ELSEVIER

Journal of Chromatography A, 815 (1998) 243–250

JOURNAL OF CHROMATOGRAPHY A

Determination of seven biogenic amines in foods by micellar electrokinetic capillary chromatography

Martin Křížek*, Tamara Pelikánová

University of South Bohemia, Faculty of Agriculture, Department of Chemistry, Studentská 13, 370 05 České Budějovice, Czech Republic

Received 3 March 1998; received in revised form 11 May 1998; accepted 25 May 1998

Abstract

Micellar electrokinetic capillary chromatography was applied to the separation of seven biogenic amines, most frequently occurring in foods. Putrescine, cadaverine, spermidine, spermine, tryptamine, histamine and tyramine were separated as *N*-substituted benzamides using an uncoated fused-silica capillary column (43 cm×75 μm I.D.). Borate buffer (15 m*M*) with sodium dodecylsulphate (40 m*M*) and methanol (25%, v/v) was found to be a suitable system for the determination of these compounds in food samples. The detection limits in the range of 0.2 to 0.7 mg l^{-1} are sufficient for this type of sample.

Keywords: Food analysis; Biogenic amines

1. Introduction

Biogenic amines represent a group of low-molecular-mass organic bases occurring in all organisms. Amines are formed and degraded during normal metabolic processes in living cells and therefore they are ubiquitous in animals, plants and microorganisms. In foods biogenic amines are usually generated by microbial decarboxylation of amino acids and can have an aliphatic, aromatic or heterocyclic structure. The most frequent biogenic amines important in food analysis are: the diamines – putrescine (PUT) and cadaverine (CAD), polyamines – spermidine (SPD) and spermine (SPM), aromatic amines – e.g. tyramine (TY) and heterocyclic amines – tryptamine (TR) and histamine (HI).

Biogenic amines are normal constituents of fermented foods such as cheese, wine, beer, sauerkraut etc. In non-fermented foods these compounds were found useful as quality indicators and markers of food decomposition.

Though limited amounts of putrescine and polyamines in foods play positive role in human nutrition [1], excessive oral intake of biogenic amines, especially of histamine and tyramine, can result in nausea, respiratory distress, heart palpitations, headache and hyper- or hypotension [2]. Histamine food poisoning is one of the commonest forms of food intoxication reported [3]. Toxic effects of biogenic amines are potentiated by monoamine oxidase inhibitor drugs or alcohol [4]. Studies aimed at biogenic amines in foods usually involve two aspects: toxicology and/or food quality.

Development of new analytical methods is usually related with the progress in instrumentation. For the determination of amines thin-layer chromatography

*Corresponding author

0021-9673/98/$19.00
PII: S0021-9673(98)00464-6

(TLC), ion-exchange chromatography (IEC), gas chromatography (GC) and especially high-performance liquid chromatography (HPLC) have been proposed. A relatively new technique, capillary zone electrophoresis (CZE), was also applied for amines determination in the last years.

As amines are strong organic bases it is very useful to take advantage of this feature for their separation from the sample matrix. This important preliminary step was discussed in detail by Moret and Conte [5].

HPLC procedures involve pre- or post-column derivatization step. Dansyl chloride has been the most widely used reagent for derivatization of amines prior to HPLC [6,7]. Light sensitivity and limited stability of dansyl chloride lead some authors to the use of different derivatisation agents. One effective derivatizing agent is benzoyl chloride, first applied by Redmond and Tseng in analysis of body fluids [8]. It was successfully used in plant tissues [9,10], in preserved forage [11] and in beers [12].

TLC of derivatized amines has continued to be used due to its quickness and simplicity. For this purpose dansyl chloride has been solely applied. TLC method is especially popular in plant biochemistry [13]. Very effective is the separation of dansylated polyamines by overpressured layer chromatography (OPLC) [14].

Good results are provided by both IEC [15] and by separation of ion pairs of amines on reversed-phase HPLC [16]. When using both these methods post-column derivatization is inevitable. For this purpose ninhydrin or *o*-phthalaldehyde are used to a large extent. The possibility of employing an amino acid analyser is a big advantage of IEC.

Gas chromatography is not so often applied for the determination of amines. Because of inherent tailing problems, derivatization is also frequently used. For separation of ethylchloroformiate derivatives of putrefactive amines from fish samples a packed column was used [17]. A sensitive method based on separation of *N*-diethylthiophosphoryl derivatives on capillary column was worked out by Kataoka et al. [18].

CZE has become a powerful separation technique in the last years. In its most applications it is used for effective separation of charged compounds. Micellar electrokinetic capillary chromatography (MECC) allows the resolution of even uncharged molecules, thus extending the application range of CZE. Reports dealing with separation of biogenic amines by capillary electrophoresis are not numerous to date. There are three possible approaches to solve this task:

1. Aromatic or heterocyclic amines can be separated in selected buffer systems without derivatization.
2. Polyamines are determined either derivatized (usually in MECC mode) or
3. their detection must be indirect.

It is evident, that separation of the whole large group of biogenic amines usually needs derivatization.

Histamine, the principal causative agent in scombroid food poisoning, was analyzed in seafood by rapid, sensitive CZE method using fused-silica capillary filled with 20 m*M* citrate buffer (pH 2.5). Histamine from methanolic fish extract migrated within 4 min [19]. Low pH buffers seem to be convenient for very fast histamine separations, because this amine is positively charged and moves towards the cathode edge across the capillary. Gallardo et al. [20] determined histamine in fish and marine products using phosphate buffer at pH 2.44. In uncoated fused-silica capillaries (57 cm×75 μm) the separation was achieved in 5 min.

Aromatic or heterocyclic amines (HI, TY, TR, benzylamine, serotonine and 2-phenylethylamine) were resolved in fused-silica capillary (44 cm×50 μm) within 11 min in 100 m*M* ammonium acetate buffer (pH 7.5) with 40% (v/v) methanol [21].

For the measurements of polyamine contents in tumor cells a method using indirect photometric detection has been worked out. The background electrolyte contained quinine sulfate and polyamines were resolved in 9 min [22].

More complex mixtures of biogenic amines require derivatization step. Fluoresceinisothiocyanate (FITC) is an agent of great importance for CZE determination of amines. Detection using xenon lamp-based fluorescence [23] or laser induced fluorescence (LIF) [24,25] is very sensitive, with detection limits reaching 0.1–1 ng g^{-1}. Separation of FITC derivatives has been applied to cheese [24], wine [25] and soy sauce [23] samples.

The method developed in our laboratory is based on separation of *N*-substituted benzamides in MECC mode on CZE apparatus. When choosing a method we decided to find an easy, quick but sufficiently

sensitive and reproducible one. The origins of the method can be traced back to our work on amines in silage using HPLC separation of these derivatives [26]. The main advantage of transferring the method from HPLC to CZE is in the increase of effectiveness due to better possibilities of automation and very low price of chemicals used. The method was applied to fish meat and sauerkraut samples.

2. Experimental

2.1. Sample preparation

A 20 g sample is homogenised and then shaken in a closed Erlenmeyer flask with 100–150 ml of 0.6 *M* $HClO_4$ for 1 h. The mixture is filtered through a filter paper, washed with $HClO_4$ and the volume is adjusted to 200 ml. Depending on expected amine content, 1–5 ml of acidic extract is transferred to a test tube, the volume is adjusted to 5 ml with $HClO_4$, spiked with 125 μl of internal standard solution (1,7-heptanediamine, 400 mg l^{-1}). The mixture is then made alkaline by adding 1 ml of 9.8 *M* NaOH solution. After briefly vortexing, 100 μl of pure benzoyl chloride is added. The test tube is shaken for 2.5 min. Then it is allowed to stand in the water bath of the laboratory ultrasound cleaner for 15–20 min. Subsequently 2.5 g of NaCl are shaken with the solution for 1 min. Then follows a two step extraction with 3 ml of diethyl ether. Combined extracts (1 ml in each step) are dried under a stream of hot air. The dry residue is dissolved in 400 μl of methanol–water (1:1, v/v).

2.2. Reagents

The amine standards were prepared from the following reagents: Putrescine dihydrochloride, cadaverine dihydrochloride, histamine dihydrochloride, tyramine hydrochloride, tryptamine hydrochloride, 1,7-heptanediamine, perchloric acid, sodium tetraborate (Fluka, Buchs, Switzerland); spermidine trihydrochloride, spermine tetrahydrochloride, benzoyl chloride 99%, sodium dodecyl sulphate 95% [L-5750] (Sigma, St. Louis, MO, USA); diethyl ether, sodium hydroxide, sodium chloride (Lachema, Neratovice, Czech Republic); methanol (Penta, Chrudim, Czech Republic). All chemicals of analytical grade. Deionized water was prepared with Premier (Premier Systems, Phoenix, AZ, USA). Standard solutions of biogenic amines were prepared at a concentration of about 400 mg l^{-1} in 0.6 *M* $HClO_4$.

2.3. Separation conditions

Analyses were carried out on Spectraphoresis 2000, a fully automated system for capillary zone electrophoresis equipped with a multi-wavelength UV–VIS scanning detector (Thermo Separation Products, Fremont, CA, USA). Separations were achieved in plain fused-silica capillary of 43 cm (36 cm effective length to the detector)×75 μm I.D. (CElect FS75 CE column, Supelco, Bellefonte, PA, USA). Data processing was performed using Spectacle and PC 1000 CE software version 3.0.

Samples were homogenised with either commercial food handblender (Philips) or Ultra-Turrax T25 homogeniser (Ika Labortechnik, Staufen, Germany).

Benzamides are UV absorbing substances, but they do not exercise apparent proteolytic character. On CZE apparatus they can be separated using micellar electrokinetic capillary chromatography.

Separations were performed in plain fused-silica capillary (43 cm×75 μm I.D., 36 cm to the detector). The applied voltage +15 kV resulted in an electrophoretic current of 48 μA and the temperature around the capillary was maintained constant by the Peltier system at 30±0.01°C. Injection of the samples was achieved by a 2.5 s vacuum application. Approximative volume of the sample injected was 15 nl. The separation buffer consisted of 15 m*M* sodium tetraborate, 40 m*M* SDS and 25% (v/v) methanol, pH 9.45. The wavelength of detection was set at 200 nm.

When a new capillary was used, it was washed for 1 h with 1.0 *M* NaOH at 30°C, followed by 1 h wash with deionized water at the same temperature. The capillary was prewashed for 3 min with running buffer before each injection and postwashed for 3 min with deionized water, 3 min with 0.1 *M* NaOH and 3 min with deionized water to maintain proper reproducibility of run-to-run injections. A simplified postwash procedure, 3 min rinsing with water

(30°C), was performed when reproducible migration times were observed during consequent analyses.

3. Results and discussion

The first step in the determination of amines in various materials including foods is usually the extraction. The first phase of the analytical procedure is the most critical in terms of obtaining adequate recoveries for all amines [5]. Next steps, derivatization and further separation of derivatives are not so closely linked with the kind of the sample matrix. If proper extraction resulting in quantitative isolation of amines is employed, next steps do not depend so much on the type of material analyzed. From this point of view it is not surprising to find a narrow group of acid chlorides to be applied in HPLC to a broad group of samples.

The choice of benzoyl chloride as derivatizing reagent has several advantages. Benzoyl chloride is an inexpensive, stable, easily accessible chemical and its purity is less critical than that of dansyl chloride. Benzamides are not sensitive to light, reaction proceeds at room temperature in alkaline media and no buffers are required [8]. It was reported, that with the exception of agmatine and spermidine, benzamides are stable for several months in methanol at −20°C [10]. We had no problems with the stability of dry benzamides when stored in the freezer for 2 weeks. Improvement in the derivatization efficiency by elevated reaction temperature and by dissolving benzoylchloride in methanol [27] was not observed. In accordance with Verkoelen et al. [28] vigorous mixing of the reagent solution resulting in the emulsification of benzoyl chloride, was found to be of critical importance to achieve optimal derivatization. Ultrasonication of the reaction mixture at the end of the derivatization process was very beneficial. In an ultrasonic bath, residues of benzoyl chloride are readily converted to benzoic acid and no important extra peaks interfere. Some authors [8,9] recommend addition of NaCl solution to the mixture prior to the organic solvent extraction. According to our experience, this step minimises water coextraction when diethyl ether is applied. When less polar organic solvent (e.g. chloroform) is used, addition of NaCl can be omitted [28].

In CZE the optimisation of the buffer composition plays the key role in the method development. There was a lot of buffer systems tested for the suitability for amines determination. Borate buffer exercising high electroosmotic flow in the capillary was found to be better than other media. The concentrations of either sodium tetraborate and SDS were changed ranging from 10 to 50 m*M*. The temperature range was from 25 to 35°C. The resolution of PUT, CAD, TR, SPD, and TY in the standard mixture was well in some borate–SDS buffer mixtures, but SPM and HI were never satisfactorily resolved. Addition of urea had no apparent influence on separation improvement. Another additive, methanol, shortened the migration time of SPM thereby making the complete separation possible. The pronounced influence of methanol addition on the SPM and HI migration is shown in Fig. 1. It should be pointed

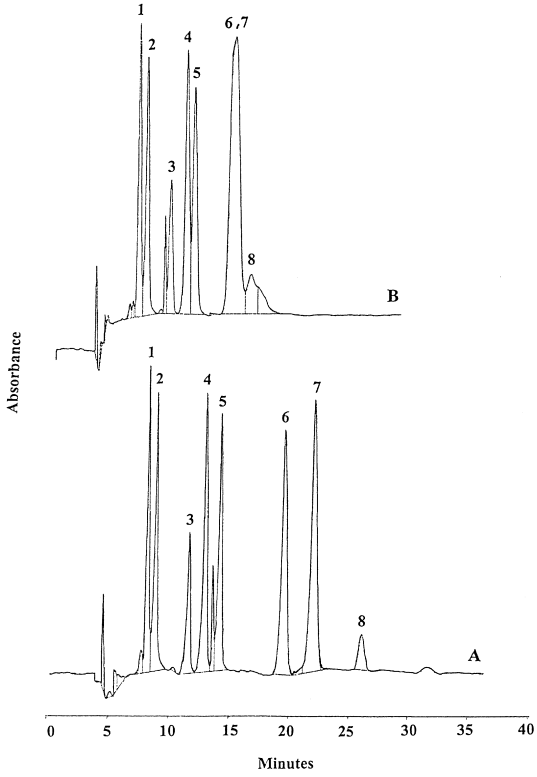

Fig. 1. The influence of addition of methanol to the aqueous buffer on compounds migration. (A) Methanol 20% (v/v), (B) methanol 15% (v/v). Numbers of amines: 1 - PUT, 2 - CAD, 3 - TR, 4 - SPD, 5 - HEP, 6 - SPM, 7 - HI, 8 - TY.

out, that the strong influence of organic modifier is demonstrated on a buffer mixture which was already optimised. There are systems without an organic modifier providing sufficient resolution though except for SPM and HI. The optimised buffer composition is described in Section 2.3. It is not surprising, that the migration order of derivatives of di- and polyamines strictly follows their polarity. Longer migration time of TY might originate in dissociated phenolic group in alkaline buffer. Figs. 2 and 3 show the separation of a standard mixture and an example of analysis of fish meat sample respectively. This sample represents a carp (*Cyprinus carpio*) muscle tissue stored at 15°C for four days. Concentrations of PUT and CAD above 10 mg kg^{-1} reveal just the moment of the onset of decomposition in this type of matrix. Contents of HI and TY are negligible from toxicological point of view.

Similarly to Michaelsen et al. [29] several method characteristics were calculated:

$$\mathrm{RMT} = t_m(\mathrm{X})/t_m(\mathrm{STD}) \quad (1)$$

where RMT is the relative migration time, t_m is the migration time, the index X means the actual amine and index STD represents the internal standard.

$$\mathrm{NPA} = A/t_m \quad (2)$$

where NPA is the normalised peak area and A is the measured peak area.

$$\mathrm{RNPA} = \mathrm{NPA(X)}/\mathrm{NPA(STD)} \quad (3)$$

where RNPA is the relative NPA and indexes X and STD have the same meaning as in the Eq. (1).

The repeatibilities of the method expressed as relative standard deviation (R.S.D.) of t_m, RMT and RNPA are shown in Table 1. As revealed from Table 1, the method performed satisfying results, with R.S.D. values of RMT below or near 1%, except for TY. The values of RNPA are acceptable, but some space for further improvement in TY reproducibility is still left. Due to small fluctuations in the electroosmotic flow velocity, the stability of migration times in CZE is usually worse than that of retention times in HPLC.

In spite of the fact that R.S.D. of t_m did not exceed 5% in seven consecutive analytical runs (with the exception of TY), the use of internal standard is beneficial as can be seen from the comparison of t_m and RMT R.S.D.s (Table 1). 1,7-Heptanediamine

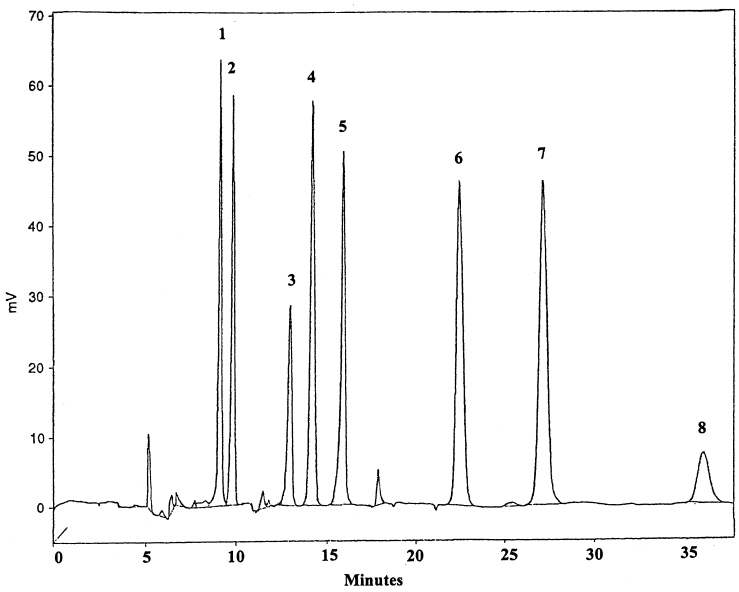

Fig. 2. Separation of standard mixture of biogenic amines as *N*-substituted benzamides. Conditions as in Section 2.3. Concentrations of the amines are in the methanolic solution for injection: PUT - 41.9 mg l^{-1}, CAD - 41.6 mg l^{-1}, TR - 40.9 mg l^{-1}, SPD - 41.6 mg l^{-1}, HEP - 42.9 mg l^{-1}, SPM - 41.9 mg l^{-1}, HI - 41.8 mg l^{-1}, TY - 41.9 mg l^{-1}. Numbers as in Fig. 1.

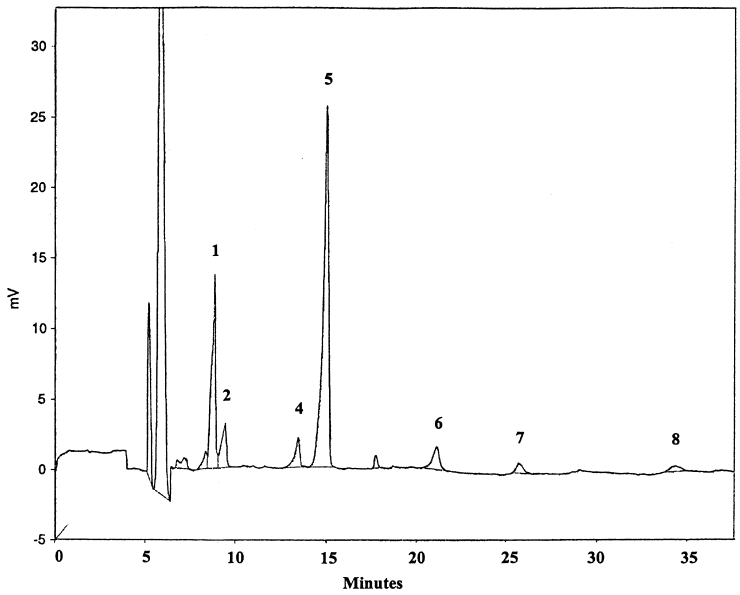

Fig. 3. Analysis of fish sample. Conditions as in Section 2.3. Content of amines in the sample: PUT - 41.7 mg kg^{-1}, CAD - 14.5 mg kg^{-1}, SPD - 6.36 mg kg^{-1}, SPM - 4.91 mg kg^{-1}, HI - 2.02 mg kg^{-1}, TY - 7.27 mg kg^{-1}. Numbers as in Fig. 1.

(HEP) seemed to be better internal standard for CZE than 1,6-hexanediamine often used in HPLC [26].

The relative response factors (RRF) were calculated from response factors (RF) as:

$$RF = NPA(X)/C(X) \quad (4)$$

$$RRF = RF(X)/RF(STD) \quad (5)$$

where C is the concentration of amine (mmol l^{-1}).

Table 2 shows the average values of t_m, RMT, RRF and N/m (number of theoretical plates per meter) calculated from five parallel determinations. The RRF values of diamines and polyamines fit well the theoretical expectations. The RRF values showed that the molar ratios of benzoyl chloride to di- and polyamines were 2.1 (for the diamines), 3.0 to SPD and 3.7 to SPM which indicates that all available amine groups were subject to benzoylation. This result is quite identical with that of Verkoelen [28]. Proposed detection wavelength 229 nm [28] was found acceptable when examining the derivatives spectra. At this wavelength a local maximum appears. We achieved higher sensitivity at 200 nm. No

Table 1
Repeatibility as relative standard deviation of t_m, RMT and RNPA values for biogenic amines

Amine	R.S.D. (%)		
	t_m	RMT	RNPA
PUT	1.62	0.78	2.33
CAD	1.67	0.78	2.58
TR	2.06	0.38	2.50
SPD	2.12	0.36	3.52
HEP (I.S.)	2.39	–	–
SPM	3.41	1.35	3.43
HI	4.43	1.42	4.16
TY	6.57	2.93	6.78

Values relative to 1,7-heptanediamine, seven repetitions.

Table 2
Average values of selected analysis parameters

Amine	t_m (min)	RMT	RRF	N/m
PUT	9.23	0.578	1.083	51 900
CAD	9.92	0.621	1.056	61 100
TR	13.05	0.818	0.840	53 600
SPD	14.27	0.894	1.482	58 400
HEP	15.97	1.000	1.000	68 500
SPM	22.76	1.425	1.863	49 400
HI	27.88	1.745	0.956	46 400
TY	35.82	2.367	0.238	35 700

Values are an average of five parallel determinations.

excessive baseline noise at this wavelength was observed. Spectral proximity of both diamines and polyamines was evident.

The N/m values are not big, but they are ten times higher as compared to HPLC separation of the same derivatives [26]. A pronounced increase of the N/m could be achieved in 50 μm I.D. capillary, but to the prejudice of sensitivity.

The linearity of the method was determined by using a standard containing seven biogenic amines at six concentration levels and with five repetitions. Linear regression analyses of RNPA values against concentrations provided data given in Table 3. The correlation coefficients range from 0.9931 to 0.9981, except for tyramine (0.9670). Recovery was tested by the standard addition procedure using one addition level for each amine. Five determinations were carried out for sauerkraut sample. Samples were spiked by 400 μg of each amine.

Detection limits in the solution for injection (DL) of each derivative were calculated from the sensitivity of response estimating standard deviation as follows

$$D = t_{(n-1,0.05)}\sigma/\sqrt{n}.\mathrm{d}C/\mathrm{d}R \quad (6)$$

$$\mathrm{DL} = 2D_{\mathrm{avg.}} \quad (7)$$

where D is the detection power and $D_{\mathrm{avg.}}$ is the average value of D calculated from different concentrations (detection limits were defined as two times the detection power), $t_{(n-1,0.05)}$ the t-distribution at 95% reliability, σ the standard deviation of the response, n the number of replicates (five), C the concentration of the amines and R the detector response [17]. The detection limits (DL) recalculated with respect to the way of sample preparation (Section 2.3) result in $\mathrm{DL_S}$ values, where $\mathrm{DL_S}$ means the detection limit in food sample. In this calculation 2.5 ml of acidic extract is taken into account (Table 3). The $\mathrm{DL_S}$ values can be lowered in some extent by modifying the mass/volume ratios in sample preparation, but for food samples it does not seem to be necessary.

Table 3
Linearity, recovery and detection limits

Amine	r	R	R.S.D. (%)	DL ($\mathrm{mg\,l^{-1}}$)	$\mathrm{DL_S}$ ($\mathrm{mg\,kg^{-1}}$)
PUT	0.9934	89.5	4.35	0.44	2.1
CAD	0.9947	95.3	1.51	0.29	1.4
TR	0.9981	101.8	2.03	0.27	1.3
SPD	0.9980	94.7	1.58	0.21	1.0
SPM	0.9962	93.6	1.93	0.29	1.4
HI	0.9931	93.2	3.17	0.43	2.1
TY	0.9670	88.7	8.82	0.73	3.5

r - correlation coefficient; R - recovery [calculated from five parallel determinations of sauerkraut samples]; R.S.D. - relative standard deviation [calculated from five parallel determinations]; DL - detection limit in the injected solution; $\mathrm{DL_S}$ - detection limit in a sample.

During experiments it was found that tyramine exercises worse separation parameters as compared with other amines. Worse recovery of TY might originate in phenolic group of this compound thus lowering the extractability of its derivative from alkaline media. The calibration curves were found to be linear up to the concentrations of 500 $\mathrm{mg\,kg^{-1}}$ (for TY only to 100 $\mathrm{mg\,kg^{-1}}$). This observation should be taken into account in sample preparation.

4. Conclusions

The determination of biogenic amines by the MECC method is easy in sample preparation and gives good results. Derivatization of amines avoids their sorption on capillary inner wall that is critical especially in di- and polyamines. Benzamides are not sensitive to light and in the freezer they can be stored for at least 2 weeks. As derivatives especially that of diamines are chemically similar it was beneficial to increase the partitioning effect by adding an organic modifier to the buffer system. The best effect was obtained with methanol. The optimised method gives complete separation of the most important biogenic amines within 35 min and the efficiency (N/m) is ten times higher as compared with HPLC separation [26].

Acknowledgements

This work was supported by the grant of the Czech Grant Agency No. 203/96/0316. This work forms a part of the project COST 917.

References

[1] S. Bardocz, Trends Food Sci. Technol. 6 (1995) 341.
[2] B. ten Brink, C. Damink, H.M.L.J. Joosten, J.H.J. Huis in t Veld, Int. J. Food Microbiol. 11 (1990) 73.
[3] S.A. Slorach, Handbook of Experimental Pharmacology, Vol. 97, Springer, Berlin, 1991, p. 511.
[4] S.L. Rice, J. Milk Food Technol. 39 (1976) 353.
[5] S. Moret, L.S. Conte, J. Chromatogr. A 729 (1996) 363.
[6] W. Ziegler, M. Hahn, P.R. Wallnoefer, Deut. Lebensm. Rundsch. 90 (1994) 108.
[7] O. Busto, Y. Valero, J. Guasch, F. Borrull, Chromatographia 38 (1994) 571.
[8] J.W. Redmond, A. Tseng, J. Chromatogr. 170 (1979) 479.
[9] H.E. Flores, A.W. Galston, Plant Physiol. 69 (1982) 701.
[10] R.D. Slocum, H.E. Flores, A.W. Galston, L. Weinstein, Plant Physiol. 89 (1989) 512.
[11] M. Křížek, Arch. Anim. Nutr. 43 (1993) 169.
[12] M. Křížek, V. Hlavatá, Kvasny Prum. 41 (1995) 265.
[13] A.F. Tiburcio, R. Kaur-Sawhney, A.W. Galston, Plant Cell Physiol. 29 (1988) 1241.
[14] L. Simon-Sarkadi, A. Kovacs, E. Mincsovics, J. Planar Chromatogr. 10 (1997) 59.
[15] L. Simon-Sarkadi, W.H. Holzapfel, Z. Lebensm.-Unters. Forsch. 200 (1995) 261.
[16] M.L. Izquierdo-Pulido, M.C. Vidal-Carou, A. Marine-Font, J. AOAC Int. 76 (1993) 1027.
[17] H. Yamanaka, K. Shiomi, T. Kikuchi, J. Food Hyg. Soc. Japan 30 (1989) 170.
[18] H. Kataoka, S. Shindoh, M. Makita, J. Chromatogr. A 695 (1995) 142.
[19] B. Mopper, C.J. Sciacchitano, J. AOAC Int. 77 (1994) 881.
[20] J.M. Gallardo, C.G. Sotelo, R.I. Perez-Martin, Z. Lebensm.-Unters. Forsch. 204 (1997) 336.
[21] W.Ch. Lin, Ch.E. Lin, E.C. Lin, J. Chromatogr. A 755 (1996) 142.
[22] R. Zhang, C.L. Cooper, Y. Ma, Anal. Chem. 65 (1993) 704.
[23] I. Rodriguez, H.K. Lee, S.F.Y. Li, J. Chromatogr. A 745 (1996) 255.
[24] G. Nouadje, M. Nertz, P. Verdeguer, F. Couderc, J. Chromatogr. A 717 (1995) 335.
[25] G. Nouadje, N. Siméon, F. Dedieu, M. Nertz, P. Puig, F. Couderc, J. Chromatogr. A 765 (1997) 337.
[26] M. Křížek, Arch. Anim. Nutr. 41 (1991) 97.
[27] S. Asotra, P.V. Mladenov, R.D. Burke, J. Chromatogr. 408 (1987) 227.
[28] C.F. Verkoelen, J.C. Romijn, F.H. Schroeder, J. Chromatogr. 426 (1988) 41.
[29] S. Michaelsen, P. Moeller, H. Soerensen, J. Chromatogr. 608 (1992) 363.