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Short communication

Determination of aromatic amine mutagens, PBTA-1 and PBTA-2, in river water by solid-phase extraction followed by liquid chromatography–tandem mass spectrometry

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Abstract

We describe a novel method for the determination of two kinds of aromatic amine mutagens, 2-[2-(acetylamino)-4-[bis(2-methoxyethyl)-amino]-5-amino-7-bromo-4-chloro-*2H*-benzotriazole (PBTA-1) and 2-[2-(acetylamino)-4-[bis(2-cyanoethyl)-ethylamino]-5-amino-7-bromo-4-chloro-*2H*-benzotriazole (PBTA-2), in river water based on liquid chromatography–electrospray ionization-tandem mass spectrometry (LC–ESI-MS–MS). A solid-phase extraction procedure was used for the extraction of PBTA-1 and PBTA-2 from river water. The procedure was rapid and the relative standard deviations were below 4%. The detection limits of PBTA-1 and PBTA-2 in river water using the proposed method were found to be 1 and 2 ng/l, respectively. The compounds were detected by this method in river water taken from two sites in the Yodo River system at the ng/l level.

Keywords: Water analysis; Solid-phase extraction; Aromatic amines

1. Introduction

It has been previously reported that water samples from the Yodo River system, which serves as the main drinking water supply for the Osaka area in Japan, are mutagenic to *Salmonella typhimurium* strains YG1024 with S9 mix [1–3]. Recently, two potent aromatic amine mutagens were isolated in samples concentrated from the Nishitakase River, a tributary of the Yodo River. The structures of the two mutagens were determined to be 2-[2-(acetylamino)-4-[bis(2-methoxyethyl)-amino]-5-amino-7-bromo-4-chloro-*2H*-benzotriazole (PBTA-1) and 2-[2-(acetylamino)-4-[bis(2-cyanoethyl)-ethylamino]-5-amino-7-bromo-4-chloro-*2H*-benzotriazole (PBTA-2) [4–6]. These two aromatic amines are potent mutagens in *Salmonella* and are presumed to be formed from disperse azo dyes used as industrial materials for dyeing through the reducing process in dyeing factories and the chlorination in sewage plants for disinfection [4,6].

Ohe et al. [7] have reported the analytical methods for the determination of PBTA-1 and PBTA-2 in

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river water, and ng/l levels of the PBTAs were detected from the Nishitakase River water. The method is as follows: water samples (40 l) were collected and then passed through a glass column with blue rayon. The adsorbed compounds were extracted and evaporated to dryness. The dissolved residue in methanol was fractionated twice by high-performance liquid chromatography (LC) and the fractions were analyzed by liquid chromatography with electrochemical detection (LC–ED). The detection limits of the method are 0.01 ng/l, and the recoveries are 52 and 56% for PBTA-1 and PBTA-2, respectively.

The analytical method for the determination of PBTA-1 and PBTA-2 in river water requires troublesome pretreatments, because the selectivity of the LC–ED system is somewhat poor, and this method is too tedious and time-consuming to be used for a detailed environmental survey of PBTA-1 and PBTA-2 in rivers.

Liquid chromatography–mass spectrometry (LC–MS) has been shown to be an efficient technique for the determination of polar compounds, such as PBTA-1 and PBTA-2. Furthermore, by using LC–MS–MS, there is the potential of simplifying the clean-up procedure for the analyses of the samples containing various environmental contaminants, because LC–MS–MS generally has a higher selectivity than other detection methods coupled with LC [8].

In this study, the method for the determination of PBTA-1 and PBTA-2 in river water based on LC–MS–MS following a solid-phase extraction is demonstrated. By applying LC–MS–MS and solid-phase extraction to the analysis of PBTA-1 and PBTA-2 in river water, the clean-up treatment was simplified and faster than the conventional method.

2. Experimental

2.1. Materials

All solvents were of HPLC grade while the other chemicals were of analytical-reagent grade. PBTA-1 and PBTA-2 were synthesized according to the methods previously described in Refs. [5,6]. Diazinon-d_{10}, the internal standard, was purchased from Hayashi Pure Chemical Industries (Osaka, Japan). Ammonium acetate and acetic acid were obtained from Wako (Osaka, Japan). Ultra-pure water was produced by a Milli-Q system (Millipore, Bedford, MA, USA). River water was collected at two sites in the Yodo River system, on 26 December 2002.

2.2. LC conditions

LC was carried out on an LC apparatus equipped with an Agilent Model 1100 series (Agilent, Yokogawa Analytical Systems, Tokyo, Japan). A TOSOH TSK-GEL ODS-80TsQA (Tosoh, Tokyo, Japan: 5 μm particle size, 150×2.0 mm I.D.) was used for the LC separation of PBTA-1, PBTA-2 and the internal standard. The separation was carried out at 40 °C using a gradient composed solution A (1 m*M* ammonium acetate solution adjusted to pH 4 by the addition of acetic acid) and solvent B (methanol). The gradient expressed as changes in solvent B was as follows: 0–10 min, a linear increase from 50 to 90% B; 10–20 min, hold at 90% B. The flow-rate was 0.2 ml/min.

2.3. Electrospray ionization (ESI) MS–MS

The ESI-MS–MS analyses were performed using a PE-Sciex API2000 (Sciex, Applied Biosystems Japan, Tokyo, Japan). Ionization of the analytes was achieved by electrospray in the positive ion mode. All the interface parameters were optimized by infusing a standard solution (5 μg/ml) of the analytes at a flow-rate of 10 μl/min. The final electrospray conditions were as follows: nitrogen curtain gas, 40 l/min; ion-spray voltage, 4800 V; declustering voltages, 76, 91 and 61 V for PBTA-1, PBTA-2 and internal standard, respectively. The collision energy was 29 eV for every target compound. LC–MS–MS acquisition was performed in the multiple reaction monitoring (MRM) mode, by following the reactions m/z 543→511, characteristic of PBTA-1, m/z 508→467 characteristic of PBTA-2, and m/z 315→170 characteristic of the internal standard, diazinon-d_{10}.

2.4. Solid-phase extraction procedure

The water samples (500 ml) were extracted by

solid-phase extraction using an Empore disk C_{18} (Sumitomo 3M, Tokyo, Japan). The Empore disk was preconditioned with 10 ml of methanol and 20 ml of distilled water. The sample solution (500 ml) was loaded onto the solid-phase extraction disk at room temperature. The disk was dried and eluted with 5 ml methanol into a glass tube. The eluate was dried in a nitrogen atmosphere and concentrated to 0.25 ml, and 2.5 μl of the internal methanol solution (diazinon-d_{10}, 1 mg/l) was added. A 10-μl aliquot was then injected into the LC–MS–MS system.

3. Results and discussion

3.1. Detection of PBTA-1 and PBTA-2

For the mass spectral investigation using ESI-MS by infusing a standard solution (5 μg/ml) of the analytes, the 543 and 508 ions (m/z), which were assigned as the $[M+H]^+$ ions, were observed as the main peaks. The product-ion mass spectra of the $[M+H]^+$ ions were then acquired. For PBTA-1 and PBTA-2, the major product ions were m/z 511 and 467, respectively. The product ions would form through bond cleavages at the amino substitutes by collision-induced dissociation as shown in Fig. 1. The fragmentation at the alkyl chain of amino group is not unusual for ESI-MS–MS measurements, and it has been observed, for example, in Ref. [9]. The collision energy was optimized at 29 eV for the target compounds.

3.2. Analytical data and application

The PBTA ions were detected by ESI-MS–MS after separation of the PBTA-1 and PBTA-2 on a reversed-phased ODS column. Fig. 2a shows the chromatograms of the PBTA-1 and PBTA-2 (5 ng/ml) standard solution and internal standard using MRM. The instrumental detection limits calculated as three times the standard deviation of the relative peak intensity [standard solution (5 ng/ml), $n=5$] were 0.20 and 0.17 ng/ml for PBTA-1 and PBTA-2, respectively.

The calibration equations (relative MS responses to internal standard versus analyte concentrations) for the PBTAs were obtained using a series of standard solutions over the concentration range 1–100 ng/ml. The correlation coefficients (r^2) of the calibration curves were 0.9993 and 0.9989 for PBTA-1 and PBTA-2, respectively.

For the recovery evaluation of the PBTAs, the solid-phase extraction procedure was carried out after the river water (500 ml for sample of point B) was spiked with 2.5 and 5.0 ng of the PBTAs. The samples ($n=4$) were prepared for the LC–ESI-MS–MS analysis as described in the Experimental section. Peaks due to PBTAs were observed prior to spiking for the river water obtained from the downstream sites of the Yodo River. The recoveries were evaluated by determining the peak areas based on the MRM chromatograms, and the mean recoveries ($n=4$) were calculated from the difference in the concentrations obtained between the spiked and the unspiked river water. The chromatograms of the river water without spiking are shown in Fig. 2b,

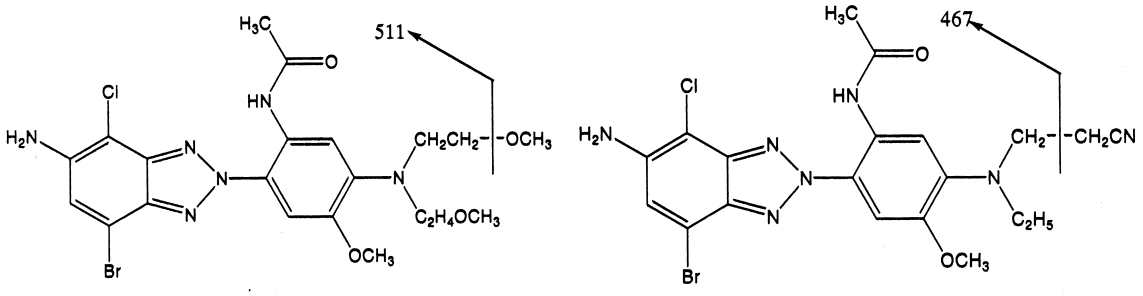

Fig. 1. Proposed ion structures for the fragment ions of the protonated PBTA-1 and PBTA-2.

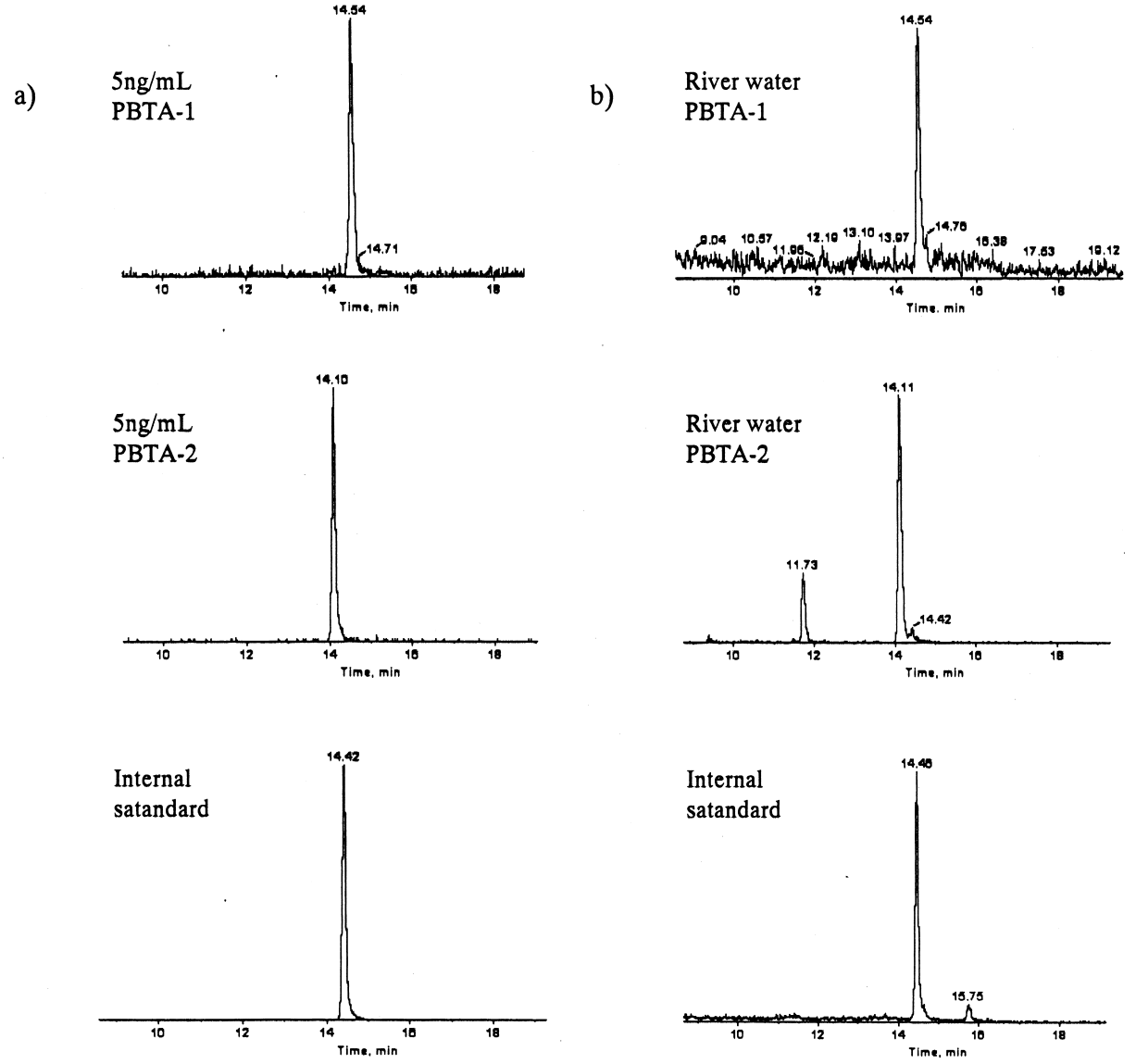

Fig. 2. LC–MS–MS chromatograms for (a) the standard solution (5 ng/ml) and (b) for the river water (sampling point: B) by MRM.

using MRM. The peaks of the PBTAs were clearly detected without interference by contaminant peaks in the river water taken at sampling point B.

In the recovery test on the 2.5 ng spiked river water (sampling point B), the mean recoveries were 87 and 106% for PBTA-1 and PBTA-2, respectively (Table 1). The detection limits of PBTA-1 and PBTA-2, defined as three times the standard deviation of the obtained concentrations from the recovery tests (2.5 ng addition, $n=4$), were 1 and 2 ng/l, respectively. These results indicate that the present method has capability to carry out the surveys of PBTAs present in river water at the low-ng/l level range. In the river water from sampling points A and B, the peaks of PBTA-1 and PBTA-2 are observed, the concentrations are shown in Table 1. In Ref. [7], in the Yodo River system, PBTA-1 and PBTA-2 were detected at ng/l concentration levels.

Table 1
Analytical data for PBTA-1 and PBTA-2 in river water using LC–MS–MS

Analyte	Concentration (ng/l)		Percent recovery (RSD; $n=4$)[a]		Detection limit (ng/l)[b]
	Site A	Site B	Spiked river water (site B)		
			2.5 ng	5.0 ng	
PBTA-1	<1	9.4	87.0 (3.4)	101 (8.3)	1
PBTA-2	2	33	106 (2.1)	104 (7.4)	2

[a] Mean recoveries ($n=4$) calculated from difference of obtained concentration between spiked and the unspiked river water ($n=4$; site B).
[b] Calculated as three times the standard deviation ($n=4$). Water samples of 500 ml were used in this method.

4. Conclusions

The present LC–MS–MS method is sufficiently selective and sensitive for rapid PBTA analyses in river water present at ng/l concentrations. The advantage of the proposed method is that the pretreatment is much simpler than that of the conventional method. The quantity of river water needed for the PBTA analysis was 500 ml. The procedure should facilitate the monitoring of PBTAs in river water at the ultra-trace level.

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