

Simultaneous Determination of 4-Nitrotoluene and Benzophenone in Ground Water and Soil by Gas Chromatography-Mass Spectroscopy

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(Received June. 1, 2002, Accepted Jan. 8, 2003)

Abstract : Benzophenone (BZP) and 4-nitrotoluene (4-NT) listed as endocrine disrupting chemicals are suspected to contaminate ground water sites and soil. Analytical method for simultaneous determination of the two chemicals in soil and ground water was developed by gas chromatography-mass spectrometry. Water (100 ml) was extracted with hexane, and soil (10 g) was extracted with methanol and hexane. Recovery in water was >72% for BZP and 90-118% for 4-NT. Recovery for 4-NT and BZP in soil was 51-59% with coefficient variation of less than 19.5%. Calibration curves showed a good linearity with $r^2=0.997$. In water and soil collected at nation-wide sites, BZP was found at 5 sites among 43 water sites at the concentration of 14.87 ± 3.83 ng/100 ml. No 4-NT was found. It is suggested that this method is appropriate to the simultaneous quantitation of 4-NT and BZP in ground water and soil samples.

Key words : 4-nitrotoluene, benzophenone, soil, ground water, gas chromatography/mass spectrometry

1. Introduction

Organic nitrocompounds are the most important constituent of explosives. Nitrotoluenes are chemicals used in the synthesis of agricultural chemicals and various dyes. Especially, 4-nitrotoluene (4-NT) is used primarily as an intermediate in the production of various dyes, explosives, pharmaceuticals, and in the production of rubber and agricultural chemicals. *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 conditions¹. 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 workshop². Benzophenone (BZP) derivatives are used as UV-absorbing agents which are contained in a large number of products such as hair sprays, shampoo, lipsticks, hair dyes, sunscreen lotions, and photoaffinity labeling for various biological materials³⁻⁵. BZP is found in paper and board materials intended for food contact⁶ and in urine as a metabolite of benzodiazepines⁷. This metabolite was determined by gas chromatography/electroncapture detector⁷, high-performance liquid chromatography/UV detector⁸⁻⁹ and gas

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chromatography/mass spectrometry (GC/MS)¹⁰.

The clean-up procedure for the GC/MS analysis of BZP and 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/L¹⁰.

4-NT and BZP are listed in the table of endocrine disrupting chemicals by National Institute of Health Sciences in Japan¹¹ and World Wildlife Fund. 4-NT and BZP are volatile compounds and they 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-NT¹². These chemicals are suspected to contaminate ground water and soil environment, and no literatures of analytical method for determining BZP and 4-NT simultaneously in soil and ground water are found. Monitoring of these compounds 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 simultaneous determination of the 4-NT and BZP in soil and ground water was developed by gas chromatography-mass spectrometry.

2. Experimental

2.1. Chemicals

Benzophenone was obtained from Sigma (St. Louis, MO, USA). 4-Nitrotoluene (>99%) and nitrobenzene-d₅ were purchased from Sigma-Aldrich Korea (Seoul, Korea). Benzophenone-d₅ and benzophenone-d₁₀ were purchased from Supelco (Bellefonte, PA, USA). Acetone, n-hexane (95%, for organic residue analysis), isopropanol, 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 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.

2.2. Soil and water sample

Non-contaminated blank soils were obtained in the vicinity of Korea Institute of Science and Technology. The soil was dried under room temperature and mixed in a porcelain dish before use for recovery test. Double distilled water was used for blank and recovery tests. Soil (46 sites) and water (43 sites) samples were collected at nation-wide sites by Korea National Institute of Environmental Research.

2.3. Instruments

A gas chromatograph/mass spectrometer (GC/MS; 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/MS by an auto liquid sampler (HP7673).

2.4. Gas chromatography-mass spectrometry

For analyzing 4-nitrotoluene and benzophenone, 50% phenylmethylsiloxane capillary column (HP-50+; 25 m, length x 0.2 mm, internal diameter x 0.31 mm, film thickness) was used. The flow rate of helium as carrier gas was 0.7 ml/min. Split mode for injection was used and its ratio was 10:1. The flow rate of septum purge was 5 ml/min. Initial oven temperature was set to 50 °C with holding time of 1 min, increased by 20 °C per min to 300 °C where it was maintained for 10 min. For the mass spectrometer, electron impact mode was selected at 70 eV. The temperature of transfer line was set at 280 °C. 4-Nitrotoluene and benzophenone were detected by using a selected ion monitoring mode. The selected ions were m/z 65 ([C₅H₅]⁺), m/z 91

$([C_6H_4CH_3]^+)$ and m/z 137 $([M]^+)$ for 4-nitrotoluene, m/z 77 $([C_6H_5]^+)$, m/z 105 $([C_6H_4CO]^+)$ and m/z 182 $([M]^+)$ for benzophenone. The ions values for quantitation were m/z 91 for 4-nitrotoluene and m/z 182 for benzophenone. Nitrobenzene- d_5 (m/z 82 $([C_6D_5]^+)$ and m/z 128 $([M]^+)$) and benzophenone- d_{10} (m/z 110 $([C_6D_5CO]^+)$ and m/z 192 $([M]^+)$) were used as internal standards.

2.5. Preparation of samples for calibration curves of 4-nitrotoluene and benzophenone

To 100 ml of distilled water or 10 g of soil, mixture of 4-nitrotoluene and benzophenone (1-250 ng) was fortified and nitrobenzene- d_5 ($1 \mu g/ml \times 50 \mu l$) and benzophenone- d_{10} ($1 \mu g/ml \times 50 \mu l$) were added as internal standards. The other steps were the same as described in Sections below.

2.6. Extraction of 4-nitrotoluene and benzophenone 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 ($1 \mu g/ml \times 50 \mu l$) and benzophenone- d_{10} ($1 \mu g/ml \times 50 \mu l$) were added as internal standards. 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 (Buchi 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 μl of final volume. One μl of the solution was injected to GC/MS by an auto liquid sampler.

2.7. Extraction of 4-nitrotoluene and benzophenone in soil

To 40 ml of centrifuge tubes 10 g of soil was added and mingled homogeneously with the same amount of anhydrous sodium sulfate. Nitrobenzene- d_5 ($1 \mu g/ml \times 50 \mu l$) and benzophenone- d_{10} ($1 \mu g/ml \times 50 \mu l$) were added as internal standards. The soil 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 x 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 into a 15 ml centrifuge. 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 poured to a centrifuge tube with tapering end and evaporated for concentration until the final volume reached to about 100~200 μl . One μl of this solution was injected to the GC/MS by the auto liquid sampler.

3. Results

3.1. Recoveries and accuracy

Recovery of 4-NT and BZP was determined at 50 ng or 100 ng of 4-NT or BZP standards spiked in 10 g soil and 100 ml water (Table 1). In water matrix, the recovery of 4-NT was ranged from 90% to 118% and the recovery of BZP was greater than 73%. Coefficient of variation was less than 13% in water samples. Recoveries of 4-NT and BZP in soil were greater than 51% and coefficient of variation was less than 19.5%. Accuracy for the quantitation of 4-NT and BZP was less than 16% for water and less than 14% for soil at concentrations of 50 or 100 ng spiked (Table 2).

Table 1. Recoveries of 4-nitrotoluene and benzophenone in water and soil

| Chemicals | Added (ng) ^a | Found (ng) | Recovery (%) | CV (%) |
|-------------------|----------------------------|---------------|-----------------|-----------|
| Water (in 100 mL) | | | | |
| 4-Nitrotoluene | 50 | 59.1 ± 7.7 | 118.2 ± 15.4 | 13.0 |
| | 100 | 89.6 ± 3.3 | 89.6 ± 3.3 | 3.7 |
| Benzophenone | 50 | 40.2 ± 0.8 | 80.5 ± 1.7 | 2.1 |
| | 100 | 72.4 ± 5.5 | 72.5 ± 5.5 | 7.6 |
| Soil (in 10 g) | | | | |
| 4-Nitrotoluene | 50 | 26.5 ± 5.2 | 52.9 ± 10.3 | 19.5 |
| | 100 | 51.5 ± 4.8 | 51.5 ± 4.8 | 9.3 |
| Benzophenone | 50 | 29.3 ± 1.0 | 58.7 ± 2.0 | 3.4 |
| | 100 | 51.2 ± 2.1 | 51.2 ± 2.1 | 4.1 |

^a Mean ± S.D. (n=5).

Table 2. Accuracy of 4-nitrotoluene and benzophenone in water and soil

| Chemicals | Added (ng) ^a | Found (ng) ^a | CV (%) | Accuracy (%) ^b |
|-------------------|----------------------------|----------------------------|-----------|------------------------------|
| Water (in 100 mL) | | | | |
| 4-Nitrotoluene | 50 | 42.2 ± 2.1 | 4.9 | -15.6 |
| | 100 | 102.1 ± 2.3 | 2.2 | +2.1 |
| Benzophenone | 50 | 46.1 ± 0.2 | 0.3 | -7.8 |
| | 100 | 105.8 ± 1.2 | 1.1 | +5.8 |
| Soil (in 10 g) | | | | |
| 4-Nitrotoluene | 50 | 43.0 ± 5.7 | 13.3 | -14.0 |
| | 100 | 107.6 ± 6.0 | 5.6 | +7.6 |
| Benzophenone | 50 | 47.0 ± 2.3 | 4.9 | -6.0 |
| | 100 | 105.1 ± 0.1 | 0.1 | +5.1 |

^a Mean S.D. (n=5)

^b Accuracy was calculated by the equation : $\{(\text{Found} - \text{Added})/\text{Added}\} \times 100$

3.2. Selection of quantitation ions and standard calibration curves

Selected ion values for the analysis of 4-NT and BZP in water and soil were m/z 65, m/z 91 and m/z 137 (molecular ion; [M]⁺) for 4-NT; m/z 77, m/z 105 and m/z 182 ([M]⁺) for BZP; m/z 82 and m/z 128 ([M]⁺) for nitrobenzene-d₅; and m/z 110 and m/z 192 ([M]⁺) for bezophenone-d₁₀ as shown in Fig. 1. Ion values used for

quantitation were m/z 91 (Fig.1) for 4-NT and m/z 182 for BZP. Retention times were 5.18 min for nitrobenzene-d₅, 6.10 min for 4-NT, 9.08 min for BZP, and 9.04 min for benzophenone-d₁₀. Ion chromatograms for the selected ions of 4-NT and BZP in water (Fig. 2) and soil (Fig. 3) were shown to be compared with those for 4-NT and BZP-free blanks, respectively.

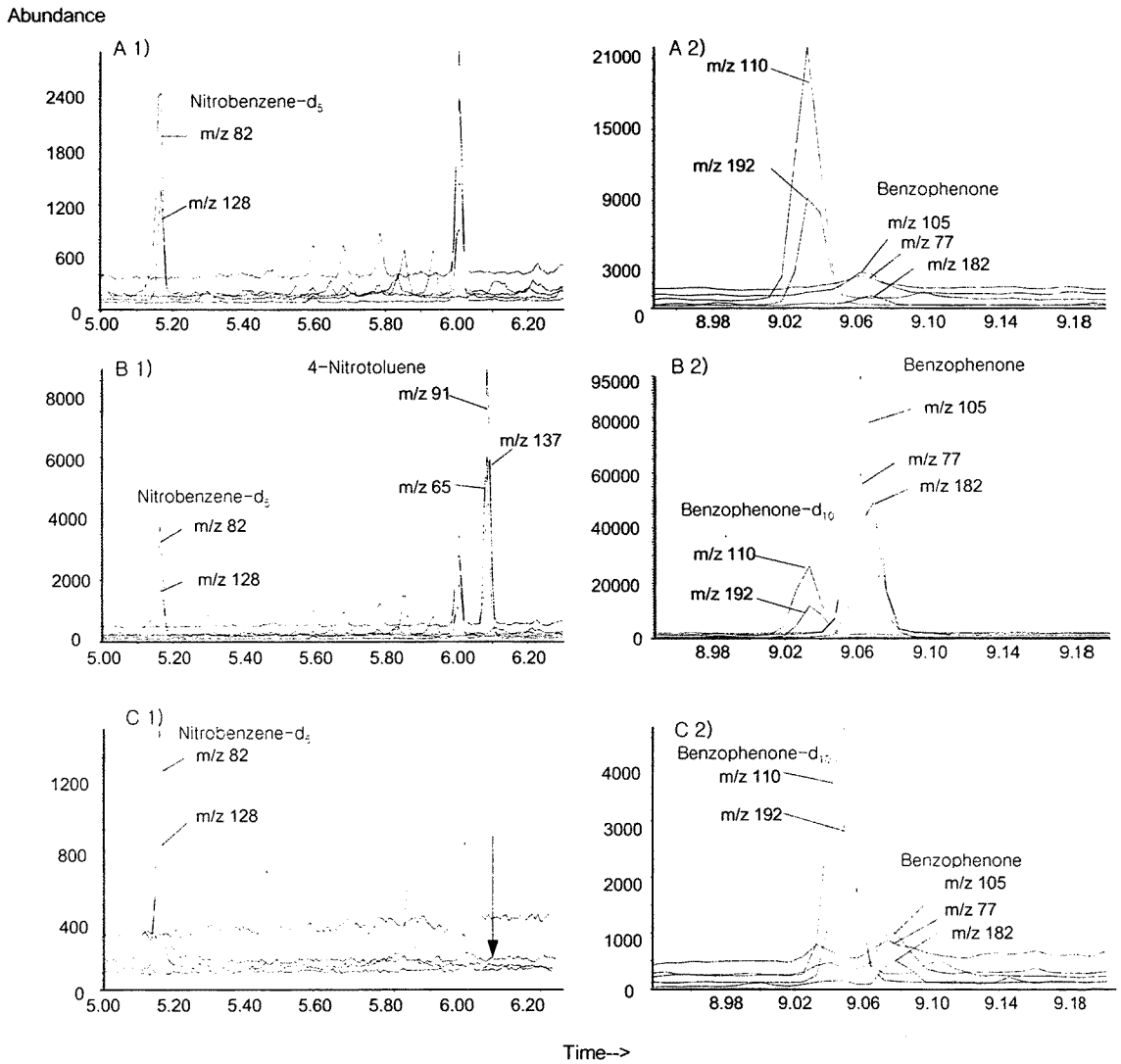


Fig. 3. Ion chromatograms obtained from soil blank (A1, A2) and soil blank fortified standards (B1, B2, each 250 ng for 4-nitrotoluene and benzophenone spiked), and a soil sample (C1, C2). The values of characteristic ions selected for the quantitation of 4-nitrotoluene and benzophenone were m/z 91 and m/z 182, respectively. Each 50 ng of internal standards was added.

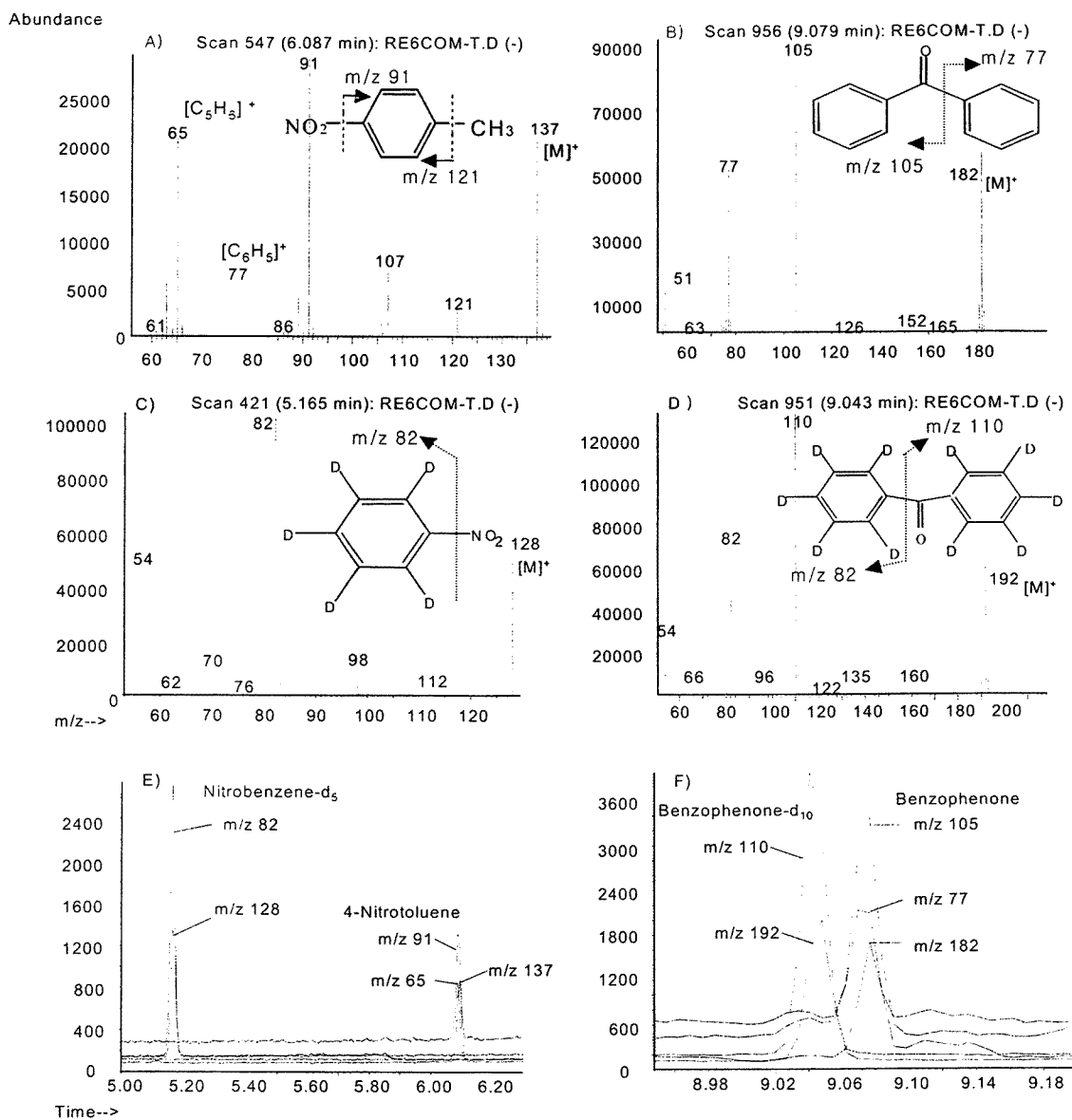


Fig. 1. Gas chromatograph/mass spectrometry (GC/MS) spectra of 4-nitrotoluene (A), benzophenone (B), nitrobenzene-d₅ (C) and benzophenone-d₁₀ (D); and ion chromatograms (E, F) obtained by GC/MS/selected ion monitoring of each authentic compounds (4-nitrotoluene, m/z 65, 91 and 137; benzophenone, m/z 77, 105 and 182). Nitrobenzene-d₅ (E; m/z 82 and 128) and benzophenone-d₁₀ (F: m/z 110 and 192) were used as internal standards for the analysis of 4-nitrotoluene and benzophenone, respectively.

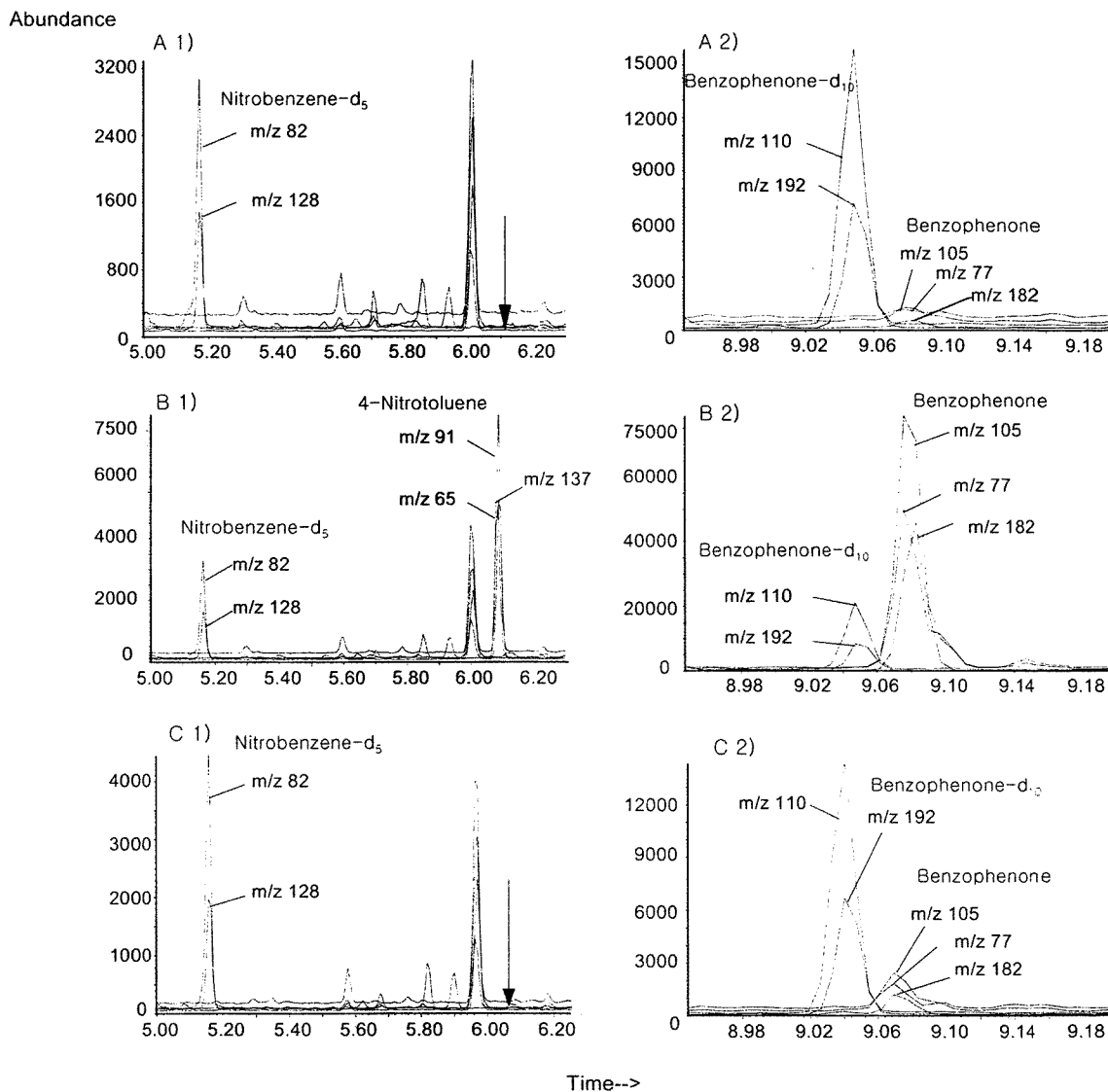


Fig. 2. Ion chromatograms obtained from water blank (A1, A2), water fortified standards (B1, B2, each 250 ng for 4-nitrotoluene and benzophenone spiked), and a river water sample (C1, C2). The values of characteristic ions selected for the quantitation of 4-nitrotoluene and benzophenone were m/z 91 and m/z 182, respectively. Each 50 ng of internal standards was added.

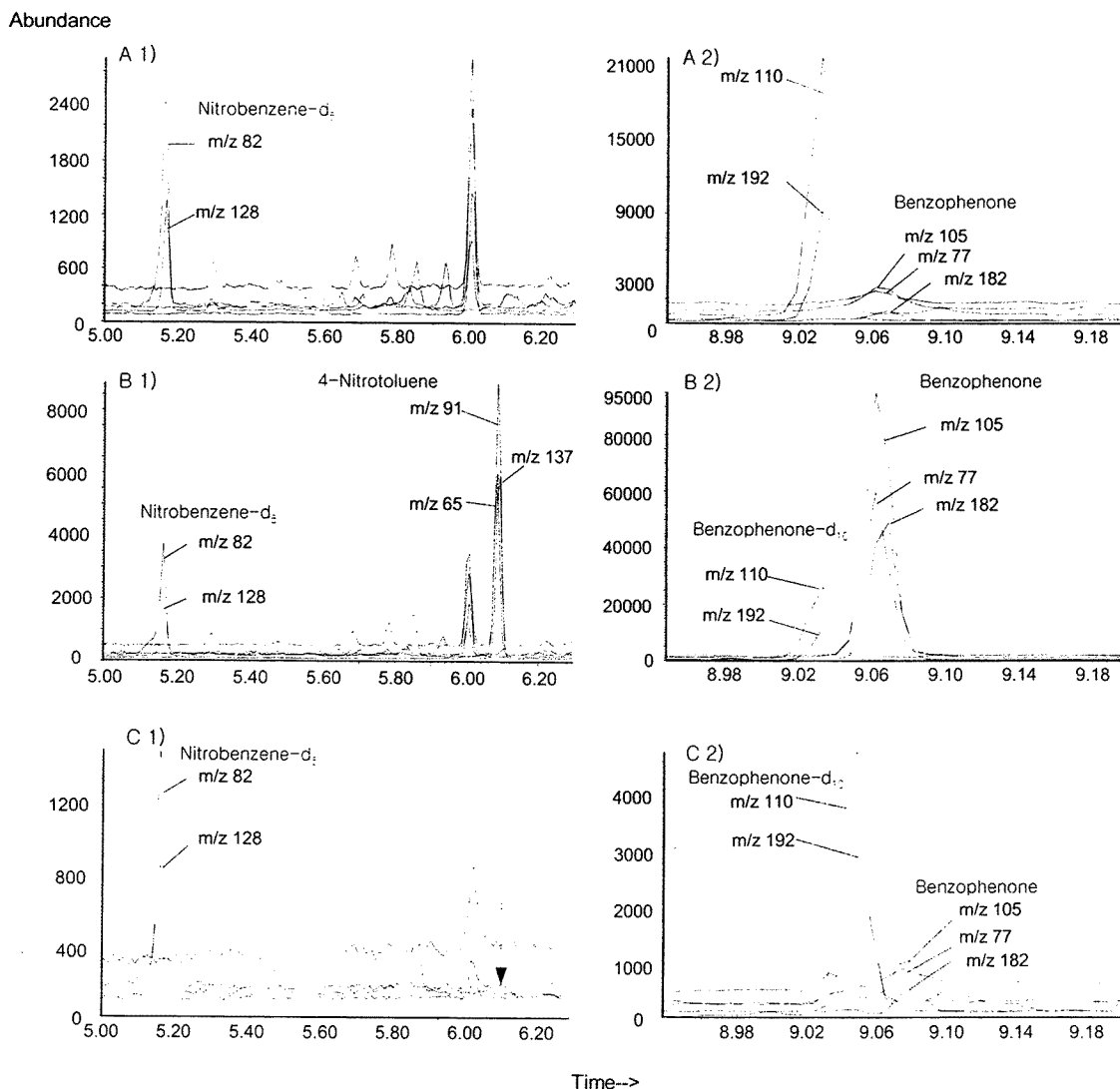


Fig. 3. Ion chromatograms obtained from soil blank (A1, A2) and soil blank fortified standards (B1, B2, each 250 ng for 4-nitrotoluene and benzophenone spiked), and a soil sample (C1, C2). The values of characteristic ions selected for the quantitation of 4-nitrotoluene and benzophenone were m/z 91 and m/z 182, respectively. Each 50 ng of internal standards was added.

For the selected ions for 4-NT (m/z 91) and BZP (m/z 182), no major interfering peaks were found. Linear equations of 4-NT and BZP calibration curves were obtained by linear regression. Each curve was plotted concentrations at x-axis with the area ratio of 4-NT or BZP to internal standards nitrobenzene- d_5 or benzophenone- d_5 at y-axis, respectively. The curves showed a good linearity ($r^2 > 0.997$, 6 points, $n=5$ per

point) at concentration ranges of 10-2,500 ng/L spiked to water or soil. Linear equations in water were determined to $y=0.004354 x - 0.00145$ ($r^2=0.9981$) for 4-NT and to $y=0.005109 x + 0.02870$ ($r^2=0.9988$) for BZP. In soil, linear equations were $y=0.003708 x + 0.03609$ ($r^2=0.9969$) for 4-NT and $y=0.004897 x + 0.04581$ ($r^2=0.9991$) for BZP.

3.3. Effect of various solvents on extraction of 4-nitrotoluene and benzophenone in soil and water

Effect of various solvents on extraction of 4-NT and BZP in water and soil samples was investigated after agitation on a shaker. Among used solvents of hexane, methylene chloride and mixture of methanol: methylene chloride (1:3, v/v), extractability presented by peak area of ion chromatograms was the highest when hexane was used for the extraction in water. The mixture solution of methanol: methylene chloride (1:3, v/v) resulted in higher peak area than methylene chloride (Fig. 4).

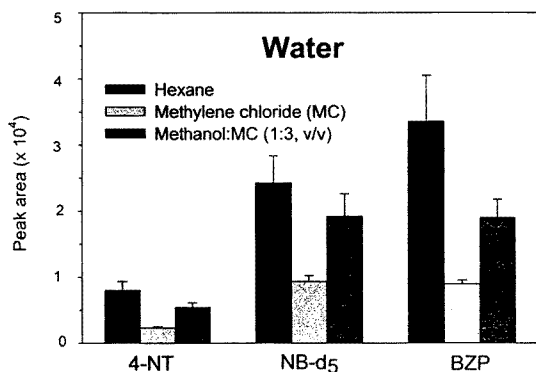


Fig. 4. Effects of various solvents on the extraction of 4-nitrotoluene and benzophenone in water. Extraction was performed as described in the Experimental section. Each result was presented as mean \pm S.E. (n=4).
Abbreviations: 4-NT, 4-nitrotoluene; NB-d₅, nitrobenzene-d₅; BZP, benzophenone.

In soil samples as described in Fig. 5, methylene chloride were better choice than hexane for the extraction of 4-NT and nitrobenzene-d₅. For BZP spiked in soil, peak areas from hexane as well as methylene chloride were higher, compared to those obtained from 2-propanol, methanol and methanol/methylene chloride (1:3, v/v) mixture as shown in Fig. 5.

In this experiment hexane was selected as extraction solvent for simultaneous analysis of 4-NT and BZP in water and soil.

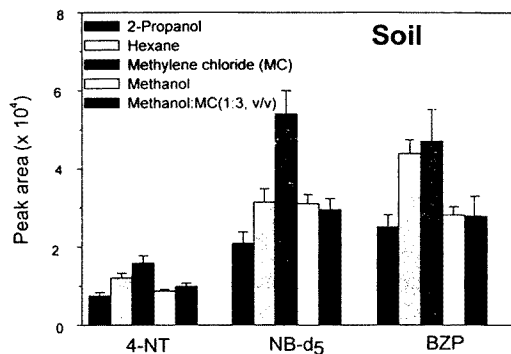


Fig. 5. Effects of various solvents on the extraction of 4-nitrotoluene and benzophenone in soil. Soil (10 g) was extracted with 20 ml of various solvents by a shaker after addition of 10 g of anhydrous sodium sulfate and internal standards. The organic layer was transferred to a round flask (100 ml) and evaporated for concentration. After transfer of the solution into a centrifuge tube, the solution was concentrated to about 200 μ l of final volume, and 1 μ l of the solution was used for the analysis. Each result was presented as mean \pm S.E. (n=4).
Abbreviations: 4-NT, 4-nitrotoluene; NB-d₅, nitrobenzene-d₅; BZP, benzophenone.

3.4. Limit of detection and limit of quantification

Limit of detection (3-fold baseline noise levels) for 4-NT was 1 ng/100 ml in water and 5 ng/10 g in soil. Limit of detection for BZP was 1 ng/100 ml in water and 1 ng/10 g in soil. Based on this detection limits, limit of quantification (11-fold baseline noise levels) for 4-NT was estimated to 4 ng/100 ml in water and 18.3 ng/10 g in soil. Limits of quantification for BZP were estimated to 4 ng/100 ml water or 10 g soil.

3.5. The analysis of 4-nitrotoluene and benzophenone in water and soil

Water, sediment and soil collected in 2000 at nation-wide sites were analyzed. Among 43 water sites, BZP was found in 5 sites (11.6%) at the mean concentration of 14.87 \pm 3.83 ng/100 ml (range: 10.3 -

21.2 ng/100 ml). In the similar study conducted in National Institute for Environmental Studies of Japan, BZP was observed in 12% of water sites collected¹⁰. No BZP was found in the other samples. 4-NT was also not found.

4. Discussion

The characteristic ions of 4-nitrotoluene and benzophenone 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 quantitation and confirmation of these two compounds (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 and m/z 182 for BZP were used for the analysis of two compounds. Although other ions also can be used for the quantitation, the ion of m/z 91 gives better sensitivity than other ions for determination of 4-NT and the ion of m/z 182 was selected for using the advantage of molecular weight.

In addition, use of high-resolution GC/MS will be the best for confirmation of these two chemicals from many interfering peaks in water and soil samples. Practically, small amount of interfering peaks exists in BZP as shown in Fig. 2 and Fig. 3 (A2 and C2). This interfering peaks can be distinguished from the peak of target compounds by using high-resolution GC/MS.

BZP and 4-NT are highly volatile and insoluble to water. In using the 50% phenylmethylsiloxane capillary column, the retention time of 4-NT was 6.10 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. Loss of BZP by complete evaporation, however, was less affected compared to 4-NT when the solvent was undertaken evaporation. Chromatographic property of 4-NT was very sensitive to the extent of aging of the column used, compared to that of BZP. 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-labelled compound of 4-NT analogue or BZP was essential for better reproducibility of data. Especially, benzophenone- d_{10} should be used for the analysis of BZP rather than benzophenone- d_5 because benzophenone- d_5 has m/z 105 of characteristic ion as base peak, resulting in the formation of the same characteristic ion of m/z 105 as in benzophenone (data not shown).

Effect of various solvent on extraction of 4-NT and BZP in water and soil samples was examined. In water samples, extractability of 4-NT and BZP was in the decreasing order of hexane, methanol: methylene chloride (1:3, v/v) and methylene chloride (Fig. 4). The same effects of solvent as in water were not observed in soil (Fig. 5). Methylene chloride gives the highest peak area in 4-NT and nitrobenzene- d_5 . In BZP, extractability by methylene chloride was similar to that by hexane in peak area of ion chromatograms. 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 two compounds in soil was lower than that in water since extraction steps with methanol and hexane were used for extraction of these chemicals in soil samples (Table 1). 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 soil¹³. Recovery of 4-NT in soil, however, was not reported by this ultrasonic method¹³. Detection limit of 4-NT is similar to the value that has been reported by Bader *et al.*, where 4-NT was analyzed by derivatization with pyridine and pentafluoropropionic anhydride². Our method is relatively simple and rapid to determine 4-NT and BZP simultaneously in soil and water samples. Advantages of this method are using small amounts of sample (100 ml of water and 10 g of soil), are time-saving and simple with a similar sensitivity, compared to the previous method¹⁰ which employed 1,000 ml of water and 20 g of soil, as well as required distillation and purification steps.

5. Conclusion

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

6. References

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