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Simultaneous determination of food-related biogenic amines and precursor amino acids by micellar electrokinetic capillary chromatography with electrochemical detection

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Abstract

A simple and reproducible method, based on micellar electrokinetic capillary chromatography (MECC) with amperometric detection (AD), for the separation and determination of the biogenic amines and their precursor amino acids was studied in this paper. The optimal conditions of separation and detection of typtamine, tyramine, tryptophan and tyrosine were 0.020 mol l^{-1} borate–NaOH (pH 10.35) containing 0.03 mol l^{-1} sodium dodecylsulphate (SDS) as running buffer, 20 kV as separation voltage, and +800 mV (vs. SCE) as detection potential. Under the optimum conditions, the four analytes were separated completely within 15 minutes, and good linearity, reproducibility and recovery results were obtained. Based on three times standard deviation of a low level sample, the detection limits for the four analytes were as low as at 10^{-7} mol l^{-1} level. This method was also successfully used in the analysis of actual rice spirit, and satisfactory assay results were obtained.

Keywords: Micellar electrokinetic capillary chromatography; Amperometric detection; Biogenic amines; Amino acids

1. Introduction

Biogenic amines (BAs) play important roles in many human physiological functions, but high concentrations of these compounds contained in food may cause toxic effects in susceptible individuals. Among these amines, tyramine is the principle substance responsible for migraine, and LSD-like physiological disease (Baker, Wong, Coutts, & Pasutto, 1987). Besides formed and degraded as a result of normal metabolic activity in animals, plants and microorganisms, BAs can be produced by the decarboxylation of precursor amino acids. Once BAs are formed, they are difficult to be destroyed by pasteurization or cooking. Therefore, it's important to control manufacture environment strictly during food ripening process so that the concentrations of the Bas can be decreased as low as possible. In order to examine the influence of different environment factors, the quantification correlation of Bas and their precursor amino acids should be found accurately and immediately. Thus, it's necessary to establish a simple, reliable and convenient method determining BAs and their precursor amino acids simultaneously.

Some methods have been developed for the analysis of these amines in body tissues and foodstuffs (Buteau, Duitschaever, & Ashton, 1984; Hayman, Gray, & Evans, 1985; Van Boekel & Arensten-Stasse, 1987), such as gas chromatography (Yamamoto, Itano, Kataoka, & Makita, 1982), Ion-exchange chromatography (Sayem-El-Daher, Simard, L'Heureux, & Roberge, 1983) and high-performance liquid chromatography (HPLC) (Auger, Boulag, Jaillais, & Delion-Vancassel, 2000; Remero, Gazquez, Bagur, & Sanchez-Vinas, 2000; Standara, Vesela, & Dradak, 2000). In recent years, capillary electrophoresis (CE) has been shown to be a powerful technique for amino analysis for its easy operation, low sampling volume and high separation efficiency (Agnes,

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Simon-Sarkadi, & Ganzler, 1999; Nouadje, Simeon, Dedieu, Nertz, Puig, & Couderc, 1997; Oguri, Watanabe, & Abe, 1997; Wang, Yang, & Mo, 2000; Zhou, Yu, Ma, Xue, Zhang, & Lin, 1995). Usually, UV, LIF, MS and electrochemistry are used as detection means together with CE separation. For UV detection, the operation is simple but the detection limit is only at about ppm level; For LIF detection, it is very sensitive but suffers from expensive instrument and complicated procedure including pre- or post- column derivation; The MS instrument is also very expensive. However, electrochemical detection (ED) is less expensive than LIF and MS and shows higher sensitivity and selectivity than UV. Furthermore, ED is very suited for CE because the detection is compatible with the small dimensions of the capillary. However, simultaneously determination of amines and their precursor amino acids by simple CE-ED method has not been yet reported.

This experiment presented the first attempt to simultaneously determine two food-related BAs (typtamine, tyramine) and their precursor amino acids (tryptophan, tyrosine) by micellar electrokinetic capillary chromatography separation with amperometric detection (MECC-AD). In MCCE, the addition of SDS significantly improved the resolution of amino acids. Good linear relationships of the four analytes were obtained in the concentration range from 10^{-6} to 10^{-3} mol l^{-1}. By using three times the standard deviation of a low level sample, the instrumental detection limits for the four analytes were calculated as low as about 10^{-7} mol l^{-1}, which were low enough and ensured this method practical when it was used in monitoring BAs and their precursor amino acids during the fermentation or deterioration process of foods. This method was also successfully used in the analysis of actual rice spirit, and satisfactory assay results were obtained. The results showed that this method was simple, reliable, convenient and very potential to be used in the biological research of BAs and their precursor amino acids.

2. Experimental

2.1. Apparatus

A laboratory-constructed capillary electrophoresis system equipped with wall-jet amperometric detection (Fang, Fang, & Ye, 1995) was employed in our research work. The separations were carried out in a polyimide-coated fused silica capillary (360 μm OD×25 μm ID × 70 cm, Polymicro Technologies, Phoenix, AZ). A high-voltage power supply (Shanghai Institute of Atomic Nuclei, Shanghai, China) was used to provide the required voltage (0 to ±30 kV).

A carbon-disc working electrode was prepared by using a 300 μm diameter pencil lead (Fang, Ye, & Fang, 1996). Prior to use, the carbon disk electrode was firstly polished with emery paper and 0.05-μm alumina powders, then ultrasonicated in deionized water, and finally carefully positioned opposing the capillary outlet with the aid of a micromanipulator.

The electrochemical cell was composed of a platinum auxiliary electrode, a carbon disk working electrode and a SCE reference electrode. A BAS LC-3D amperometric detector (Bioanalytical System, West Lafaye, IN, USA) provided potential control and current output. A chart recorder (XWTD-164, Shanghai Dahua Instrument Factory, China) was used to obtain the electropherograms.

2.2. Materials and methods

Tryptamine (trpa), tyramine (tyra), tryptophan (trp), and tyrosine (tyr) were kindly supplied by Shanghai Second Pharmaceutical Factory (Shanxi, China) and Shanghai Dazhong Pharmaceutical Factory (Shanghai, China) respectively, and were used without further purification. The detected sample was rice spirit, which is a kind of wine brewed by rice and wheat (Zhejiang Huzhou Huanzhong Brewage Co. Ltd., China, manufacturing date: 1 June 2001). Other chemicals were of analytical reagent grade.

An accurately weight of tryptamine, tyramine, tryptophan, and tyrosine were firstly dissolved with appropriate amount of water separately, then diluted with the buffer solution to obtain the stocking solutions with a concentration of 5.0×10^{-4} mol l^{-1}. A borate–NaOH buffer solution (*pH* 10.35) was prepared by mixing 0.1 mol l^{-1} sodium tetraborate and 0.05 mol l^{-1} sodium hydroxide. 0.03 mol l^{-1} SDS-containing carrier was prepared by adding the required amount of SDS to the buffer.

The sample of rice spirit was prepared simply by dilution with the running solution (1/5 v/v). All experimental solutions including sample solutions were filtered through a polypropylene filter (0.22 μm) and degassed by ultrasonic prior to use.

2.3. Procedure

Before the first run, the capillary was repeatedly pretreated with 0.5 mol l^{-1} NaOH, and the run buffer 10 min for each using a laboratory-built high-pressure system. After every fifth run, the capillary was washed with 0.05 mol l^{-1} NaOH and the run buffer 4 min for each to ensure a constant electro-osmotic flow.

The optimum separation conditions were: CE separation voltage 20 kV, a 0.02 mol l^{-1} borate–NaOH containing 0.03 mol l^{-1} SDS run buffer at pH 10.35, detection potential +800 mV (vs. SCE), injection time 10 s at 20 kV. All experiments were performed at room temperature about 22 °C.

3. Results and discussion

3.1. Selection of working electrode and hydrodynamic voltammograms

In MECC-AD analysis, it is very important that a suitable working electrode is selected. In this experiment, the carbon disc electrode (pencil lead) and the carbon-paste electrode were investigated as working electrodes. The results showed that the carbon-disc electrode was better than the carbon-paste electrode in the current responses of the four analytes in our experiment under the same conditions. So the carbon-disc electrode was selected as the working electrode.

Typical cyclic voltammograms for the four analytes are shown in Fig. 1. In blank borate–NaOH solution (pH 10.35), no anodic response was observed in the potential range of 0.00–1.00 V. After the separate addition of the four analytes to the solution, four anodic peaks were observed at 0.59, 0.41, 0.45, and 0.58 V corresponding to tryptophan, tyrosine, tyramine, and tryptamine, respectively. Thus, it was apparent that all four analytes could be oxidized when the work potential was above 0.59 V. Fig. 2 shows the hydrodynamic voltammograms for tryptamine, tyramine, tryptophan, and tyrosine. When the potential was lower than +600 mV, tryptamine and tryptophan couldn't be detected out, whereas the potential was greater than +600 mV, their peak currents increased with the increase of potential, especially the peak currents of tryptamine. But when the potential was above +900 mV, both the base current and the noise increased promptly. Thus +800 mV was selected as working potential, where the base current was not too high and the working electrode could maintain both constancy and reproducibility in a longer time.

3.2. Effect of concentration and pH value of running solution

Three buffers, namely Tris-perchloric acid, boric acid–NaOH and borate–NaOH were tested. The experimental results showed that under the same pH condition (pH 10.0–11.0), the best separation was obtained with borate–NaOH, and the analysis of the four analytes was carried out within 15 min. However,

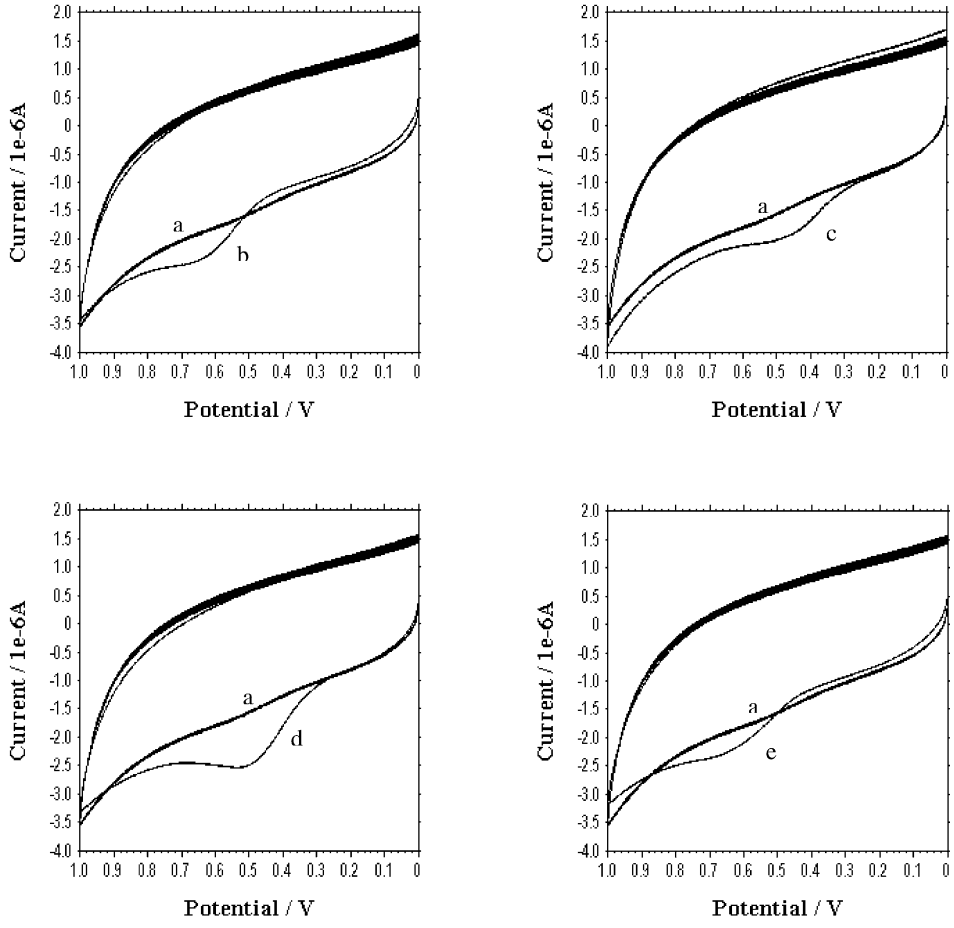

Fig. 1. The cyclic voltammograms of the four analytes at carbon disk electrode in 0.02 mol l^{-1} borate–NaOH buffer (pH 10.35). Reference electrode: SCE; auxiliary electrode: platinum wire. (a): blank solution; (b–e): 5.0×10^{-4} mol l^{-1} tryptophan, tyrosine, tyramine and tryptamine, respectively. Scan rate: 0.1 v/s.

the Tris-perchloric acid and boric acid–NaOH system gave incomplete separations. So the borate–NaOH buffer was chosen in our experiment.

From Figs. 3 and 4, it was found that the migration time and resolution of the four analytes were great affected by the pH value and the concentration of the running buffer solution, because the retention behaviors of the studied analytes depend on several properties including hydrophobicity and dissociation degree in solution. The experiment demonstrated that when the pH was more than 9.0, the four analytes could be separated perfectly. But in fact increasing the pH value would result in peak shape broadening and migration time prolonging. In order to get a good resolution and peak shape, 10.35 was chosen as the optimum pH condition. In addition, the effect of concentration of buffer was examined by using 0.01, 0.02, 0.03 and 0.04 mol l^{-1} borate–NaOH buffer (pH 10.35). The results showed that with the increase of the concentration of borate–NaOH buffer, the peak currents of the four analytes increased, and migration time increased too. This was due to the fact that the increased ionic strength of the borate–NaOH solution resulted in the decrease of the mobility of the solutes in the capillary and a lengthening of the migration time. The CE current in the capillary also increased, which led to Joule heating and peak broadening. From the comprehensive consideration of the sensitivity, the migration time, and the resolution,

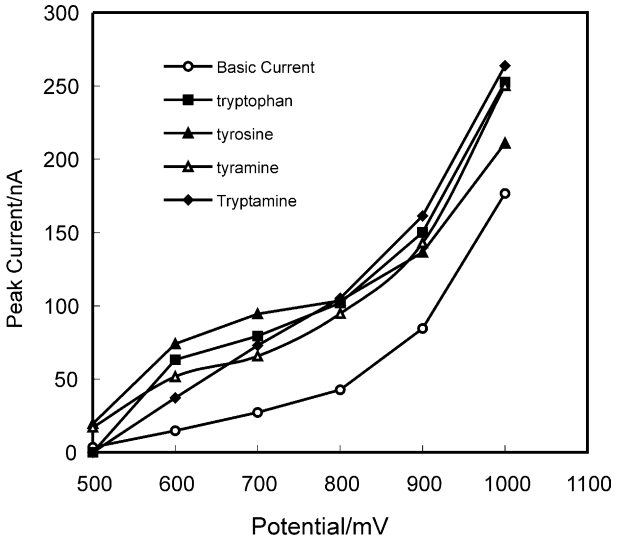

Fig. 2. The hydrodynamic voltammograms obtained from the experiment for tryptophan, tyrosine, tyramine, and tryptamine. The CE conditions were the optimum.

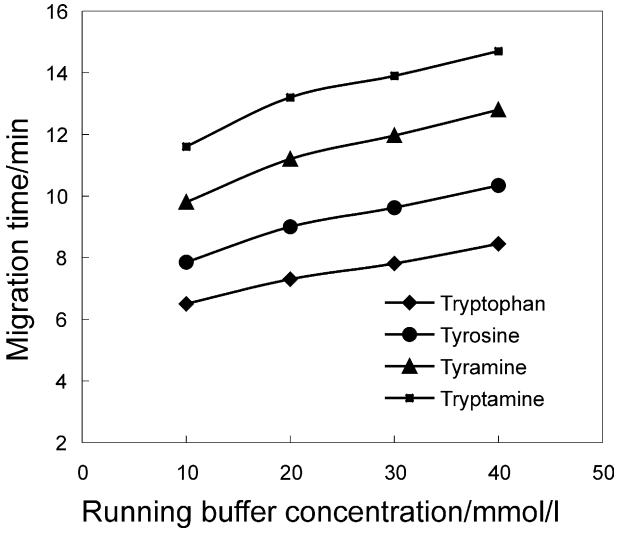

Fig. 4. Effect of running buffer concentration on migration time. The concentration of analytes is 1.0×10^{-4} mol l^{-1}. Other conditions were the same as the optimum.

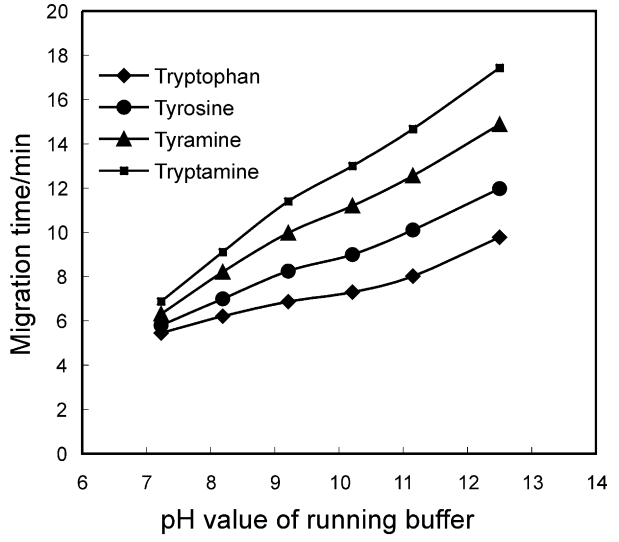

Fig. 3. Effect of running buffer pH on migration time. The concentration of analytes is 1.0×10^{-4} mol l^{-1}. Other conditions were the same as the optimum.

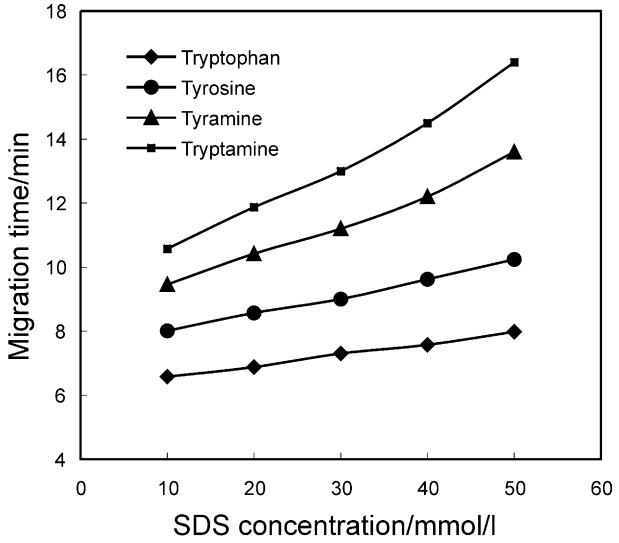

Fig. 5. Effect of SDS concentration on migration time. The concentration of analytes is 1.0×10^{-4} mol l^{-1}. Other conditions were the same as the optimum.

0.02 mol l^{-1} borate–NaOH (pH 10.35) was selected as the running buffer solution.

3.3. Effect of SDS concentration

To obtain satisfactory resolution, SDS, one of the most usual surfactants, was added in the running buffer. The results were shown as Fig. 5. As the SDS concentration increased, the migration time of the analytes was prolonged corresponding to their solubility in micelles. The migration time of tryptamine and tyramine increased more than those of tryptophan and tyrosine, because tryptophan and tyrosine are more negatively charged than tryptamine and tyramine. In order to obtain the best resolution in least time possible, 0.03 mol l^{-1} SDS was selected as optimum.

3.4. Effect of separation voltage

The separation of the four analytes was also affected by separation voltage. With the increase of separation voltage, the electroosmosis was increased, the migration time was decreased and the peak shapes became sharper correspondingly, but too high voltage would lead to peak shape broadening. When the separation voltage was too low, the samples in the capillary would diffuse and lead to peak broadening in the electropherograms. In order to obtain higher efficiency and save analysis time, the separation voltage of 20 kV was employed to achieve a good compromise.

3.5. Effect of sample injection time

The effect of the injection time was examined under the optimum electrophoresis conditions except the injection time was 4, 6, 8, 10, 12, and 14 s respectively. When the injection time was increased from 4 to 10 s, the peak currents increased correspondingly. But when the injection time surpassed 12 s, the current peaks were tailed and the peak currents were decreased. So 10 s under 20 kV was chosen as the optimum injection time.

The optimum analytical conditions were therefore: detection potential +800 mV (vs. SCE), separation voltage 20 kV, sampling time 10 s at 20 kV, and 0.02 mol l^{-1} borate–NaOH run buffer (pH 10.35) containing 0.03 mol l^{-1} SDS.

Under the selected optimum conditions, the electropherograms for the mixture of the four standard analytes are shown in Fig. 6. It is evident that the four analytes were separated completely in 15 min.

3.6. Reproducibility, linearity and detection limits

The reproducibility was calculated by analyzing the standard solutions of the four analytes at a concentration of 1.0×10^{-4} mol l^{-1} ($n=5$). The RSD of the

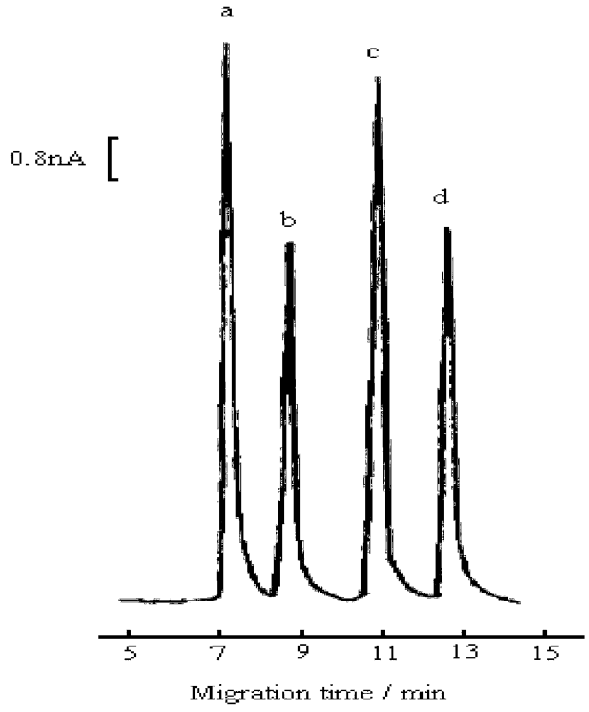

Fig. 6. Electropherograms of a mixture of the four standard analytes. (a) tryptophan; (b) tyrosine; (c) tyramine; (d) tryptamine with same concentration of 1.0×10^{-4} mol l^{-1}. The conditions were the same as the optimum.

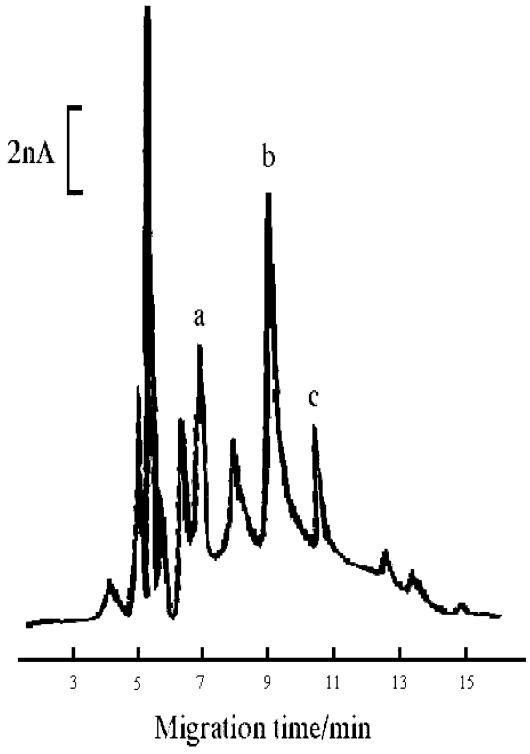

Fig. 7. Electropherograms of the rice spirit sample. (a) tryptophan, (b) tyrosine, (c) tyramine. The conditions were the same as the optimum.

Table 1
The regression equation and dection limits[a]

Compounds	Regression equation Y(nA); C (mol l^{-1})	Correlation coefficient	Linear range (mol l^{-1})	Detection limit (mol l^{-1})
Tryptophan	$Y=0.15+1.05\times10^{5}C$	$R=0.9996$	1.0×10^{-6}–1.0×10^{-3}	1.5×10^{-7}
Tyrosine	$Y=0.04+6.82\times10^{4}C$	$R=0.9995$	2.0×10^{-6}–1.0×10^{-3}	2.1×10^{-7}
Tyramine	$Y=0.06+1.00\times10^{5}C$	$R=0.9998$	1.0×10^{-6}–1.0×10^{-3}	1.8×10^{-7}
Tryptamine	$Y=0.10+7.04\times10^{4}C$	$R=0.9995$	2.0×10^{-6}–1.0×10^{-3}	2.3×10^{-7}

[a] MECC-AD conditions as the optimum.

Table 2
Assay results for the four analytes in rice spirit sample

Compounds	Sample amount (μg ml^{-1})	Added amount (μg)	Found amount (μg)	Recovery	RSD (%) ($n=3$)
Tryptophan	49.1	20.4	71.2	102.4	1.5
Tyrosine	98.7	20.1	120.2	101.2	3.1
Tyramine	58.6	20.0	80.1	102.0	2.3

migration time were lower than 2%, and the RSD of the peak current values were less than 3%.

To determine the linearity for tryptamine, tyramine, tryptophan, and tyrosine at the carbon-disc electrode in MECC-AD, a series of mixed standard solutions of the four analytes were tested. The linear ranges, regression equations, correlation coefficients and instrumental detection limits are listed in Table 1. Good linear relationships of the four analytes were obtained in the concentration range from 10^{-6} to 10^{-3} mol l^{-1}. By using three times the standard deviation of a low level sample, the instrumental detection limits for the four analytes were calculated as low as about 10^{-7} mol l^{-1}.

3.7. Study of feasibility

In order to validate the feasibility of the method established above, it was applied to actual sample determination. Wine has been commonly reported to be a health risk for some consumers because it contains BAs. Thus a Chinese wine–rice spirit was chosen as the actual sample in this experiment.

The typical electropherograms of the rice spirit sample are shown in Fig. 7. Peak *a* was identified as tryptophan, peak *b* as tyrosine, and peak *c* as tyramine by spiking each of the four standard analytes to the sample.

The assay results are summarized in Table 2. The results showed that tyramine, tryptophan, and tyrosine were detected out in the rice spirit sample. Since the quantity of tryptophan was greatly lower than tyrosine in sample, its degradation compound of tryptamine was not detected out. Tyramine, one of the most toxic amine, was not beyond the proposed limit (500–800 μg/g). So this kind of rice spirit was eligible.

Recoveries were tested under the same conditions stated above. The results were given in Table 2 too.

4. Conclusions

Simultaneous determination of BAs and their precursor amino acids by MECC-AD was performed in this experiment; the results showed that this method was of high separation efficiency, good selectivity, short analysis time, convenience of sample analysis. Under the optimum conditions, typtamine, tyramine, tryptophan and tyrosine were separated completely within 15 min, and good linearity, reproducibility and recovery results were obtained. Based on three times standard deviation of a low level sample, the detection limits for the four analytes were as low as at 10^{-7} mol l^{-1} level. This method was successfully used in the analysis of actual rice spirit, and satisfactory assay results were obtained. This method is very potential not only in the food quality controls but also in the research of the relationship between BAs and their precursor amino acids during biogenic processes.

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