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기체크로마토그래피/질량분석기에 의한 수질, 토양 및 저질 시료중의 benzophenone 분석법에 관한 연구

전희경 · 최해연 · 류재천★
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Determination of benzophenone in water, soil and sediment by gas chromatography/mass spectrometry

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요 약 : 본 연구는 GC/MS를 이용하여 수질, 토양 및 저질 시료 중의 benzophenone (BP)을 분석하는 방법을 확립하고자 하였다. BP는 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 BP 회수율은 71.4% 이상이었으며 토양에서의 회수율은 86.5-94.7%를 보였고 재현성은 19.8% 이하였다. 검정곡선은 상관계수 (r^2) 값이 수질과 토양 모두에서 0.998이상의 좋은 직선성을 보여주었다. 수질 시료의 경우 43지역 중 3지역의 수질에서 30-200 ng/L 농도 범위로 BP가 검출되었으며 토양과 저질 시료에서는 모든 지역에서 검출되지 않았다. 이 분석방법은 환경 중에 미량으로 존재하는 BP의 분석과 모니터링에 유용하게 사용할 수 있는 적합한 방법이라 사료된다.

Abstract : Benzophenone (BP) which is one of endocrine disrupting chemicals is suspected to contaminate waters (river, lake and industrial drainage) and soils (ground soil and sediment). Analytical method for determination of BP in soil and water was developed by gas chromatography/mass spectrometry. Water sample (100 mL) was extracted with n-hexane, and soil (10 g) was extracted with methanol and n-hexane. Recovery for BP was >71.4% in water and 86.5-94.7% in soil with coefficient variation of less than 19.8%. Calibration curves showed a good linearity (r^2>0.998). In water, soil and sediment collected at nation-wide sites, BP was detected at 5 sites among 43 water sites at the concentration range of 30-200 ng/L. No BP was found in the soil and sediment samples. It is suggested

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that this method will be useful to the determination of BP in the environmental matrices such as waters, soils and sediments in minute quantities.

Key words : benzophenone, endocrine disruptor, water, soil, gas chromatography/mass spectrometry

1. Introduction

Benzophenone (BP) 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 photo-affinity labeling for various biological materials.[1-3] BP is found in paper and board materials intended for food contact[4] and in urine as a metabolite of benzodiazepines.[5] This metabolite was determined by gas chromatography/electron capture detector (GC/ECD)[5], high-performance liquid chromatography/UV detector (HPLC/UVD)[6-7] and gas chromatography/mass spectrometry (GC/MS).[8]

The clean-up procedure for the GC/MS analysis of BP developed by Japan National Institute of Environmental Studies is tedious and labor-intensive because a large amount of samples such as 1L of water were used, and distillation and purification steps with silica gel column from water and soil samples were required even if the detection limit in water reached to 10 ng/L.[8]

BP is listed in the table of endocrine disrupting chemicals by National Institute of Health Sciences in Japan[9] and World Wildlife Fund. BP is semi-volatile compounds and it may be exposed to water and soil through ambient air. This chemicals is suspected to contaminate ground water and soil environment. In a previous paper[10], a method base on GC/MS was proposed for determination of benzophenone in sediment sample of the river. Monitoring of this compounds in the environment including water and soil is necessary for evaluating the extent of environmental contamination and applying to the risk characterization and assessment. Our laboratory involved in the determination of some chemical contaminants from environmental matrices such as ground water, soils and sediments.[11-16] In this work, analytical method for determination of the BP in water, soil and sediment was developed by gas chromatography/mass selective detector, and was applied to environmental samples of matrices collected from nation-wide sites.

2. Experimental

2.1. Chemicals

Benzophenone was obtained from Sigma (St. Louis, MO, USA). Benzophenone-d_{10} was purchased from Supelco (Bellefonte, PA, USA). 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 ℃ 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.

2.2. Water, soil and sediment samples

Non-contaminated blank soil was obtained in the vicinity of Korea Institute of Science and Technology. The samples of water (43 sites), soil (35 sites) and sediment (11 sites) were collected at nation-wide sites, and supplied by Korea National Institute of Environmental Research. These soils were 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.

2.3. 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 Chem-Station (G1701AA, Version A.03.00, Hewlett Packard, USA). Samples were applied to the GC/MSD by an auto liquid sampler (HP7673).

2.4. Gas chromatography/mass spectrometry

For analyzing benzophenone, 50% phenylmethylsiloxane capillary column (HP-50+; 20 m, length × 0.25 mm, internal diameter m 0.25 μm, film thickness) was used. The flow rate of helium as carrier gas was 0.7 mL/min. Sample injection (1 μL) was in splitless mode. The flow rate of septum purge was 5 mL/min. The GC column temperature was programmed from 50 ℃, ramped at 20 ℃/min to 260 ℃, ramped at 25 ℃/min to 290 ℃, 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 ℃. Benzophenone was detected by using a selected ion monitoring mode. The selected ions was m/z 182 (molecular ion; $[M]^+$), 105 ($[C_6H_4CO]^+$) and 77 ($[C_6H_5]^+$) for benzophenone. The ions values for quantification were m/z 182 for benzophenone. Benzophenone-d_{10} (m/z 110 ($[C_6D_5CO]^+$) and m/z 192 ($[M]^+$)) was used as internal standards.

2.5. Preparation of samples for calibration curves of benzophenone

To 100 mL of distilled water or 10 g of soil and sediment, benzophenone (1-250 ng) was fortified and benzophenone-d_{10} (100 ng; 10 ppm × 10 L) was added as internal standard. The other steps were the same as described in Section 2.6 and 2.7.

2.6. Extraction of 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.

Benzophenone-d_{10} (100 ng; 10 ppm × 10 μL) was added as internal standard. After addition of 50 mL of n-hexane, the separating 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, Switzland). 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 the GC/MSD by an auto liquid sampler.

2.7. Extraction of benzophenone in soil and sediment

To 40 mL of centrifuge tubes, 10 g of soil and sediment were added and mingled homogeneously with the same amount of anhydrous sodium sulfate.

Benzophenone-d_{10} (100 ng; 10 ppm × 10 μL) was added as internal standard. The soil and sediment was extracted with 20 mL of methanol by a shaker (Edmund Buchler 7400, Tubingen, Germany) for 20 min and centrifuged (RT 6000B, Sorvall Inc., New town, CT, USA) for 15 min at 1,660 × 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

Table 1. Recoveries of benzophenone in water and soil (n=5)

Matric	Added	Found	Recovery (%)	SD (%)[a]	RSD (%)[b]
Water (μg/L)					
	0.05	0.04	71.4	12.45	17.44
	0.10	0.08	78.9	15.24	19.30
	0.50	0.49	98.1	2.92	2.98
Soil (μg/kg)					
	1.00	0.87	86.5	17.13	19.80
	5.00	4.74	94.7	2.23	2.36

[a] SD : Standard deviation

[b] RSD : Relative standard deviation

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 ℃) 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 μL. One μL of this solution was injected to the GC/MSD by the auto liquid sampler.

3. Results

3.1. Recoveries

To further validate the precision and accuracy of the method recovery testing was carried out by spiking a known amount of the standard to distilled water and soil, which do not contain the test chemicals. Recovery of BP 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 the water, the recovery of BP was more than 71.4% and the relative standard deviation (RSD) values were below 19.3% in all cases. Recoveries in soil of BP was ranged from 86.5 to 94.7 % and the RSD of all recovery experiments was less than 19.8%. The precision of the method is therefore very good.

3.2. Selection of quantification ions

Selected ion values for analysis of BP in water and soil were m/z 77, 105 and 182 for BP and m/z 82, 110 and 192 for benzophenone-d_{10} as shown in *Fig*. 1. Ion values used for quantification were m/z 182 for BP. Retention times were 8.45 min for BP and 8.42 min for benzophenone-d_{10}.

Ion chromatograms for the selected ions of BP in water (*Fig*. 2) and soil (*Fig*. 3) were shown to compare with those for BP-free blanks. For the selected ions for BP (m/z 182), no major interfering peaks were found.

3.3. Linearity of standard calibration curves

The linear range of GC-MS method for the determition of BP was tested by increasing amounts of stamdards

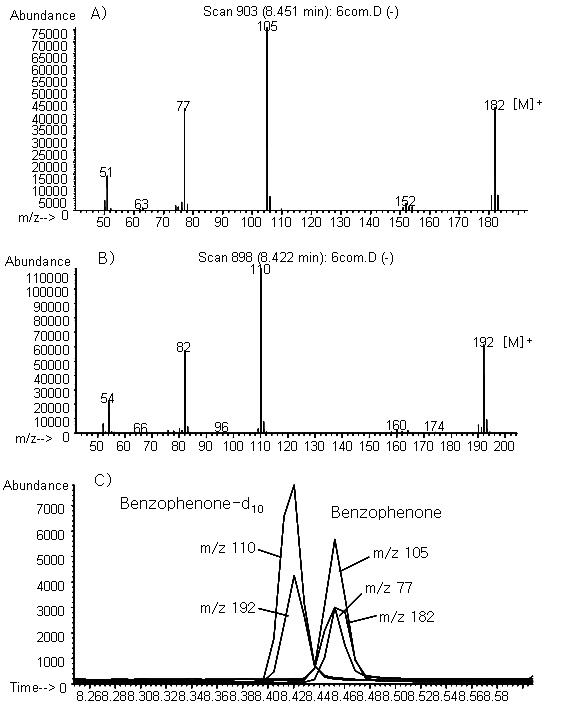

Fig. 1. Gas chromatograph/mass spectrometry (GC/MS) spectra of benzophenone (A) and benzophenone-d10 (B); and ion chromatograms (C) obtained by GC/MS/selected ion monitoring of benzophenone (m/z 77, 105 and 182). Benzophenone-d10 (m/z 110 and 192) were used as internal standards for the analysis of benzophenone.

Table 2. Calibration and detection limit of benzophenone 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	0.0078	0.0197	0.9998	0.01	0.04
Soil (μg/kg)	0.1 - 25	0.0072	0.1405	0.9982	0.1	0.4

[a] LOD : Limit of detection
[b] LOQ : Limit of quantification

at 1, 5, 10, 25, 50, 100, 250 ng/ 100 mL water (or 10g soil) and fixed amount (100 ng/ 100 mL or 10 g) of internal standards in distilled water or blank soil, and extracted and concentrated as described above (Section 2.6 and 2.7). The curve was plotted concent- rations at

x-axis with the area ratio of BP to internal standards benzophenone-d_{10} at y-axis. The curves showed a good linearity at concentration ranges spiked (*Table* 2). Linear equation for BP was determined to y=0.0078 x + 0.0197 (r^2=0.9998) in water, and to y=0.0072 x + 0.1405 (r^2 =0.9982) in soil by linear regression (7 points, n=3 per point).

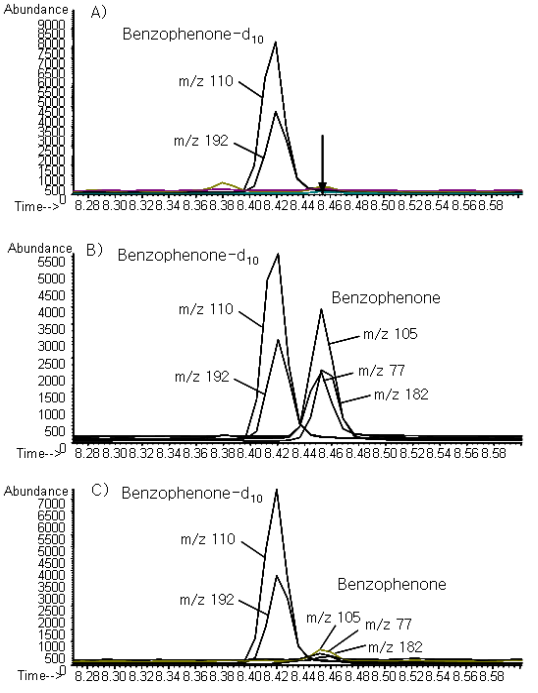

Fig. 2. Ion chromatograms obtained from water blank (A), water fortified standards (B, 250 ng for benzophenone spiked), and a river water sample (C). The values of characteristic ions selected for the quantification of benzophenone was m/z 182. Internal standards of 100 ng was added.

3.4. Limit of detection and limit of quantification

The limits 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 BP concentration that can be quantified in a sample with acceptable precision under the stated operational conditions of the method.

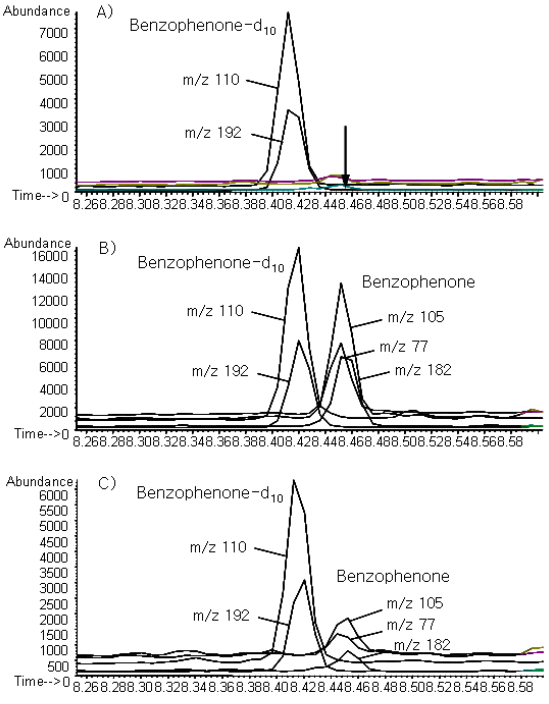

Fig. 3. Ion chromatograms obtained from soil blank (A), soil fortified standards (B, 250 ng for benzophenone spiked), and a soil sample (C). The values of characteristic ions selected for the quantification of benzophenone was m/z 182. Internal standards of 100 ng was added.

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 0.01 μg/L in water and 0.1 μg/kg in soil for BP. Based on this detection limits, LOQ for BP was estimated to 0.04 μg/L in water and 0.4 μg/kg in soil (*Table* 2).

3.5. The analysis of benzophenone in groud water, soil and sediment

The ground water, sediment and soil collected in year 2002 at nation-wide sites were analyzed. Among 43 water sites, BP was found in 3 sites (7.0%) at the concentration range of 30-200 ng/L. In the similar study conducted in National Institute for Environmental Studies of Japan, BP was detected nearly 12% in collected water sites.[8] No BP was found in the soil and sediment

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[8] which employed 1,000 mL of water and 20 g of soil, as well as required distillation and purification steps.

4. Discussion

BP is semi-volatile and generally insoluble to water. To isolate BP, the 50% phenylmethyl-siloxane capillary column was adopted, and the retention time of BP was 8.45 min in our condition. The characteristic ions of 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 quantification and confirmation (*Fig*. 1). It is useful to select more than 3 ions for a compound in the process of its confirmation. Characteristic ion of m/z 182 for BP was used for the quantification. Although other ions also can be used for the quantification, the ion of m/z 182 gives better sensitivity than other ions for determination of BP and was selected for using the advantage of molecular weight.

In extraction procedure, evaporation of the organic layer should be very cautious because complete drying of the solvent will remain little residual BP. For this reason, 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 isotope-labelled compound (deuterated form) of BP was essential for better reproducibility of data. Especially, benzophenone-d_{10} should be used for the analysis of BP rather than benzophenone-d_5 because benzophenone-d_5 has m/z 105 as base peak, resulting in the formation of the same characteristic ion of m/z 105 as in benzophenone.

In soil, 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. 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 in the extraction step. Our method is relatively simple and rapid to determine BP in water, soil and sediment samples.

5. Conclusion

An analytical method for determining BP from environmental matrices such as water, soil and sediment samples was developed by gas chromatography/mass spectroscopy. This method is simple, time-saving and reliable enough to analyze BP in small amounts of environmental samples such as soil and ground water. This analytical procedure will be useful for monitoring the exposure of BP to the environment.

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