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Original Research

Determining Eight Biogenic Amines in Surface Water Using High-Performance Liquid Chromatography–Tandem Mass Spectrometry

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Abstract

A method for determining eight biogenic amines in surface water was established using high-performance liquid chromatography–triple quadrupole mass spectrometry to improve on the detection sensitivity of current methods. The limits of detection and quantification were 0.05 and 0.2 μg L^{-1}, respectively, for histamine, cadaverine, spermine, and spermidine, 1.0 and 3.0 μg L^{-1}, respectively, for putrescine, 1.5 and 5.0 μg L^{-1}, respectively, for tyramine and tryptamine, and 2.0 and 5.0 μg L^{-1}, respectively, for 2-phenylethylamine. The recovery of biogenic amines from spiked water samples was between 97.3 and 108.6% at levels between 0.05 and 15 μg L^{-1} ($R > 0.999$). The relative standard deviations were in the range of 0.10-0.29%. This method can achieve rapid and accurate determination of biogenic amines in surface water, and it meets the minimum residue measurement requirements.

Keywords: HPLC-MS/MS, surface water, biogenic amines

Introduction

Biogenic amine (BA) is the generic name for low-molecular-weight nitrogen-containing organic compounds. BAs are not only precursors in the synthesis of hormones, nucleic acids, proteins, and so on, but also precursors of carcinogens and nitroso compounds [1, 2]. Trace BAs are normal biologically active components in living bodies, including the human body. They have important physiological functions in cells, but become toxic when accumulated in high levels in the human body. Putrescine (PUT), cadaverine (CAD), 2-phenylethyleamine (2-PHE), tyramine (TYR), and tryptamine (TRP) are common BAs as well as highly toxic, water-soluble chemicals. After the death of an organism, bacterial decomposition of the body produces a certain amount of BAs, which can cause water pollution [1].

In 2008 the China State Bureau of Quality and Technical Supervision issued a standard high-performance liquid chromatography (HPLC) method for determining PUT, CAD, spermidine (SPD), spermine (SPM), and histamine (HIS) in water samples [3]. However, this standard method using pre-column derivatization HPLC has a number of drawbacks, including multi-step extraction, the formation of multiple derivatives, a time-consuming process, low

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detection sensitivity (in the range 2.0-40.0 mg L^{-1}), and false positive result [3-5]. To date, only HPLC methods have been reported for the detection of BAs in water.

In view of this, this study used HPLC as the separation system and triple quadrupole mass spectrometry as the detection system. High-performance liquid chromatography (HPLC) is good for separation of complex samples. Relative molecular mass and structural information can be provided by mass spectrometry of high selectivity and high sensitivity. By combining advantages of chromatogram and mass spectrum, we directly determined eight BAs in surface water without the formation of derivatives.

Materials and Methods

Materials

Ammonium acetate was purchased from Hangzhou Chemical Reagent Co. Ltd., and HIS, CAD, SPM, SPD, PUT, TYR, TRP, 2-PHE, and HPLC-grade methanol were purchased from Sigma, USA. Formic acid and organic phase filtration membranes were obtained from the Alfa Aesar A Johnson Matthey Company. Water was purified using a Milli-Q ultrapure water purification system (Millipore Co., Germany).

Instrumentation

The HPLC-MS/MS system consisted of an Agilent 1290 Series liquid chromatograph (with a line degasser and autosampler) and an Agilent 6460 triple quadrupole mass spectrometer (Agilent Technologies, Inc., USA) with electrospray ionization (ESI).

Separation of the BAs was performed on a hydrophilic interaction chromatography column (50 mm × 2.1 mm, 2.6 μm, Agilent Technologies). The mobile phase was 5 mmol L^{-1} aqueous ammonium acetate and methanol (6:4 by volume). The mobile phase flow rate was 0.2 mL min^{-1}, the sample injection volume was 5 μL, and the column temperature was 30ºC [6, 7].

For MS, the ESI conditions were as follows: gas temperature 350ºC, gas flow rate 11 L^{-1} min, nebulizer 50 psi, capillary voltage 4000 V (positive) and 3500 V (positive), sheath gas temperature 100°C, nozzle voltage 500 V [8].

Sample Preparation

MCX Solid phase extraction column was conditioned by passing 2 mL of methanol followed by 2 mL of Milli-Q purified water. 10 ml Surface water samples were filtered through 0.45 micron disposable filters. The filtrate was added to an MCX solid phase extraction column. And then 2 mL 3% (by volume) formic acid aqueous solution was run through the column and the effluent discarded (The process flow rate was <1 mL min^{-1}). The analytes later were eluted with 3 mL of 3% ammonia methanol solution (the process flow rate was <1 mL min^{-1}). The eluate was collected in a vial. Eluate was then gently dried by blowing nitrogen for 20 min; methanol was added to 1 mL in the vial and the analytes were filtered through 0.22 μm filter into vials for test [9, 10].

Results and Discussion

Optimization of Ion Detection Conditions

The selected ion monitoring mode in HPLC-MS/MS was used to optimize the conditions for determining BAs.

First, we used the SIM mode to determine the retention time of the Bas and confirm the mass number of the target compound precursor ions. Second, we optimized the capillary outlet voltage (fragmentor) to ensure transmission efficiency of the parent ion. Then we made the daughter ion scan to get optimal response of product ions by using optimized capillary outlet voltage to choose a quantitative ion and optimization collision energy [11]. Multiple reaction mode (MRM) quantitative analysis was conducted using the optimized capillary outlet voltage and collision energy [12]. The ESI positive ion mode was used to qualitatively and quantitatively determine the eight BA ions. The optimized MRM conditions are shown in Table l.

Linear Relationship and Detection Limit

Standard working solutions for the eight BAs at concentrations ranging from 0.05 to 15 μg L^{-1} were used to determine the linear relationship between ion peak area and BA concentration and detection limit. MRM mass spectrograms of the BAs in surface water are shown in Fig. 1. Final test results were negative for surface water blank BAs after adding the mixed standard separation of the eight BAs on the chromatogram was determined to be good. There was no interference from miscellaneous peaks. To quantitatively determine the linear relationship for each BA, the ion peak area was plotted as the vertical axis and the BA concentration was plotted as the horizontal axis to obtain the linear regression equation. There was a good linear relationship between BA concentration and the quantitative ions. Limit of detection (LOD) and limit of quantification (LOQ) were measured based on signal-to-noise ratios of about 3 and 10, respectively. The fitted linear regression equations are shown in Table 2. The correlation coefficients of the standard curves were ≥0.9990, reflecting good correlation. HPLC-MS/MS detection of BAs compared with HPLC with a lower detection limit.

Precision and Recovery

Recovery was determined using the standard addition recovery test. Three concentrations of BA standards were added to surface water samples: 1, 3, and 5 μg L^{-1}. The recovery studies were repeated three times and the

Table 1. Detection conditions of eight biogenic amines using MRM mode.

BA	Precursor Ion (m/z)	Product Ion (m/z)	Dwell (s)	Fragmentor (v)	Collision Energy (v)	Cell Accelerator Voltage (v)
Histamine	112.2	95.0	50	80	13	7
		68.1	50	80	21	7
Cadaverine	102.2	58.1	50	80	21	7
		43.1	50	80	13	7
Putrescine	89.2	72.1	50	85	5	7
		30.0	50	85	21	7
Spermine	203.2	129.1	50	320	9	7
		84.1	50	320	35	7
Spermidine	146.3	112.1	50	320	13	7
		72.1	50	320	13	7
2-phenylethylamine	122.2	105.0	50	70	9	7
		77.0	50	70	33	7
Tyramine	138.2	121.0	50	80	5	7
		77.0	50	80	33	7
Tryptamine	161.2	144.0	50	80	9	7
		117.0	50	80	35	7

recovery mean was calculated. The results are shown in Table 3. The recoveries of the eight BAs in the surface water matrices were between 97.3 and 108.6%, and the relative standard deviations (RSDs) were between 0.10 and 0.29%. The percentage recoveries and RSDs obtained using this method meet the minimum requirements for residue analysis.

Surface Water Sample Test

Under the HPLC-MS/MS experimental conditions, blank samples of surface water were added to 15 μg L^{-1} of standard working solution. Ion chromatogram peak of the eight kinds of BAs in the spiked sample does show a good peak shape. All peaks have good symmetry: HIS

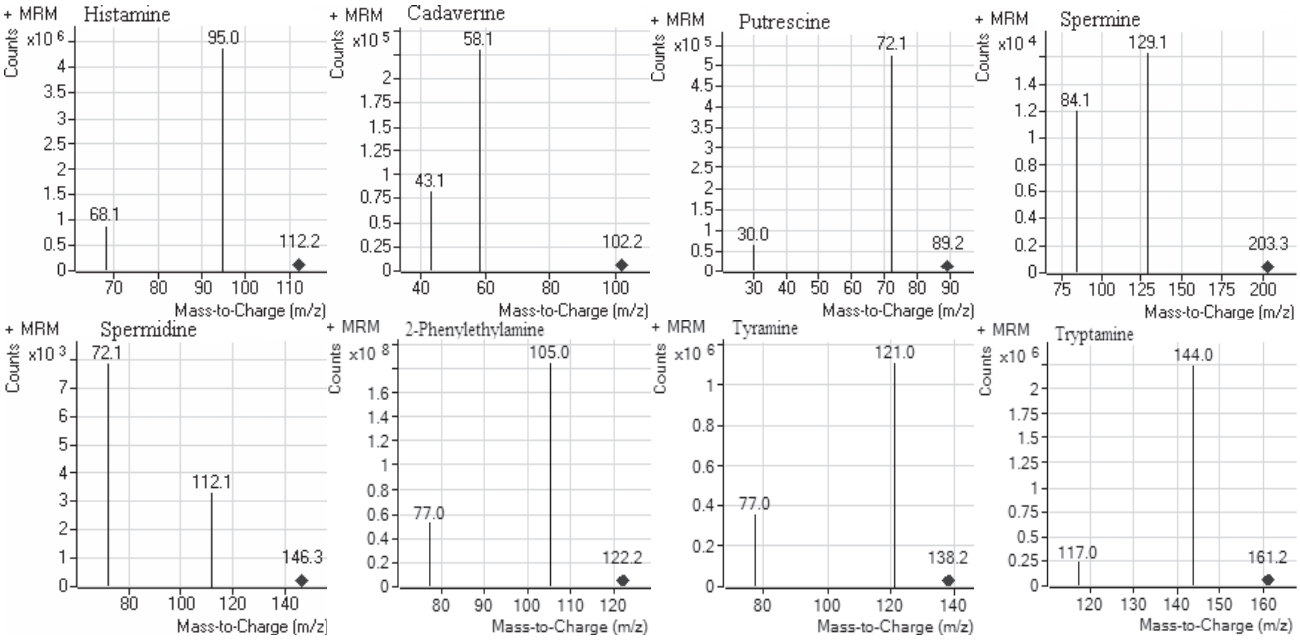

Fig. 1. MRM mass spectrogram of biogenic amines in surface water.

Table 2. Linear relationships, LODs, and LOQs for the eight biogenic amines.

BA	Linear range (μg/L)	linear equation	r^2	LOD (μg/L)	LOQ (μg/L)
Histamine	0.05-15	Y = 3609.21 X + 19691	0.9990	0.05	0.2
Cadaverine	0.05-15	Y = 1985.61X + 4385	0.9991	0.05	0.2
Putrescine	0.05-15	Y = 1506.28 + 2066	0.9995	1.0	3.0
Spermine	0.05-15	Y = 216.70X + 151	0.9992	0.05	0.2
Spermidine	0.05-15	Y = 87.69X + 181	0.9996	0.05	0.2
2-phenylethylamine	0.05-15	Y = 82019.07X + 188077	0.9991	2.0	5.0
Tyramine	0.05-15	Y = 6430.10X + 22160	0.9990	1.5	5.0
Tryptamine	0.05-15	Y = 5159.23X + 30809	0.9995	1.5	5.0

parent ion 112.2, qualitative and quantitative ions 68.1 and 95.0; CAD parent ion 102.2, qualitative and quantitative ions 43.1 and 58.1; PUT parent ion 89.2, qualitative and quantitative ions 30.0 and 72.1; SPM parent ion 203.2, qualitative and quantitative ions 84.1 and 129.1; SPD precursor ion 146.3, qualitative and quantitative ions 112.1 and 72.1; 2-PHE parent ion 122.2, qualitative and quantitative ions 77.0 and 105.0; TYR parent ion 138.2, qualitative and quantitative ions 77.0 and 121.0; TRP precursor ion 161.2, qualitative and quantitative ions pair 117.0 and 68.1 [13-17]. We used MS is the triple quadruple (QQQ) mass spectrometer because of its capability to run a large variety of different experiments. The QQQ instrument is comprised of three quadrupoles arranged in a linear row, where the first (Q1) and third (Q3) act as mass filters and the quadrupole in the middle (Q2) acts as a collision cell for molecular fragmentation and generation of MS/MS information. Product ion scan, where Q1 acts as a mass filter to selectively isolate a single m/z, which is fragmented in Q2. The fragments are scanned in Q3 for the generation of a fragment mass spectrum. This scan mode creates an MS/MS spectrum for the precursor ion selected by Q1 [18-21].

Table 3. Spike recovery tests (n = 6).

BA	Spiked level (μg/kg)	Recovery mean (%)	Repeatability (RSD, %)
Histamine	1	98.9	0.29
	3	105.5	0.21
	5	103.7	0.17
Cadaverine	1	97.0	0.16
	3	99.6	0.19
	5	101.1	0.15
Putrescine	1	99.2	0.22
	3	99.1	0.27
	5	103.9	0.25
Spermine	1	97.4	0.12
	3	99.6	0.26
	5	97.3	0.28
Spermidine	1	94.3	0.29
	3	105.1	0.25
	5	108.6	0.27
2-phenylethylamine	1	105.8	0.13
	3	106.6	0.10
	5	107.1	0.27
Tyramine	1	104.3	0.18
	3	106.5	0.21
	5	108.3	0.19
Tryptamine	1	106.3	0.23
	3	107.5	0.26
	5	108.3	0.19

Conclusions

This was an experimental study on the use of HPLC-MS/MS as a rapid detection method for determining BAs in surface water samples. The sample pretreatment for LC-MS is quick and easy. Other methods for determining BAs were excluded because of false positives [22]. The standard addition recovery test showed that HPLC-MS/MS has higher sensitivity and a detection limit down to lower level compared with the current conventional liquid phase standard procedure.

1. The HPLC-MS/MS method can achieve accurate qualitative and quantitative information about trace BAs in surface water samples without using the derivatization process. This improves the efficiency for testing large quantities of samples before treatment, while addressing the false positive problem. It meets the residue testing requirements of BAs in surface water samples.
2. With the HPLC-MS/MS method, if the measured result is outside the linear range of the method, the sample solution can be diluted to within the linear range

[23]. As the method uses ESI, when the concentration of the sample solution is too high, the ion abundance ratio exceeds the standard abundance ratio range due to the ion suppression phenomenon. Therefore, for this method to be suitable for the accurate qualitative and quantitative determination of the actual concentrations of trace BAs, high-concentration samples should be diluted before injection to obtain good detection results.

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