MSA concentration (6 mM), while PUT, CAD, HIS, and AGM require a gradient from 6 to 11 mM. More retained species, such as SPM and SPMD, need a strong isocratic step at 40 mM MSA. As it can be seen, all the investigated analytes are eluted within 30 min, and in spite of the absence of any eluent generator or cation trap column, an almost flat baseline has been obtained, which allows an accurate determination of TMA, PUT, and CAD, even in the presence of a high concentration of metal cations.

In Table 1 are reported the typical chromatographic parameters characterizing the separation of a 10-mg/kg standard mix of biogenic amines with the optimized multilinear gradient. As shown in Fig. 2 and from the data of Table 1, the eight biogenic amines can be separate efficiently, with asymmetry factors comparable to the literature methods [23, 24], but in a short period of time and with a better resolution, in particular for PUT, CAD, HIS, and AGM.

The use of an acid mobile phase [22] is crucial for the elution of the analytes by weak exchange columns (e.g., CS17) without the need of using organic solvents, which are essential with strong cation exchanger-based columns.

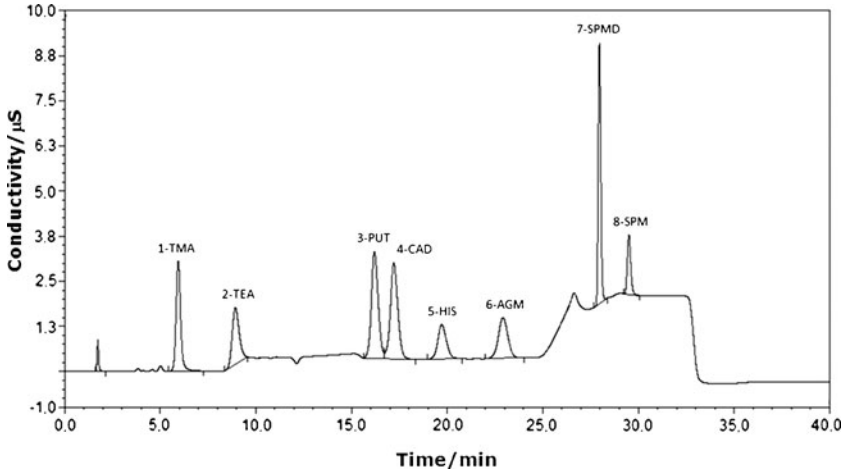

Fig. 1 Chromatogram of a mixed standard solution containing (1) trimethylamine (*TMA*), (2) triethylamine (*TEA*), (3) putrescine (*PUT*), (4) cadaverine (*CAD*), (5) histamine (*HIS*), (6) agmatine (*AGM*), (7) spermidine (*SPMD*), and (8) spermine (*SPM*) at a concentration of 10 mg/L each. Column IonPac CS17 (250×4 mm I.D.; particle size, 7 μm, Dionex). Multilinear gradient elution with methanesulfonic acid (6–40 mM) at 1 mL/min. Injection volume, 25 μL. Electrochemical suppression of conductivity performed by applying a current of 100 mA

In the use of weak exchange columns, it should be also considered that the ionization of the stationary phase sites, and therefore the separation of the biogenic amines, are influenced not only by the pH of the mobile phase (see above), but also by the pH of the sample extraction solution. Moreover, the pH of the extraction solution also affects [19, 27, 28] the recoveries of these analytes from complex food matrices. Biogenic amines are typically extracted with aqueous acid solutions (e.g., HCl, $HClO_4$, and MSA); therefore, we have evaluated the influence of the MSA concentration of the extraction solution on the separation efficiency and recovery. Standard mixtures of biogenic amines solubilized in MSA at various concentrations in the range 2–100 mM were injected and separated by the optimized multilinear gradient method, and relevant chromatographic parameters, sensitivity values, and recoveries are summarized in Table 2.

The comparison of data with those of Table 1, obtained by injections of a standard solution in water, shows that the retention times are not affected (intra-day retention time repeatability ≤1.03 %) by the concentration of MSA in the amine standard solution, whereas area, peak heights, and resolution decrease with increasing pH of the extraction solution. The use of 2 mM MSA provides the best results in terms of chromatographic parameters, but recoveries resulted much low, while with 20 mM MSA, recoveries greater than 80 % were obtained without affecting chromatographic parameters significantly. These extraction conditions were then used in the preparation of real samples.

Finally, the effect of the column temperature on the retention times, peak efficiencies, peak symmetries, and selectivity was tested by the analyses of standard mixtures

Table 1 Chromatographic parameters for the determination of biogenic amines by multilinear gradient ion chromatography and conductivity detection

Biogenic amine[a]	t_R (min)	Peak width (min)	Resolution	Asymmetry	Number of plates	Sensitivity (μS·min kg/mg)	*k*
TMA	6.0	0.42	5.49	1.18	3,241	0.07	2.45
TEA	8.0	0.61	11.14	2.25	2,875	0.08	4.18
PUT	16.4	0.65	1.49	1.04	10,185	0.11	8.40
CAD	17.4	0.72	3.23	1.10	9,362	0.11	8.99
HIS	19.8	0.80	3.74	1.16	9,758	0.04	10.40
AGM	23.0	0.81	8.76	1.05	10,714	0.06	12.22
SPMD	28.0	0.24	5.24	1.15	210,645	0.07	15.07
SPM	29.5	0.33	2.88	1.21	124,826	0.06	15.94

[a] Mixed standard solutions at a concentration of 10 mg/L each in water

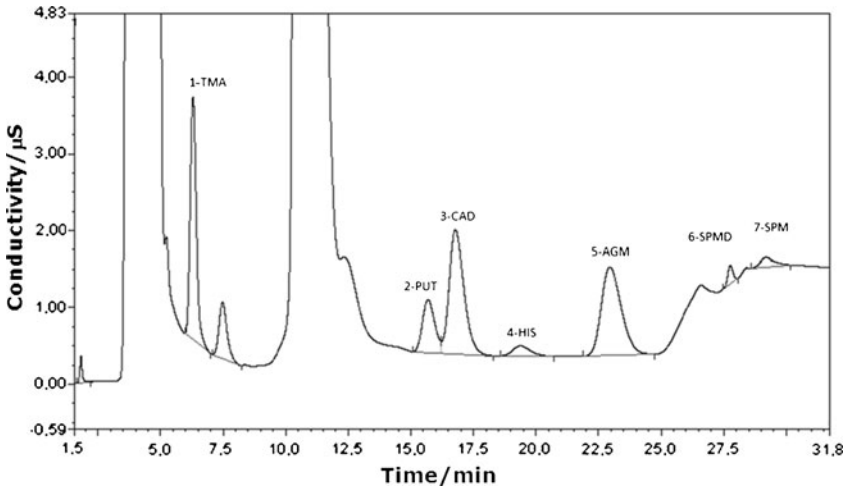

Fig. 2 Chromatogram of an anchovy sample. (1) trimethylamine (*TMA*), (2) putrescine (*PUT*), (3) cadaverine (*CAD*), (4) histamine (*HIS*), (5) agmatine (*AGM*), (6) spermidine (*SPMD*), and (7) spermine (*SPM*). Experimental condition as in Fig. 2

of biogenic amines, thermostating the column at temperatures in the range 25–40 °C. Chromatographic parameters were not significantly different in the range of temperature investigated, and then the room temperature was selected for the method application.

Method validation

The method was submitted to an in-house validation procedure, to assess precision, recovery, selectivity, sensitivity, and limits of detection and quantification, according to the recent European legislations (Decision 657/2002/EC and Regulation 882/2004/EC) [29, 30], which describe the analytical parameters to be tested in order to assure the method reliability.

For the assessment of the method selectivity, 20 independent blank samples (tinned tuna fish, anchovies, and cheeses) were processed, confirming that the proposed method is able to distinguish the analytes from other matrix components, since in the retention time window of interest (±2.5 % of the retention time of each biogenic amine), no interfering peaks were observed.

The comparison between blank and spiked samples confirmed the method selectivity against matrix components in a wide range of real samples. Besides, the method selectivity was also tested by the analysis of standard solutions of monovalent and divalent inorganic cations at concentration values typically present in food and beverages. These substances showed retention times significantly different from those of the biogenic amines investigated.

The linearity test was performed by three series of analyses in three different days by injecting standard solutions of biogenic amines in the range 0.5–200 mg/L. A good linearity was found, with correlation coefficients higher than

Table 2 Effect of the MSA concentration of the extraction solution on the chromatographic parameters

Biogenic amine[a]	MSA, 2 mM			MSA, 20 mM			MSA, 100 mM		
	Peak width (min)	Height (µS)	Resolution	Peak width (min)	Height (µS)	Resolution	Peak width (min)	Height (µS)	Resolution
TMA	0.52	3.36	1.83	0.55	2.54	1.60	0.63	2.16	1.02
TEA	0.65	2.03	1.46	0.67	1.63	1.13	0.71	1.31	0.97
PUT	0.82	3.22	1.54	0.82	2.55	1.52	1.04	1.91	1.21
CAD	0.90	2.93	3.17	0.81	2.26	3.23	1.12	1.79	2.74
HIS	1.03	1.01	8.71	1.07	0.80	8.70	1.21	0.68	7.82
AGM	0.86	1.06	8.71	0.88	0.98	8.70	0.93	0.69	7.82
SPMD	0.34	7.58	3.29	0.34	5.87	3.32	0.34	5.65	3.26
SPM	0.54	1.99	3.29	0.53	1.26	3.32	0.54	1.26	3.26

[a] Mixed standard solutions at a concentration of 10 mg/L each

Table 3 Calibration parameters for the determination of biogenic amines by multilinear gradient ion chromatography and conductivity detection

Biogenic amine	LOD[a]	LOQ[b]	R[c]	Intra-day precision (RSD%)[d]		
	μg/kg			0.5 mg/kg	5 mg/kg	10 mg/kg
TRI	36	198	0.9998	0.26	0.10	0.09
TEA	68	227	0.9995	0.32	0.26	0.24
PUT	25	130	0.9997	0.22	0.12	0.06
CAD	26	120	0.9996	0.25	0.08	0.19
HIS	55	108	0.9999	0.27	0.07	0.07
AGM	48	105	0.9997	0.23	0.25	0.25
SPMD	23	65	0.9996	0.23	0.10	0.03
SPM	65	110	0.9999	0.21	0.18	0.05

[a] Detection limit determined by standard injections at a signal-to-noise ratio of 3

[b] Quantification limit estimated from the chromatograms of anchovies at a signal-to-noise ratio of 10

[c] Correlation coefficient. Linear range from LOQ to 200 mg/kg

[d] Within-laboratory relative standard deviation under repeatability conditions

0.9995. The goodness of fit of the data to the calibration curve is obtained in terms of response factor distribution (signal-to-concentration ratio, y_i/x_i) whose reference range is (y/x)mean±10 %. Furthermore, any systematic instrumental bias can be ruled out since the confidence interval of intercept includes the zero value, at 95 % confidence level (ν=4). The calibration parameters evaluated for each amine are reported in Table 3.

Detection limits (LODs) were calculated by injecting standard solutions in the range 0.02–1 mg/L. LOD values (signal-to-noise ratio of 3) were in the order of micrograms per kilogram for all the biogenic amines, which resulted well below the legal limit of 100 mg/kg set for histamine in fish products [1]. Quantification limits (LOQs), calculated at a signal-to-noise ratio of 10 by the analysis of a blank not ripened caciocavallo cheese sample spiked at a fortification level of 0.5 mg/kg, were in the range 65–227 μg/kg.

The method was tested for intra-day assay within laboratory precision by performing four independent determinations of anchovy samples fortified with mixed standard solutions of biogenic amines at 0.5, 5, and 10 mg/kg. The injection-to-injection repeatability data (Table 3), calculated as standard deviation, were lower than 0.32 % for all the analytes.

Recoveries were determined by analyses of sets of blank samples (anchovies, salamini, tuna fish, olives, red wine, and caciocavallo cheese) fortified with each biogenic amine at concentrations of 50 and 100 mg/kg in matrix, corresponding to 5 and 10 mg/kg in the final extract. Four replicates were performed at each fortification level. Recovery percentages were evaluated by comparing the concentration of spiked samples, determined by the calibration regression line, with the nominal fortification level. The results of recovery experiments are summarized in Table 4. Recoveries and percent RSD

Table 4 Recoveries of biogenic amines in spiked real samples

Biogenic amine	Fortification level (mg/kg)	Anchovies	Salami	Tinned tuna fish	Olives	Red wine	Caciocavallo cheese
		Recovery (%)±SD (n=4)					
TRI	50	98.1±1.4	84.4±0.4	95.5±0.1	93.2±0.3	99.2±1.5	97±1
	100	95.6±0.4	86.6±0.8	96.7±0.1	93.4±0.1	97±2	94±1
TEA	50	96.5±0.7	86±1	97.6±0.5	87.8±0.9	98.5±0.6	93.6±0.5
	100	97.5±0.9	87±1	97.5±0.8	88±1	98.8±0.8	95.4±0.8
PUT	50	97.3±1.5	87.0±0.8	95.4±0.14	94.7±0.2	92±1	92±2
	100	95.4±0.7	88.6±0.8	98.4±0.1	96.8±0.1	90.2±0.7	95.2±0.6
CAD	50	93±2	88±1	91.4±0.2	93.2±0.1	96±3	103±1
	100	93±1	86±2	96.8±0.2	95.8±0.1	92.8±1.4	92±2
HIS	50	97.3±1.5	84±2	93.6±0.2	93.8±0.3	96.4±1.5	99.8±0.3
	100	97.1±0.8	87±2	94.6±0.1	98.5±0.1	93±2	98±1
AGM	50	94±1	86±2	96.2±0.6	96±2	90.8±0.1	85.6±0.1
	100	95±1	84±2	97.0±0.7	97±1	91.5±0.1	86.1±0.2
SPMD	50	97±2	87±2	91.1±0.2	90.8±0.1	95±2	99.5±0.5
	100	98±1	87±2	94.7±0.1	91.5±0.1	94±2	95±1
SPM	50	92.1±1.4	82±2	93.6±0.2	85.6±0.1	90.0±0.8	96.8±0.7
	100	97±1	87±2	93.0±0.2	86.1±0.2	91.1±0.8	94±2

ranging from 82±2 to 103±1 and 0.1–3 %, respectively, were obtained, demonstrating the method reliability in the analysis of complex real samples.

The proposed method can be considered as a useful tool for the quantification of histamine and the other biogenic amines in food samples, as suggested by Decision 657/2002/EC [29], which establishes that alternative methods with respect to the official ones can be employed usefully in the confirmation analysis, if the analytical requirements of precision, recovery, specificity, and linearity are fulfilled. The method performance parameters, such as linear range, detection and quantitation limits, recovery, and precision, were comparable or even better than those obtained by the most recent [31, 32] mass spectrometry-based methods, which require additional time-consuming sample preparation steps and expensive instrumentations.

Analyses of real samples

In order to assess the feasibility and the potential of the proposed method for routine analyses, several fresh or processed foods (tinned tuna fish, anchovies, salami, and fresh and matured cheeses) and fermented beverages (red and white wines) were monitored. Ten samples were collected and analyzed for each food matrix or wine, and each of them was injected in triplicate. Quantification of BAs was carried out by interpolation on the calibration curve, and the correspondent results, according to the recovery value, are summarized in Table 5. A typical chromatogram of an anchovy sample, which shows the natural presence of the biogenic amines caused by the spoilage process, is displayed in Fig. 2. Apart from storage conditions, the contamination by BAs also depends on both the protein/amino acid content and the fermentation process or production technology used. Green and black olives produced by different manufacturing processes showed no presence of BAs, apart from a sample (produced by the Sivigliano method) that was contaminated by putrescine at a level of 14.63±0.02 mg/kg, which probably originated from an abnormal fermentation caused by microorganisms with high concentrations of arginine decarboxylase [11]. Samples of white wine showed a minimal contamination by putrescine (2.38±0.07 mg/kg), whereas in the red wine samples, in addition to higher contents of putrescine (8.66±0.03), low amounts of histamine (1.48±0.09) were found. These BAs are the most present in wine, and their concentration, which are low after the alcoholic fermentation, increases during the malolactic fermentation [33]. The investigations carried out on fresh and matured cheeses (mozzarella, canestrato, and caciocavallo cheeses) evidenced the absence of biogenic amines in fresh products and an increase of putrescine, cadaverine, and histamine levels in matured canestrato and caciocavallo cheeses. In particular, canestrato cheese with the surface treated by pimaricin (an antifungal agent) showed an insignificant content of BAs with respect to the untreated one (data not shown). Tinned tuna fish samples presented low concentrations of these contaminants, probably because of the rapid processing of the raw material, as confirmed by the presence of low levels of trimethylamine, spermidine, and spermine. On the contrary, in the samples of fresh anchovies were found very high values of trimethylamine that can be ascribed to inappropriate storage conditions. Finally, it should be pointed out that the problem of histamine cannot be limited to seafood chain products, since levels three times higher (300 mg/kg) than legal limit for fish (100 mg/kg) were also found in caciocavallo cheese.

Conclusions

The optimized extraction step of the biogenic amines from real samples, the separation conditions by using a proper MSA multilinear gradient, and a weak ionic

Table 5 Content of biogenic amines in real samples

Biogenic amine	Tinned tuna fish	Anchovies	Salami	Olives	Red wine	White wine	Caciocavallo cheese	Canestrato cheese
	Concentration (mg/kg)±SD (n=30)							
TMA	3.2±1.3	120±1	1.7±0.2					
PUT		20±2	9±1	14.63±0.02	80.66±0.03	20.38±0.07	20.0±0.4	10.7±0.1
CAD		71±6	10±1				100.5±1.4	10.00±0.04
HIS		20.7±0.2			10.48±0.09		340±4	20.10±0.08
AGM	10.7±0.9	16.1±0.9	8.3±0.8					
SPMD	0.4±0.1	6.3±0.2	3.4±1.3					
SPM	2.2±0.6	10.1±0.4	4.6±0.7					

exchange column have provided an excellent resolution and separation, with high recovery values of the analytes in fresh and processed food samples. This method resulted to be simple, sensitive, selective, reproducible, and fast with respect to HPLC methods that require derivatization reactions for the determination of biogenic amines. The results of the validation procedure have demonstrated that the proposed method is very useful for confirmation analyses of biogenic amines. Moreover, the simultaneous quantification of TMA and SPM can allow the evaluation of freshness indicators [34, 35] that are very important for food quality control.

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