

## Optimization of Analytical Conditions for a Rapid Determination of Aniline in Environmental Water by Liquid Chromatography/Tandem Mass Spectrometry

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A rapid determination of aniline in environmental water was examined based on liquid chromatography/tandem mass spectrometry (LC/MS/MS). Environmental water samples were diluted 20-fold with Mill-Q water and measured by LC/MS/MS after adding a surrogate substance (aniline-*d*<sub>5</sub>). In the results of the present study, the calibration curve of aniline showed good linearity in the range of 0.05 – 2.0 µg/L. Since the RSD (repeatability) by measuring repeatedly an aniline standard solution (0.05 µg/L, *n* = 7) was 3.2%, the repeatability of this work was very excellent. In addition, the recovery rate of aniline in environmental water was in the range of 99.0 – 102% with RSD 3.4 – 7.7%, and very good recovery test results were obtained. From these results, this analytical method was confirmed to be effective for aniline measurements of environmental water samples. Also, it is possible to conduct rapid analyses of aniline in environmental water without any solid-phase extraction process, compared to the solid-phase extraction-GC/MS method.

**Keywords** Aniline, LC/MS/MS, rapid analytical

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### Introduction

Aniline is mainly used as a raw material for the synthesis of 4,4'-methylenedianiline, which is an intermediate of 4,4'-diphenylmethane diisocyanate of urethane raw materials. It also has been used for dyes, mordant agents, rubber chemicals (sulfur accelerator), pharmaceuticals (pneumonia, suppuration disease, antipyretics), organic synthesis, fungicides, paints and the like. However, aniline shows a toxic effect on aquatic organisms, such as *Daphnia magna*,<sup>1,2</sup> etc. For this reason, the Ministry of the Environment has added aniline to required monitoring materials that were used for the conservation of aquatic organisms (guideline values: freshwater 0.02 mg/L, sea area 0.1 mg/L).<sup>3</sup>

So far, the analytical method for the determination of aniline in environmental water using gas chromatography,<sup>4,5</sup> and the gas chromatography/mass spectrometry<sup>6</sup> (referred to as "GC/MS") has been investigated. Ministry of the Environment has adopted a solid-phase extraction-GC/MS as an official method of aniline measurements in environmental samples; this method has the advantage of high sensitivity and selectivity.<sup>3,7</sup> However, in this analysis method, there are disadvantages in that aniline is lost by volatilization when the solid-phase column is applied into the reduced pressure state at the time of the dehydration process. For this reason, in order to accurately quantify aniline, analysts will be required for operating a careful pretreatment.

In recent years, liquid chromatography/tandem mass spectrometry (referred to as "LC/MS/MS") has been attracting

attention as a new analysis method of water supply, environment, pharmaceutical and product areas. This method has the advantage of a higher measurement sensitivity for numerous measurable substances compared to GC/MS. There are many reports of an analytical method using LC/MS/MS, such as haloacetic acid<sup>8,9</sup> pesticides,<sup>10</sup> organic tin,<sup>11</sup> PFOS/PFOA (organic fluorine compounds)<sup>12</sup> and LAS (anionic surfactant).<sup>13</sup>

However, there is little information on the application of LC/MS/MS with the aid of an isotope dilution method into environmental samples, since the analyte in the sample containing a high concentration of matrix (nonvolatile salts) could not be appropriately ionized by the ion source, such as electrospray ionization, in the LC/MS/MS apparatus, and it is impossible to directly inject environmental samples (especially seawaters) into LC/MS/MS for accurate analysis.

Therefore, in this study, the rapid analytical method was applied for the determination of aniline with a 2 µg/L (1/10 of the guideline values) concentration in environmental water using LC/MS/MS and the isotope dilution method, for the purpose of problem-solving, which is present in the analysis method using GC/MS.

### Experimental

#### Reagent

1) *Aniline standard stock solution* (1000 mg/L). The aniline standard (manufactured by Wako Pure Chemical Industries, Ltd.), 100 mg, was dissolved into 100 mL of methanol.

2) *Aniline standard solution for the calibration curve* (0.05 – 2 µg/L). The aniline standard stock 1 mL had a constant volume of up to 100 mL with Mill-Q water. It was then diluted to

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6 steps in the 0.05 – 2 µg/L range with Milli-Q water.

3) *Aniline-2,3,4,5,6- $d_5$  standard stock solution (100 mg/L: referred to as “aniline- $d_5$ ”).* The aniline- $d_5$  standard (manufactured by Wako Pure Chemical Industries, Ltd.), 10 mg, had a constant volume of up to 100 mL of methanol. This surrogate substance of aniline was used as an internal standard.

4) *Mobile phase.* Ammonium acetate (Wako Pure Chemical Industries, Ltd., special grade) used in the mobile phase was used. Acetonitrile (Kanto Chemical Co., LC/MS analysis grade) used in the mobile phase was used. The purified water used in the mobile phase was Milli-Q water.

#### Optimization of LC/MS/MS measurement conditions

The LC/MS/MS measurement conditions used in this study were optimized as follows. The Agilent Co., Ltd. 1260 LC section of was used and the Agilent Co., Ltd. 6460 MS/MS section was used. The separation column used was an InertSustain C18 (2.1 × 150 mm 3 µm: GL Sciences Inc.); its temperature was 40°C. The mobile phase was a mixed solution of an A: 0.5 mM ammonium acetate aqueous solution (containing 0.1% formic acid) and an B: acetonitrile. In addition, the ratio of mobile phases was set to A:B = 95:5, and isocratic measurement was used. The flow rate was 0.2 mL/min and the injection volume was 20 µL.

The measurement conditions of the MS/MS section were optimized as follows. The measurement mode was the ESI method (positive), the fragment voltage (fragment voltage is the voltage added to the ion species generated within the ESI ion source) was 30 V for aniline, and 100 V for aniline- $d_5$ . The multiple reaction monitoring (MRM) transitions were  $m/z$  94 to 51 (collision voltage 35 V) for the quantitative ion of aniline,  $m/z$  94 to 77 (collision voltage 20 V) for the quality ion of aniline and  $m/z$  99 to 54 (collision voltage 35 V) for the quantitative ion of aniline- $d_5$ . It is to be noted that the collision voltage is a voltage that adds the mass selected by Q1 to accelerate decomposition in the collision cell.

#### Calibration curve, linearity, calculation of limit of quantification and RSD

The linearity of the calibration curve (0.05 to 2.0 µg/L) of the aniline standard solution obtained under the LC/MS/MS measurement conditions was evaluated. The aniline standard solution (0.05 µg/L) was 7-times repeatedly measured to calculate the mean value, standard deviation ( $\sigma$ ), limit of quantification (10 S/N) and RSD (repeatability).

#### Pretreatment method of sample

The pretreatment method of samples was carried out as follows. One milliliter was taken to a 2-mL glass vial from the sample, which was diluted 20-fold with Milli-Q water. Then, a 10-µL surrogate substance standard solution (aniline- $d_5$ : 100 µg/L) was added to the diluted sample. The pretreated samples were analyzed under the above-mentioned LC/MS/MS measurement conditions. Also, the same procedure was applied to the standard solution for the calibration curve measurement.

#### Addition recovery test of the environmental water

In order to evaluate whether this analytical method can be applied to a variety of environmental samples (river water and seawater), it was conducted to evaluate an addition recovery test of aniline ( $n = 7$  for 2 days) under the measurement conditions previously described. Incidentally, the samples used in the addition recovery tests were prepared as follows.

The aniline standard solution (2000 mg/L; 1 mL) was added to the 1 L of environmental water. The added environmental

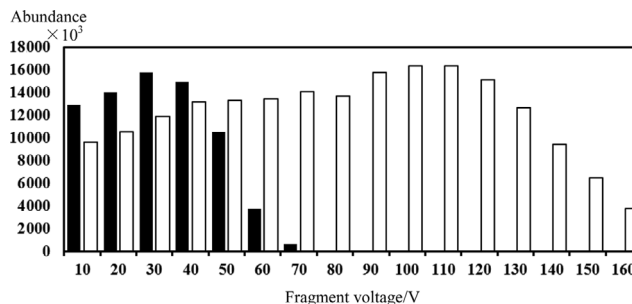


Fig. 1 Relationship between the measured intensity of precursor ions (aniline and aniline- $d_5$ ) and the fragment voltage. ■, The measured intensity of an aniline; □, the measured intensity of an aniline- $d_5$ .

water had a constant volume to 1000 mL. The recovery tests were examined for the environmental water of river water (Kushida river) and seawater (Ise bay). Aniline in the prepared test solution contained 2.0 µg/L (1/10 of the guideline values).

## Results and Discussion

#### Optimization of the MS/MS measurement conditions and compatibility of aniline- $d_5$ as an internal standard

Figure 1 shows the relationship between the measured intensity of the precursor ions of aniline and aniline- $d_5$  and the fragment voltage. According to this figure, when the fragment voltage was 30 V, the measured intensity of the precursor ion of aniline was the highest. It seems that it is difficult to detect the precursor ions of aniline at a high fragment voltage, because the precursor ions of aniline are prone to fragmentation and are unstable. On the other hand, the precursor ion of aniline- $d_5$  was the highest concerning to the measurement intensity when the fragment voltage was 100 V, unlike in the case of aniline. This is because the D atom present in aniline- $d_5$  has twice the mass of the H atom, so that intramolecular transfer reaction and fragmentation are less likely to occur.<sup>14</sup> Therefore, since the precursor ion of aniline- $d_5$  is more stable relative to the precursor ion of aniline, it could be considered that the measured intensity increased with a high fragment voltage.

As a result of the SCAN measurement of product ions, the product ions of aniline were detected at  $m/z$  51 and 77; the product ions of aniline- $d_5$  were detected at  $m/z$  54 and 82. These measurement results indicate that the product ions having the structure of  $[M-(NH_2)]^+$  ( $m/z$  77) and  $[M-(NH_2)-(C_2H_5)]^+$  ( $m/z$  51) were formed because the precursor ions,  $[M+H]^+$ , of aniline were impinged with nitrogen molecules in the collision cell. In addition, the measurement intensity of the product ion (94/77) of aniline was highest for a collision voltage 20 V, and the measurement intensity for the product ion (94/51) of aniline was highest for a collision voltage 35 V. This tendency was similar to the case of aniline- $d_5$ , and it was found that the process of producing product ions of aniline and aniline- $d_5$  showed the same behavior.

Therefore, it could be judged that aniline- $d_5$  can be used as an internal-standard substance by adjusting the fragment voltage of the precursor ion of aniline- $d_5$ , so as to exhibit the same behavior as the precursor ion of aniline.

#### Calibration curve, limit of quantification and repeatability

It was confirmed that the calibration curve (0.05 to 2.0 µg/L)

Table 1 Results of the addition of recovery test (river water and seawater:  $n = 7$ , 2 days)

Sample	Added/ $\mu\text{g L}^{-1}$	$n$	Analysis/ $\mu\text{g L}^{-1}$	RSD, %	Recovery, %
River water 1	2.0	7	1.98	4.2	99.0
River water 2	2.0	7	2.04	7.7	102
Seawater 1	2.0	7	1.89	5.9	94.5
Seawater 2	2.0	7	1.90	3.4	95.0

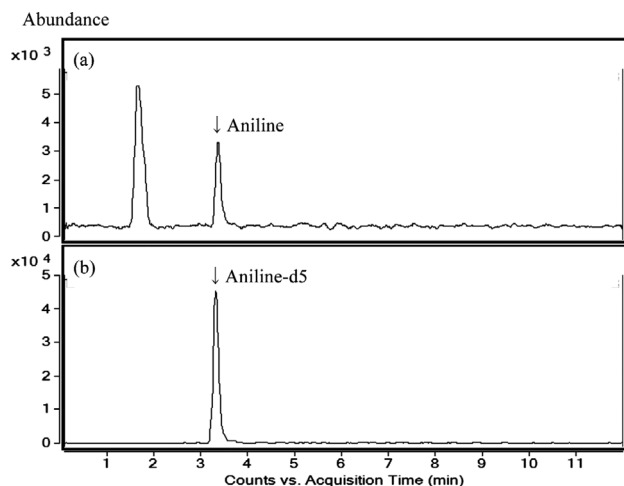


Fig. 2 MRM chromatogram obtained by measuring a seawater sample spiked an aniline standard. (a) shows MRM chromatogram of aniline and (b) shows MRM chromatogram of aniline- $d_5$ .

for the aniline standard solution obtained under the LC/MS/MS measurement conditions has a multiple correlation coefficient of 0.999 or more, and has good linearity. In addition, as a result of repeated measurements of an aniline standard solution ( $0.05 \mu\text{g/L}$ ), since the RSD was 3.2% ( $n = 7$ ), very good repeatability was obtained. The limit of determination ( $10 S/N$ ) of aniline was  $0.016 \mu\text{g/L}$ .

#### Results of the addition recovery test of environmental samples and comparison between this analysis method and GC/MS method

Table 1 gives the results of adding the recovery tests (7 parallel for 2 day) for river water and seawater in which aniline was added of a concentration of  $2.0 \mu\text{g/L}$  (1/10 of the indicator value). From this table, the recovery efficiencies of aniline in river water and seawater were 99.0–102% with RSD being 3.4–7.7%, and very good recovery test results were obtained. In addition, Fig. 2 shows an MRM chromatogram obtained by measuring a seawater sample in which aniline was added. From this figure, because the peak of aniline measured in 3.3 min and the peak of coexisting substances (mainly salts) measured in 1.8 min could be separated, and it was possible to obtain

excellent aniline and aniline- $d_5$  peak shapes with high selectivity. Furthermore, the lower limit of quantification ( $10 S/N$ ), calculated from the measurement result of river water No. 1, was  $0.83 \mu\text{g/L}$ , which satisfied the quantification limit value of  $2 \mu\text{g/L}$ .

From the above, it is possible to use this analysis method to measure aniline at a concentration of  $2 \mu\text{g/L}$  (1/10 of the guideline value). It was confirmed that this analysis method has a performance equal to, or better than, the limit of quantification of  $2 \mu\text{g/L}$ , indicated by the public notice method (solid phase extraction-GC/MS method) by the Ministry of the Environment. Compared to the public notice method, this analysis method can greatly accelerate aniline analysis, because the solid-phase extraction process requiring about 3 h of work time becomes unnecessary. Furthermore, because the isotope dilution method in combination with LC/MS/MS gives very little application into the analysis of harmful substances in environmental samples, the present work will become a promising method which one can analyze aniline in water samples.

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