LC/MS/MS Method for Detection of N-methyldidecane-1-ylamine in Environmental Water

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Summary

A determination method for N-methyldidecane-1-ylamine in environmental water by liquid chromatography tandem-mass spectrometry (LC/MS/MS) was developed. The method detection limit (MDL) and method quantification limit (MQL) were 0.00055 µg/L and 0.0014 µg/L, respectively. The average recoveries from river water and sea water were 98% with a relative standard deviation (RSD) of 6.2% and 85% with a RSD of 2.4%, respectively. The concentrations of N-methyldidecane-1-ylamine in rivers and five locations in northern Ise Bay in Nagoya City were below the MDL. The concentrations of N-methyldidecane-1-ylamine added to river water and sea water were almost stable over a three-day preservation period, while the concentrations decreased by 50% after seven days of preservation. In order to discuss the mechanism underlying the aforesaid decrease, the preserved solutions were analyzed by liquid chromatography/high-resolution mass spectrometry. Two compounds were identified as the oxidation products of N-methyldidecane-1-ylamine in the preserved solutions.

Key words: N-methyldidecane-1-ylamine, LC/MS/MS, environmental water

INTRODUCTION

N-methyldidecane-1-ylamine is used as a cationic surfactant, as it imparts softness or antistatic properties to textiles and human hair, and is hence a major ingredient of textile softeners and hair rinses1 2. The production of cationic surfactants in Japan stands at 50,000 tons per year, which constitutes 5% of the total surfactant production1 2. The production amount of N-methyldidecane-1-ylamine has increased significantly in the recent years (135 tons in 2012, 224 tons in 2013, 530 tons in 2014, and 517 tons in 20152).

N-Methyldidecane-1-ylamine has been recognized to be harmful to the human body, as it can cause chemical burns on the skin and in the eyes4 5. This chemical is designated as a Priority Assessment Chemical Substance, the environmental risks of which should be evaluated by the Chemical Substance Control Law6. However, risk assessment using the environmental measurement data for this chemical has not yet been carried out, as this chemical started to be used in Japan in 2011. For reliable risk assessment, accurate and sensitive detection of N-methyldidecane-1-ylamine at a concentration level of 0.001 µg/L, and the non-observed effect concentration (NOEC) of this chemical7 8, are imperative.

To date, the reason why the µg/L levels of N-methyldidecane-1-ylamine have not been measured except for that of the oxide is not known7 8. The reason might be due to the high hydrophobicity (log P ow 8.88) or highly nucleophilic property of tertiary amine of N-methyldidecane-1-ylamine. Recently, nucleophilic compounds and hydrophobic compounds have been successfully analyzed by liquid chromatography-tandem mass spectrometry (LC/MS/MS)9 12. The authors reported a provisional measurement method of N-methyldidecane-1-ylamine in environmental water21. In the presented paper, the authors discuss an LC/MS/MS method determining 0.001 µg/L levels of N-methyldidecane-1-ylamine in environmental water and its application to the surveillance of environmental water quality in Nagoya City.

EXPERIMENTAL

Reagents

Standards of N-methyldidecane-1-ylamine (didecylmethylamine, >95% purity) were purchased from Tokyo Chemical Industry Co. (Tokyo, Japan). Formic acid, acetone, and hexane for pesticide residue analysis, and sodium chloride and anhydrous sodium sulfate for polychlorinated biphenyl and phthalic acid ester analysis were purchased from Wako Pure Chemical Industries (Osaka, Japan). Acetonitrile for LC/MS analysis was purchased from Kanto Chemicals (Tokyo, Japan). Standard solutions of N-methyldidecane-1-ylamine were prepared in acetonitrile. Sodium chloride was baked at 600℃ for 2 h, and sodium sulfate was washed sequentially with 30 mL of acetone and 30
mL of hexane.

**Analytical conditions**

A Waters Alliance2695/Quattro micro API (Milford, MA, USA) LC-MS-MS instrument was used for quantitative analysis. A Waters Acquity Xevo G2-S QTof (Milford, MA, USA) liquid chromatograph/high-resolution mass spectrometer (LC/HRMS) was used for qualitative analysis. An Ascentis Express C18 (10 cm × 2.1 mm, 2.7 µm, Sigma-Aldrich Co., St. Louis, MO, USA) column was used for both the quantitative and qualitative measurements; the mobile phase was acetonitrile and formic acid/pure water (0.1:99.9), with gradient elution of 50-95% acetonitrile for 17.5 min at a flow rate of 0.2 mL/min. N-methyldidecane-1-ylamine was measured by selected reaction monitoring (SRM). The MS/MS conditions and ToFMS conditions for the quantitative and the qualitative measurements are respectively listed in Table 1 and Table 2.

**Sampling and Sample preparation**

Environmental water samples were collected from ten rivers and at the mouth of Ise Bay in Nagoya City. River waters were collected at the centers of the rivers and seawaters were at the surface with a stainless-steel bucket. The sampled waters were subsequently transferred into 1-litre glass bottles and stored in refrigerator at 4°C. All the water samples were analyzed within three days from the sampling.

Fig. 1 shows the analytical scheme for N-methyldidecane-1-ylamine. Glass bottle was washed with 20 mL of acetone to extract N-methyldidecane-1-ylamine adsorbed on the glass surface of the bottle. The extracted solution was dissolved into a 100 mL sample of water in separating funnel. Twenty-five milliliters of 25% NaCl aqueous solution and 50 mL of acetone were added to the 100 mL of sample water, and then extracted twice with 50 mL portions of hexane. The extract was washed with 50 mL of acetonitrile, dehydrated over anhydrous Na₂SO₄, and concentrated to 5 mL on a rotary evaporator. Subsequently, the concentrated solution was diluted to 10 mL with acetonitrile.

| Table 1 | MS/MS conditions for the quantitative measurements |
|---------|--------------------------------------------------|
| Monitor ions | ESI-positive-SRM |
|            | quantitation | confirmation |
| Cone voltage (V) | 312 > 172    | 312 > 85 |
| Collision Energy (eV) | 25            | |
| Capillary voltage (kV) | 3              | |
| Source Temp. (°C) | 110           | |
| Desolvation Temp. (°C) | 450         | |
| Desolvation Gas (L/hr) | 700           | |
| Cone Gas (L/hr) | 50           | |

| Table 2 | ToFMS conditions for the qualitative measurements |
|---------|--------------------------------------------------|
| Monitor ions | ESI-positive and ESI-negative |
| Leucine enkephalin | m/z 50-500 |
| Lock mass | Cone voltage (V) | 25 |
| Mass accuracy (ppm) | Collision Energy (eV) | 25 |
| Resolution | Capillary voltage (kV) | 3 |
| Desolvation Temp. (°C) | Source Temp. (°C) | 110 |
| Desolvation Gas (L/hr) | 450 |
| Cone Gas (L/hr) | 700 |

![Scheme for the analysis of N-methyldidecane-1-ylamine in environmental water](image)

Fig. 1 Scheme for the analysis of N-methyldidecane-1-ylamine in environmental water
RESULTS AND DISCUSSION

Evaluation of the measurement method

The SRM chromatogram of N-methyldidecane-1-ylamine is shown in Fig.2-(1). The calibration curve of the standard solution shows linearity in the range 0.0500–100 ng/mL with an R² value of 0.996. Table 2 shows the recovery of N-methyldidecane-1-ylamine from river water and sea water, method detection limit (MDL), and method quantification limit (MQL). The present method allows for N-methyldidecane-1-ylamine detection in environmental water above an MDL of 0.001 µg/L, with good reproducibility. The recovery efficiency of N-methyldidecane-1-ylamine (Table 3) from river water and sea water is 98.3% (relative standard deviation (RSD) 6.2%) and 85.2% (RSD 2.4%), respectively.

Effect of experimental parameters on quantitation accuracy

A comparatively large amount of N-methyldidecane-1-ylamine was eluted from the anhydrous sodium sulfate (NaSO₄), the dehydrating reagent of hexane solution.

Hence, two procedures were adopted for the removal of N-methyldidecane-1-ylamine from anhydrous Na₂SO₄: 1) washing with hexane and 2) sequential washing with hexane and acetone. As illustrated in Figs. 2-(2) and (3), a peak due to N-methyldidecane-1-ylamine was observed in the SRM chromatogram of the blank with anhydrous Na₂SO₄ washed using hexane but not in the chromatogram of the blank with the Na₂SO₄ washed using hexane and acetone.

NaCl used for salting-out was also a source of contamination of N-methyldidecane-1-ylamine. As illustrated in Fig. 2-(4) and (5), the peak of the target compound was observed (signal to noise ratio (S/N) of 2–3) in the chromatogram of the blank with the non-pretreated NaCl, while the contaminant peak disappeared in the chromatogram of the blank with NaCl baked at 60°C for 2 h.

The recovery of N-methyldidecane-1-ylamine was affected by coexisting compounds in the extraction solution from river water and sea water. Table 3 shows the recovery efficiency of N-methyldidecane-1-ylamine from water samples using acetonitrile/hexane partitioning. The recovery efficiencies were higher in the case of river water than for sea water.

N-methyldidecane-1-ylamine surveillance in environmental water in Nagoya City

Using the proposed method, N-methyldidecane-1-ylamine was measured at 10 locations in rivers and at 5 locations in Northern Ise Bay, Nagoya City in June 2017. Fig. 2-(6) is an SRM chromatogram of N-methyldidecane-1-ylamine of Horikawa river sample. N-methyldidecane-1-ylamine was not found above a limit of detection (LOD) of 0.0050 ng/L in any of the samples.

N-methyldidecane-1-ylamine discharged in environmental water was suspected to be subsequently degraded under the conditions listed in Table 4. In experiments (1) to (9) listed in Table 4, the preservation stability of 0.050 µg/L N-methyldidecane-1-ylamine in various solvents was examined for seven days by measuring the recoveries. The concentrations of N-methyldidecane-1-ylamine in hexane preserved in a glass tube and plastic tube (1) and (2), in river water and seawater in glass bottles (3) and (4), and in autoclaved river water and seawater preserved in glass bottles (5) and (6) decreased to 0–26% of the original value during preservation for seven days, while the concentrations in methanol, methanol saturated with non-contaminated Na₂SO₄, and acetonitrile (7), (8), and (9) remained unchanged during the preservation period. The latter observation suggested that the concentration of N-methyldidecane-1-ylamine does not change in a solvent that shows affinity for the lone pair of electrons on the nitrogen atom. The concentration decrease in autoclaved river water and seawater was most probably due to the oxidation of N-methyldidecane-1-ylamine and not due to biological processes.

Degradation product of N-methyldidecane-1-ylamine

Ultra-performance liquid chromatography/high-resolution mass spectrometry (UPLC/HRMS) was applied to identify the degradation product of N-methyldidecane-1-ylamine. Fig. 3 shows the total ion chromatogram (TIC) of 1.0 mg/L N-methyldidecane-1-ylamine in hex-
Table 3  Recovery rate of N-methyldidecane-1-ylamine from river water and sea water

| Sample                      | River Water | Sea Water |
|-----------------------------|-------------|-----------|
| Sample Volume (mL)          | 100         | 100       |
| Added Amount (µg)           | 0           | 0.0005    |
| Number of experiment        | 1           | 7         |
| Concentration (µg/L)        | ND          | 0.00492   |
| Recovery Rate (%)           | 98.3        | 85.2      |
| C.V. (%)                    | 6.2         | 2.4       |
| MDL* (µg/L)                 | 0.00055     | 0.00173   |
| MQL** (µg/L)                | 0.000140    | 0.00407   |

* MDL: method detection limits were calculated with the following equations:
MDL (river water) = \( t(n-1, 0.05) \times \sigma_{n-1,M} \times 2 = 1.9432 \times 0.000140 \times 2 = 0.000545 \)
MDL (sea water) = \( t(n-1, 0.05) \times \sigma_{n-1,M} \times 2 = 2.1318 \times 0.0000407 \times 2 = 0.00173 \)

** MQL: method quantification limits were calculated with the following equations:
MQL (river water) = 10 x \( \sigma_{n-1,M} = 10 \times 0.000140 = 0.00140 \)
MQL (sea water) = 10 x \( \sigma_{n-1,M} = 10 \times 0.0000407 = 0.00407 \)

Table 4  Changes in concentration of N-methyldidecane-1-ylamine in prepared sample solutions preserved during 7 days at 4℃

| Experiment* | Addition Concentration | Preservation period ( % ) |
|-------------|------------------------|---------------------------|
| (1) µg/L    | 0.005                  | 100.2 101.7 96.7 0.0 0.0 |
| (2) µg/L    | 0.005                  | 102.4 102.5 102.6 0.0 0.0 |
| (3) µg/L    | 0.003                  | 104.1 101.4 53.8 - 25.5 |
| (4) µg/L    | 0.003                  | 101.4 83.5 27.5 - 20.6 |
| (5) µg/L    | 0.005                  | 99.9 84.9 80.8 - 29.4 |
| (6) µg/L    | 0.005                  | 98.2 87.5 59.0 - 31.1 |
| (7) µg/L    | 0.005                  | 102.7 96.2 97.5 96.1 94.9 |
| (8) µg/L    | 0.005                  | 102.4 96.5 101.4 98.0 92.6 |
| (9) µg/L    | 0.005                  | 104.1 99.9 96.2 100.2 98.6 |

* Experiment
(1) preserved in hexane in glass tube
(2) preserved in hexane in plastic tube
(3) preserved in river water in glass tube
(4) preserved in seawater in glass tube
(5) preserved in autoclaved river water in glass tube
(6) preserved in autoclaved seawater in glass tube
(7) preserved in methanol in glass tube
(8) preserved in methanol saturated with non-contaminated Na₂SO₄ in glass tube
(9) preserved in acetonitrile in glass tube
ane for the storage duration of 0–7 days. Sharp peaks were observed at retention times of 4 min (peak A, N-methyldidecane-1-ylamine), 8 min (peak B), 11 min (peak C), and 13 min (peak D).

Accurate mass spectra of peak B, C and D were analyzed to determine the elemental compositions. Table 5 shows the elemental composition candidates of peaks B, C, and D. From the protonated ion and sodium adduct ion in the mass spectrum, peak D was estimated to be bis-(2-ethylhexylphthalate) and/or bis-(n-octylphthalate). Accurate mass value of the precursor ion (probably protonated ion) of peak B, $m/z$ 301.1422, corresponded to formulas $\text{C}_{21}\text{H}_{18}\text{NO}^+$ and $\text{C}_{21}\text{H}_{18}\text{O}^+$. If $\text{C}_{21}\text{H}_{18}\text{NO}^+$ was a protonated ion, the intact molecule of $\text{C}_{21}\text{H}_{20}\text{NO}$ could be produced by oxidative dehydrogenation and direct oxidation of tertiary amine to produce N-oxide from N-methyldidecane-1-ylamine. If $\text{C}_{18}\text{H}_{16}\text{NO}^+$ were the protonated ion of the degradation product, the intact molecule of $\text{C}_{18}\text{H}_{20}\text{O}$ must be produced by deamination of N-methyldidecane-1-ylamine. However the deamination is not feasible as the tertiary amine of N-methyldidecane-1-ylamine. Then $\text{C}_{21}\text{H}_{20}\text{NO}$ was supposed to be formula of Peak B. Accurate mass value of the precursor ion (probably protonated ion) of peak C, $m/z$ 385.2920, corresponded to $\text{C}_{21}\text{H}_{22}\text{NO}^+$, $\text{C}_{21}\text{H}_{22}\text{N}_2\text{O}_2^+$ and $\text{C}_{21}\text{H}_{22}\text{O}^+$. $\text{C}_{21}\text{H}_{22}\text{N}_2\text{O}_2^+$, $\text{C}_{21}\text{H}_{22}\text{NO}^+$, the protonated intact molecule of $\text{C}_{21}\text{H}_{22}\text{NO}_x$ could be produced by oxidative dehydrogenation and direct oxidation.

Fig. 3 TICs (m/z 50 to 600 in positive mode) illustrating changes in concentration of N-methyldidecane-1-ylamine, prepared at 1.0 mg/L in hexane, preserved at 4°C for 7 days.

(1) 0 day, (2) 1 day, (3) 2 days, (4) 3 days, (5) 5 days, (6) 7 days of preservation
A: N-methyldidecane-1-ylamine; B-D: peaks of unknown species
of tertiary amine to produce N-oxide from N-methylididecane-1-yl-amine. However, \( \text{C}_9\text{H}_{18}\text{N}_2\text{O}_2 \) and \( \text{C}_9\text{H}_{18}\text{O}_2 \), respectively corresponding to \( \text{C}_9\text{H}_{18}\text{N}_2\text{O}_2^+ \) and \( \text{C}_9\text{H}_{18}\text{O}_2^+ \), were not produced by oxidative dehydrogenation and direct oxidation of the tertiary amine. Then formula of peak C was supposed to be \( \text{C}_9\text{H}_{18}\text{O}_2 \), possibly a degradation product of \( \text{N-methylididecane-1-ylamine} \).

From the results that \( \text{N-methylididecane-1-ylamine} \) were not found in the environmental waters and that the compound was decomposed in all the water and hexane solutions in preservation (Table 4), it is suggested that \( \text{N-methylididecane-1-ylamine} \) might be oxidized in environmental water as well as in storage process in water and hexane solutions.

**CONCLUSION**

A method based on LC/MS/MS for \( \text{N-methylididecane-1-ylamine} \) determination in environmental water was developed and applied to environmental samples. The concentration level of \( \text{N-methylididecane-1-ylamine} \) levels in river and sea waters in Nagoya City were below 0.001 \( \mu \text{g/L} \) of the LOD. \( \text{N-methylididecane-1-ylamine} \) could be adsorbed on glass surface and was degradable in water, sterilized water, and hexane during preservation. Two possible formulas resulting from the oxidation products of \( \text{N-methylididecane-1-ylamine} \) were identified by LC/HRMS. However, the structures of the chemicals could not be determined due to their low concentrations. For \( \text{N-methylididecane-1-ylamine} \) measurement in environmental water: (1) the sample water should be preserved in a container washed with acetone or methanol, (2) the sample solution should be in a solvent that prevents from oxidation, and (3) measurement should be carried out within a few days of sampling. We intend to carry out further studies to elucidate the degradation mechanism and the environmental behavior of \( \text{N-methylididecane-1-ylamine} \).

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[Table 5: Chemical candidates estimated for the peaks of A to D in Fig. 3 with the accurate mass spectra]

| Peak | Retention time (min) | Accurate mass of precursor ion (m/z) | Possible elemental composition | Suggested chemical |
|------|---------------------|-------------------------------------|------------------------------|-------------------|
| A    | 4.50               | 312.3643                            | \( \text{C}_{21}\text{H}_{44}\text{N} \) | \( \text{N-methylididecane-1-ylamine} \) (original chemical) |
| B    | 8.22               | 301.1422                            | \( \text{C}_{21}\text{H}_{40}\text{NO} \) | an oxidized compound of the original chemical |
| C    | 11.33              | 385.2920                            | \( \text{C}_{21}\text{H}_{42}\text{NO}_2 \) | an oxidized compound of the original chemical |
| D    | 13.88              | 413.2659                            | \( \text{C}_{23}\text{H}_{40}\text{O}_4 \) | di-octyl- and di-\{(2-ethylhexyl)-esters of phthalic acid} |