

Determination of 4-Nitrotoluene Exposure in Water, Soil and Sediment by Gas Chromatography-Mass Spectrometry

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기체크로마토그래피/질량분석기에 의한 토양, 저질 및 수질시료 중 4-Nitrotoluene의 노출평가

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요 약

본 연구는 GC/MS를 이용하여 수질, 토양 및 저질 시료 중의 4-Nitrotoluene (4-NT)을 분석하는 방법을 확립하고자 하였다. 4-NT은 수질 시료 (100 mL)에서 n-hexane으로 추출하였으며, 토양 및 저질 시료 (10 g)에서는 먼저 메탄올로 추출한 후 hexane으로 다시 추출하여 농축시켜 분석하였다. 수질 시료 중의 4-NT의 회수율은 99.1% 이상이었으며 토양에서의 회수율 (recovery)은 약 110%를 보였고 재현성 (relative standard deviation)은 11.99% 이하였다. 검정곡선은 상관계수 (R^2) 값이 수질과 토양 모두에서 0.994 이상의 좋은 직선성을 보여주었다. 본 연구에서 채취한 수질 43개 지역, 토양 35개 지역 및 저질 11개 지역의 모든 시료에서 4-NT은 검출한계 이하로 나타났다. 이 분석방법은 환경 중에 미량으로 존재하는 4-NT의 분석과 모니터링에 유용하게 사용할 수 있는 적합한 방법이라 사료된다.

Key words : 4-nitrotoluene, nitrobenzene-d₅, endocrine disrupter, water, soil, sediment, gas chromatography/mass spectrometry

INTRODUCTION

Nitrotoluenes are used in the synthesis of agricultural chemicals and various dyes (Dunnick, 1993). The nitrotoluenes are high production volume chemicals with over 30 million pounds of o-nitrotoluene

and over 10 million pounds of p-nitrotoluene produced in the US every year (US EPA, 2000). Organic nitrocompounds are the most important constituent of explosives and some of them have been evaluated to be highly toxic or even carcinogenic (Dunnick *et al.*, 2003). Human contact with explosives may originate from a variety of sources, including occupational exposure during the production of ammunition as well as environmental exposure due to the contamination of soil and ground water reservoir (Bader *et*

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al., 1998). Especially, 4-nitrotoluene (4-NT) is used primarily as an intermediate in the production of various dyes (cotton, wool, silk, leather and paper), explosive, pharmaceuticals, and in the production of rubber and agricultural chemicals (NTP, 2002). *Mycobacterium* sp. strain in soil utilizes 4-NT as the sole source of nitrogen, carbon and energy, and metabolizes 4-NT into ammonia or 6-amino-*m*-cresol under certain conditions (Spiess *et al.*, 1998). Recently, nitroaromatic compounds including 4-NT were analyzed in human urine for biomonitoring by gas chromatography-mass spectrometry, and several urinary metabolites were found in urine sample obtained from workers of an ammunition dismantling workshop (Bader *et al.*, 1998). Our laboratory involved in the determination of some chemical contaminants from environmental matrices such as ground water, soils and sediments (Lee *et al.*, 2000; Eo *et al.*, 2000; Park *et al.*, 2003; Jeon *et al.*, 2005a, b).

The clean-up procedure for the GC/MS analysis of 4-NT developed by Japan National Institute of Environmental Studies is tedious and labor-intensive because a large amount of samples (such as 1 L of water) are used and distillation and purification steps with a silica gel column from water and soil samples are required even if the detection limit in water reached to 10 ng/L (NIES, 1998).

4-NT is listed as endocrine disrupting chemicals by National Institute of Health Sciences in Japan (Jobling *et al.*, 1995) and World Wildlife Fund. 4-NT is volatile compound and it may be exposed to water and soil through ambient air. It was reported that absorption of 4-NT through the skin readily occurs, resulting in higher bioavailability of 4-NT (Woollen *et al.*, 1986). Monitoring of this compound in the environment including ground water and soil is necessary for evaluating the extent of environmental contamination and applying to the risk characterization and assessment. In this work, analytical method for determination of the 4-NT in water, soil and sediment was developed by gas chromatography-mass spectrometry.

EXPERIMENTAL METHODS

Chemicals

4-Nitrotoluene (>99%) and nitrobenzene-*d*₅ were purchased from Sigma-Aldrich Korea (Seoul, Korea). Acetone, *n*-hexane (95%, for organic residue analysis), methanol, methylene chloride, and anhydrous sodium sulfate were of analytical grade and purchased from J.T. Baker (Phillipsburg, NJ, USA). Sodium chloride was obtained from Mallinckrodt (Mexico). Sodium chloride and anhydrous sodium sulfate were baked in a furnace at 500°C for 8 hr before use. High purity of helium (Shinyang Oxygen Inc., Seoul, Korea) as a carrier gas was used for the gas chromatographic separation. Distilled water prepared by Milli-Q water system apparatus (Milford, MA, USA) was used. The other agents were of analytical grade.

Water, soil and sediment samples

Non-contaminated blank soil was obtained in the vicinity of Korea Institute of Science and Technology. Real water (43 sites), soil (35 sites) and sediment (11 sites) samples were collected at nationwide sites by Korea National Institute of Environmental Research. These soils were dried at room temperature to a constant weight and mixed in a porcelain dish before use for recovery test. Double distilled water was used for blank and recovery tests.

Instruments

A gas chromatograph/mass selective detector (GC/MSD; HP 6890 plus/HP 5973, Hewlett Packard, USA) was used and the instrument was controlled by ChemStation (G1701AA, Version A.03.00, Hewlett Packard, USA). Samples were injected into the GC/MSD by an auto liquid sampler (HP7673).

Gas chromatography-mass spectrometry

For analyzing 4-nitrotoluene, 50% phenylmethyl-

siloxane capillary column (HP-50+; 20 m, length \times 0.25 mm, internal diameter \times 0.25 μ m, film thickness) was used. The flow rate of helium as carrier gas was 0.7 mL/min. Sample injection (1 μ L) was in splitless mode. The flow rate of septum purge was 5 mL/min. The GC column temperature was programmed from 50°C, ramped at 20°C/min to 260°C, ramped at 25°C/min to 290°C, held for 10 min. For the mass selective detector, electron impact mode was selected at 70 eV. The temperature of transfer line was set at 280°C. 4-Nitrotoluene was detected by using a selected ion monitoring mode. The selected ions was m/z 137 (molecular ion; $[M]^+$), 91 ($[C_6H_4CH_3]^+$) and 65 ($[C_5H_5]^+$) for 4-nitrotoluene. The ions values for quantification were m/z 91 for 4-nitrotoluene. Nitrobenzene- d_5 (m/z 82 ($[C_6D_5]^+$) and m/z 128 ($[M]^+$)) was used as internal standards.

Preparation of samples for calibration curves of 4-nitrotoluene

To 100 mL of distilled water or 10 g of soil and sediment, 4-nitrotoluene (1~250 ng) was fortified and nitrobenzene- d_5 (100 ng; 10 ppm \times 10 μ L) was added as an internal standard. The other steps were the same as described in below.

Extraction of 4-nitrotoluene in water

One hundred mL of water samples was added to 250 mL of a separatory funnel and 10 g of sodium chloride was added and dissolved by gentle shaking. Nitrobenzene- d_5 (100 ng; 10 ppm \times 10 μ L) was added as an internal standard. To the funnel 50 mL of *n*-hexane was added. The separatory funnel was agitated rigorously in a vertical shaker (D0647, Dongyang Inc., Seoul, Korea) for 20 min. After the separation of *n*-hexane layer by discarding an aqueous layer, 50 mL of 5% sodium chloride solution was used for cleansing the organic layer. The organic layer was transferred to a 100 mL round flask and evaporated to about 3 mL with rotary evaporator (Büchi 461, Switzerland). This solution was transferred to a 15 mL centrifuge tube with tapering end

and the round flask was washed twice with small amount of *n*-hexane for combining washed solution to the centrifuge tube. The combined solution was evaporated to 100~200 μ L of final volume. One μ L of the solution was injected to the GC/MSD by an auto liquid sampler.

Extraction of 4-nitrotoluene in soil and sediment

To 40 mL of centrifuge tubes 10 g of soil and sediment was added and mingled homogeneously with the same amount of anhydrous sodium sulfate. Nitrobenzene- d_5 (100 ng; 10 ppm \times 10 μ L) was added as an internal standard. The soil and sediment was extracted with 20 mL of methanol by a shaker (Edmund Buchler 7400, Tübingen, Germany) for 20 min and centrifuged (RT 6000B, Sorvall Inc., New town, CT, USA) for 15 min at 1,660 \times g. The methanol layer was transferred to a 100 mL round flask and evaporated to the final volume of about 3 mL which was transferred to a 15 mL centrifuge tube. To the centrifuge tube 1 mL of 5% sodium chloride solution was added and mixed in a vortex-mixer. After 5 mL of *n*-hexane was added, the tube was extracted by a shaker, centrifuged and put into a freezer (-30°C) for the separation of the organic layer. The organic layer was transferred to a centrifuge tube with tapering end and evaporated for concentration until the final volume reached to about 100~200 μ L. One μ L of this solution was injected to the GC/MSD by the auto liquid sampler.

Table 1. Recoveries of 4-nitrotoluene in water and soil (n=5)

Matrix	Added (ng)	Found (ng)	Recovery (%)	SD (%) ^a	RSD (%) ^b
Water (in 100 mL)	5	4.95	99.1	8.00	8.07
	10	12.43	124.3	4.07	3.27
	50	56.63	113.3	3.68	3.25
Soil (in 10 g)	10	11.30	113.0	13.54	11.99
	50	56.12	112.2	5.18	4.61

^aSD: Standard deviation, ^bRSD: Relative standard deviation

RESULTS

Recoveries and accuracy

To further validate the precision and accuracy of the method recovery testing was carried out by spik-

ing a known amount of the standard to distilled water and soil, which do not contain the test chemicals. Recovery of 4-NT was determined at 5, 10 and 50 ng spiked in 100 mL water and 10 and 50 ng spiked in 10 g soil. The results obtained are shown in Table 1. In water matrix, the recovery of 4-NT was more than 99.1% and the relative standard deviation (RSD)

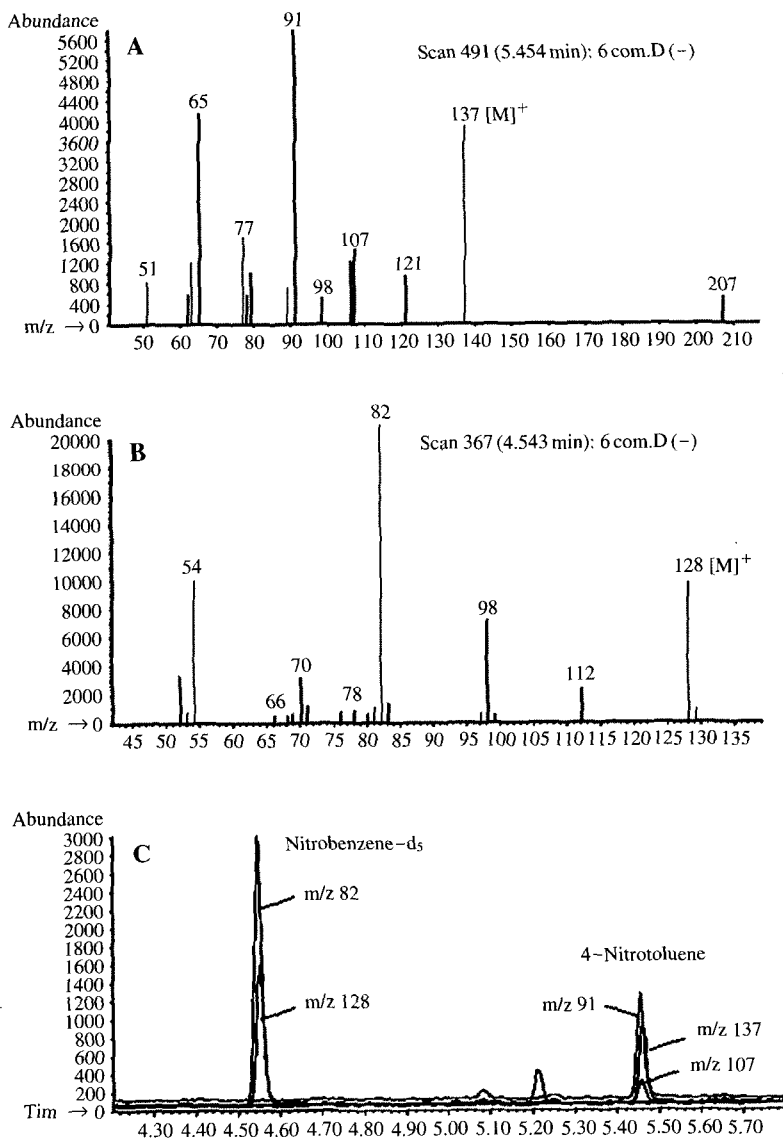


Fig. 1. Gas chromatograph/mass spectrometry (GC/MS) spectra of 4-nitrotoluene (A) and nitrobenzene-d₅ (B), and ion chromatograms (C) obtained by GC/MS/selected ion monitoring of 4-nitrotoluene (m/z 65, 91 and 137). Nitrobenzene-d₅ (m/z 82 and 128) were used as an internal standard for the analysis of 4-nitrotoluene.

values were below 8.07% in all cases. Recoveries in soil of 4-NT was about 110% and the RSD of all recovery experiments was less than 11.99%. The precision of the method is therefore very good.

Selection of quantification ions

Selected ion values for analysis of 4-NT in water

and soil was m/z 65, m/z 91 and m/z 137 ($[M]^+$) for 4-NT and m/z 82 and m/z 128 ($[M]^+$) for nitrobenzene- d_5 as shown in Fig. 1. Ion values used for quantification was m/z 91 for 4-NT. Retention times were 4.54 min for nitrobenzene- d_5 and 5.45 min for 4-NT.

Ion chromatograms for the selected ions of 4-NT

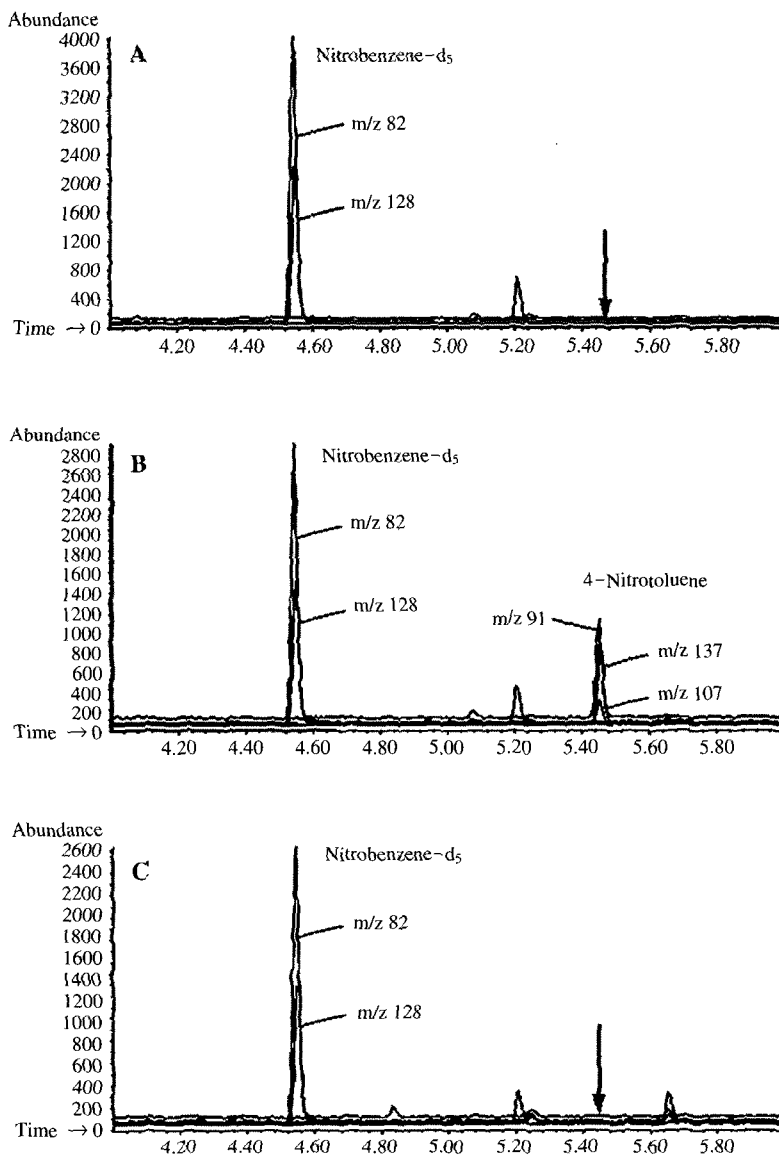


Fig. 2. Ion chromatograms obtained from water blank (A), water fortified standards (B, 250 ng for 4-nitrotoluene spiked), and a river water sample (C). The values of characteristic ions selected for the quantification of 4-nitrotoluene was m/z 91. Internal standard of 100 ng was added.

in water (Fig. 2) and soil (Fig. 3) were shown to compare with those for 4-NT-free blanks. For the selected ions for 4-NT (m/z 91), no major interfering peaks were found.

Linearity of standard calibration curves

The linear range of GC-MS method for the determination of 4-NT was tested by increasing amounts

of standards at 1, 5, 10, 25, 50, 100, 250 ng/ 100 mL water and fixed amount (100 ng/100 mL) of internal standards in distilled water, and the analytes were extracted and concentrated as described above. Linear equations of 4-NT calibration curves were obtained by linear regression. The curve was plotted concentrations at x-axis with the area ratio of 4-NT to internal standard nitrobenzene- d_5 at y-axis. The experiments were repeated in soil (10 g) matrices.

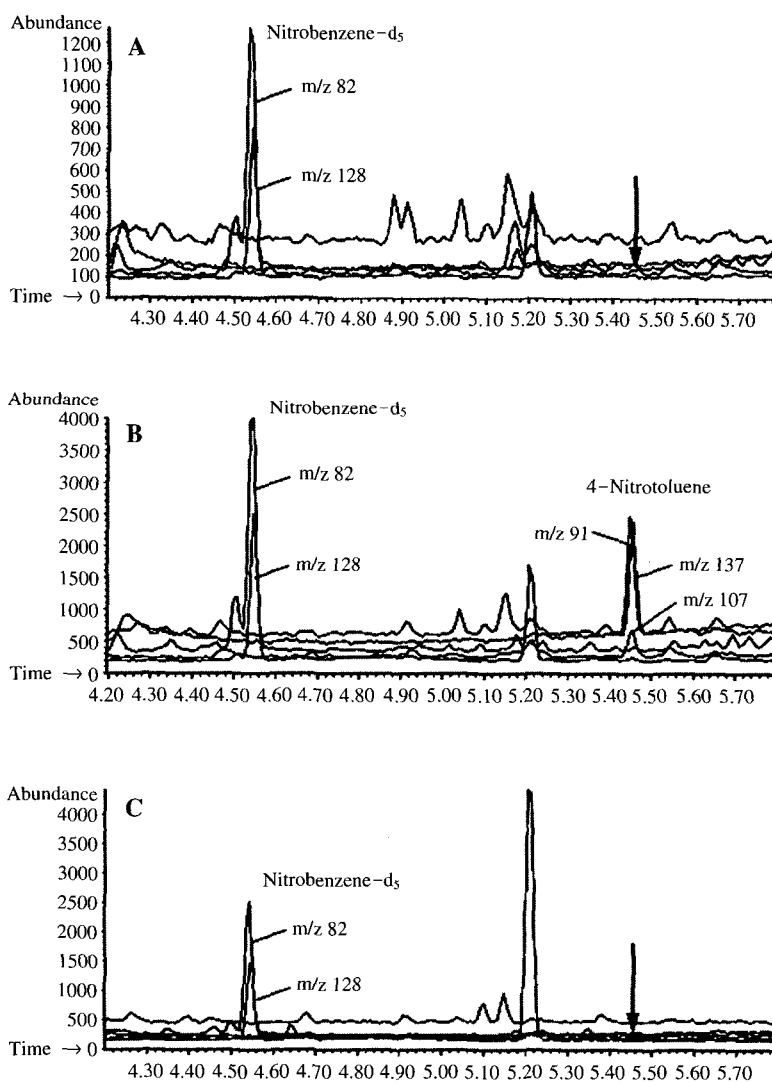


Fig. 3. Ion chromatograms obtained from soil blank (A), soil fortified standards (B, 250 ng for 4-nitrotoluene spiked), and a soil sample (C). The values of characteristic ions selected for the quantification of 4-nitrotoluene was m/z 91. Internal standard of 100 ng was added.

Table 2. Calibration and detection limit of 4-nitrotoluene in water and soil

Matrix	Conc. range	y = ax + b			LOD ^a	LOQ ^b
		a	b	r ²		
Water (µg/L)	0.01 ~ 2.50	0.0066	0.0094	0.9996	0.01	0.04
Soil (µg/kg)	0.1 ~ 25	0.0091	0.0568	0.9945	0.5	1.83

^aLOD: Limit of detection, ^bLOQ: Limit of quantification

The curves showed a good linearity ($r^2 > 0.994$, 7 points, $n = 3$ per point) at concentration ranges spiked (Table 2). Linear equation in water was determined to $y = 0.0066x + 0.0094$ ($r^2 = 0.9996$) for 4-NT. In soil, linear equation was $y = 0.0091x + 0.0568$ ($r^2 = 0.9945$) for 4-NT.

Limit of detection and limit of quantification

The limit of detection (LOD), defined as the peak having a signal-to-noise ratio of 3, was measured by integrating peak area for analyte in 10 independent performances with distilled water and soil. The limit of quantification (LOQ) is the lowest 4-NT concentration the can be quantified in a sample with acceptable precision under the stated operational conditions of the method. LOQ was determined as the analyte concentration corresponding to a signal-to-noise ratio of 10. The method described showed very good sensitivity with detection limit in the low 1 ng/100 mL in water and 5 ng/10 g in soil for 4-NT. Based on this detection limits, LOQ for 4-NT was estimated to 4 ng/100 mL water and 18.3 ng/10 g soil (Table 2).

The analysis of 4-nitrotoluene in real water, soil and sediment

Water, sediment and soil collected in 2002 at nation-wide sites were analyzed. 4-NT was not detected because it has the value below detection limit in all samples. Advantages of this method are using small amounts of sample (100 mL of water and 10 g of soil and sediment), are time-saving and simple with a similar sensitivity, compared to the previous method (Potard *et al.*, 1999) which emp-

loyed 1,000 mL of water and 20 g of soil, as well as required distillation and purification steps.

DISCUSSION

The characteristic ions of 4-nitrotoluene were selected based on their spectra of which the ions of base peak and molecular weight or ions with high intensity such as base peaks were used for quantification and confirmation (Fig. 1). It is useful to select more than 3 ions for a compound in the process of its confirmation. Characteristic ions of m/z 91 for 4-NT was used for the quantification of target compounds. Although other ions also can be used for the quantification, the ion of m/z 91 gives better sensitivity than other ions (molecular ion) for determination of 4-NT.

4-NT is highly volatile and insoluble to water. In using the 50% phenylmethylsiloxane capillary column, the retention time of 4-NT was 5.45 min (Fig. 1). In extraction procedure, evaporation of the organic layer should be very cautious because complete drying of the solvent will remove the residual 4-NT. Chromatographic property of 4-NT was very sensitive to the extent of aging of the column used. By injection of about 100 samples, peak for 4-NT became broad and showed its tailing, therefore requiring cutting of the front part or the exchange of the column. In our procedure, an aliquot (about 0.1 mL) of the organic layer (hexane for water; methanol and hexane for soil) was left to protect the removal of 4-NT during the evaporation. For this purpose, centrifuge tubes have the tapering tip (about 0.5 mL volume) at the end of the tube, with scaled by every

0.1 mL. The use of internal standards of radio-labeled compound (deuterated form) of 4-NT analogue was essential for better reproducibility of data.

In soil, a light solvent like hexane may be not mixed with soil and absorbed well to soil. The penetration of a solvent into soil and solubility of chemicals to the solvent may become important factors for the extraction of soil. Even if methylene chloride will be better choice of solvent for extraction of 4-NT, separation between the solvent and soil is not efficient compared to methanol and other solvents. This is why we used methanol as the first choice of the solvent for soil analysis. For soil analysis, using methanol at first step without hexane extraction as the second step did not get good results if small amount of water could not be removed completely. This problem was solved by addition of hexane extraction step.

Recovery of nitrobenzene, a structural analogue of 4-NT, was reported to be 59.8% by methylene chloride, 38.7% by methylene chloride/acetone (1 : 1, v/v), 46.9% by hexane/acetone (1 : 1, v/v) after ultrasonic extraction in soil (US EPA, 1996). Recovery of 4-NT in soil, however, was not reported by this ultrasonic method (US EPA, 1996). Detection limit of 4-NT is similar to the value of previous report where 4-NT was analyzed by derivatization with pyridine and pentafluoropropionic anhydride (Bader *et al.*, 1998). Our method is relatively simple and rapid to determine 4-NT in water, soil and sediment samples.

CONCLUSION

Analytical method for determining 4-NT in water, soil and sediment samples was developed by gas chromatography/mass spectroscopy. This method is simple, time-saving and reliable enough to analyze 4-NT in small amounts of environmental samples such as soil and ground water. This method may be applied for monitoring the exposure of 4-NT to the environment.

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