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Simultaneous Determination of Seven Biogenic Amines in Foodstuff Samples Using One-Step Fluorescence Labeling and Dispersive Liquid–Liquid Microextraction Followed by HPLC-FLD and Method Optimization Using Response Surface Methodology

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Abstract A simple, sensitive and selective method based on one-step fluorescence labeling and ultrasound-assisted dispersive liquid–liquid microextraction (UA-DLLME) was developed for the determination of biogenic amines (BAs) in foodstuff samples by high-performance liquid chromatography (HPLC) with fluorescence detection (FLD). In this work, fluorescence probe 2-(11H-benzo[a]carbazol-11-yl) ethyl carbonochloridate (BCEC-Cl) was applied to label BAs. What followed was the UA-DLLME procedure that was carried out using chloroform and acetone as extraction and disperser solvents, respectively. A response surface methodology (RSM) based on a Box–Behnken design (BBD) was employed to optimize the main parameters affecting the fluorescence labeling and DLLME efficiency. Under the optimal conditions, this method offered low limits of detection (LODs) of 1.1–7.8 ng/mL and limits of quantification (LOQs) of 3.5–26.1 ng/mL. Finally, the method was successfully used for the determination of trace BAs in real samples and exhibited powerful potential in the high-throughput sample screening.

Keywords Biogenic amines · Fluorescence labeling · Ultrasound-assisted dispersive liquid–liquid microextraction · Foodstuff samples · Box–Behnken design

Hongliang Wu and Guoliang Li contributed equally to this work.

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Introduction

Biogenic amines (BAs) are low-molecular-weight organic alkalis with aliphatic, aromatic or heterocyclic structures, formed mainly by decarboxylation of amino acids or by amination and transamination of aldehydes and ketone (Linares et al. 2011). BAs can be found in a wide range of foods and fermented beverages, including cheese, sausage, yogurt, milk, wine, beer, fish, soy sauces, aged meat, etc. (Aflaki et al. 2013; Önal 2007). Recent studies indicate BAs are hazardous and toxic microcomponents (Latorre-Moratalla et al. 2007) and can cause undesirable health problems, such as difficulty in breathing, itching, rash, vomiting, fever, hypertension headache, heart palpitation, hypertension, nausea, emesis, and death in very severe cases (Li et al. 2014; Zotou and Notou 2012). In addition, the amounts of BAs usually

increase in the course of food spoilage or spontaneous microbial fermentation in nonstandard food storage conditions or inadequate fermentation treatments (Jastrzębska et al. 2011). Thus, BAs have been normally served as one potentially index of freshness or spoilage of food (Koral et al. 2013; Linares et al. 2011; Martuscelli et al. 2013). So it is vital to develop a sensitive, reliable and routine method for BA determination ensuring food safety.

For BA analysis, there are lots of methods that have been developed, such as gas chromatography (Ali Awan et al. 2008; Hwang et al. 2003), thin-layer chromatography (Lapa-Guimarães and Pickova 2004; Latorre-Moratalla et al. 2009), capillary electrophoretic method (Bricio et al. 2004; Sun et al. 2003) and high-performance liquid chromatography (HPLC) (Lázaro et al. 2013; Wang et al. 2014; Kabashima et al. 2008). Among the cited techniques, HPLC is considered the most suitable and frequently used for the separation and quantification of biogenic amines (Innocente et al. 2007; Lázaro and Conte-Junior 2013). Moreover, because of the lack of suitable chromophoric or fluoropheric moieties in BA molecules, chemical pre- or postcolumn derivatization strategy has been widely accepted to enhance the selectivity and sensitivity.2-(11H-benzo[a]carbazol-11-yl) ethyl carbonochloridate (BCEC-Cl) was synthesized according to our reported study (You et al 2007a) and its application in aliphatic amines (You et al. 2007b) and amino acids (You et al. 2007a; Zhao et al. 2008) . The labeling reagent possesses strong photoluminescence property and can rapidly react with primary and secondary amino compounds with good selectivity and generate fewer by-products (Zhao et al. 2007).

In addition, real samples usually need to be purified due to the complexity sample matrix and the low concentration of BAs (Ahmar et al. 2013). For the analysis of BAs in various food matrices like milk, cheese, sausage and yogurt, the traditional liquid–liquid extraction (LLE) is the most commonly used technique as pretreatment procedure. However, LLE has many drawbacks, such as being time-consuming, requiring large amounts of toxic organic solvents and suffering from loss of target analytes (Moradi et al. 2013). To overcome these drawbacks, alternative pretreatment procedures have been developed, such as solid-phase extraction (SPE) (Ali Awan et al. 2008; De Mey et al. 2012), cloud point extraction (CPE) (Paleologos et al. 2003), solid-phase microextraction (SPME) (Saaid et al. 2009a) and dispersive liquid–liquid microextraction (DLLME) (Almeida et al. 2012). Among these methods, DLLME was an emerging, simple and widely used technology in analytical fields (Jia et al. 2013). Contrary to other pretreatment procedures, DLLME presents many advantages such as high enrichment ability, simple operation, low organic solvent consumption, time-saving, low cost and high recovery (Donthuan et al. 2014).

The purposes of this study were to set up a novel analytical method for the simultaneous determination of seven BAs followed by HPLC-fluorescence detection (FLD) using one-step fluorescence labeling and dispersive liquid–liquid microextraction and optimize the main parameters affecting the efficiency of fluorescence labeling and DLLME by response surface methodology (RSM).

Materials and Methods

Reagents

2-(11H-benzo[a]carbazol-11-yl) Ethyl carbonochloridate (BCEC-Cl) was synthesized in our laboratory (You et al. 2007a). Tryptamine (Try), histamine (His), cadaverine (Cad), 2-phenylethylamine (Phe), 1,6-hexamethylenediamine (Hex), tyramine (Try) and spermine (Spe) were purchased from Sigma (St. Louis, MO, USA). Water was purified on a Milli-Q system (Millipore, Bedford, MA, USA). HPLC-grade acetonitrile was purchased from Yucheng Chemical Reagent Co. (Yucheng, Shandong Province, China). Other reagents were of analytical grade from Jining Chemical Reagent (Jining, Shandong Province, China).

Apparatus

Chromatographic separations were performed with an Agilent 1100 Series HPLC (Agilent Technologies, Palo Alto, CA, USA). The mass spectrometer (MSD Trap SL, model G2445D) from Bruker Daltonik (Bremen, Germany) was equipped with an atmospheric pressure chemical ionization (APCI) source (model G1947A). Ion source conditions were as follows: APCI in positive ion detection mode, nebulizer pressure 60 psi, dry gas temperature 350 °C, dry gas flow 5 L/min, APCI Vap temperature 350 °C, corona current 4,000 nA, and capillary voltage 3,500 V. An ultrasonic cleaner (KQ3200E, Kunshan Ultrasonic Instrument, Jiangsu, China) set at 40 kHz (equivalent to the wavelength of 37.5 mm) was used to emulsify the solutions.

Preparation of Standard Solutions

Individual stock solutions (1×10^{-2} mol/L) of BAs were prepared in acetonitrile/water mixed solution (*v*/*v*, 1:1). The mixed standard solutions (2×10^{-5} mol/L) for HPLC analysis were prepared by diluting the stock solutions with acetonitrile/water. The labeling reagent solution (5×10^{-3} mol/L) was prepared by dissolving 16.15 mg of BCEC-Cl in 10 mL acetonitrile. Then, all standard solutions were stored at 4 °C until use.

Sample Preparation

All foodstuff samples (beer, cheese, yogurt, ham sausage and rice wine) were purchased from a supermarket (Qufu City,

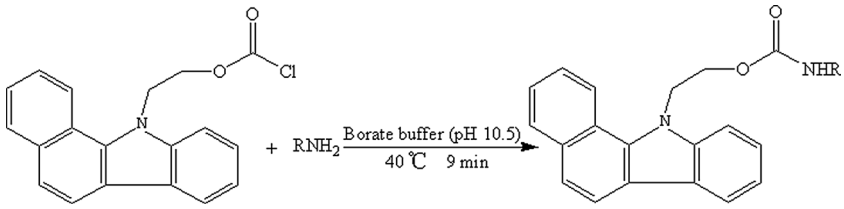

Fig. 1 The derivatization scheme for BCEC-Cl with biogenic amines

Shandong Province, China) east of Qufu Normal University. According to several reported studies, the extractions of BAs were performed with minor revision (Saaid et al. 2009b). All solid samples were treated as follows: 5 g of sample was extracted twice with 10 mL 5 % trifluoroacetic acid (TCA) in an ultrasonic cleaner (10 min each extraction) and then centrifuged for 5 min at 5,000 rpm. Collecting the supernatants, the extracts were defatted twice with 20 mL *n*-hexane and then filtered through 0.45-μm nylon filters (Automatic Science, Instrument Co., Ltd., Tianjin, China). For beer, 6 mL beer was centrifuged for 5 min at 5,000 rpm and filtered through 0.45-μm nylon filters. Moreover, all extracts need to be adjusted to pH 10.5 with NaOH solution before utilized. They were then sealed and stored at 4 °C until analysis.

Fluorescence Labeling and Ultrasound-Assisted Dispersive Liquid–Liquid Microextraction Procedure

In a conical test tube, 50 μL of fluorescence reagent was added to 6 mL sodium borate buffer (pH 10.5) involving 30 μL mixed standard solution (or 6 mL real sample extract solvent). The solution was shaken for 10 s, sealed and then allowed to stand for 9 min at 40 °C. When fluorescence labeling was accomplished, 30 % acetic acid (*v*/*v*) was added into the solution until neutral. Afterwards, DLLME process was immediately performed; 105 μL chloroform (as extraction solvent) and 1,030 μL acetone (as disperser solvent) were well mixed. The mixture solvent was rapidly injected into the sample solution by using the 1mL glass syringe, and then the tube was immersed in an ultrasonic water bath for 3.5 min. After centrifugation at 5,000 rpm for 5 min (800B centrifuge, Shanghai Anting Instruments Inc., China), the organic phase containing the targeted analytes was deposited at the bottom of the vial and withdrawn with a 100 μL glass syringe, and then the organic phase was injected into the HPLC system for direct analysis.

HPLC Separation

Chromatographic separation was achieved on a Hypersil C18 (4.6 mm×200 mm, 5 μm) column with a gradient

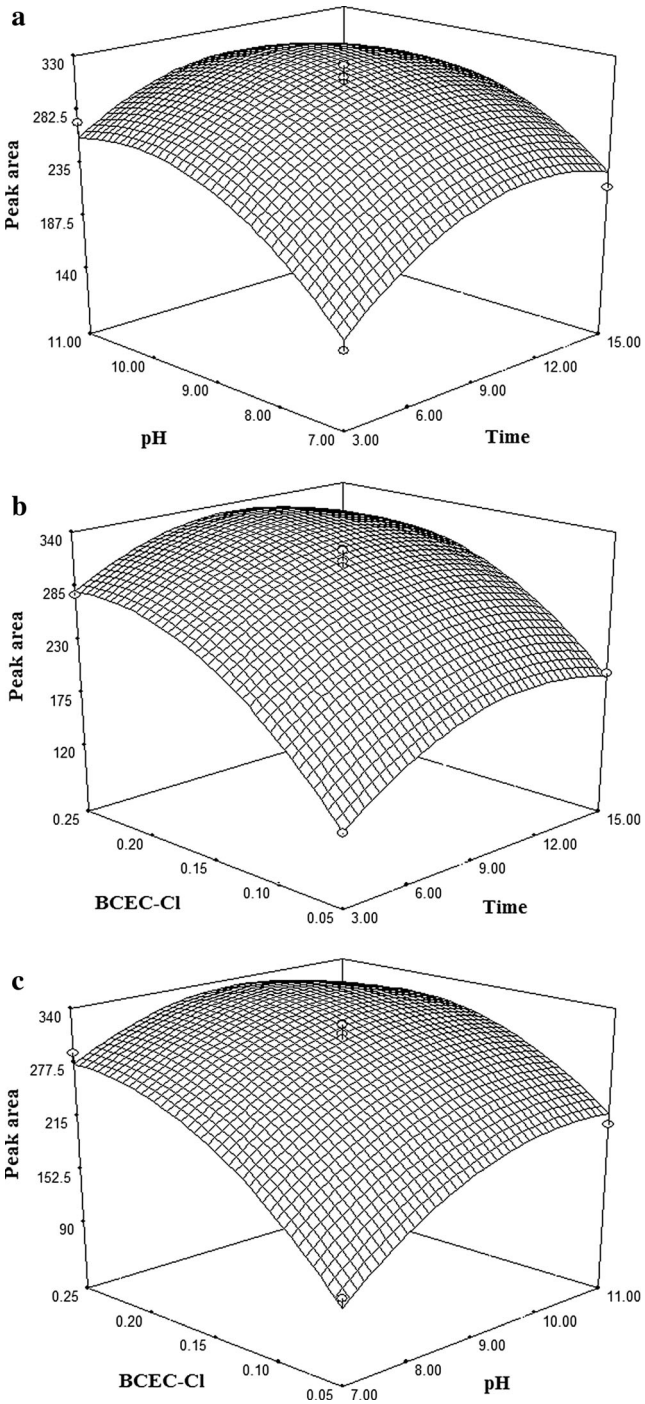

Fig. 2 Response using the Box–Behnken design obtained by plotting **a** reaction time versus pH; **b** concentration of BCEC-Cl versus reaction time; and **c** pH versus concentration of BCEC-Cl

elution. Mobile phase A was 5 % acetonitrile and B was 100 % acetonitrile. The gradient conditions were selected at 65–70 % (B) from 0 to 10 min, 70–78 % (B) from 10 to 16 min, 78–80 % (B) from 16 to 20 min, 80–100 % (B) from 20 to 25 min and keeping constant until 32 min. Then, the column was equilibrated with the initial mobile phase for 10 min before injecting the next sample. The flow rate was maintained at 1 mL/min and the column temperature was set to 30 °C. The injection volume was 10 μL for each analysis. The fluorescence detector was set with the excitation wavelength (Ex) of 279 nm and the emission wavelength (Em) of 380 nm (You et al. 2007a).

Results and Discussion

Optimization of Fluorescence Labeling Conditions

BCEC-Cl has the same acyl chloride reaction with BAs as benzoyl chloride does (Paleologos et al. 2003). Compared with the traditional reagents, it has larger conjugated substructure which contributes to improve the fluorescence sensitivity. The molecular structure of BCEC-Cl and the derivatization scheme with BAs are shown in Fig. 1. Before RSM optimization, the effect of reaction temperature was tested from 30 to 60 °C. It can be found that the peak area reached the maximum value, when the reaction temperature was 40 °C. Therefore,

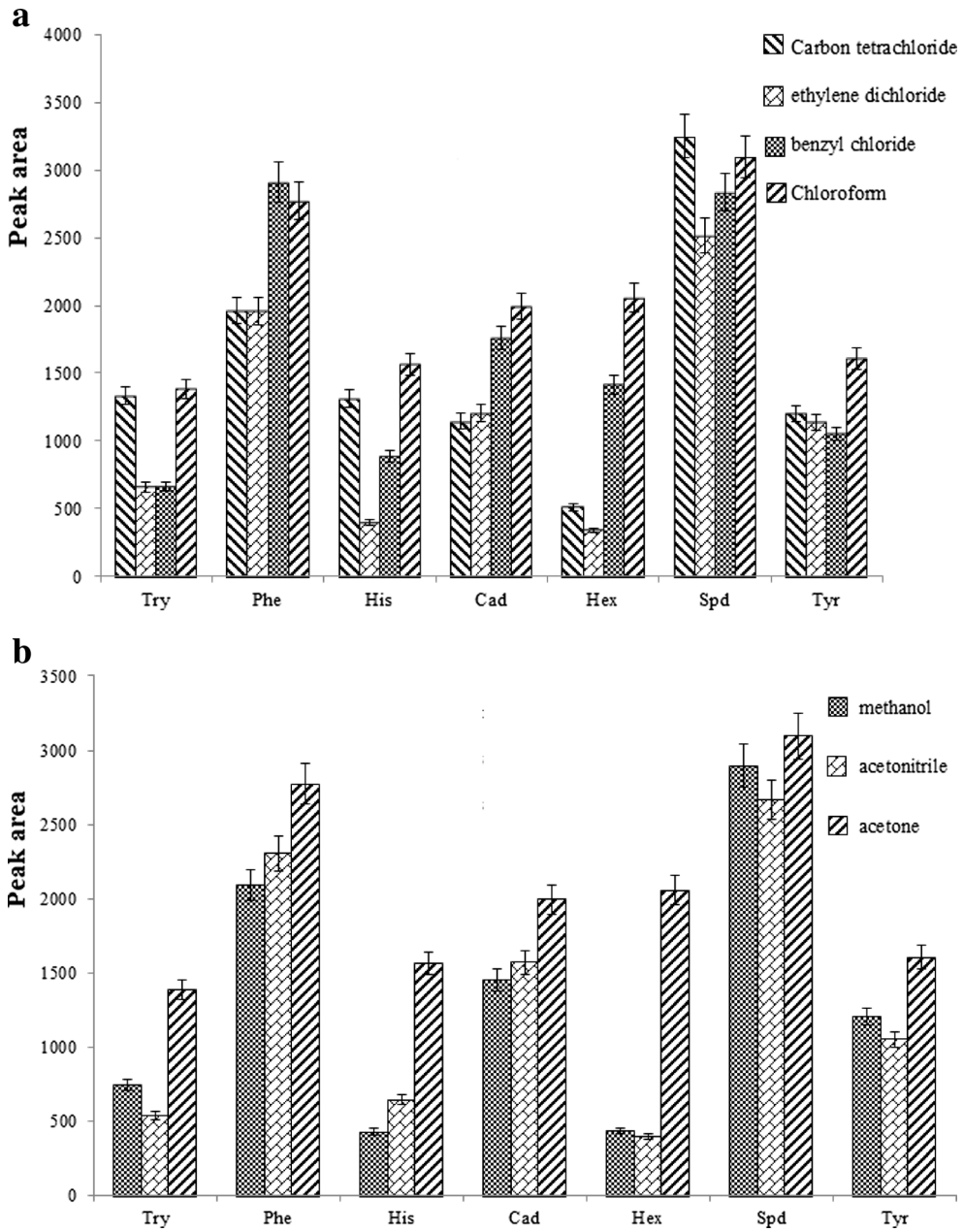

Fig. 3 Effect of different extraction solvents on the extraction efficiency (**a**); effect of different disperser solvents on the extraction efficiency (**b**). *Try* tryptamine, *His* histamine, *Cad* cadaverine, *Phe* 2-phenylethylamine, *Hex* 1,6-hexamethylenediamine, *Tyr* tyramine, *Spe* spermine

this temperature was used in this work. Then, the labeling time, reaction buffer pH and concentration of BCEC-Cl were chosen as the main variables and further optimized by RSM. The experimental results are listed in Table S1. A regression equation that could predict the optimum point within the given range was obtained by applying multiple regression analysis on the experimental data. The second-order polynomial model was the following:

$$Y=311+22.61X_1+37.70X_2+66.41X_3-14.23X_1X_2-13.00X_1X_3-25.23X_2X_3-40.53X_1^2-41.35X_2^2-46.38X_3^2$$

Where, Y is the predicted average peak areas; X_1, X_2 and X_3 are the coded values of labeling time, pH of buffer and concentration of BCEC-Cl, respectively.

The surface response plot was seen as a visual process of the predicted model equation to study the effects of parameters and their interactions on fluorescence labeling reaction. Response surface plots are shown in Fig. 2. Figure 2a depicts the combined effect of labeling time and pH of buffer on the response. The response initially increases when there is an increase in labeling time and pH of buffer. With further increase in labeling time and pH of buffer, a slightly declined on the response was observed. Figure 2b describes the combined effect of labeling time and concentration of BCEC-Cl at the fixed value of pH. Figure 3c depicts the combined effect of buffer pH and BCEC-Cl concentration on the response value at the fixed value of labeling time.

The results of the analysis of variance (ANOVA) indicated all the linear parameters and quadratic parameters of the quadratic equation were significant at the level of $p<0.01$. The value of R^2 was 0.98, meaning that there was a satisfactory agreement between experimental and predicted values. The optimum conditions given by the model were as follows: 9 min labeling time, pH 10.5 borate buffers and 0.18 mmol/L BCEC-Cl. Under the best conditions, the maximum predicted value of the peak area was 326. And the actual peak area under the proposed conditions was 341 ($n=6$), very close to the predicted value. The excellent correlation between predicted and measured values verified the response model was adequate to reflect the expected optimization.

Types of Extraction Solvent and Disperser Solvent

In DLLME, extraction and disperser solvents have a significantly influence on extraction efficiency. According to our preexperiment, chloroform, ethylene dichloride, benzyl chloride and carbon tetrachloride were selected as extraction solvents, and their extraction efficiencies were evaluated in presented study (Fig.3a). As can be seen from Fig.3a, chloroform has the highest extraction efficiency than the other tested solvents. For the disperser solvent, the main criterion was that it ought to be miscible with the aqueous phase as well as with the extraction solvent. To seek a more acceptable disperser solvent, methanol, acetone and acetonitrile were investigated as disperser solvents. Figure 3b shows the best extraction efficiency was found when acetone was selected as disperser solvent. Therefore, chloroform and acetone were selected as optimal extraction and disperser solvents in the following work, respectively.

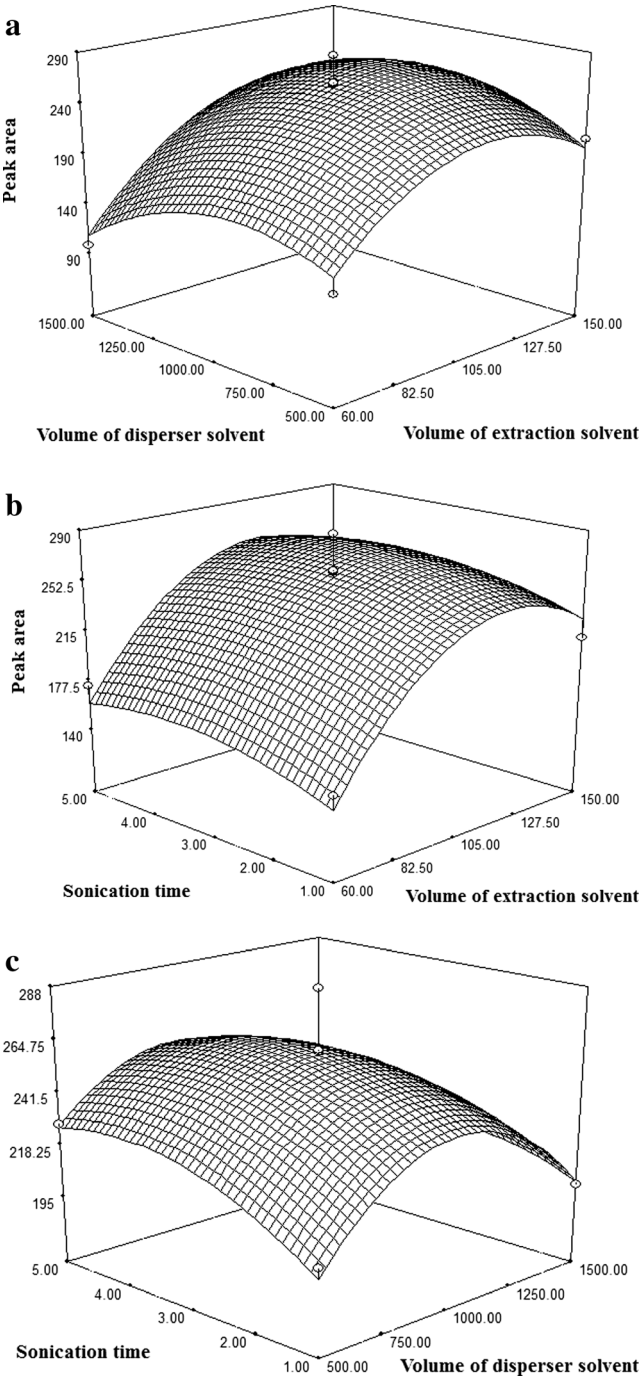

Fig. 4 Three-dimensional surface plot showing the significant interaction effects of the DLLME parameters: **a** volume of the extraction solvent versus volume of disperser solvent; **b** volume of the extraction solvent versus sonication time; and **c** volume of disperser solvent versus sonication time

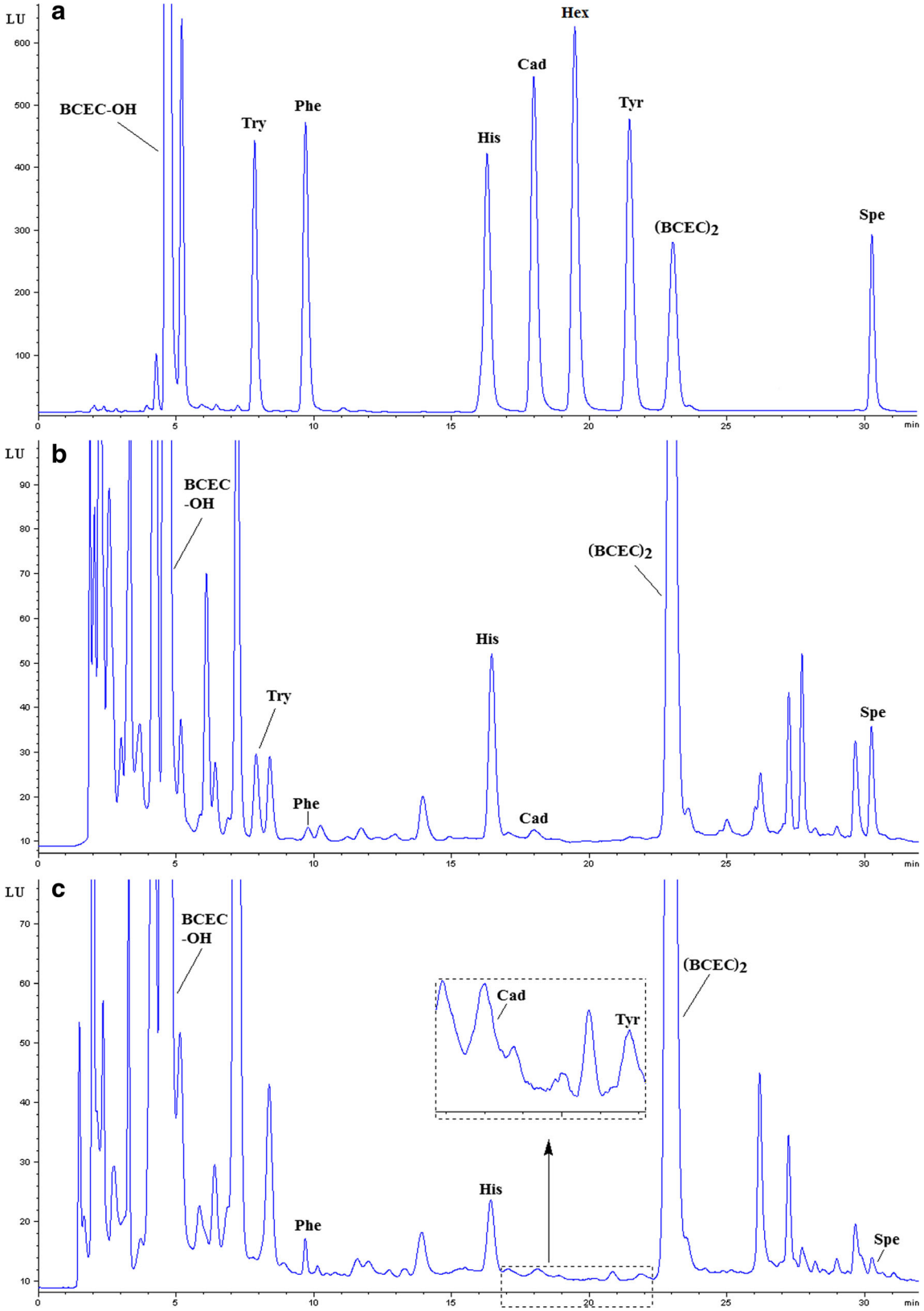

Fig. 5 The representative chromatograms for seven biogenic amine standards (**a**), ham sausage sample (**b**) and rice wine (**c**). Chromatographic conditions: column temperature at 30 °C; excitation wavelength λ_{ex} 279 nm, emission wavelength λ_{em} 380 nm; Hypersil C18 (4.6 mm× 200 mm, 5 μm) column; flow rate=1 mL/min; peak labels: *Try* tryptamine, *His* histamine, *Cad* cadaverine, *Phe* 2-phenylethylamine, *Hex* 1,6-hexamethylenediamine, *Tyr* tyramine, *Spe* spermine

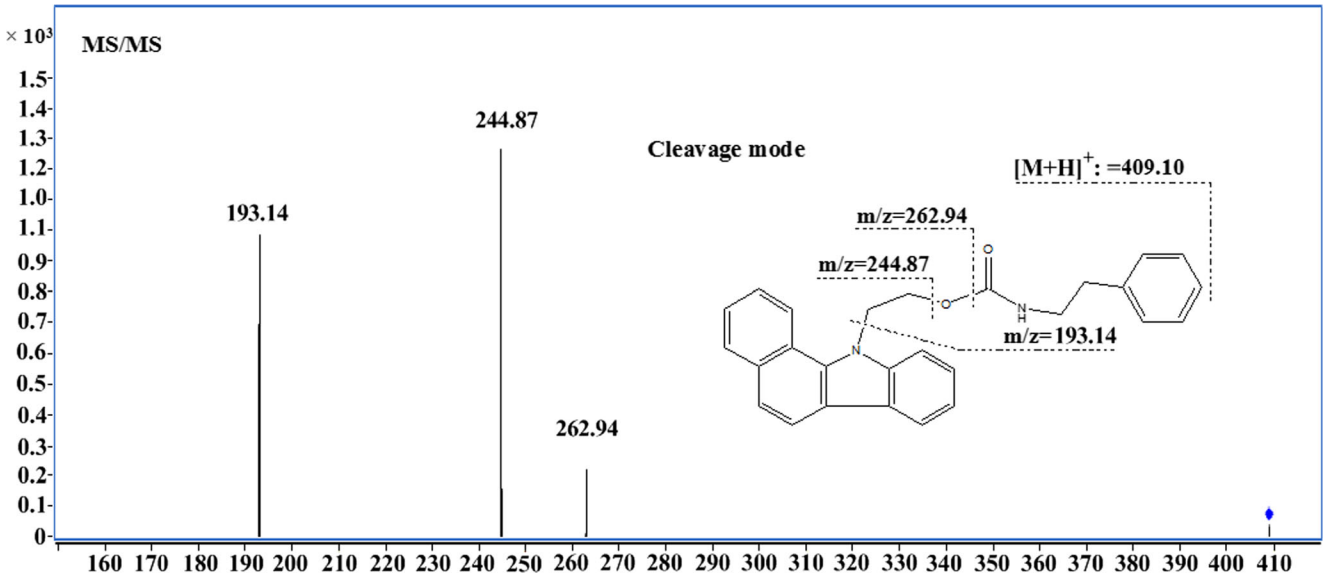

Fig. 6 The MS data and cleavage mode for labeled 2-phenylethylamine

Optimization of DLLME

In order to obtain the highest extraction efficiency, the main parameters affecting the DLLME efficiency including extraction solvent volume, disperser solvent volume and sonication time were optimized by RSM. The predicted model that reflected the empirical relationship between the response and the three mentioned variables was obtained by analyzing the experimental data (Table S2) and expressed by the following second-order polynomial equation:

$$Y=260.2+39.51X_1+5.490X_2+6.900X_3-13.80X_1X_2-2.020X_1X_3-7.880X_2X_3-55.01X_1^2-39.16X_2^2-14.64X_3^2$$

Y is the predicted response value and X_1, X_2 and X_3 are the coded values of the extraction solvent volume, disperser solvent volume and sonication time mentioned, respectively. Three-dimensional response surface curves are shown in Fig. 4. In Fig. 4a, when the extraction solvent volume increased from 60 to 150 μL, the response value rapidly increased and reached a maximum value and then slightly decreased as the further increasing extraction solvent. Similarly, Fig. 4b, c describes the interaction effect of sonication time and disperser solvent volume and extraction solvent volume on the response value, respectively.

The result of ANOVA indicated that the model was significant at the level of $p<0.01$. The coefficient of determination (R^2) was 0.93, indicating a high potential of the model to reflect the experimental data. The optimal conditions were given by RSM as follows: extraction solvent volume=105 μL; disperser solvent volume=1,030 μL and sonication time=3.5 min. Under the proposed conditions, the peak area was predicted to be 261. The suitability of the optimal derivatization conditions above was also tested by executing six experiments, and the average peak area was 274, which was very close to the predicted value. The excellent correlation between predicted

Table 1 Linear regression equation, correlation coefficients (R), LOD, LOQ, reproducibility of retention time and peak area, intra- and inter-day precision

Analytes	Linearity		LOD (μg/L)	LOQ (μg/L)	Repeatability RSD (%) (n=6)		Intra-day precision RSD (%) (n=6)	Inter-day precision RSD (%) (n=6)
	$Y=AX+B$	R			Retention time	Peak area		
Tryptamine	$Y=20.586X-17.90$	0.9997	3.2	10.7	0.09	3.1	3.7	4.7
Phenylethylamine	$Y=19.340X-4.600$	0.9994	1.2	4.1	0.06	2.2	4.1	5.7
Histamine	$Y=7.8570X-6.250$	0.9988	2.2	7.4	0.11	3.2	2.9	4.3
Cadaverine	$Y=29.667X-14.50$	0.9995	1.1	3.5	0.08	2.2	3.4	5.1
Hexamethylenediamine	$Y=33.628X-18.50$	0.9999	1.2	3.9	0.08	1.3	3.8	4.9
Tyramine	$Y=26.791X-9.000$	0.9998	1.4	4.6	0.10	1.9	4.1	5.3
Spermine	$Y=4.1817X-21.24$	0.9997	7.8	26.1	0.07	1.2	2.9	4.6

X the injected amounts (pmol), Y the peak area detected with fluorescence detector

and measured values verified that the response model was adequate to reflect the expected optimization.

HPLC Separation and Mass Spectrometry Identification

In the present study, four reversed-phase columns including Hypersil C18 (4.6 mm×200 mm, 5 μm) column, Eclipse XDB-C8 (4.6 mm×150 mm, 5 μm) column, Hypersil BDS C8 (4.6 mm×200 mm, 5 μm) column and Spherisorb C18 (4.6 mm×200 mm, 5 μm) column were tested and compared in detail. The results showed the Hypersil C18 (4.6 mm× 200 mm, 5 μm) column was considered the most suitable one. The effect of mobile-phase solvents including acetonitrile and methanol was evaluated and better results were obtained using acetonitrile. Finally, the optimum mobile phases were found to be mobile-phase A acetonitrile/H_2O (5:95; *v*/*v*) and mobile-phase B 100 % acetonitrile. The optimal flow rate and column temperature were 1 mL/min and 30 °C, respectively. The typical chromatograms of the seven standards and the real samples are shown in Fig. 5.

The characteristic chromatographic peaks were dually verified by retention time and on-line APCI/mass spectrometry (MS) in positive ion mode. The fragmentation pattern of representative Phe derivative is given in Fig. 6. Data from the MS and MS/MS spectra provided an intense quasi-molecular ion ($[M+H]^+$, *m*/*z*) at *m*/*z* 409.10 and the characteristic fragment ions at *m*/*z* 244.87, *m*/*z* 262.94 and *m*/*z* 193.14, respectively. The characteristic fragment ion at *m*/*z* 244.87 came from the cleavage of the CH_2–OCO bond, and the fragment ion at *m*/*z* 262.94 was from the cleavage of the CH_2O–CO bond. The collision-induced dissociation spectrum of molecular ions (MS, $[M+H]^+$ ion) was characteristic fragment ions and selected as the specific reaction for monitoring fluorescence labeling BAs.

Analytical Performance of the Proposed Method

Under the optimal experimental conditions, the proposed method was evaluated by linearity, limits of detection (LODs), limits of quantification (LOQs), recovery and accuracy. According to the experimental data analysis, the linear regression equations for seven BAs were established with good coefficients of >0.9988 (Table 1). The LODs (S/N=3) and the LOQs (S/N=10) ranged from 1.1 to 7.8 and 3.5 to 26.1 μg/L, respectively. The method precision was determined by intra- and inter-day, which was expressed as relative standard deviation (RSD). RSDs of the intra- and inter-day precision were less than 4.1 and 5.7, respectively. The RSD values for the retention time and peak area were lower than 0.11 and 3.2, respectively. These results indicated that this DLLME method established in this study had a good precision. The method recoveries were in the range of 91.2–108.3 % and RSD values were less than 3.7 %. These data further suggested that the proposed approach was a simple and precise procedure for the determination of trace BAs.

The comparison of the proposed method with the reported methods is summarized in Table 2. The proposed method including fluorescence labeling and DLLME can be completed in only 12.5 min, which was less time-consuming than

Table 2 The overall comparison of the new methods and reported methods

Methods	Reagents	Sample treatments	Reaction conditions	LODs	Reference
LC-LC-FLD	OPA/MCE	Online SCX precolumn	0.4 mol boric acid buffer, pH 10.5	10 ng/mL	Hyötyläinen et al. (2001)
HPLC-FLD	OPA/MCE	–	0.4 mol borate buffer, pH 10.5, 90 min	3,540–7,090 ng/mL	Pereira et al. (2008)
HPLC-UV	DNS-Cl	PVPP pretreatment	Na_2CO_3-$NaHCO_3$ buffer, pH 10, 60 min, 60 °C	90–300 ng/mL	Pineda et al. (2012)
HPLC-UV	DNS-Cl	LPME	$NaHCO_3$ buffer, pH 9.5, 30 min	10–30 ng/mL	Saaid et al. (2009a)
UPLC-MS	DNS-Cl	SPE	Na_2CO_3 buffer, 45 min, 40 °C	3–15 ng/mL	Jia et al. (2012)
HPLC-UV	DBS-Cl	SPE	0.95 mol $NaHCO_3$, 20 min, 40 °C	100–940 ng/g	De Mey et al. (2012)
HPLC-UV	CNBF	–	$Na_2B_4O_7$ buffer, pH 9.5, 30 min, 60 °C	10.2–404.7 ng/mL	Kim et al. (2011)
HPLC-UV	BZO-Cl	DLLME-SFO	Borate buffer, pH 10, 30 min, 30 °C	5–10 ng/mL	Jia et al. (2013)
CE-UV	BZO-Cl	LLE	2.0 mol NaOH solution, 15 min, 25 °C	200–2,500 ng/mL	Ozdestan and Uren (2009)
HPLC-UV	BZO-Cl	CPE	2.0 mol NaOH solution, 15 min, 30 °C	15–100 ng/mL	Paleologos et al. (2003)
HPLC-FLD	BCEC-Cl	UA-DLLME	Borate buffer, pH 10.5, 9 min, 40 °C	1.1–7.8 ng/mL	This work

Reagents: *OPA/MCE* *o*-phthalaldehyde/2-mercaptoethanol, *DNS-Cl* dansyl chloride, *DBS-Cl* dabsyl chloride, *CNBF* 4-chloro-3,5-dinitrobenzotrifluoride, *BZO-Cl* benzoyl chloride, *BCEC-Cl* 2-(11H-benzo[a]carbazol-11-yl) ethyl carbonochloridate (BCEC-Cl); sample treatments: *SCX* strong cation-exchange material, *PVPP* polyvinylpolypyrrolidone cross-linked, *LPME* liquid-phase microextraction, *SPE* solid-phase extraction, *DLLME-SFO* dispersive liquid–liquid microextraction-solidification of floating organic droplets, *LLE* liquid–liquid extraction, *CPE* cloud point extraction, *UA-DLLME* ultrasound-assisted dispersive liquid–liquid microextraction (UA-DLLME)

other reported methods, such as dabsyl chloride (DBS-Cl)-SPE (De Mey et al. 2012), dansyl chloride liquid-phase microextraction (DNS-Cl-LPME) (Saaid et al. 2009a), benzoyl chloride (BZO-Cl)-LLE (Ozdestan and Uren 2009) and BZO-Cl-CPE (Paleologos et al. 2003). Furthermore, the DLLME procedure diminished the usage for organic solvent only in microliter level and simultaneously reduced the cost of BA analysis. In addition, it can be found that the developed method provided relatively lower LOD (Table 2). Thus, the proposed method was proved to be simple, rapid, reliable and accurate method for BA analysis.

Application to Real Sample Analysis

Under the optimized experimental conditions, the established method was successfully applied for the analysis of seven BAs in different real samples such as beer, rice wine, cheese, yogurt and ham sausage. The analytical results, along with the

Table 3 Analytical results for seven biogenic amines in food samples (n=6)

Samples	Analytes	Original (μg/g or μg/mL)	Added (μg/g or μg/mL)	Found (μg/g or μg/mL)	Recovery (%)	RSD (%) n=6
Cheese	Tryptamine	13.18	20	32.98	99.1	2.9
	Phenylethylamine	14.65	20	33.52	94.4	3.2
	Histamine	4.79	5	9.58	95.8	1.7
	Cadaverine	5.53	10	15.14	96.2	2.4
	Hexamethylenediamine	1.57	5	6.49	98.4	3.6
	Tyramine	22.58	30	51.24	95.5	2.7
	Spermine	62.43	70	135.32	104.1	3.1
Yogurt	Tryptamine	0.51	1	1.48	97.1	2.5
	Phenylethylamine	1.24	5	6.18	98.8	1.4
	Histamine	0.55	1	1.61	106.1	3.3
	Cadaverine	0.18	1	1.15	96.9	2.9
	Hexamethylenediamine	ND	1	0.93	93.0	2.2
	Tyramine	ND	1	0.94	94.1	3.1
	Spermine	1.36	5	6.59	104.7	3.7
Rice wine	Tryptamine	ND	1	1.07	107.1	2.8
	Phenylethylamine	3.58	5	8.73	103.0	2.0
	Histamine	17.56	20	36.59	95.2	2.4
	Cadaverine	1.18	5	6.05	97.5	2.8
	Hexamethylenediamine	ND	1	0.93	93.1	2.4
	Tyramine	1.09	5	5.99	98.1	3.2
	Spermine	18.35	20	37.47	95.6	2.6
Ham sausage	Tryptamine	13.75	20	34.00	101.3	1.6
	Phenylethylamine	1.76	5	6.58	96.4	2.5
	Histamine	59.05	60	115.82	94.6	2.5
	Cadaverine	2.63	5	7.25	92.4	3.4
	Hexamethylenediamine	ND	1	1.03	103.4	2.8
	Tyramine	ND	1	0.98	98.1	1.9
	Spermine	104.28	110	223.37	108.3	2.7
Beer	Tryptamine	2.77	5	7.54	95.4	2.9
	Phenylethylamine	2.45	5	7.29	96.8	2.2
	Histamine	6.55	10	17.10	105.5	3.2
	Cadaverine	2.22	5	7.18	99.2	3.5
	Hexamethylenediamine	1.28	5	6.06	95.6	2.8
	Tyramine	18.69	20	36.92	91.2	2.4
	Spermine	1.86	5	6.50	92.8	2.1

The unit for solid sample is “micrograms per gram”; for liquid samples, the unit is “micrograms per milliliter”

ND no detectable

recovery for the spiked samples to establish the accuracy of the proposed method, are listed in Table 3. The results showed cheese contained higher contents of relevant biogenic amines than do other samples. The most abundant biogenic amines in the tested samples were Cad, Phe, Spe and His. No concentration of rice wine, yogurt, and ham sausage was detected for Hex. Tyr was absent from both yogurt and ham sausage, as was Try only from rice wine. The typical chromatograms of ham sausage and rice wine are illustrated in Fig. 5b, c, respectively.

Conclusion

In this study, a one-step fluorescence labeling and ultrasound-assisted dispersive liquid–liquid microextraction (UA-DLLME) method followed by HPLC-FLD for the simultaneous determination of seven BAs in foodstuff samples was developed and optimized. In order to obtain the optimal experiment conditions, various parameters affecting the efficiency of fluorescence labeling and DLLME were optimized by RSM based on Box–Behnken design. The proposed method possesses the advantages of both fluorescence labeling and UA-DLLME, including rapidity, simplicity, low cost, high sensitivity and excellent selectivity. Moreover, this method has the powerful potential of practical applications for the analysis of BAs in many other samples.

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Conflict of Interest Hongliang Wu declares that he has no conflict of interest. Guoliang Li declares that he has no conflict of interest. Shucheng Liu declares that he has no conflict of interest. Zhongyin Ji declares that he has no conflict of interest. Qiulong Zhang declares that he has no conflict of interest. Na Hu declares that she has no conflict of interest. Yourui Suo declares that he has no conflict of interest. Jinmao You declares that he has no conflict of interest. This article does not contain any studies with human or animal subjects.

References

Aflaki F, Ghoulipour V, Saemian N, Sheibani S (2013) Biogenic amine contents in non-alcoholic beers: screening and optimization of derivatization. Food Anal Methods 7:713–720

Ahmar H, Fakhari AR, Tabani H, Shahsavani A (2013) Optimization of electromembrane extraction combined with differential pulse voltammetry using modified screen-printed electrode for the determination of sufentanil. Electrochim Acta 96:117–123

Ali Awan M, Fleet I, Paul Thomas CL (2008) Determination of biogenic diamines with a vaporisation derivatisation approach using solid-phase microextraction gas chromatography–mass spectrometry. Food Chem 111:462–468

Almeida C, Fernandes JO, Cunha SC (2012) A novel dispersive liquid–liquid microextraction (DLLME) gas chromatography-mass spectrometry (GC–MS) method for the determination of eighteen biogenic amines in beer. Food Control 25:380–388

Bricio Santos BMS, Ríos A, Valcárcel M (2004) Direct automatic determination of biogenic amines in wine by flow injection-capillary electrophoresis-mass spectrometry. Electrophoresis 25:3427–3433

De Mey E, Drabik-Markiewicz G, De Maere H, Peeters MC, Derdelinckx G, Paelinck H, Kowalska T (2012) Dabsyl derivatisation as an alternative for dansylation in the detection of biogenic amines in fermented meat products by reversed phase high performance liquid chromatography. Food Chem 130:1017–1023

Donthuan J, Yunchalard S, Srijaranai S (2014) Ultrasound-assisted dispersive liquid–liquid microextraction combined with high performance liquid chromatography for sensitive determination of five biogenic amines in fermented fish samples. Anal Methods 6:1128

Hwang B, Wang J, Choong Y (2003) A rapid gas chromatographic method for the determination of histamine in fish and fish products. Food Chem 82:329–334

Hyötyläinen T, Savola N, Lehtonen P, Riekkola M-L (2001) Determination of biogenic amines in wine by multidimensional liquid chromatography with online derivatisation. Analyst 126: 2124–2127

Innocente N, Biasutti M, Padovese M, Moret S (2007) Determination of biogenic amines in cheese using HPLC technique and direct derivatization of acid extract. Food Chem 101:1285–1289

Jastrzębska A, Kurzawa M, Piasta A, Szłyk E (2011) Determination of histamine in some foods by isotachophoretic method with simple sample preparation. Food Anal Methods 5:1079–1087

Jia S, Kang YP, Park JH, Lee J, Kwon SW (2012) Determination of biogenic amines in Bokbunja (Rubus coreanus Miq.) wines using a novel ultra-performance liquid chromatography coupled with quadrupole-time of flight mass spectrometry. Food Chem 132: 1185–1190

Jia S, Ryu Y, Kwon SW, Lee J (2013) An in situ benzoylation-dispersive liquid-liquid microextraction method based on solidification of floating organic droplets for determination of biogenic amines by liquid chromatography-ultraviolet analysis. J Chromatogr A 1282: 1–10

Kabashima T, Kabashima T, Yu Z, Tang C, Nakagawa Y, Okumura K, Shibata T, Lu J, Kai M (2008) A selective fluorescence reaction for peptides and chromatographic analysis. Peptides 29:356–363

Kim JY, Kim D, Park P, Kang H-I, Ryu EK, Kim SM (2011) Effects of storage temperature and time on the biogenic amine content and microflora in Korean turbid rice wine, Makgeolli. Food Chem 128: 87–92

Koral S, Tufan B, Ščavničar A, Kočar D, Pompe M, Köse S (2013) Investigation of the contents of biogenic amines and some food safety parameters of various commercially salted fish products. Food Control 32:597–606

Lapa-Guimarães J, Pickova J (2004) New solvent systems for thin-layer chromatographic determination of nine biogenic amines in fish and squid. J Chromatogr A 1045:223–232

Latorre-Moratalla ML, Bover-Cid S, Aymerich T, Marcos B, Vidal-Carou MC, Garriga M (2007) Aminogenesis control in fermented sausages manufactured with pressurized meat batter and starter culture. Meat Sci 75:460–469

Latorre-Moratalla ML, Bover-Cid S, Veciana-Nogues T, Vidal-Carou MC (2009) Thin-layer chromatography for the identification and semi-quantification of biogenic amines produced by bacteria. J Chromatogr A 1216:4128–4132

Lázaro CA, Conte-Junior CA (2013) Chromatographic methods for biogenic amines determination in foods of animal origin. Braz J Vet Res Anim Sci 50:430–446

Lázaro CA, Conte-Júnior CA, Cunha FL, Máralém ET, Mano SB, Franco RM (2013) Validation of an HPLC methodology for the identification and quantification of biogenic amines in chicken meat. Food Anal Methods 6:1024–1032

Li G, Dong L, Wang A, Wang W, Hu N, You J (2014) Simultaneous determination of biogenic amines and estrogens in foodstuff by an improved HPLC method combining with fluorescence labeling. LWT Food Sci Technol 55:355–361

Linares DM, Martin MC, Ladero V, Alvarez MA, Fernandez M (2011) Biogenic amines in dairy products. Crit Rev Food Sci 51:691–703

Martuscelli M, Arfelli G, Manetta AC, Suzzi G (2013) Biogenic amines content as a measure of the quality of wines of Abruzzo (Italy). Food Chem 140:590–597

Moradi M, Yamini Y, Seidi S, Ghambarian M, Esrafili A (2013) Ultrasound-assisted emulsification microextraction using low density solvent for analysis of toxic nitrophenols in natural waters. Int J Environ Anal Chem 93:199–212

Önal A (2007) A review: current analytical methods for the determination of biogenic amines in foods. Food Chem 103:1475–1486

Ozdestan O, Uren A (2009) A method for benzoyl chloride derivatization of biogenic amines for high performance liquid chromatography. Talanta 78:1321–1326

Paleologos EK, Chytiri SD, Savvaidis IN, Kontominas MG (2003) Determination of biogenic amines as their benzoyl derivatives after cloud point extraction with micellar liquid chromatographic separation. J Chromatogr A 1010:217–224

Pereira V, Pontes M, Camara JS, Marques JC (2008) Simultaneous analysis of free amino acids and biogenic amines in honey and wine samples using in loop orthophthalaldeyde derivatization procedure. J Chromatogr A 1189:435–443

Pineda A, Carrasco J, Peña-Farfal C, Henríquez-Aedo K, Aranda M (2012) Preliminary evaluation of biogenic amines content in Chilean young varietal wines by HPLC. Food Control 23:251–257

Saaid M, Saad B, Ali AS, Saleh MI, Basheer C, Lee HK (2009a) In situ derivatization hollow fibre liquid-phase microextraction for the determination of biogenic amines in food samples. J Chromatogr A 1216:5165–5170

Saaid M, Saad B, Hashim NH, Mohamed Ali AS, Saleh MI (2009b) Determination of biogenic amines in selected Malaysian food. Food Chem 113:1356–1362

Sun X, Yang X, Wang E (2003) Determination of biogenic amines by capillary electrophoresis with pulsed amperometric detection. J Chromatogr A 1005:189–195

Wang Y, Ye D, Zhu B, Wu G, Duan C (2014) Rapid HPLC analysis of amino acids and biogenic amines in wines during fermentation and evaluation of matrix effect. Food Chem 163:6–15

You J, Liu L, Zhao W, Zhao X, Suo Y, Wang H, Li Y (2007a) Study of a new derivatizing reagent that improves the analysis of amino acids by HPLC with fluorescence detection: application to hydrolyzed rape bee pollen. Anal Bioanal Chem 387:2705–2718

You J, Zhao W, Liu L, Zhao X, Suo Y, Wang H, Li Y, Ding C (2007b) Determination of amines using 2-(11H-benzo[a]carbazol-11-yl) ethyl chloroformate (BCEC-Cl) as labeling reagent by HPLC with fluorescence detection and identification with APCI/MS. Talanta 72:914–925

Zhao W, Liu L, Zhu F, Zhao X, Liu S, Suo Y, You J (2007) Mass spectrometric identification of aliphatic amines using 2-(11h-benzo[a]carbazol-11-yl) ethyl chloroformate as fluorescence labeling reagent. Chin J Anal Chem Chin 35:1251–1256

Zhao W, Liu L, Zhao X, Ding C, Suo Y, You J (2008) Application of 2-(11h-benzo[a]carbazol-11-yl) ethyl carbonochloridate as a precolumn derivatization reagent of amino acid by high performance liquid chromatography with fluorescence detection. Chin J Anal Chem 36:1071–1076

Zotou A, Notou M (2012) Enhancing fluorescence lc analysis of biogenic amines in fish tissues by precolumn derivatization with naphthalene-2,3-dicarboxaldehyde. Food Anal Methods 6:89–99