Simultaneous Gas Chromatography-Mass Spectrometric Determination of Total and Individual Phthalic Esters Utilizing Alkaline Hydrolysis and Silyl Derivatization Technique

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Environmentally important phthalic esters have been analyzed by GC-MS in terms of individual phthalic esters or total phthalic esters directly or after derivatization. Derivatization improves the chromatographic characteristics of the highly polar phthalic esters. This study focused on the GC-MS determination of the total phthalic esters and the individual phthalic esters simultaneously. The phthalic esters were hydrolyzed to phthalate and corresponding alcohols in 1 M NaOH solution at 90 °C for 30 min followed by extraction with ethyl acetate after acidifcation. The phthalic acid and alcohols were simultaneously silyl derivatized using bis(trimethylsilyl)trifluoroacetamide (BSTFA) to their corresponding silyl ester and ethers in the mixture of 60% acetone and 40% ethyl acetate at room temperature within 30 min. Because of the high reactivity of BSTFA with the phthalic acid and alcohols effective silyl derivatization was possible simultaneously. GC-MS analysis of the silyl derivatives of phthalic acid and alcohols was performed. The total phthalic ester content was estimated from the analytical result of phthalic silyl ester, while the individual phthalic ester was quantified from the analytical results of alcoholic silyl ethers. This technique was applied to spiked tab water and real seawater samples from the Lake Shihwa in Korea. The results were checked against the results from the direct GC-MS analysis of the phthalic esters and reasonable recoveries with high sensitivity were achieved. The recoveries were higher than 75% with low relative standard deviation (below 10%).

Key Words : Total phthalic esters, Silyl derivatization, Plasticizer

Introduction

The phthalic esters such as dibutylphthalic ester (DBP) and di(2-ethylhexyl)phthalic ester (DEHP) have been widely used as plasticizers for the last several decades. Linear or branched alkyl chains are attached to the backbone of the phthalate in the phthalic esters. The alkyl chains bound to the phthalic acid determine the physical and chemical properties of various phthalic esters.[1] In order to control the flexibility of polymeric materials such as polyvinyl chloride (PVC), polyethylene (PE), polyvinyl acetates (PVA), many kinds of phthalic esters have been added during plastic manufacturing processes.[2-4] Annual production of phthalic esters was four million ton in the world in 2003. Among them dibutylphthalic ester (DBP) and di(2-ethylhexyl)phthalic ester (DEHP) were about 80% of all phthalates produced.[4,5]

Despite of the low persistency and bioaccumulation, it may cause a series of cancer and disease by the endocrine disrupting mechanism.[2,6-9] DBP reduced the fetal weight and caused birth defects in mice.[10,11] DEHP was found in the liver cancer cell of rodents. It acts as a tumor promoter in the skin and liver of mice.[12,13] Most countries including USA and EU have classified them as toxic substances and endocrine disrupters.[1,14,15]

The phthalic esters are not bound chemically to polymeric backbones in plastics. They are simply mixed with the polymer physically and retained in the polymeric matrix. So the plasticizer could be leached out from the polymeric products in water or vaporized at elevated temperature.[16-19] As a result of widespread use of plastic products like polyvinyl chloride (PVC), the phthalic esters are found in high concentration in the environments like water, atmosphere and soil.

Various analytical methods for the determination of the phthalic esters from various environmental samples have been developed. Mostly they are focused on the identification and quantification of individual phthalic ester using high performance liquid chromatography (HPLC),[20-22] gas chromatography with flame ionization detectors (GC-FID),[23] gas chromatography with electron capture detectors (GC-ECD),[24] and gas chromatography with mass spectrometry (GC-MS).[5,9,11,14] Although these methods have been successfully applied for the environment sample analysis, contamination problems are one of the biggest issues in the phthalate analysis.[25,26] The phthalic esters are found easily from the majority of equipments and reagents used in laboratories, which means that the background contamination problem is serious.[27] So, careful decontamination is required during sample treatment and analysis. In order to reduce the chance of recontamination during the analytical process and increase sensitivity, numerous analytical methods determining total phthalic ester were developed recently.[28] The phthalic esters

were hydrolyzed to phthalate using strong basic solution such as NaOH or KOH, and then the phthalic acid was extracted with organic solvents after acidification. It was derivatized with suitable derivatizing agents followed by instrumental analysis. Common derivatization methods are methylation,[29] esterification[30,31] and amination.[32] These methods showed high sensitivity and selectivity due to the removal of contamination sources. Only the phthalic acid from various sources was targeted for analysis in this case. However, understanding of the contamination levels of both total phthalic esters and individual phthalic ester are important in the study of environmental fate and toxicity. To determine the contamination levels of individual phthalic ester, the untreated extracts should be reanalyzed or the alcohols hydrolyzed from the phthalic esters should be analyzed separately. They are time consuming and tedious to do so. High sensitivity and selectivity cannot be expected due to the severe tailing of highly polar alcoholic analytes if analyzed directly. A simple analytical technique capable to determine the total phthalic ester and individual phthalic ester contamination levels simultaneously is highly desired. Here we report a simple satisfactory method of simultaneous GC-MS determination of the total phthalic ester and individual phthalic esters from environmental samples. A simultaneous silyl derivatization technique of the phthalic acid and alcohols formed by the alkaline hydrolysis of phthalic ester samples was studied.

Experimentals

Reagents and standards. The compounds, dibutylphthalate (DBP), dicyclohexyl phthalate (DCHP), di(2-ethylhexyl) phthalate (DEHP), phthalic aicd (PA), butanol, cyclohexanol and *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) were purchased from Aldrich. 2-Ethylhexanol was purchased from Junsei (Japan). Gas chromatographic internal standard, (dibutyl phthalate-d_4) were purchased from Cambridge Isotope Laboratories Inc. (USA). Dichloromethane, hexane,

acetone, and ethyl acetate were from Burdick & Jackson (USA). The stock and working standard solutions were prepared in acetone.

Chromatographic and mass spectrometric conditions. The gas chromatographic analysis was performed using a GC-MS (Shimadzu GC-2010) equipped with an AOC-20i+s type auto-injector and a Shimadzu MS QP-2010 mass spectrometer. A silica capillary column of DB-5MS ((5%-phenyl)-methylpolysiloxane, 0.25 mm i.d, 0.25 μm film, 30 m long, J&W, USA) was selected for the separation of analytes. Two different temperature programs were applied for the analysis of phthalic esters and their derivatives. For the phthalic esters: the initial temperature was set at 70 °C, and then increased to 120 °C in the rate of 15 °C/min, and 10 °C/min from 120 °C to 280 °C. For the derivatives of phthalic esters: the initial temperature was set at 50 °C for 2 min, and then increased to 100 °C in the rater of 5 °C/min, and 20 °C/min from 100 °C to 280 °C. The flow rate of He was maintained at 0.9 mL/min. The injection port was maintained at 280 °C. One μL of sample was injected in splitless mode. The ionization potential of the mass spectrometer was set at 70 eV with the source temperature at 200 °C and the electron multiplier voltage at 1200 eV. The analytes were analyzed in selected ion mode. The information for the retention time, quantification ion and confirmation ions is in Table 1. Internal standard method was applied for the quantification of the analytes.

Extraction and derivatization. For the extraction and derivatization efficiency experiment 50 μL of each phthalic ester standard of 20 μM was spiked in 3 mL of 1 M NaOH and hydrolyzed at 90 °C for 30 min.[29] After completion of alkaline hydrolysis the solution was acidified with HCl in order to convert sodium phthalate to phthalic acid. The phthalic acid and alcohols formed by hydrolysis and acidification were extracted twice using 2 mL of organic solvent. In order to extract the phthalic acid and alcohols simultaneously, the extracting solvent and the acidity of the aqueous medium were optimized. The organic phase containing target

Table 1. Ions for selected ion monitoring of phthalic esters and their corresponding silyl derivatives

	Analyte[a]	Retention time (min)	Quantification ion (abundance)	Confirmation ion (abundance)	Detection limit (pg)
Phthalic ester	dibutyl phthalic ester (DBP)	15.52	149 (100)	223 (5.5), 205 (4.4)	
	dihexyl phthalic ester (DHP)	19.56	149 (100)	251 (11), 233 (3.3)	
	dicyclohexyl phthalic ester (DCHP)	21.87	149 (100)	249 (17), 167 (32)	
	diethylhexyl phthalic ester (DEHP)	21.96	167 (36)	149 (100), 279 (9.8)	4.0[b] [24]
	DBP-d_4	15.53	153 (100)	227 (6.3)	
	DEHP-d_4	30.31	153 (100)	171 (41)	
Silyl derivative	butyl silyl ether (BSE)	8.53	131 (100)	103 (23), 145 (72)	
	hexyl silyl ether (HSE)	12.12	159 (100)	103 (42), 115 (5.0)	
	cyclohexyl silyl ether (CHSE)	14.52	157 (97)	129 (100), 172 (23)	5.0
	ethylhexyl silyl ether (EHSE)	16.58	187 (80)	103 (100), 202 (0.1)	1.0
	phthalic acid silyl ester (PASE)	20.49	295 (17)	147 (100), 310 (2.0)	1.0
	DBP-d_4	21.62	153 (100)	227 (6.3)	
	DEHP-d_4	24.37	153 (100)	171 (41)	

[a]: The analytes were determined by phthalic ester form and silyl derivative form after hydrolysis of phthalic esters. [b]: Detection limit of DEHP was obtained using electron capture detector.

analytes was transferred to a vial, and then concentrated to 0.2 mL approximately using gentle flow of nitrogen gas. It was subjected to silyl derivatization and GC-MS analysis for the evaluation of extraction efficiency.
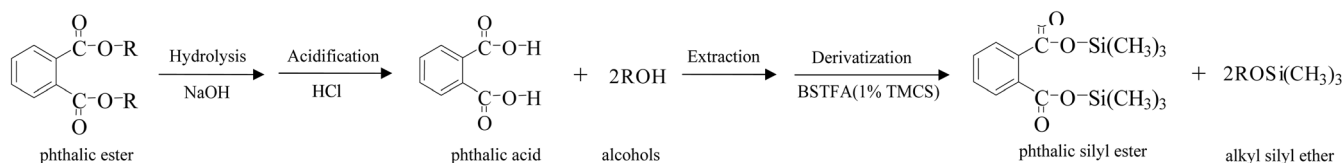
In order to optimize silyl derivatization condition, the effect of the solvent on the reaction rate was studied in acetone, ethyl acetate, dichloromethane and hexane medium, respectively. The derivatization was completed quantitatively in the medium of acetone or acetone-ethyl acetate mixture. Based on this result, 1 mL of acetone was added to the concentrated extract, and then 100 μL of BSTFA was added for derivatization. The solution was shaken vigorously using a vortex mixer for a moment, and enough time was allowed for the completion of the reaction. The solution was concentrated to 0.5 mL with gentle flow of nitrogen gas. After the addition of 50 μL internal standard (45 μM) to the derivatized samples, the volume was set to 1 mL. The samples were subjected to GC-MS analysis.
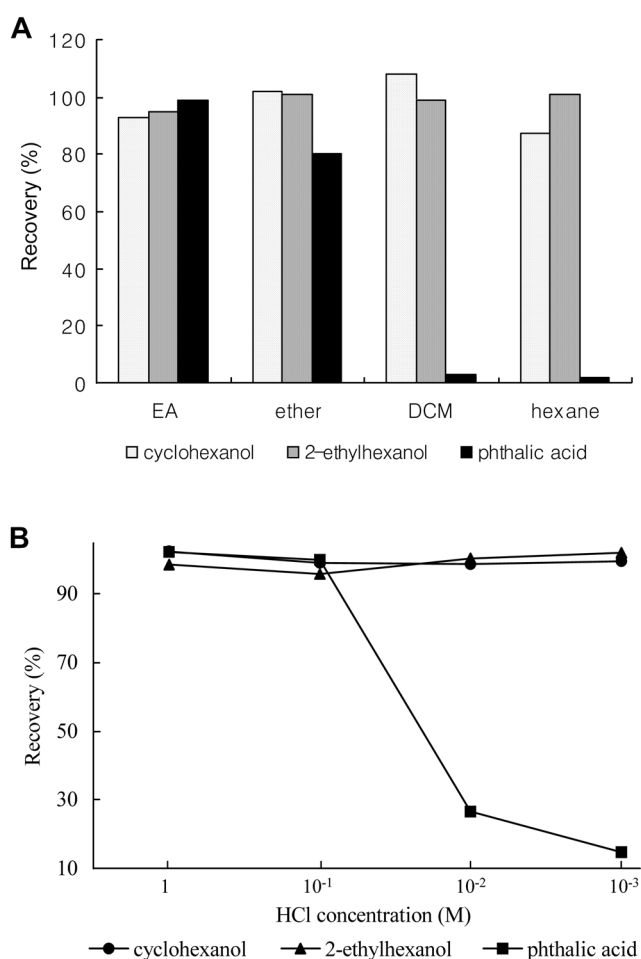
Results and Discussion

The reaction scheme of the hydrolysis of phthalic esters in basic medium, acidification and silyl derivatization of the phthalic acid and alcohols is shown in Figure 1. According to the scheme, one mole of phthalic acid and two moles of alcohol will be produced from one mole of phthalic ester. Most of the phthalic esters have identical alkyl groups bound to the phthalic acid. So variation in the alkyl groups results in different phthalic esters. For examples, di(2-ethyl hexyl) phthalic ester (DEHP) has two identical 2-ethyl hexyl groups instead of two different butyl groups in dibutyl phthalic ester (DBP). Based on this point, simultaneous determination of total phthalic ester and individual phthalic esters was thought to be possible if suitable simultaneous derivatization process for the phthalic acid and alcohols was developed. For satisfactory results, the hydrolysis efficiency of the phthalic esters in samples, the extraction yields of the phthalic acid and alcohols from the acidified solution, and the derivatization conditions should be optimized.

DEHP and cyclohexyl phthalic ester (CHP) were selected as probe molecules. DEHP is the most abundant compound[8] and CHP is known as one of the inert compounds among the phthalic esters.[5] In this work after acceptable results were obtained from a series of experiments for DEHP and CHP, the same analytical procedures was applied to other phthalic esters and also to real environmental samples.

Extraction of phthalic acid and alcohols. The resulting hydrolysis products of the phthalic esters are phthalic acid and alcohols. Under the strong basic hydrolyzing conditions

Figure 2. Optimization of extraction conditions. A: extraction recoveries of phthalic acid and alcohols in various solvent. B: Effect of acidity on the extraction yield of phthalic acid and alcohols.

the phthalic acid moiety must be turned to anionic phthalate, which is not favored form for the extraction to organic solvents. The phthalates should be turned into uncharged neutral species by acidification before the extraction. Furthermore the polarity of extracting solvent should be considered because of the high polarity of the phthalic acid and alcohols. Figure 2a shows the extractability of extracting solvents from 0.1 M HCl aqueous solution of analytes. The acidity of 0.1 M HCl was chosen considering the pK_a value of the phthalic acid ($pK_{a1} = 2.95$). The extraction efficiency increased with increasing the polarity of the extraction solvent. Among the solvents tested, ethyl acetate (EA) showed the highest extractability for all analytes examined. In some case of alcoholic analytes such as cyclohexanol and

Figure 1. Reaction schemes of hydrolysis and silyl derivatization on phthalic esters.

ethyl hexanol, acceptable extraction recoveries were obtained even when non-polar hexane was used. However the extractability of the phthalic acid decreased rapidly as the polarity of the solvent decreased. Hexane or dichloromethane (DCM) was not suitable for the extraction of the phthalic acid. For simultaneous extraction of the phthalic acid and alcohols, ethyl acetate was selected as an extracting solvent in the subsequent experiments.

Figure 2b shows the effect of the acidity of aqueous medium on the extraction yield of the phthalic acid and alcohols. The extraction efficiencies of the alcoholic analytes were not dependent on the acidity within the range examined. However the extraction yield of the phthalic acid decreased abruptly to below 30% at the acid concentration of 0.01 M or less. The phthalic acid has a high first dissociation constant (pK_{a1} = 2.95) and ionizes easily in aqueous solution. A considerable amount of the phthalic acid exists in a form of charged phthalate anion resulting in low extraction efficiency at low acidity. Enough acid concentration is required in order to suppress the dissociation of the phthalic acid. Based on the results the extractions were carried out from 0.1 M HCl solution in the subsequent experiments.

Derivatization techniques. The phthalic acid is highly polar and thermally unstable. It can be transformed easily to phthalic anhydride and water when heated over 230 °C. So it cannot be injected directly to the gas chromatographic analyzer. In order to increase the thermal stability and reduce the tailing of the peaks caused by hydroxyl group, derivatization techniques such as esterification have been applied in previous analytical studies.[29] The techniques were mainly focused on the analysis of the phthalic acid for the determination of the total phthalic ester content. Individual phthalic ester should be analyzed separately when required. To realize the idea of the simultaneous determination of the total phthalic ester as phthalic acid and individual phthalic ester, the selection of the derivatizing reagent is critical. *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) has been known as an excellent silyl reagent for the derivatization of hydroxyl groups. For example, the silyl derivatizations of sterols,[32] fatty acid[33] and phenol[34] were successfully demonstrated under moderate reaction conditions with high reaction yields. In this work, BSTFA was used as a derivatizing reagent for the simultaneous silylation of the phthalic acid and alcohols for the GC-MS analysis. The reaction conditions were optimized in terms of reaction temperature and medium.

In general, many kinds of silyl reactions were performed at high temperature to increase the reaction rate and yield. But it is inconvenient and time consuming to handle the samples at elevated temperature. To understand the temperature effect on the silyl reactions of the phthalic acid and alcohols, the reactions were carried out at 80 °C and room temperature in acetone medium. The reactions were completed quantitatively even at room temperature (about 25 °C) if enough reaction time was allowed (2 hours, data not shown). So the reaction rate in several medium having different polarity was determined at room temperature. As shown in
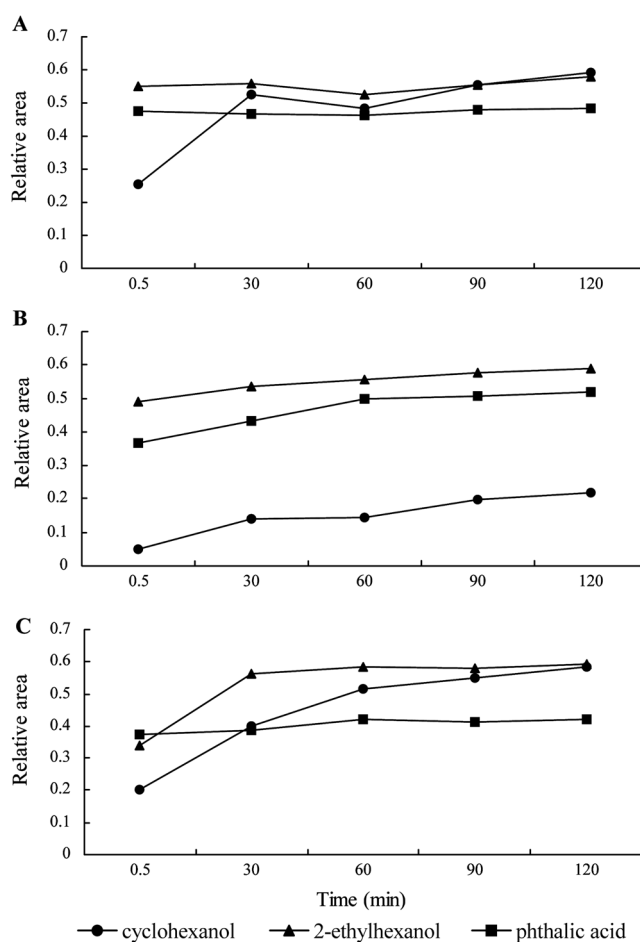
Figure 3. Kinetic studies of silylation reactions of phthalic acid and alcohol in different reaction medium. (A) acetone, (B) ethyl acetate, (C) dichloromethane.

Figure 3, the silyl reaction rate was very dependent on the nature of the reaction medium. The silylation of the phthalic acid and ethylhexanol was completed in acetone as soon as the silyl reagent, BSTFA was added. Even the silylation of cyclohexanol was completed within 30 min. In contrast the reactions in the other solvents like ethyl acetate or dichloromethane were not only sluggish but also non-quantitative. Only 80% of the phthalic acid was derivatized into phthalic silyl ester in dichloromethane although enough reaction time allowed. Less than 50% of the cyclohexanol was converted to its corresponding silyl ether in ethyl acetate medium. Among the target analytes examined, the cyclohexanol showed the slowest reaction rate compared to the phthalic acid or ethyl hexanol. It is likely that the bulky cyclohexyl group attached to the hydroxyl group reveals steric hindrance toward the trimethylsilyl group resulting in slower reaction rate. Anyway, quantitative conversion was achieved in the acetone medium within 30 min even in the case of the cyclohexanol. It implied that the acetone is most suitable reaction medium for the simultaneous silyl derivatization of the phthalic acid and alcohols among the solvents examined. Similar results were reported for the silyl derivatization of alkyl phenolic compounds in our previous work.[34]
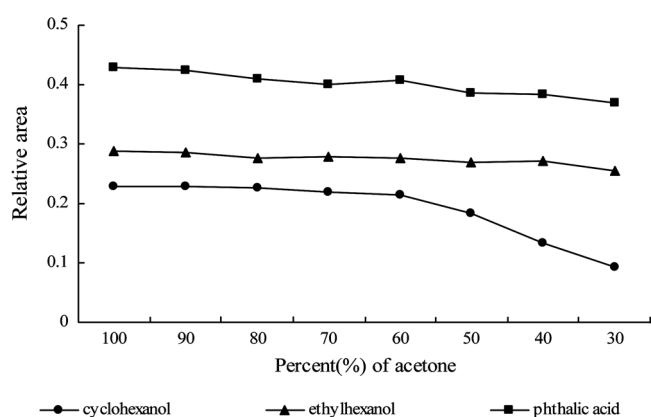
Figure 4. Effect of acetone content on silylation reaction rates in ethyl acetate medium.

Although the acetone may serve as an excellent reaction medium for the fast and quantitative silyl reaction of the phthalic acid and alcohols, it is not suitable solvent for the extraction from the samples containing water, *e.g.* environmental water, sediment, food, vegetable or biological samples.

The phthalic acid and alcohols should be extracted using a water immiscible organic polar solvent like ethyl acetate from aqueous samples after the base hydrolysis and acidification. After evaporation of the extracting solvent the acetone should be added for effective derivatization. However, this solvent switching process is tedious and time consuming. To overcome the inconvenience, the use of mixed solvent system in the derivatization reaction was carefully examined with increasing the acetone content in the ethyl acetate medium. We have reported the silyl reactions of phenolic compounds were accelerated by the addition of acetone to less polar extracting solvent in previous work.[34] As it can be seen in Figure 4 the derivatization yields at 30 min increased slowly until the acetone content became 60% and leveled off afterwards. Cyclohexanol required higher content of acetone for the completion of the reaction, whereas the others are not critical in the acetone content. This trend can be explained from the results of the solvent effect on the derivatization rate above. Since quantitative derivatization of all analytes within 30 min is possible as long as the acetone content exceeds 60%, the derivatization
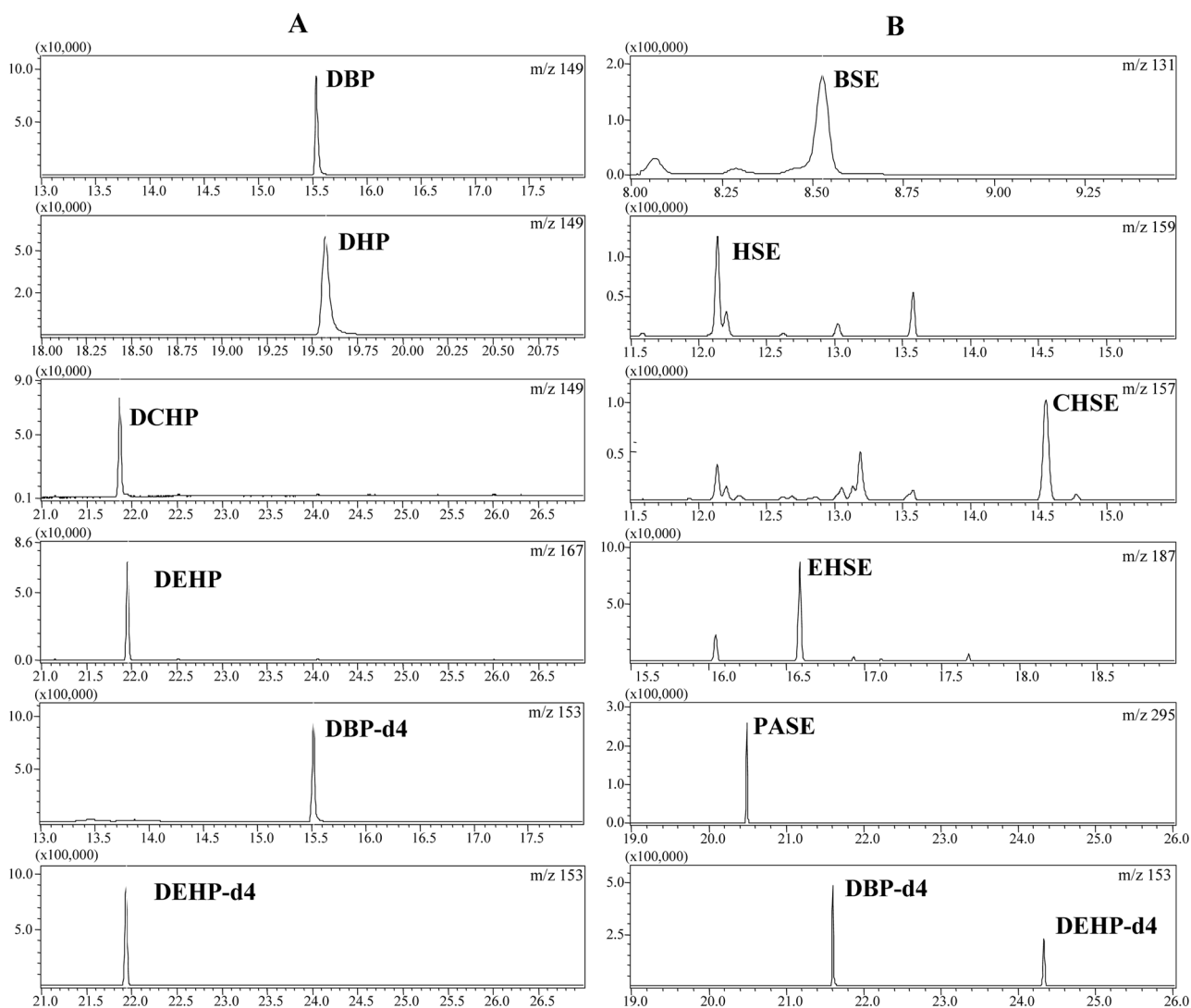
Figure 5. Selected ion chromatograms of pththalic esters (A) and silyl derivatives of phthalic acid and alcohols (B).

using BSFTA can be carried out in the ethyl acetate extraction medium after the addition of acetone as required.

Figure 5 shows typical chromatograms of both phthalic esters and silyl derivatives of the hydrolysis products of the phthalic esters. Chromatogram A is for the phthalic esters without hydrolysis and derivatization. Chromatogram B was obtained after silyl derivatization of the phthalic acid and alcohols from the extracts of the hydrolysis product. The same concentration levels of the phthalic ester were used for both experiments. Compared to the chromatogram A, more symmetrical and sharper peaks were observed in the chromatogram B with shorter retention time in most cases. It is likely due to the low polarity and high vapor pressure of the derivatized analytes. The height of the alcoholic ether peaks in the chromatogram B was much higher than corresponding phthalic ester peaks in the chromatogram A. In fact the detection limit was lower several times than those of corresponding phthalic esters as shown in Table 1. For examples, the detection limit of the silyl ether of ethyl-hexanol (corresponding to DEHP) was 1 pg, while it was 4 pg for DEHP in direct analysis. Consequently the content of the total phthalic esters and individual phthalic esters can be determined simultaneously from the peaks of their corresponding silyl derivatives with improved detection limit.

Applications. In order to evaluate the utility of this method, it was applied to the analysis of spiked tab water and contaminated costal seawater sample taken from Shihwa Lake in South Korea. The phthalic esters in 100 mL of the water samples were extracted with hexane, and the extract was concentrated to about 5 mL using a rotary evaporator. Then it was equally divided into two portions. One portion was used for the direct determination of the phthalic esters. The other portion was used for the simultaneous determination of the phthalic acid (equal to total phthalic ester) and alcohols (corresponding individual phthalic ester) after hydrolysis. The hydrolysis was carried out in 1 M NaOH solutions. The target analytes were extracted with ethyl-acetate after adjusting the acidity of the reaction medium to

pH 1 with hydrochloric acid. After dehydration with anhydrous Na_2SO_4, acetone was added to extracts for proper condition of the derivatization of the phthalic acid and alcohols. After the silyl derivatization with BSTFA it was concentrated to below 0.5 mL with gentle flow of dry nitrogen. Then the internal standard was added. Final volume of the solution was set to 1 mL and followed by GC-MS analysis.

The results are shown in Table 2 for both spiked tab water and real seawater sample. PE and SD denote direct GC-MS analysis of the phthalic esters and GC-MS analysis after silyl derivatization, respectively. Very large RSD values for the analytical results of the real seawater sample determined by the phthalic ester form directly were obtained because of the extremely low concentrations near the detection limits for certain phthalic esters. In contrast better RSD values were obtained when the silyl derivatiztion technique applied because of the improved sensitivity.

For spiked tab water, in general, the recoveries of the target analytes estimated from the alcoholic silyl derivatives were slightly lower than those obtained by the direct analysis of the phthalic ester. It is likely due to the multi-step procedure in the silyl derivatization method (SD). Nevertheless the results still satisfy the analytical requirements with reasonable recovery (> 75%) and low relative standard deviation (< 10%). The similarities (denoted in the ratio of SD/PE) between the results obtained by both procedures were over 0.81 and 0.94 for individual phthalic ester and total phthalic esters respectively.

For the real seawater sample (Table 2), in general, the similarities (SD/PE) were over than 1 for both low level and high level of the analytes detected. It might be due to the low method detection limit (MDL) and the effect of phthalic mono-esters existing in the real sample. In particular the reliability of the data increased significantly compared to that of the direct phthalic ester analysis in low concentration levels of the phthalic esters. For example, the relative standard deviations of DHP and DCHP obtained by the

Table 2. Comparison of analytical results obtained using different determination methods

Analyte	Form determine[a]	Spiked tab water[b]			Real sample		
		Con. (nM)	RSD (%)	Comparison of two different methods[c] (SD/PE or PA/PE)	Con. (nM)	RSD (%)	Comparison of two different methods[c] (SD/PE or PA/PE)
DBP	PE	486	8.6	0.83	413	17.1	0.93
	SD	401	5.2		386	11.3	
DHP	PE	477	3.6	0.85	3.84	47.3	1.52
	SD	406	6.7		5.83	7.88	
DCHP	PE	485	5.0	0.80	2.99	68.0	1.30
	SD	390	4.6		3.89	22.6	
DEHP	PE	489	8.7	0.83	254	8.92	1.19
	SD	397	6.1		302	3.64	
Total	PE	1937		0.81	674		1.04
	SD	1594			698		
	PA	1832	3.9	0.94	721	5.6	1.07

[a]: PE denotes "determined by phthalic ester form directly", SD denotes "determined by the corresponding silyl derivatization form after hydrolysis.", and PA denotes "determined by phthalic silyl ester form after hydrolysis". [b]: Phthalic ester standards mixture (500 nM each) was spiked into clean tab water. [c]: Comparison of analytical results determined by two different methods (SD/PE or PA/PE)

direct phthalic ester analysis are higher several times than those obtained by the silyl derivatization analysis (Table 2).

Conclusions

In conclusion quantitative silyl derivatization for both phthalic acid and alcohols was possible within 30 min at room temperature using an acetone containing medium. So it was possible to determine the total phthalic ester and individual phthalic ester simultaneously by GC-MS utilizing the silyl derivatization technique. It is much easier and time saving in monitoring the total phthalic ester and individual compounds of interest. Compared to the direct phthalic ester analysis, this method gave improved sensitivity and reproducibility. The utility of this approach in the analysis of various environmental samples is currently under way in our laboratory.

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