

Determination of 4,4'-, 3,4'-, and 2,2'-Diaminodiphenylethers in Sediment Samples from the Sea Using Liquid Chromatography-Tandem Mass Spectrometry

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Abstract A selective method has been developed for determining the concentration of 4,4'-, 3,4'-, and 2,2'-diaminodiphenylethers in sediment samples from the sea, using liquid chromatography/tandem mass spectrometry (LC/MS/MS). 4,4'-diaminodiphenylether is a suspected toxic compound, and categorized as “Class I Designated Chemical Substance” in Japan. We have investigated the levels of 4,4'-diaminodiphenylether in sediments to evaluate long-term water pollution. The methods detection limits for the 4,4'-, 3,4'-, and 2,2'-diaminodiphenylethers were 2.0, 1.7, and 4.8 ng/g-dry, respectively.

Keywords 4,4'-, 3,4'-, and 2,2'-diaminodiphenylethers · Sediment · Solid phase extraction (SPE) · Liquid chromatography/tandem mass spectrometry (LC/MS/MS)

Introduction

4,4'-Diaminodiphenylether (CAS No.; 101-80-4, mw: 200.24) is a suspected toxic compound, used as a raw material in polymerizations and as a cross-linking agent (Akutsu et al. 1998). In Japan, this 4,4'-diaminodiphenylether is categorized as “Class I Designated Chemical Substance” based on “the Act on Confirmation, etc. of Release Amounts of Specific Chemical Substances in the Environment and Promotion of Improvements to the Management Thereof” in 2008. The “Pollutant Release and Transfer Register (PRTR)” was established in 1999, reaffirming “the Act on Confirmation, etc. of Release

Amounts of Specific Chemical Substances in the Environment and Promotion of Improvements to the Management Thereof”. The “Class I Designated Chemical Substance” classification is applied to the PRTR system. Therefore, quantities of these types of chemical are routinely checked, in terms of the amounts of these chemicals transferred to and from businesses. All relevant information must be reported to the Japanese Ministry of the Environment. 4,4'-Diaminodiphenylether is regarded as a toxic, mutagenic and genetic compound, so levels in the environment need to be investigated.

Liquid chromatography/tandem mass spectrometry (LC/MS/MS) methods for the analysis of chemicals in environmental water samples have been reported (Tong et al. 2009). Numerous classes of chemical will have been accumulated in the sediments of both river and sea waters. Therefore, it would be possible to evaluate long-term water pollution by investigating the levels of certain chemical in sediments. Research into the levels of chemicals in sediments, using LC/MS/MS, have been reported (Diaz-Cruz and Barcelo 2007; Nunez et al. 2008; Labadie and Hill 2007; Kinani et al. 2010). The measurement of 4,4'-diaminodiphenylether in food samples has also been reported (Aznar et al. 2009; Mortensen et al. 2005), however research on the levels of 4,4'-diaminodiphenylether in sediments have not yet emerged.

In this study, we have determined levels of 4,4'-, 3,4'- and 2,2'-diaminodiphenylethers in sediment samples from the sea using LC/MS/MS. This compound exists as isomers, so it is important to detect them selectively. For our research, we selected the obtainable 3,4'- and 2,2'-diaminodiphenylethers as isomers. The present study demonstrates the advantage of the selective method for the simultaneous quantification of 4,4'-, 3,4'-, and 2,2'-diaminodiphenylethers in sediment.

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Materials and Methods

The 4,4'-, 3,4'- and 2,2'-diaminodiphenylether standards (99%; 4,4'-, 97%; 3,4'-, 98%; 2,2'-) were purchased from Sigma-Aldrich (St. Louis, USA). The 4,4'-diaminodiphenylether-*d*₁₂ (99.2% atom D), internal standard and surrogate, was purchased from Cambridge Isotope Laboratories, Inc. (MA, USA). Ammonium formate, 2 M hydrochloric acid (HCl), and 25% ammonia were used as received from Wako Pure Chemical Industries (Osaka, Japan). Methanol and ultra pure water of a LC/MS grade were also from Wako Pure Chemical Industries (Osaka, Japan).

Sediment samples from the Japanese Sea were collected. Sediment sampling was performed in October 2010. 20 ng of 4,4'-diaminodiphenylether-*d*₁₂ was added as a surrogate to each 1 g-dry sediment sample. The sample was mixed and stirred with 30 mL 2 M HCl, and shaken for 10 min. This sample solution was then treated ultrasonically for 10 min and centrifuged at 3,000 rpm for 10 min. The supernatant solution was transferred to another tube. This process was repeated twice. The resultant solution was diluted to 1 L with water. 500 mL of this solution was passed through a solid phase extraction cartridge (an Oasis MCX Plus, pre-conditioned with 10 mL methanol, 10 mL ultrapure water and 0.1 M HCl; Waters, Milford, MA) at a flow rate of 10 mL/min. 10 mL 0.1 M HCl and 10 mL water was also passed through the cartridge. The 4,4'-, 3,4'-, 2,2'-diaminodiphenylethers, and 4,4'-diaminodiphenylether-*d*₁₂, were eluted with 5 mL 25% ammonia/methanol (5/95) through the Oasis MCX Plus cartridge. This solution was evaporated to one drop with a nitrogen flow and adjusted to 1 mL with methanol. This methanol solution was finally analyzed by LC/MS/MS.

The 4,4'-, 3,4'-, 2,2'-diaminodiphenylethers, and 4,4'-diaminodiphenylether-*d*₁₂, were measured using LC/MS/MS. The LC was carried out with an Alliance 2695 instrument (Waters). An Atlantis T3 column [2.1 mm i.d. × 150 mm, 5 µm (Waters)] was used under a gradient solvent method [A; methanol, B; 10 mmol/L ammonium formate, A: 10 → 95, B: 90 → 5, linear gradient (0–10 min), A : B = 95 : 5 (10–20 min), A: B = 10: 90 (20–30 min)]. The flow rate was 0.2 mL/min and the injection volume was 10 µL. The column temperature was 40°C. A Quattro micro API quadrupole mass spectrometer (Waters), with an ESI interface, was employed. The interface was operated in the positive ion mode. The capillary and cone voltages were 3.5 kV and 25 V, respectively. The source and desolvation temperatures were 120 and 350°C, respectively, and the cone and desolvation gas flows were 50 and 650 L/h, respectively. The collision energy was 20 eV and the SRM mode was used for quantification. The analysis time for one sample by LC/MS/MS was 30 min.

Results and Discussion

Both the precursor and product ion mass spectra acquired for 1 mg/L samples of the 4,4'-, 3,4'-, 2,2'-diaminodiphenylethers, and 4,4'-diaminodiphenylether-*d*₁₂, in the positive ion scanning mode. The ions were intense at *m/z* 201 (4,4'-, 3,4'-, 2,2'-diaminodiphenylethers) and 209 (4,4'-diaminodiphenylether-*d*₁₂), so these ions were selected as the precursor ions for further collision with argon gas. Their product ions were detected in the product ion scanning mode. The product ions from the precursor ion at *m/z* 201 were *m/z* 108 (4,4'-, 2,2'-diaminodiphenylethers) and 93 (3,4'-diaminodiphenylether). The product ion from the precursor ion at *m/z* 209 was *m/z* 112 (4,4'-diaminodiphenylether-*d*₁₂). Therefore, *m/z* 201 → 108, *m/z* 201 → 93 and *m/z* 209 → 112 were monitored for the SRM. Figure 1 shows the SRM chromatograms for 50 ng/mL samples of the 4,4'-, 3,4'-, 2,2'-diaminodiphenylethers, respectively, and a 10 ng/mL sample of 4,4'-diaminodiphenylether-*d*₁₂.

A calibration curve for the 4,4'-, 3,4'-, 2,2'-diaminodiphenylethers, and their correlation coefficients, were calculated from their concentrations and the relative peak area ratios for the 4,4'-, 3,4'-, 2,2'-diaminodiphenylethers to the 4,4'-diaminodiphenylether-*d*₁₂ standard. These are shown in Fig. 3. The curves exhibited a strong linearity in the range of 1–100 ng/mL (*R*² = 0.9988–0.9994).

In an effort to extract the 4,4'-, 3,4'-, 2,2'-diaminodiphenylethers from the sediment samples efficiently, we used dichloromethane. A standard solution of the sediment sample was mixed and stirred with acetone or methanol, shaken, treated ultrasonically, and centrifuged. The supernatant solution was then mixed with dichloromethane, and shaken. This dichloromethane solution was evaporated to 100 µL using a nitrogen stream, adjusted to 1 mL with

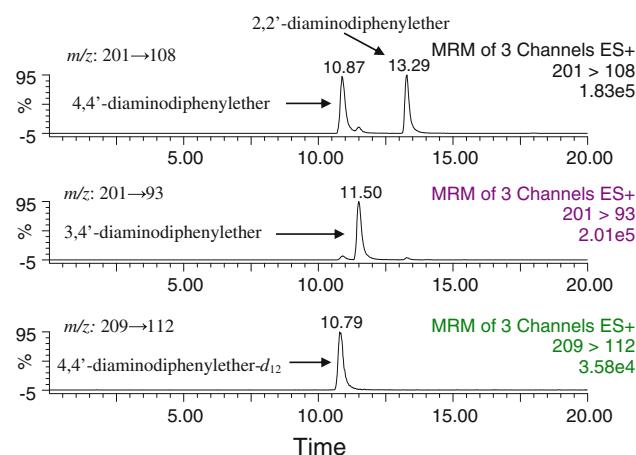


Fig. 1 LC/MS/MS-SRM chromatograms for the 4,4'-, 3,4'-, 2,2'-diaminodiphenylethers (50 ng/mL), and 4,4'-diaminodiphenylether-*d*₁₂ (10 ng/mL)

methanol, and analyzed by LC/MS/MS. However, their recoveries were low due to their high water solubility. Other solvents were also unable to achieve high recoveries. These results indicate that 4,4'-, 3,4'- and 2,2'-diaminodiphenylethers may remain in the water solution. It was impossible to extract these chemicals using dichloromethane or any other solvent. We went on to extract these chemicals using solid phase extraction cartridges.

The standard sediment sample solution was mixed and stirred with 2 M HCl instead of acetone. The 2 M HCl enabled the extraction of the 4,4'-, 3,4'-, 2,2'-diaminodiphenylethers via protonation of the amino groups. For extraction using the usual solid phase cartridge (C18), the supernatant 2 M HCl solution was adjusted to around pH 7, where a precipitate appeared. This result indicates an extraction using solid phase cartridges is necessary to avoid adjustments in pH. We therefore used cation exchange solid phase extraction cartridges.

To select the best solid phase cartridge for the effective and selective investigation of the 4,4'-, 3,4'-, 2,2'-diaminodiphenylethers, standard solution extractions were performed using three cartridges: (1) Oasis MCX Plus; surface functionality: N-vinylpyrrolidone and benzene-sulfonic acid copolymer; (2) Oasis WCX Plus; surface functionality: N-vinylpyrrolidone and benzoic acid copolymer, and (3) Sep-Pak Plus CM; $-\text{CO}_2^-\text{Na}^+$ ((1)–(3); Waters, Milford, MA)). 5 mL 50 ng/mL 4,4'-, 3,4'-, 2,2'-diaminodiphenylether solutions were passed through the solid phase cartridges, pre-conditioned with 10 mL methanol, 10 mL ultrapure water and 0.1 M HCl. 10 mL 0.1 M HCl and 10 mL ultrapure water were also passed through these cartridges and the chemicals were eluted with 5 mL 25% ammonia/methanol (5/95). This procedure was carried out three times with each cartridge. The recoveries were measured for each cartridge. The Oasis MCX Plus cartridge was selected for extraction because the recovery was 102%–110%, the best of all three cartridges.

To examine the quantity of sediment sample required using the Oasis MCX Plus cartridges, 1 and 5 g samples of dry sediment were employed. 2 μg quantities of the 4,4'-, 3,4'-, 2,2'-diaminodiphenylethers were extracted with 2 M HCl. 2 μg 4,4'-diaminodiphenylether- d_{12} was added to the supernatant solutions and made up to 1 L with water. 100 mL of these solutions were passed through the Oasis MCX Plus cartridges at 10 mL/min with 5 mL 25% ammonia/methanol (5/95) as eluent, dried using a nitrogen stream and adjusted to 2 mL with methanol. These methanol solutions were analyzed using LC/MS/MS. 10 g glass beads were also similarly examined. The recoveries are shown in Fig. 2. The chemical recoveries for the 1 g dry sediment samples were 72%–84% better than that for the 5 g dry samples.

To validate the optimum quantity for the sediment samples in the extracted water solutions, 1, 1.5, and 2.5 g dry sediment samples were added. 100 or 200 ng 4,4'-, 3,4'-, 2,2'-diaminodiphenylether samples were extracted with 30 mL of 2 M HCl. 100 or 200 ng 4,4'-diaminodiphenylether- d_{12} was added to the supernatant solutions, and made up to 1 L with water. Either 500 mL or 1 L of these solutions was passed through the Oasis MCX Plus cartridge at 10 mL/min, with 5 mL of 25% ammonia/methanol (5/95) as eluent, dried using a nitrogen stream and adjusted to 1 mL with methanol. These methanol solutions were analyzed using LC/MS/MS. The recoveries are shown in Fig. 3. The results suggest that 500 mL water is the optimum volume to be passed through the Oasis MCX Plus cartridges. Also, the 1 g dry sediment sample option was

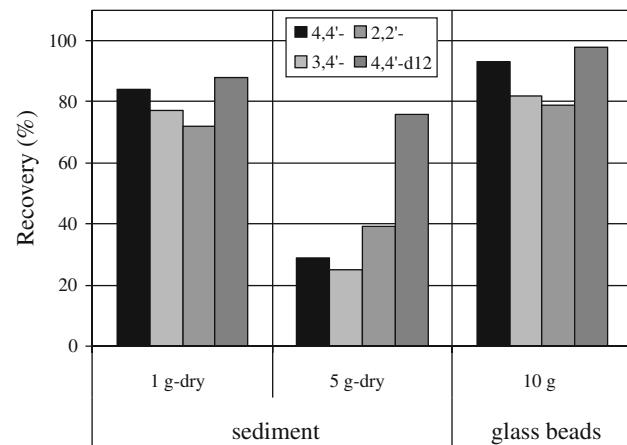


Fig. 2 Recoveries of the 4,4'-, 3,4'-, 2,2'-diaminodiphenylethers and 4,4'-diaminodiphenylether- d_{12} , from both sediment and glass beads

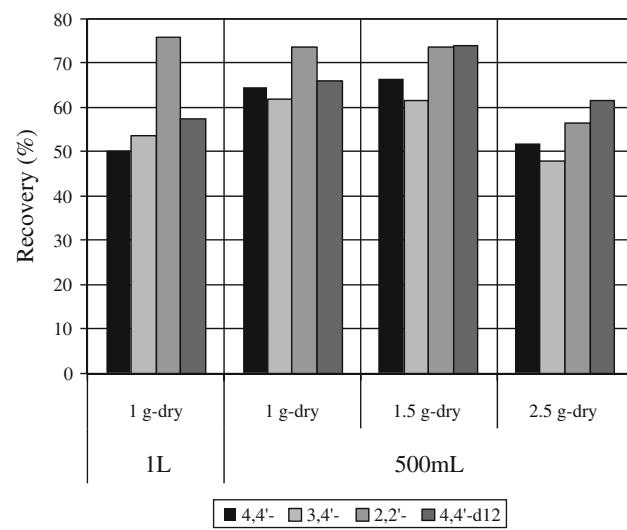
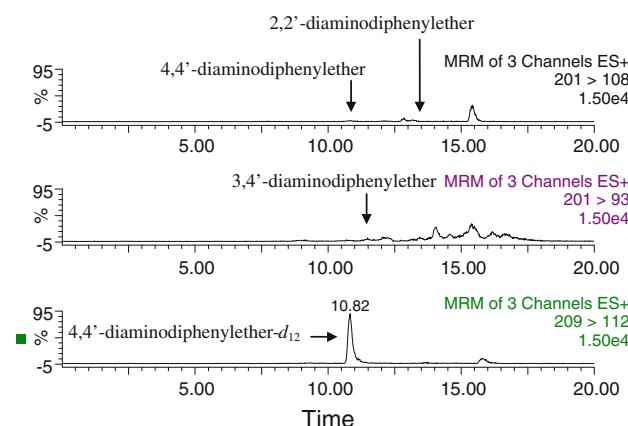


Fig. 3 Recoveries of the 4,4'-, 3,4'-, 2,2'-diaminodiphenylethers and 4,4'-diaminodiphenylether- d_{12} , in 500 mL or 1 L solutions extracted from 1, 1.5 and 2.5 g dry sediment samples using SPE cartridges

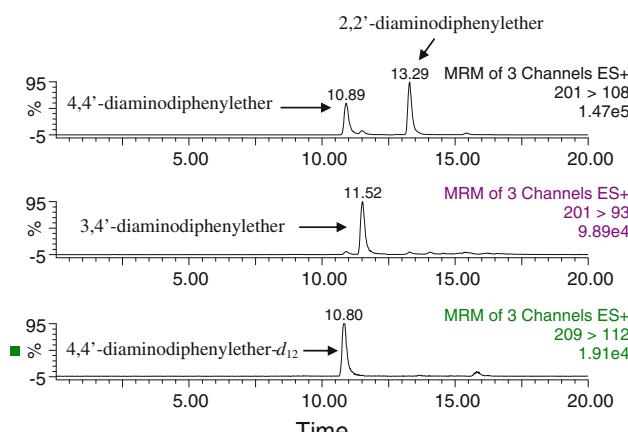
Table 1 Recoveries of 4,4'-, 3,4'-, 2,2'-diaminodiphenylethers in sediment (n = 5)

Chemicals	Spiking level (ng/g-dry)	Detected concentration (ng/g-dry)	Recovery (%)	Precision (R.S.D.) (%)
4,4'-Diaminodiphenylether	0	n.d.	–	–
	100	98	98	5.3
3,4'-Diaminodiphenylether	0	n.d.	–	–
	100	90	90	7.6
2,2'-Diaminodiphenylether	0	n.d.	–	–
	100	106	106	9.5

n.d. not detected



(a) LC/MS/MS-SRM chromatograms in sediment from the sea.



(b) LC/MS/MS-SRM chromatograms in sediment from the sea added 100 ng of 4,4'-, 3,4'-, 2,2'-diaminodiphenylethers.

Fig. 4 LC/MS/MS-SRM chromatograms for the 4,4'-, 3,4'-, 2,2'-diaminodiphenylethers and 4,4'-diaminodiphenylether-d₁₂ in the sediment samples from the sea

selected for the extraction of the 4,4'-, 3,4'-, 2,2'-diaminodiphenylethers.

The methods detection limits (MDL) were determined using sediment samples spiked with the 4,4'-, 3,4'- and 2,2'-diaminodiphenylethers and 4,4'-diaminodiphenylether-d₁₂. Samples of sediment (1 g, dry) were spiked with 20 ng of these components to give a 20 ng/g concentration. The MDL was calculated as two times the t-ratio (1.9432; 6

degrees of freedom, 5% level of significance) multiplied by the standard deviation for seven replicated determinations. The detection limits for this method were 2.0 (4,4'-), 1.7 (3,4'-), and 4.8 (2,2'-) ng/g-dry, respectively. The MDL that the Japanese Ministry of the Environment demands for the risk assessment of the environment is 2.5 ng/g-dry for 4,4'-diaminodiphenylether. Therefore, our method satisfies the requirement for the sensitive and selective detection of 4,4'-diaminodiphenylether in the environment.

Recoveries for the 4,4'-, 3,4'-, 2,2'-diaminodiphenylethers, expressed as the overall mean from a series of five analyses, are shown in Table 1. The recoveries of these chemicals in sediment samples from the sea were 98% (4,4'-), 90% (3,4'-), and 106% (2,2'-), respectively. The relative standard deviations were 5.3% (4,4'-), 7.6% (3,4'-), and 9.5% (2,2'-), respectively.

Sediment samples from three different sites in the bay in Fukuoka Prefecture, were sampled in October 2010. Representative SRM chromatograms for sediment samples from the sea, with and without the addition of a 100 ng standard, are shown in Fig. 4. The 4,4'-, 3,4'-, 2,2'-diaminodiphenylethers were not detected in the sediment samples from the sea.

Our method enables the detection of the 4,4'-, 3,4'-, 2,2'-diaminodiphenylethers in sediment samples, both simultaneously and selectively. The methods detection limits are low and show good reproducibility. Our method is simple, allows high recoveries and can be successfully applied to real sediment samples.

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