SPME-GC-MS를 이용한 DBCP 및 n-Butylbenzene의 분석

박현미 · 김영만 · 이대운* · 이강봉★
한국과학기술연구원, 특성분석센터
*연세대학교, 화학과
(2001. 10. 7 접수)

Determination of DBCP and n-Butylbenzene using SPME with GC-MS

Hyun-Mee Park, Young-Man Kim, Dai-Woon Lee* and Kang-Bong Lee★
Advanced Analysis Center, Korea Institute of Science and Technology,
P. O. Box 131, Cheongryangri, Seoul 130-650, Korea
*Department of Chemistry, Yonsei University, Seodaimungu Sinchondong 134,
Seoul 120-749, Korea
(Received Oct. 7, 2001)

요 약 : 85 μm-polyacrylate(PA) 와 100 μm-polydimethylsiloxane(PDMS) fiber를 사용하여 물중의 DBCP와 n-butylbenzene을 추출한 후 GC-MS로 분석하는 방법을 연구하였다. SPME 과정에 영향을 미치는 인자들, 즉 추출 시간, 주입 길이, 주입 온도, 탈착 시간 및 탈착 온도들에 대한 최적화 조건을 조사하였다. 검량선의 직선성(correlation coefficient, R)은 0.99 이상이었으며, 본 분석법의 검출한계는 1.5 와 10.8 μg/L이었고, 분석법의 재현성은 RSD 10.4와 14.4 % 였다.

Abstract : Solid phase microextraction(SPME) with 85 μm-polyacrylate (PA) and 100 μm-polydi -methylsiloxane(PDMS) fibers, coupled to gas chromatography-mass spectrometry was used to determine 1,2-dibromo-3-chloropropane(DBCP) and n-butylbenzene in water. The conditions affecting the SPME process(i.e, extraction time, injection length, injection temperature, desorption time and temperature) were optimized. The linearity of the calibration curve (correlation coefficient, R) was over 0.99 and the limits of detection of the method were between 1.5 and 10.8 μg/L. Repeatability of the method was between 10.4 and 14.4 %.

Key words : solid phase microextraction(SPME), GC/MS, DBCP, n-butylbenzene

1. Introduction

1,2-Dibromo-3-chloropropane(DBCP) and n-butylbenzene which are volatile organic compounds(VOCs), have been used as an insecticide and an intermediate of petroleum chemistry, respectively. As a result, they are often found in environmental media such as water, soil and sediments. Little is known about the possible effects of these substances on the environment and human health but some recent studies have shown that they may cause hormone disrupting activity[1]. Animals that were exposed to DBCP by inhalation revealed degenerative changes in the seminiferous tubules, with relative reductions in the

★ Corresponding author
Phone : +82-(0)2-958-5957, Fax : +82-(0)2-958-5969
E-mail : leekb@kist.re.kr

numbers of sperm, and an increase in the percentage of sperm with abnormal morphology[2]. Sexual abnormalities of the Lake Apopka alligators were found to be attributed to leakage of the nematicide DBCP from a manufacturing facility[3]. The first report concerning its detrimental effects on male fertility in production workers was published in 1977[4] and it was no longer used since it was known to reduce dramatically human sperm. Also the toxicity of n-butylbenzene was not certainly reported, but it was found that it reduced the binding of the titrated natural estrogen to the receptor[5].

Gas chromatography has been used for determining VOCs in water samples but when the levels of concentration are low, a previous enrichment step is usually needed. The preconcentration techniques which are commonly applied to determine VOCs are purge and trap[6,7] and solid phase microextraction(SPME)[8-10].

SPME is a solventless technique for the concentration of organic pollutants that allows the extraction of small volumes of sample. The analytes are extracted by absorption over the fiber which is directly exposed to the sample or to the headspace. Finally the fiber is introduced into the gas chromatograph injector where the analytes are thermally desorbed [11-14].

In this study, we aim to develop a method for determining DBCP and n-butylbenzene in water using SPME as the preconcentraion technique and gas chromatography with mass spectrometric detection. The experimental conditions for SPME were optimized and the method was validated with the range of concentration in the linearity, detection limit, repeatability and calibration.

2. Experimental

2.1. Reagents and standards

The standards of DBCP and n-butylbenzene were purchased from Ultra Scientific (Northkingstown, RI, USA). DBCP has 97 % purity and n-butylbenzene was 99% pure. A stock standard solution of 500 mg/L of each compound was prepared in methanol. Working standard solutions of 5 mg/L were prepared weekly in

methanol. Stock and working standards were stored at 4 ℃ in the refrigerator. The aqueous solutions were prepared daily by diluting the working solution with water(Milli-Q). Methanol was supplied by J. T. Baker (Houston, TX, USA) and was pesticide residue grade. Water was obtained from a Milli-Q water purification system(Millipore, Bedford, MA, USA).

2.2. Instrumentation

Gas chromatographic analyses were performed on a Varian CP-3800 gas chromatograph (Walnut Creek, CA, USA) equipped with a split-splitless injector and an Varian Saturn 2000 mass spectrometer. DB-5MS fused silica capillary column(cross linked 5 % methylsilicone) of 30 m x 0.25 mm x 0.25 μm was selected to separate the analytes. The temperature program used for the analyses was as follows: the initial temperature was 33 ℃ which was held for 5 min and then increased to 250 ℃ at 10 ℃/min rate. This temperature was held for 3 min. The injector and transfer line temperatures were 250 ℃ and 210 ℃, respectively. The helium flow rate was maintained at 1.0 mL/min. The samples were injected in the splitless mode and the splitter was opened after 2 min. The conditions for electron impact ionization(EI) of mass spectrometer were an ion energy of 70 eV and the mass scan range was 30~500 m/z.

2.3. SPME procedure

The SPME device and the fibers(85 μm-PA, 100 μm-PDMS) were purchased from Supelco (Bellefonte, PA, USA). Before the initial application, the fiber was conditioned in the hot injection port at 300 ℃ for 3 hours according to the supplier's instructions. Before the analysis, a fiber blank was run to confirm no contamination peaks.

Water samples(5.0 mL) spiked with an appropriate amount of standards were put in 8 mL vials. Sample was saturated with NaCl, and the pH was not adjusted. The fiber was directly introduced into the sample solution for 40 min at room temperature. The samples were continuously stirred with a magnetic stirrer. Finally, the compounds were thermally desorbed from

the fiber in the injector at 250 ℃ for 2.0 min.

3. Results and discussion

3.1. Optimization of SPME

The fibers selected were 85 μm-PA and 100 μm-PDMS which are available commercially, and these two fiber coatings gave good results in earlier studies. The efficiency of the extraction process of SPME is dependent on a fiber coating/sample matrix distribution constant, K_{fs}. Therefore, it is important to choose the appropriate coating for a given application. The performance of two different coatings for analysis of DBCP and n-butylbenzene is illustrated in *Fig.* 1. This shows that the efficiency of PA fiber is relatively the same for both compounds and PDMS fiber is much more efficient for n-butylbenzene than DBCP.
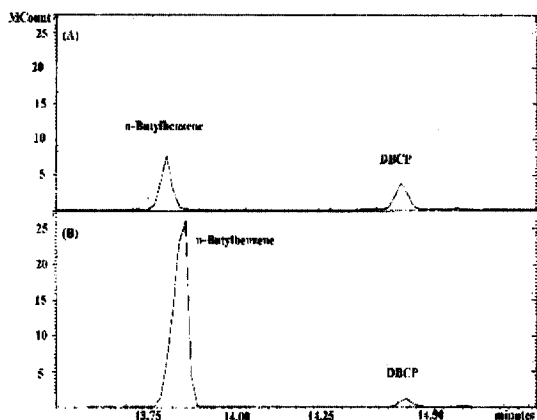

Fig. 1. Total ion chromatogram of GC-MS of n-butylbenzene and DBCP extracted with (A) PA-coating (B) PDMS-coating.

The main parameters(*e.g.*, the time of extraction, injection length, the time and temperature of desorption and the addition of NaCl to the sample) that can affect the SPME process were optimized under full scan acquisition mode. The extraction temperature, one of the main parameters, was not investigated in this report since the compounds analyzed are volatile. Standard solutions containing 2 g/L of DBCP and n-butylbenzene in Milli-Q water were used to evaluate the effect of

these parameters. All the materials like glassware and stirring bar used had to be also carefully cleaned and three blanks were run to verify the absence of two compound peaks.

3.1.1. Extraction time and injection length

The extraction time profile was studied by monitoring the area counts as a function of the extraction time. The standard solution was exposed for times which ranged from 10 to 120 min(*Fig.* 2). The temperature of extraction was set at room temperature and NaCl was not added to the sample. The sample was continuously stirred to decrease the time required for the analytes to reach the equilibrium. After the extraction, the compounds were desorbed from the fibers which had been kept throughout the run at 250 ℃ in the injector of the gas chromatograph. The absorption equilibrium in 100 μm-PDMS fiber was reached at 40 min for both compounds, but the equilibrium in 85 μm-PA fiber was reached over 120 min for two compounds. This fact indicates that polar fiber would take longer time to reach the absorption equilibrium for nonpolar compounds. However, an extraction time of 40 min was selected for subsequent analysis. The shorter extraction time than the equilibrium time can be selected for the quantification if the stirring rate, the extraction time and the temperature are held constant.
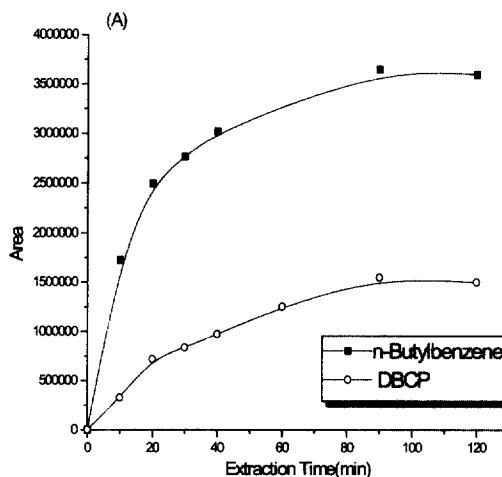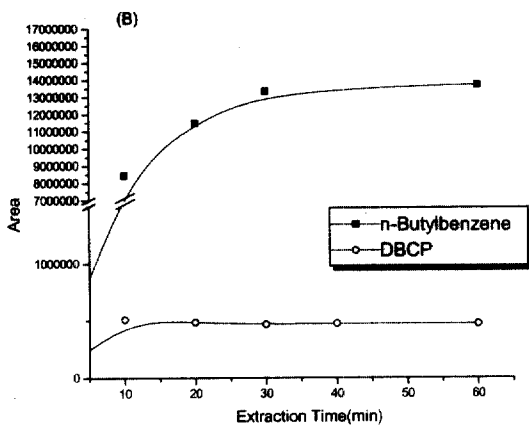
Fig. 2. Extraction time profile of n-butylbenzene and DBCP for (A) 85 μm-PA (B) 100 μ m-PDMS.

Various injection length of fiber from the top of the GC injector were examined at 4-6 cm with 1 cm interval. The peak areas for DBCP and n-butylbenzene were not sensitive to the injection length of fibers(not shown), and the fiber of 4 cm injection length was selected for the further experiment.

3.1.2. Desorption temperature and time

The amounts of analyte desorbed were monitored as a function of desorption temperature by exposing the fiber in the injector at temperatures ranging from 100 ℃ to 250 ℃. As desorption temperature raised, peak areas of both compounds became larger and maximum at 250 ℃. Thus the desorption temperature of 250 ℃ was selected for further experiments.

Desorption time was selected by examining the range of 1-3 min, and result shows that the peak area of the DBCP and n-butylbenzene increase as desorption time increases, and it reached at maximum at 2 min of desorption time. The peak areas did not increase at any longer desorption time. Thus, 2 min was selected as an optimum desorption time.

3.1.3. Salt effect

NaCl is often added to the sample in order to increase the ionic strength and enhance the amount of analyte extracted by salt out effect. Saturated sample

with NaCl(360 g/L) was analyzed and the results were displayed in *Table* 1. Thus, it can be observed that DBCP showed an increase in extraction yield with the addition of NaCl salt regarding to both of PDMS and PA fibers, but n-butylbenzene showed an decrease in extraction yield with the addition of NaCl salt in both of PDMS and PA fibers. It seems that these behavior of DBCP and n-butylbenzene are due to the difference in water solubility between two compounds.

Table 1. Relative area ratio of DBCP and n-butylbenzene peaks under no NaCl solution and NaCl saturated solution(360 g/L)

Compound	PA		PDMS	
	No addition of NaCl	NaCl saturated	No addition of NaCl	NaCl saturated
n-Butylbenzene	27.5a	9.0	26.1	3.8
DBCP	2.4	5.2	1.0	4.3

a : Relative peak area under assumption that peak area of DBCP without addition of NaCl is unit area.

3.2. SPME-GC-MS

The method was validated by analyzing water samples spiked with the standards once the SPME parameters were optimized. Typical GC-MS chromatograms of n-butylbenzene and DBCP using PA and PDMS fibers were shown in *Fig.* 1.

The linearity of the response in the range of 0.5~25 μg/L was monitored by correlation coefficient, R of calibration curve. R was over 0.99 which is shown to be a good linearity. The limit of detection(LOD) were calculated using 3σ method and they were between 1.5 and 10.8 μg/L. The repeatability of the method, expressed as a percentage of RSD(relative standard deviation) and calculated at a spiking level of 5 μ g/L(n=5) were between 10.3 % and 14.4 %. The recovery was obtained from the addition of standards to the tap water and river water. Recovery of n-butylbenzene was 94.0 % for tap water and 80.3 % for river water. Recovery of DBCP was 95.9 % for tap water and 100.5 % for river water. These validation parameters were summarized in *Table* 2.

Table 2. Results of calibration, LOD and RSD using 85 μm-PA fiber and 100 μm-PDMS fibers

Compound	Linear range (μg/L)	Calibration	LOD(μg/L)		RSD(%, n=5)	
			PA	PDMS	PA	PDMS
n-Butylbenzene	0.5-25	Y=12804.7+2954.4x R=0.9910	1.5	10.8	10.4	14.4
DBCP	0.5-25	Y=312.6+435.8x R=0.9983	1.7	8.7	11.3	11.7

4. Conclusion

In this study, SPME was used to determine 1,2-dibromo-3-chloropropane(DBCP) and n-butylbenzene in water. Parameters that affect the SPME process were optimized. Optimized extraction time was 40 min at room temperature. Adding NaCl to water increased the extracted amount of DBCP but decreased the amount of n-butylbenzene. Injection length was 4 cm, desorption was carried at 250 ℃ for 2 min. This method was evaluated by the linearity of the calibration, limit of detection and repeatability. The linearity of the calibration curve (correlation coefficient, R) was over 0.99 and the limits of detection of the method were between 1.5 and 10.8 μg/L. Repeatability of the method was between 10.4 and 14.4 %.

5. References

1. G.A. Glegg and S.J. Rowland, Mar. Pollut. Bull., 32, 486(1996).
2. W.M. Kluwe, Toxicol. Appl. Pharmacol., 59, 84(1981).
3. R.W. Risebrough, Human and Ecological Risk Assessment, 5, 869(1999).
4. B. Christine, R. Wiger and G. Brumborg, Reproductive Toxicology, 9, 461(1995).
5. S. Jobling, T. Reynolds and J.P. Sumpter, Environ. Health Perspect., 103, 582(1995).
6. H. Tom and H.V. Langenhove, J. Chromatogr. A, 893, 367(2000).
7. A.S. Allonier and A. Bermond, Talanta, 51, 467(2000).
8. M.J. Jager, D.P. McClintic and D.C. Tilotta, AppliedSpectroscopy, 54, 1617(2000).
9. A. Peñalver, E. Pocurull, F. Borrull and R.M. Marcé, Trends in Anal. Chem., 18, 557(1999).
10. A.M. Hanssan, E. Benfenati and R. Fanelli, Tox. Environ. Chem., 55, 73(1996).
11. J. Pawliszyn, "Solid Phase Microextraction : Theory and Practice", Wiley-VCH, New York, 1997.
12. M.T. Kelly and M. Larroque, J.Chromatogr. A, 841, 177 (1999).
13. C. Aguilar, S. Penalver and E. Pocerull, J. Chromatogr. A, 795, 105(1998).
14. E. Penalver, E. Pocurrull and R.M. Marce, J. Chromatogr. A, 872, 191(2000).
15. H. Prosen and L.Z. Kralj, Trends in Anal. Chem., 18, 272(1999).
16. C.K. Arthur, L.M. Kilam and Pawliszyn, J. Environ. Sci. Technol., 26, 979(1992).
17. D.W. Potter and J. Pawliszyn, J. Environ.Sci. Technol., 28, 298(1994).
18. Y. Yang, S.B. Hqwthorne and D.J. Miller, Anal. Chem., 70, 1866(1998).
19. C.H. Giles, A.P. D'Silva and I.A. Easton, J. Colloid Interface Sci., 47, 766(1974).
20. A. Peñalver, E. Pocurull and R.M. Marcé, J. Chromatogr. A, 839, 253(1999).