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Determination of biogenic amines by capillary electrophoresis[1]

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Abstract

A method for determining biogenic amines in food using micellar electrokinetic capillary chromatography has been developed. Derivatization of the amines was performed with AccQ (6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate; Waters, Milford, MA, USA) reagent. The influence of buffer composition on the separation (including pH, SDS concentration and various additives) was investigated. The separation of seven biogenic amines (histamine, tyramine, tryptamine, spermine, spermidine, cadaverine and putrescine) could be achieved within 25–30 min with good repeatability. The biogenic amine profiles in three different food samples (wine, salami and chive) were determined and quantitated.

Keywords: Food analysis; Wine; Buffer composition; Biogenic amines; Aminoquinolyl-*N*-hydroxysuccinimidyl carbamate; Histamine; Tyramine

1. Introduction

Biogenic amines can be found in a variety of foods and beverages, especially in protein-rich foods (fish and meat) and in fermented foods (cheese, salami) and beverages (wine, beer). Consumption of high amounts of these amines can result in symptoms of intoxication such as headache, nausea, rushes, hypo- or hypertension. Their formation is a consequence of decarboxylation of amino acids by some microorganisms. The study of biogenic amines is of interest not only for their toxicological risk, but also as an indicator of food quality [1,2].

Different methods have been used to determine biogenic amines [3]. Since most amines show neither natural UV absorption nor fluorescence, most methods require that amines should be derivatized before detection, or indirect detection can be used. Different chemical reagents have been used for the amine analysis, e.g., ninhydrin in amino acid analysers as a post-column derivatization reagent [4,5], dansyl-chloride (Dns-Cl) with pre-column derivatisation [6], *o*-phthalaldehyde (OPA) [7,8], fluorescein isothiocyanate (FITC) [9] and 9-fluorenylmethyl chloroformate [10,11].

For the separation of biogenic amines thin-layer chromatography and overpressured layer chromatography are frequently used analytical methods [12,13,6]. Gas chromatography can be used for determination of volatile derivatives of the amines [14,15]. Ion-exchange chromatography on an amino acid analyser [4,5], and high-performance liquid chromatography (HPLC) methods [16,10] have

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given sensitive results, but sample preparation and separation are often time-consuming. Capillary electrophoretic determinations of biogenic amines have also been described. Zhou et al. [17] developed a capillary zone electrophoretic method with indirect ultraviolet detection using quinine sulphate in background electrolyte for separation of three polyamines in serum, while Nouadje et al. [9] used a ball-lens laser-induced fluorescence detector to determine fluorescein thiocarbamyl biogenic amines in dairy products using micellar electrokinetic capillary chromatography. Oguri et al. [7] described a high-performance capillary electrophoresis (HPCE) separation method with on-line mode in-capillary derivatization with OPA and *N*-acetylcysteine.

AccQ-Fluor (6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate; Waters, Milford, MA, USA) derivatizing reagent has been developed first for amino acid analysis. It reacts rapidly with primary and secondary amines, and converts them to stable, UV and fluorescent active derivatives. The reactions of AccQ reagent with amines and with water are shown in Fig. 1. One of the greatest advantages of this reaction is its tolerance towards salts in the samples. The derivatisation procedure is very simple and quick, the time needed for derivatisation is only 15 min. Derivatized amines fluoresce strongly at 395 nm, and can be monitored (with reduced sensitivity) at 254 nm with a UV detector [18].

In the present work micellar electrokinetic capillary chromatography has been employed for separating aliphatic, aromatic and heterocyclic biogenic amines in foods. Derivatizations of amines were carried out using AccQ-Fluor reagent. The influences of different separation conditions were investigated, and the analytical reproducibility was determined.

2. Experimental

2.1. Chemicals

All of the chemicals used in this study were of analytical grade. SDS (sodium dodecyl sulphate) was purchased from Fluka (Buchs, Switzerland). For the preparation of the electrolytes double-distilled Milli-Q water (Waters) was used. Boric acid and urea were obtained from Bio-Rad Labs. (Richmond, CA, USA). Acetonitrile and methanol were of HPLC grade and were purchased from Chemolab (Budapest, Hungary). AccQ was obtained from Waters. Biogenic amines standards (histamine, tyramine, putrescine, tryptamine, spermine, spermidine and cadaverine) were purchased from Sigma (St. Louis, MO, USA).

2.2. Apparatus

A laboratory-built capillary electrophoretic system

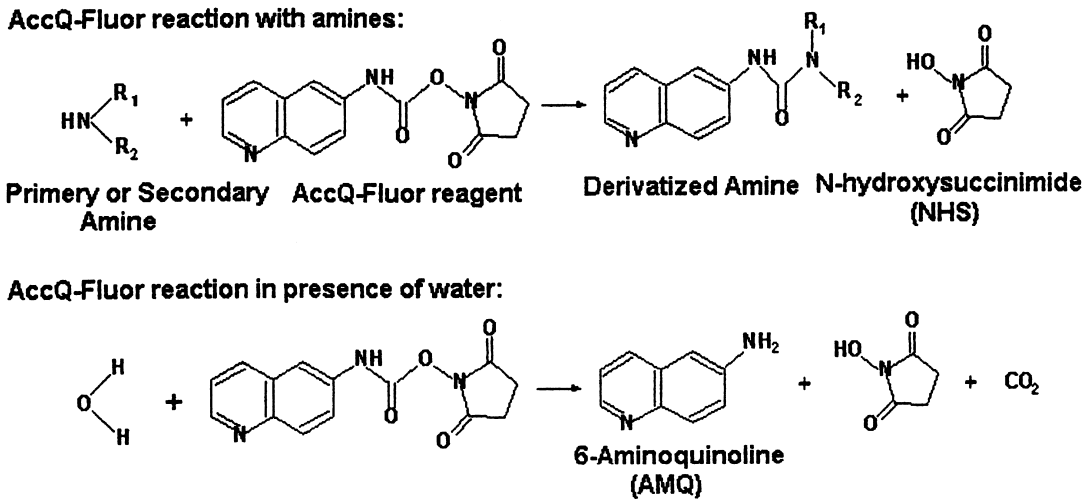

Fig. 1. Reactions of AccQ-Fluor reagent.

consisted of a high-voltage power supply (CZE 1000 PN 30, Spellman High Voltage Corp., Plainview, NY, USA), a Spectra 100 UV–Vis detector (Thermo-Separation Products, San Jose, CA, USA), a DTK Personal Computer (Parity, Budapest, Hungary) equipped with an analogue-to-digital converter board (Data Translations, Framingham, MA, USA), a data acquisition and an analysis software (Caesar, Analytical Devices, Alameda, CA, USA) were used.

All the analyses were performed at room temperature; the capillaries were cooled using a laboratory fan. Fused-silica capillaries (Polymicro Technologies Phoenix, AZ, USA) were 55 cm (30 cm to the detector)×50 μm I.D. UV detection was performed at 254 nm.

2.3. Preparation of food samples

Food samples were extracted with 6% perchloric acid (5 g/15 ml) for 1 h using a shaking equipment (VEB MLW, Labortechnik, Ilnemann, Germany). The extractant was centrifuged and filtered through a 0.45-μm membrane filter (Millipore, Bedford, MA, USA). For selective enrichment of biogenic amines 30 ml of sample was applied to an ion-exchange (Dowex 50 WX 8, mesh size 50–100) column. The column was washed with 40 ml of 0.1 *M* potassium phosphate buffer (pH 8.0) followed by 40 ml of 1 *M* HCl. Biogenic amines were eluted with 15 ml of 6 *M* HCl. The samples were dried on a heated waterbath at 90°C, and redissolved in 1.5 ml 0.1 *M* HCl. In the case of wine samples the sample preparation was performed directly, no perchloric acid extraction was made.

2.4. Derivatization procedure

Five μl of extractant was added to 150 μl borate buffer and briefly vortexed. Fifty μl of AccQ-Fluor reagent (10 m*M* in acetonitrile) was added to the sample, vortexed, and heated at 55°C in a heating block for 10 min.

2.5. Electrophoretic procedure

Buffers were made in Milli-Q water. One hundred m*M* boric acid, 50 m*M* SDS and 10% acetonitrile were dissolved, and the pH was adjusted to 8.9 with addition of 1 *M* NaOH. Buffer solutions were filtered through a 0.2-μm membrane filter.

Prior to each analysis the capillary was washed successively with 100 μl of 0.1 *M* NaOH, 100 μl of deionized water and 100 μl of buffer. Samples were injected from the anodic end of the capillary hydrodynamically for 5 s. Separations were carried out at 15 kV constant voltage at room temperature.

3. Results and discussion

3.1. Separation conditions

The influence of separation conditions on the separation of seven biogenic amines (histamine, tyramine, tryptamine, spermine, spermidine, cadaverine and putrescine) derivatized with AccQ reagent was investigated. The derivatives bear no charge, therefore micellar electrokinetic capillary chromatography was applied for their separation.

In the presence of SDS only the separation of the seven amine derivative was incomplete. Various concentrations of organic modifiers (methanol in concentrations of 10–15%, urea in concentrations of 5–10 *M* and 7.5–12.5% acetonitrile) were added to the buffer in order to increase selectivity. (Buffer composition: 100 m*M* boric acid, 50 m*M* SDS, pH 8.9). Methanol and urea did not improve separation selectivity. Acetonitrile (ACN) however did result in better resolution. Fig. 2 shows the influence of acetonitrile content in the buffer on the migration times. Increasing ACN concentration produced better separations for all biogenic amines except for tryptamine and cadaverine. The optimum selectivity was achieved using 10% ACN concentration in the buffer.

The influence of SDS concentration in the buffer was also examined (Fig. 3). SDS was applied in various concentrations above the critical micelle concentration (30, 50, 70, 90 m*M*). (Buffer composition: 100 m*M* boric acid, 10% acetonitrile, pH 8.9). Increasing SDS concentration affected the migration order only in the case of histamine and the first reagent peak. It had, however, significant effect on the migration time. Increasing SDS concentration causes long separation times. This effect can be explained by the higher Joule heating due to the

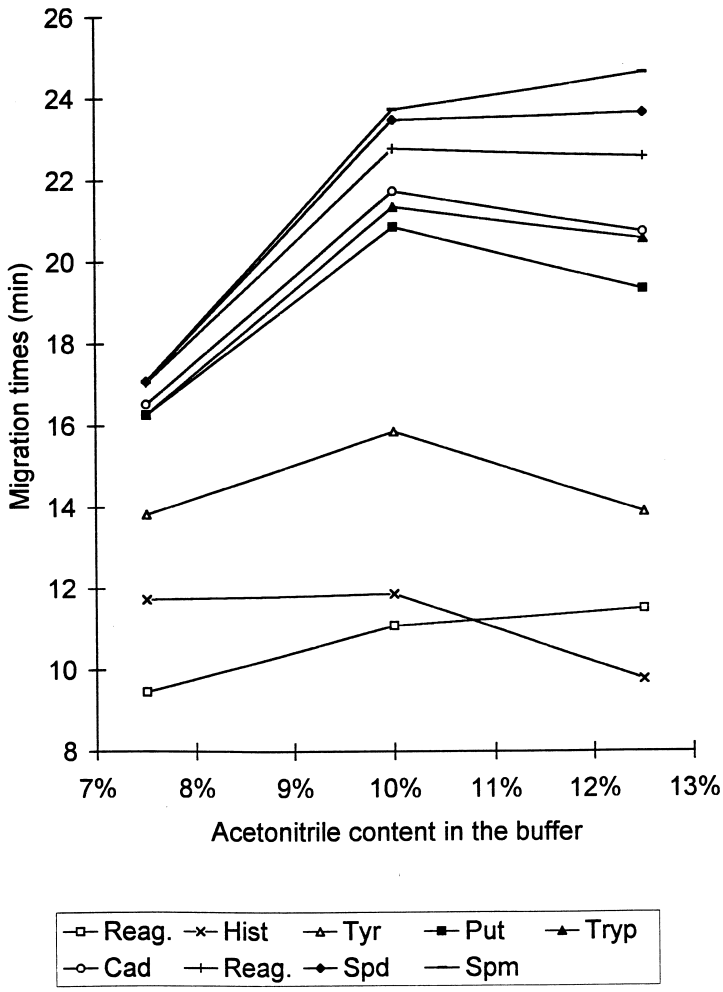

Fig. 2. Influence of the acetonitrile concentration on the migration times of biogenic amines. Conditions: 100 m*M* boric acid, 50 m*M* SDS, pH 8.9, 15 kV, 254 nm, fused-silica capillary 55 cm (30 cm to the detector)×50 μm I.D., room temperature.

increased conductivity of the buffer, by the changes in the surface charge due to the presence of counter-ions and by the increased ratio of the volume of the micellar phase to that of the aqueous phase [19]. Fifty m*M* SDS in the buffer resulted in good separation of the histamine and the system peak. Using higher SDS concentration in the buffer long separation times were observed.

The effect of the electrolyte pH on the separation at pH values of 8.5, 8.7, 8.9 and 9.2 was also investigated (Fig. 4). (Buffer composition: 100 m*M* boric acid, 50 m*M* SDS, 10% acetonitrile) Increasing pH resulted a change in migration order of histamine and the first reagent peak between pH 8.9 and pH 9.2. Two optimum values were obtained (pH 8.9 and 9.2).

Based on these data the optimal condition for the separation of biogenic amines is as follows: 100 m*M* boric acid, 50 m*M* SDS, 10% acetonitrile, pH 8.9. Fig. 5. shows the separation of seven biogenic amine standards under optimised conditions. In the Figure the chemical structure of the biogenic amines is also shown. As it can be seen, the migration order of the aliphatic biogenic amines follows the order of their hydrophobicity. Increasing chain lengths result in increasing migration time. The biogenic amines

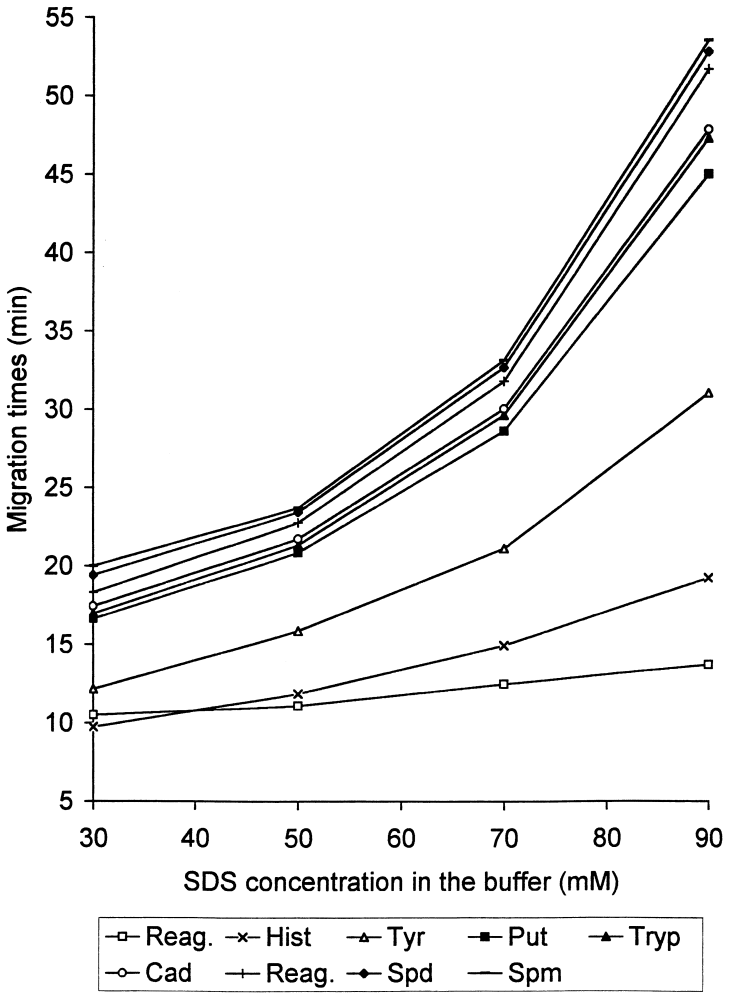

Fig. 3. Effect of SDS concentration on the migration times of biogenic amines. Conditions: 10% acetonitrile, pH 8.9, other conditions identical to Fig. 1.

containing heterocyclic or aromatic ring separate also according to their hydrophobicity.

3.2. Linearity and reproducibility

The data of linearity and of reproducibility and detection limits are presented in Table 1. These data have been determined using the optimised separation conditions.

The reproducibility (expressed as R.S.D. values of relative migration times and R.S.D. values of relative peak areas) were calculated by analysing biogenic amine standard solutions (in concentration of 1 m*M*, $n=5$). In general R.S.D. values of relative migration times (RMTs) were lower than 2%, only histamine gave worse reproducibility. R.S.D. values of the relative peak areas (RPAs) were between 3% and 5%.

The linearity of the method was determined by using standard mixtures at five concentration levels across the concentration range of 0.05–1 μmol/ml. Values of correlation coefficients ranged between 0.9880 and 0.9989, indicating good linearity.

Detection limits for the seven biogenic amines were between 1 and 40 μ*M* in the food extract after enrichment (approx. 20-fold) on the ion-exchange

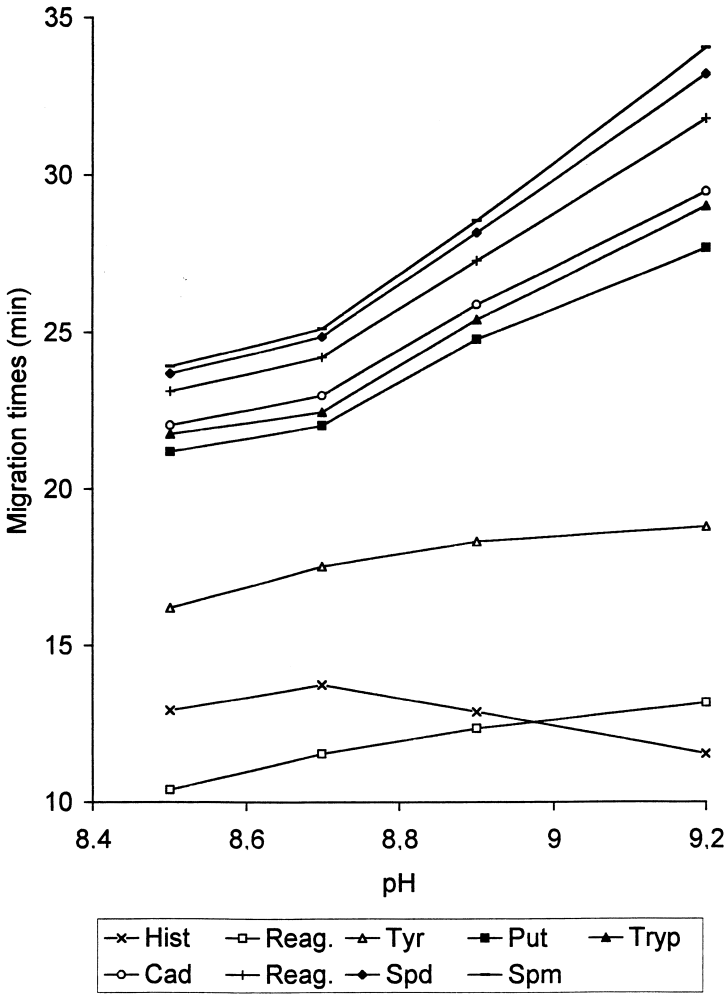

Fig. 4. Effect of pH on the separation of biogenic amines. Conditions: 50 m*M* SDS, 10% acetonitrile, other conditions identical to Fig. 1.

column. Based on these data the lowest limits of quantitation can be calculated for different foods prior to enrichment. These range from 0.05 μ*M* for tryptamine to 2 μ*M* for histamine in wines, and from 0.15 μmol/kg to 6 μmol/kg in food samples, respectively. One hundred-times more sensitivity could be achieved with the application of fluorescence detection [18].

3.3. Analysis of food samples

Wine, chive and sausage samples were analysed using the optimised HPCE method. Biogenic amines were identified by adding standards to the samples.

All amines could be detected in wine samples. Spermidine was present at the highest concentration. Histamine and tyramine, the most toxic amines, were found at low concentration. (Limit proposed for histamine in alcoholic beverages is 5–10 μg/ml). The most complex electropherogram was obtained in this case, indicating that the wine sample contained several low-molecular-mass amines beside the seven biogenic amines examined (Fig. 6). Chive sample contained tyramine, putrescine, cadaverine, spermidine and spermine. In sausage sample all amines were found at high concentration, especially histamine cadaverine and tryptamine. This sample may be considered high risk product with regard to the

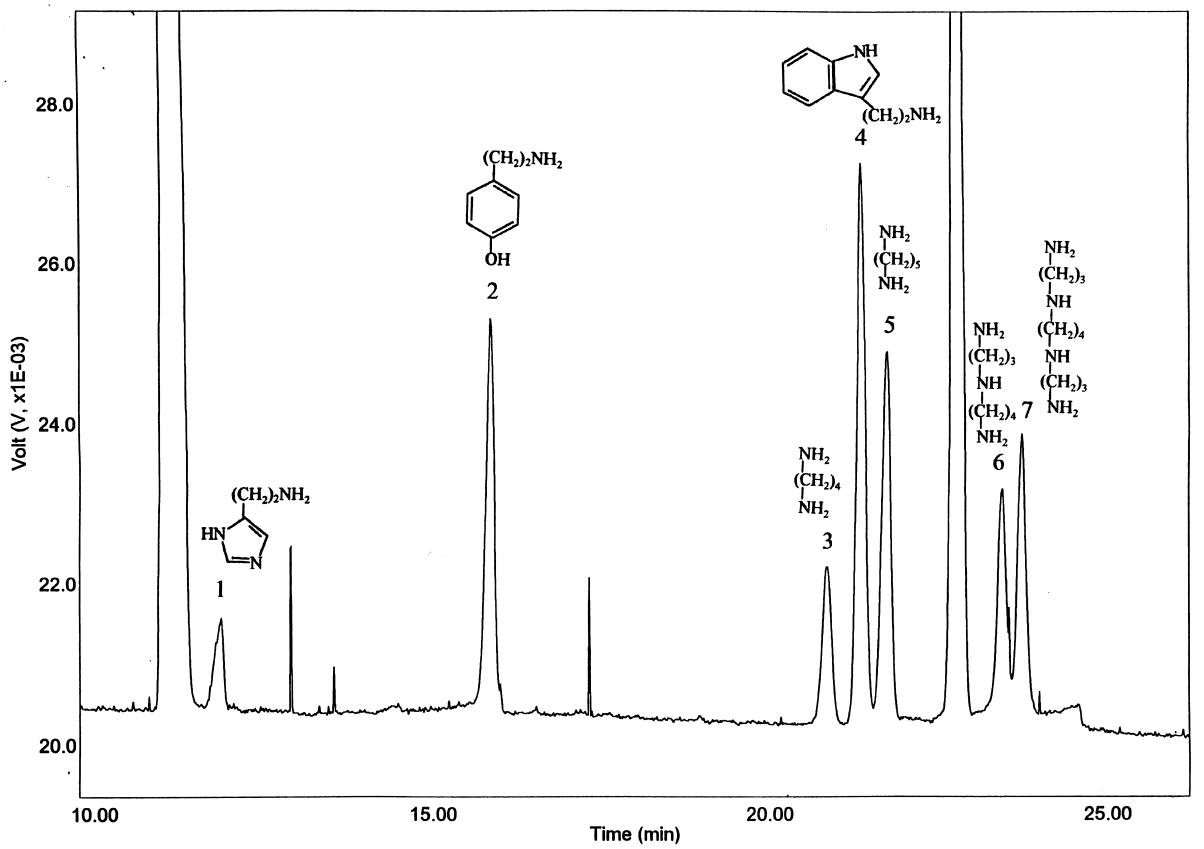

Fig. 5. Electropherogram of seven biogenic amine standards. Peaks: 1=Histamine, 2=tyramine, 3=putrescine, 4=tryptamine, 5=cadaverine, 6=spermidine, 7=spermine. Conditions: 50 m*M* SDS, 10% acetonitrile, pH 8.9, other conditions identical to Fig. 1. Above the peaks the chemical structure of the underivatised biogenic amines can be seen.

Table 1
Reproducibility and linearity values

Biogenic amines	RMT	R.S.D. (%) RMT	R.S.D. (%) RPA	Linearity			Detection limit (μM) ($S/N=3$)
				Slope	Intercept	Correlation coefficient	
Histamine	0.59	3.44	4.19	0.173	−0.013	0.9880	40
Tyramine	0.73	1.93	3.16	0.374	−0.011	0.9984	2.5
Putrescine	0.97	0.39	4.21	0.185	−0.011	0.9976	5
Tryptamine[a]	1			0.514	−0.017	0.9989	1
Cadaverine	1.03	1.13	4.93	0.337	−0.016	0.9974	2.5
Spermidine	1.12	1.28	4.20	0.204	−0.014	0.9967	25
Spermine	1.15	1.76	3.32	0.247	−0.019	0.9923	25

[a] Other amines are relative to tryptamine.
RMT: Relative migration time.
RPA: Relative peak area.
S/N: Signal-to-noise ratio.

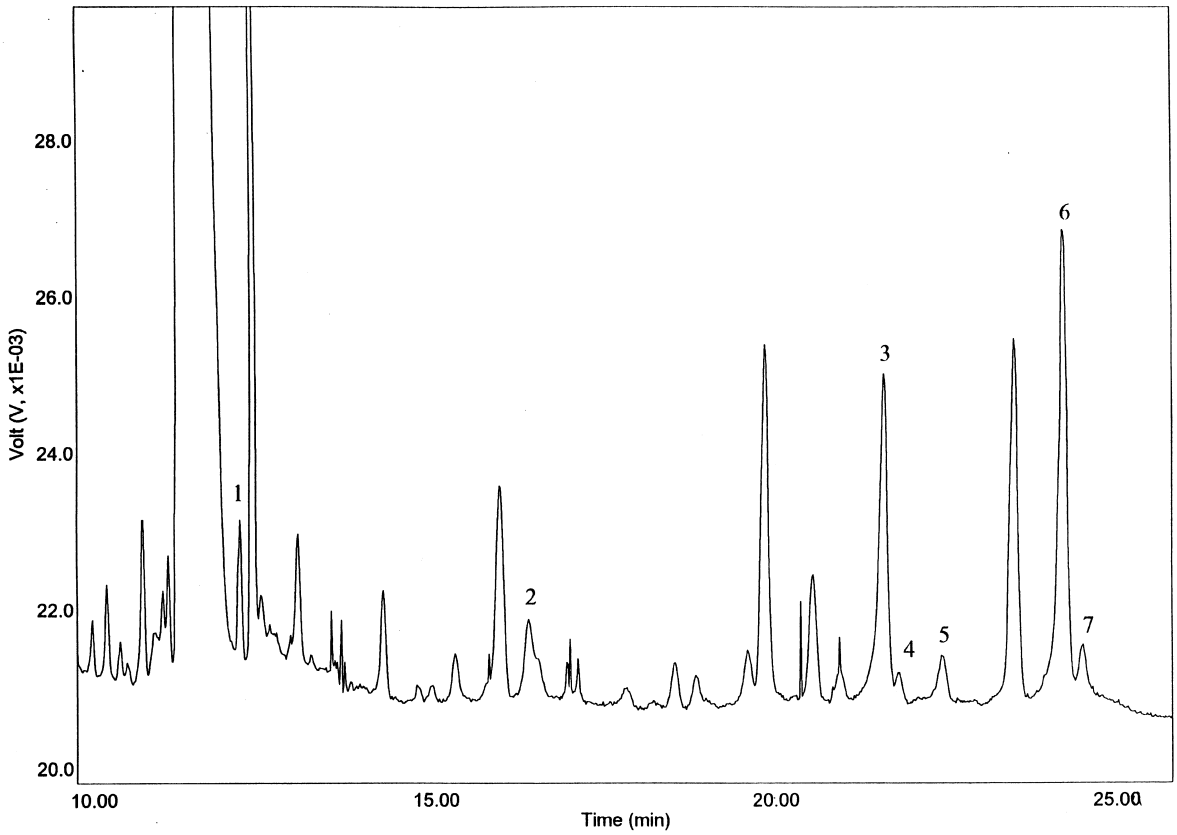

Fig. 6. Representative electropherogram of a wine sample. Peaks: 1=Histamine, 2=tyramine, 3=putrescine, 4=tryptamine, 5=cadaverine, 6=spermidine, 7=spermine. Conditions as in Fig. 5.

presence of biogenic amines. Limits from 100 to 1000 μg/g histamine and 500–800 μg/g tyramine in foods have been proposed, and their interactions with other amines could also potentiate the amine toxicity [20,21]. The results obtained from the analysis of food samples are shown in Table 2.

Table 2
Biogenic amine content in food samples

Biogenic amines	Wine (μg/ml)	Chive (μg/g)	Sausage (μg/g)
Histamine	0.82	nd	637.73
Tyramine	0.56	8.07	121.95
Putrescine	14.12	254.42	29.49
Tryptamine	0.13	nd	359.84
Cadaverine	7.78	5.30	453.26
Spermidine	78.66	236.27	20.40
Spermine	1.21	64.93	94.72

nd: Not detected.

4. Conclusions

A rapid, simple and reproducible HPCE method for determining biogenic amines in foodstuffs has been developed. The derivatization step with AccQ reagent is very simple and quick requiring only limited sample preparation. Complete separation of seven amines was achieved within 30 min with good repeatability and linearity. Examples of analysis show that this method can be applied to the determination of biogenic amines in different foodstuffs.

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