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Journal of Chromatography A, 790 (1997) 177–183

JOURNAL OF CHROMATOGRAPHY A

Determination of histamine and some other amines by high-performance capillary electrophoresis with on-line mode in-capillary derivatization

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Received 8 April 1997; received in revised form 23 June 1997; accepted 24 June 1997

Abstract

We have developed a method for the determination of histamine (His), tyramine (Tyr) and cadaverine (Cad) using high-performance capillary electrophoresis with fluorescence detection and an on-line mode in-capillary derivatization with *o*-phthalaldehyde (OPA)/N-acetylcysteine (NAC) as derivatization reagent. HPCE separation of His, Tyr, Cad and Spermidine (Spd) was influenced by sodium dodecyl sulfate (SDS) and phosphate–borate buffer (pH 10) concentration. After optimization of the method, a 4-component amine solution containing His, Tyr, Cad and Spd could be separated and detected by using 2 m*M* OPA/NAC–20 m*M* SDS–20 m*M* phosphate–borate buffer (pH 10) as a run buffer at an applied voltage of 25 kV, with detection at 340 nm. Although a practical sensitivity level can be obtained by using fluorescence detection (λ_{ex}=340 nm, λ_{em}=450 nm) instead of ultraviolet–visible detection, Spd was not detected at all. The precision (relative standard deviation; n=15) of this method for within- and between-days is less than 2.9% (peak area) and 1.3% (migration time), respectively. Linearity for these analytes, except for Spd, was established over a concentration range of 0.02 to 1.00 μmol/ml and detection limits (S/N=3) range from 1 nmol/ml for His and Tyr to 5 nmol/ml for Cad. The determination of His and some amines in aging raw fish meat samples (room temperature, 48 h) was carried out using the described method with fluorescence detection.

Keywords: Derivatization, electrophoresis; Food analysis; Histamine; Tyramine; Cadaverine; Amines

1. Introduction

Histamine (His), tyramine (Tyr), cadaverine (Cad), spermidine (Spd) and some other amines exist widely in many foods and biological materials [1,2]. His is well known as an inflammatory substance released from mast cells, and His and some other amines are produced by enzymatic degradation of amino acids during putrefaction or fermentation of food. Some of them are also known to potentiate His toxicity and to act synergistically in allergy-like food poisoning caused by the intake of His contaminated foods. Their levels are shown to be a good index of food decomposition. A number of investigations have dealt with the development of methods for the analysis of His and other related amines by using enzyme isotope assay [3], gas chromatography (GC) [4], thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) [5]. Food hygiene chemists in Japan sometimes carry out determinations of His or other amines in food according to the official standard methods estab-

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0021-9673/97/$17.00
PII S0021-9673(97)00719-X

lished by the Pharmaceutical Society of Japan [6]. This method includes a HPLC technique using a pre-column derivatization with 5-(dimethylamino)-1-naphthalene-sulfonyl chloride (Dansyl-Cl). It is well known that a pre-column derivatization method requires a batchwise operation and post-column method necessitates extra attachments to the standard HPLC system. On the other hand, high-performance capillary electrophoresis (HPCE) has become a useful and powerful separation technique. However, derivatization methods in HPCE are limited to pre-column methods which require batchwise operations when amines are determined. In order to obtain reliable data, batchwise operations for tagging free amino compounds are at a disadvantage. Recently, we have developed a method named ''on-line mode in-capillary derivatization '' as an alternative derivatization method for HPCE [7,8]. The on-line mode in-capillary derivatization HPCE method involves simultaneous separation and derivatization in a capillary filled with a run buffer containing a derivatization reagent such as 1-methoxycarbonylindolizine-3,5-dicarbaldehyde (IDA) [7], or *o*-phthalaldehyde (OPA)/N-acetylcysteine (NAC) [8].

In this paper, we studied the determination of His and some other related amino compounds, Tyr, Cad and Spd using an on-line mode in-capillary derivatization HPCE method with OPA/NAC as a labeling reagent linked either with ultraviolet–visible (UV–Vis) detection or fluorescence detection. The migration behavior of each amine in a capillary was studied and the separation of these amines with an on-line mode in-capillary derivatization HPCE method was optimized. The simultaneous determination of these amines in aging samples (room temperature, 48 h) of seafoods is also described.

2. Experimental

2.1. Reagents and materials

Sodium dodecyl sulfate (SDS) and OPA and NAC were of biochemical and superfine reagent grade, respectively. These reagents were purchased from Wako, Tokyo, Japan, and used without further purification. The other reagents used were of the highest or HPLC grade commercially available. All aqueous solutions were prepared by using water purified with a Milli-Q purified system (Millipore, Milford, MA, USA). His, Tyr and Cad and Spd (Wako) were each dissolved in water to make 0.1 m*M* and 4 m*M* solutions. The 4-component amine standard solution (the concentration of each amine was the same as that in the individual amine solution) was also prepared by dissolving in water. The run buffers were prepared as follows. The pH of each phosphate–borate buffer (mixing equal molar amounts of sodium dihydrogenphosphate and sodium tetraborate) with concentrations of 10, 20, 40 and 60 m*M* was adjusted to pH 10 with 1 *M* NaOH. These buffer solutions were stored at room temperature prior to use. Then, equal molar amounts of OPA and NAC were added to a phosphate–borate buffer solution giving a 2 m*M* solution with an SDS concentration of 5, 10, 15, 20 or 25 m*M*. A portion of each solution was filtered with disposable syringe filter unit DISMIC-13cp (Advantec, Tokyo, Japan). These run buffers were prepared just before use.

2.2. Apparatus

The HPCE system consisted of a Jasco Model CE-800 (Jasco, Tokyo, Japan) equipped with an UV–Vis detector Model CE-971-UV (Jasco) or with a FP-920 fluorescence detector (Jasco), with the flow cell-unit for HPLC replaced by a capillary cell-unit for HPCE. All data were printed with an intelligent data processor Model 807-IT (Jasco). A capillary tube of fused-silica (75 cm effective length$\times$50 μm I.D.) was used throughout the work. The window (1.0 cm for the fluorescence detector and 0.5 cm for the UV detector, respectively) for detection was made by removing the polyimide coating at a position 25 cm from the cathodic end. Sample solutions were introduced into the capillary tube from the anodic side by hydrostatic injection by raising the tube 10 cm higher than the level of the cathodic electrode for 10 s. The electropherograms were recorded by monitoring the fluorescence intensity at 450 nm (excitation at 340 nm) or absorbance at 340 nm. The applied voltage was 25 kV throughout the work.

2.3. On-line mode in-capillary derivatization HPCE

Two reservoirs were placed at the anodic site and

the cathodic site in the HPCE system. The anodic and cathodic reservoir contained 1 ml of run buffer containing 2 m*M* OPA/NAC and 10 ml of 60 m*M* phosphate–borate buffer (pH 10), respectively. Before a sample solution was injected into a capillary at the cathodic site, any remaining residue in the capillary was swept out with a run buffer. The capillary was subsequently filled with the same buffer by suction at the anodic site of the capillary. When the HPCE system was not in use, the run buffer in the capillary and in both reservoirs was replaced with 20 m*M* phosphate–borate buffer (pH 10) or water.

2.4. Extraction of His and some amines from food samples

Extraction of His and some other amines in food samples was carried out according to the authentic standard method, ''Standard Methods of Analysis for Hygiene Chemists'' [6], as follows. A 5 g amount of sample was homogenized with 20 ml of water and 10 ml of hydrochloric acid, then diluted to 50 ml with water. A 5 ml portion of the supernatant was neutralized with 1 *M* sodium hydroxide (pH 6–7), followed by addition of 25 ml of 0.1 *M* sodium acetate buffer (pH 5.6). The solution was applied on an Amberlite CG-50 (Type I, 100–200 mesh; Organo, Tokyo, Japan) ion-exchange column (10×1.0 cm I.D.) which was equilibrated with 0.1 *M* sodium acetate buffer. After 80 ml of 0.025 *M* sodium acetate buffer (4 times dilution of 0.1 *M* sodium acetate buffer) had passed through the column, amines were eluted with 1 *M* hydrochloric acid. A 20 ml fraction was collected and neutralized with 1 *M* sodium hydroxide, then diluted to 50 ml with water in a volumetric flask.

3. Results and discussion

3.1. Comparison of UV–Vis detection and fluorescence detection

To compare UV–Vis detection and fluorescence detection for the 4-component amine standard solution using an on-line mode in-capillary derivatization HPCE method with 2 m*M* OPA/NAC–20 m*M* SDS–20 m*M* phosphate–borate buffer (pH 10) as a run buffer, amines were analyzed under the same HPCE conditions in a system that was equipped either with an UV detector or a fluorescence detector. Fig. 1 shows typical electropherograms obtained by using UV–Vis detection (Fig. 1A) and fluorescence detection (Fig. 1B) (printed with the intelligent integrator at 16 mV full scale level). In the case of His,

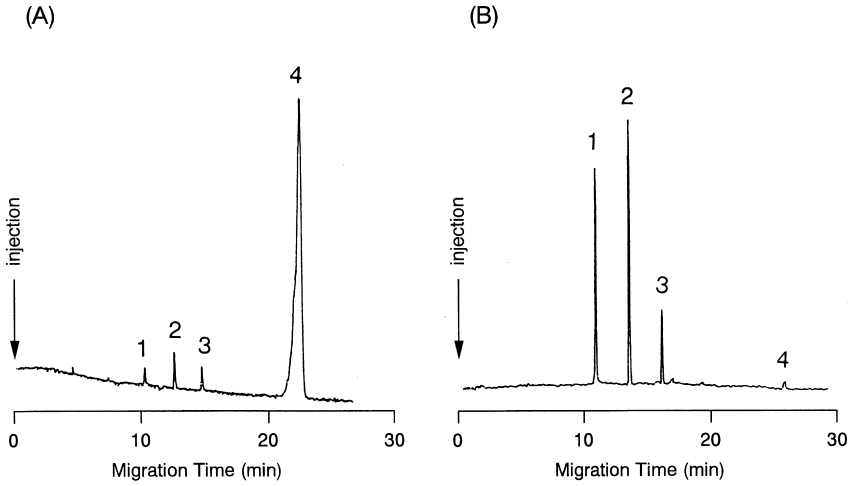

Fig. 1. Both electropherograms of the 4-component amine solution were recorded at 16 mV full scale with (A) ultraviolet detection (λ=340 nm) and (B) fluorescence detection (λ_{ex}=340 nm, λ_{em}=450 nm). The run buffer is 2 m*M* OPA/NAC–20 m*M* SDS–20 m*M* phosphate–borate buffer (pH 10). The other HPCE conditions for the on-line mode in-capillary derivatization method are given in Section 2.3. Peaks: 1=His, 2=Tyr, 3=Cad, 4=Spd.

Tyr and Cad fluorescence detection gave about a ten-times stronger response compared to UV–Vis detection. However, fluorescence intensity of Spd is remarkably diminished when compared with absorbance. It is not clear why the fluorescence intensity of Spd is remarkably poor. However, it is generally well known that cystine–OPA/2-mercaptoethanol (2ME) derivative shows only about 5% of the fluorescence of other natural amino acids. On the other hand, Benson and Hare [9] overcame this shortcoming by increasing the concentration of 2-ME 10-fold. In this system, intensity might be improved if the concentrations of NAC in the run buffer was increased or if 2ME was used instead of NAC. Although Spd is one of the important tumor marker amines [10], the main purpose of this paper is a determination of His using on-line mode in-capillary derivatization HPCE, and thus we have not investigated Spd any further because good response signals of His have been obtained.

3.2. Optimization of HPCE separation and migration behavior

In the determination of His and some other amines by using HPCE with an on-line mode in-capillary derivatization, the pH of the run buffer is an influencing factor because the run buffer acts both as a separation buffer and derivatization buffer. OPA/NAC was used as the derivatization reagent in this studies, and the pH of the run buffer was fixed at 10 which was the optimum pH for this reagent. The run buffer (sodium dihydrogenphosphate and sodium tetraborate) used in this study was selected following a consideration of His's peak response and the efficiency of separation of His from other amines on a HPCE electropherogram. Concentrations of the reagents OPA and NAC were also fixed at 2 m*M*. Some additives such as SDS, β-cyclodextrin (β-CD), ion-pairing reagent, urea and organic solvent were examined by addition to the run buffer at pH 10. After testing these additives, SDS was deemed the most effective for use in the on-line mode in-capillary derivatization HPCE method. The optimization of HPCE separation and migration behavior were studied using a 4-component amine standard solution. The migration parameters of the analytes were expressed as migration time, not as capacity factor because there were some cases where it was difficult to recognize the migration time corresponding to the aqueous and micellar phases on the electropherogram. All of these studies were carried out with UV–Vis detection.

3.2.1. Effect of SDS concentration

The effect of SDS concentration on migration time of each amine was investigated at concentrations of 0, 5, 10, 15, 20 and 25 m*M* SDS while maintaining the buffer concentration at 20 m*M* (pH 10) and containing 2 m*M* OPA/NAC. The results are shown in Fig. 2. Although the separation of each analyte is much improved by adding SDS to the run buffer, the baseline noise of the electropherogram increased with increasing concentration of SDS. The optimized concentration of SDS was found to be 20 m*M*.

3.2.2. Effect of buffer concentration

The effect of the concentration of phosphate–borate run buffer at pH 10 on the separations was examined at 10, 20, 40 and 60 m*M* while keeping 20 m*M* SDS in the run buffer. As shown in Fig. 3, the migration order of the amines remains unchanged, and separation is much improved with increasing concentration of the buffer. As the result of the studies, the optimized concentration of phosphate–borate buffer is 20 m*M*.

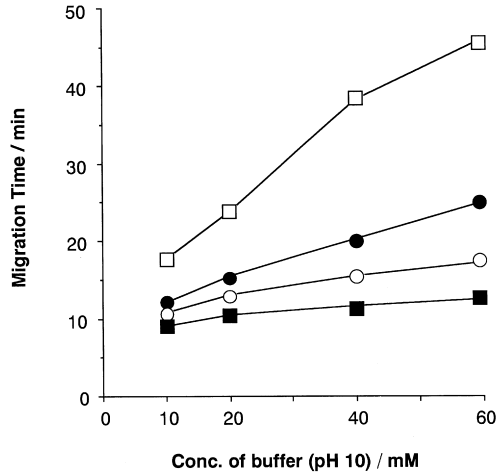

Fig. 2. Effect of SDS concentration on migration time. Keys: ■=His, ○=Tyr, ●=Cad, □=Spd. The other HPCE conditions are the same as in Fig. 1A.

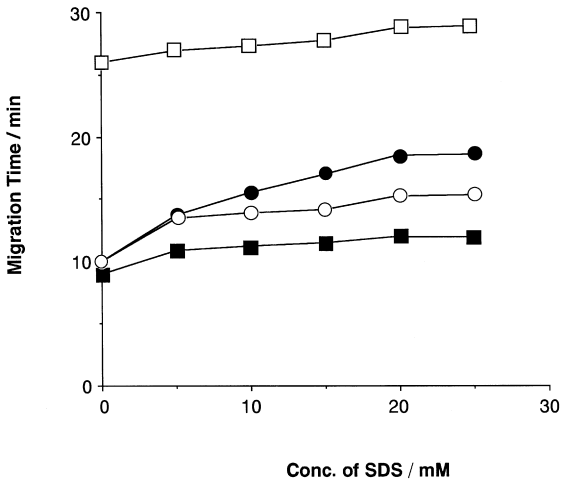

Fig. 3. Effect of phosphate–borate buffer (pH 10) concentration on migration time. Other conditions as in Fig. 2.

On the basis of these data, subsequent experiments were carried out by using 2 m*M* OPA/NAC–20 m*M* SDS–20 m*M* phosphate–borate buffer (pH 10), and the amines, except for Spd, were detected with fluorescence detection because a practical sensitivity for Spd can only be obtained by with UV–Vis detection.

3.3. Analytical reproducibility

Analytical reproducibility, i.e., peak-area count and migration time, was investigated by a daily series of five identical injections of a 4-component amine standard solution over a 3 day period. Spd was not studied because it was hardly detected with fluorescence detection (Fig. 1A). The reproducibilities of peak-area response count and migration time of each amine within- and between-days were less than 2.9% and 1.3% R.S.D. (relative standard deviation) at 0.1 μmol/ml, respectively. These data are listed in Table 1.

3.4. Linearity of peak-area response and detection limit

The linearity of the peak area for each amine over the concentration range 0.02–1.00 μmol/ml was calculated by least squares regression method for $y=ax+b$, where y is peak area and x is concentration of polyamine. Correlation factors (r) for His, Tyr and Cad were 0.999, 0.999 and 0.979, respectively, indicating relatively good linearity. The detection limits were obtained from a signal-to-noise ratio (S/N) of 3, and ranged from 1 nmol/ml for His and Tyr to 5 nmol/ml for Cad. These parameters are listed in Table 2.

Based on these data, the lower limit of quantitation (LOQ) is about 0.02 μmol/ml and the present method gives sensitive and reproducible assay data of His and other amines.

3.5. Applications

Before an on-line mode in-capillary derivatization HPCE method can be performed for determination of

Table 1
Reproducibility of the assay within- and between-days

Day	Peak area counts ($\cdot 10^5$)			Migration time (min)		
	His	Tyr	Cad	His	Tyr	Cad
1	1.30±0.016	1.34±0.019	0.57±0.008	12.5±0.029	15.5±0.044	18.76±0.035
2	1.29±0.019	1.32±0.018	0.57±0.009	12.7±0.014	15.4±0.061	18.45±0.033
3	1.26±0.015	1.31±0.024	0.54±0.008	12.9±0.033	15.8±0.019	18.75±0.020
Mean±S.D.	1.28±0.026	1.32±0.025	0.56±0.016	12.9±0.161	15.8±0.205	18.7±0.129
R.S.D.[a] (%)	2.0	1.9	2.9	1.2	1.3	0.69

Each value represents mean±S.D. (standard deviation, $n=5$). Concentration of each amine is at the 0.1 μmol/ml level. HPCE and other experimental conditions as in Fig. 1B.
[a] Relative standard deviation ($n=15$).

Table 2
Linearity of peak area response and detection limit

Amines	Linearity[a] ($\cdot 10^3$)			Detection limit (nmol/ml)
	a	*b*	*r*	(*S*/*N*=3)
Histidine	1475.9	−0.34	0.999	1
Tyramine	1335.1	0.089	0.999	1
Cadaverine	235.4	0.85	0.979	5

[a] *y* (peak area count)=*ax* (concentration of amine; 0.02–1.00 μmol/ml)+*b*. *r*=Correlation factor. HPCE and other experimental conditions as in Fig. 1B.

Table 3
Histamine (His), tyramine (Tyr) and cadaverine (Cad) content in seafood

Sample[a]	Amine (mg/g)		
	His	Tyr	Cad
Mackerel	2.89	n.d.[b]	15.3
Tuna	4.87	19.7	n.d.[b]
Saury	3.11	n.d.[b]	22.0

[a] Samples were allowed to stand at room temperature for 48 h.
[b] n.d.=Not detected (Tyr<10 μg/g, Cad<50 μg/g).
HPCE and other experimental conditions as in Fig. 1B.

His, Tyr and Cad in some seafoods, a sample extraction procedure is necessary for some seafoods (see Section 2.4). Although the efficient food sample preparation method used here is authorized officially by the Pharmaceutical Society of Japan, it was reevaluated by adding 1 μmol/g (or ml) amounts of His, Tyr and Cad to 5 ml of water and to 5 g of raw tuna meat, respectively. After cleanup steps, a portion of extracted solution including His and some other amines was directly introduced into the capillary in the HPCE system. The recovery of each amine from spiked water and meat sample was more than 97% and 91%, respectively. The accuracy of the present method is also less than 3.2% R.S.D. (n=3). These data are sufficient for determinations of His and some other amines in seafood samples. Fig. 4 shows the electropherograms for raw fish meat (mackerel, tuna and saury) purchased from a neighborhood supermarket, that was stored at room temperature for 48 h. Although a peak of His is detected in all samples, peaks for Tyr or Cad may be present or not, as shown in Fig. 4. These differences in amine content in each food are also easily identified as listed in Table 3.

4. Conclusions

In a previous paper, we developed the on-line mode in-capillary derivatization HPCE method with IDA or OPA/NAC as a derivatization reagent. Here, we tested and applied the method using an OPA/NAC reagent on determinations of His and some other amines. Although Spd is not detected at a practical concentration level using fluorescence detection, a high sensitivity and a good reproducibility for the direct determination of His and some other

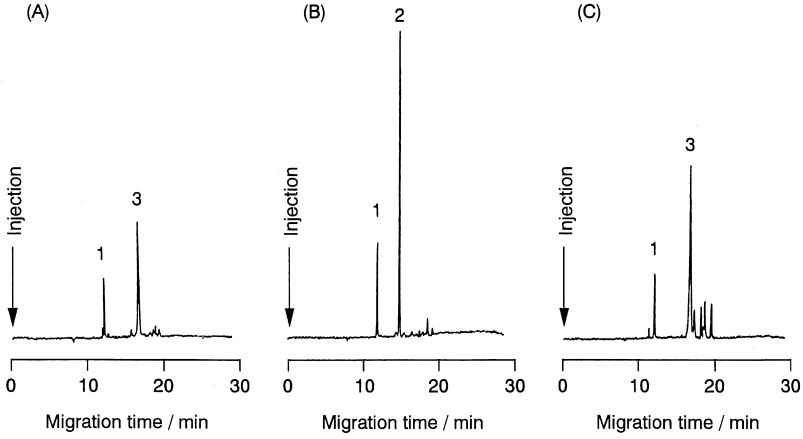

Fig. 4. Typical electropherograms of (A) mackerel, (B) tuna and (C) saury. These seafood samples had been standing at room temperature for 48 h. Other HPCE conditions as in Fig. 1B.

amines (Tyr and Cad) can be attained without clean-up and without batchwise operations prior to HPCE analysis. This method is applicable to the analysis of His, Tyr and Cad in an extracted solution from spoiled foods.

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