Environ. Eng. Res. Vol. 13, No. 4, pp. 216~223, 2008
Korean Society of Environmental Engineers

Determination of 11 Phenolic Endocrine Disruptors using Gas Chromatography/Mass Spectrometry-Selected Ion Monitoring in Five Selected Wastewater Influents

Hyub Kim†

Department of Research and Development, Korean Pharmacopuncture Institute, Seoul 157-200, South Korea

Received September 2008, accepted November 2008

Abstract

An efficient method for the simultaneous determination of eleven phenolic endocrine-disrupting chemicals (EDCs) present in wastewater influent samples was described. The 11 phenolic EDCs including alkylphenols, chlorophenols, and bisphenol A were determined by gas chromatography/mass spectrometry-selected ion monitoring (GC/MS-SIM) following two work-up methods for comparison; isobutoxycarbonyl (isoBOC) derivatization and *tert*-butyldimethylsilyl (TBDMS) derivatization. The wastewater influent samples containing the 11 EDCs were adjusted to pH 2 with H_2SO_4 and then cleaned up with *n*-hexane. Next, they were subjected to solid-phase extraction (SPE) with XAD-4 resin and subsequently converted to isoBOC or TBDMS derivatives for sensitivity analysis with gas chromatography/mass spectrometry-selected ion monitoring (GC/MS-SIM). Following isoBOC derivatization and TBDMS derivatization, the recoveries were 86.6-105.2% and 97.6-142.7%, the limits of quantitation (LOQ) for the 11 phenolic EDCs for SIM was 0.001-0.050 ng/mL and 0.003-0.050 ng/mL, and the SIM responses were linear with the correlation coefficient varying by 0.9717-0.9995 and 0.9842-0.9980, respectively. When these methods were applied to five selected wastewater influent samples, for isoBOC derivatization and TBDMS derivatization the ranges of concentration detected were 0.2-99.6 ng/mL and 0.4-147.4 ng/mL, respectively.

Keywords: Endocrine disrupting chemicals, IsoBOC, TBDMS, Wastewater influent samples

1. Introduction

The phenols, a group of compounds that are ubiquitous in environmental samples mainly because of their heavy use in the chemical industries, currently constitute an important class of ground water contaminants. Although their anti-estrogenic actions well described, alkylphenols, chlorophenols and bisphenol A can also induce endometriosis and estrogen-dependant tumors, implying possible estrogenic effects. As food and feed may contain some of these widely used products as result of diffuse environmental pollution and direct uptake by animals via food or air, a potential bioaccumulation and transfer through the food chain is possible. Concern about the endocrine-disrupting effects of these compounds has recently been increasing. Due to their toxicological potential and ubiquitous environmental presence, 11 phenols were classified as “endocrine disrupting chemicals” (EDCs) by the Japan Environmental Health and Safety Division of the Environment Agency’s Environmental Health Department. In Japan, the maximum admissible concentration of the phenolic EDCs in aqueous samples is 0.01 ug/L (nonylphenol 0.1 ug/L) for each compound (Japan Environment Agency, 1998).[1)] The other side, domestic was controlling only 4 phenolic EDCs just between 63 EDCs such as standard of WWF (World Wildlife Fund).

Surveys of alkylphenols, chlorophenols, and bisphenol A in samples of wastewater influent have been widely carried out.[2-6)] However, few reports of multi-component profiling analysis in wastewater influent samples have been published.[2,4,5)] The standard method (SPEED 98) consists of solvent extraction in a separatory funnel, silica gel clean-up for environmental aqueous samples, and analysis by GC/MS-SIM.[1)] However, these methods are only capable of simultaneously analyzing a few phenols, especially when environmental samples were analyzed, and the sample preparation was tedious and time-consuming. The simultaneous detection and identification of compounds was now a commonly encountered challenge in environmental screening as well as in the control of their overuse. Many analytical app-

† Corresponding author
E-mail: khlyj2004@dreamwiz.com
Tel: +82-2-2658-9051-3, Fax: +82-2-2658-9136

roaches have been used for the trace-level analysis of phenols, most of which include high performance liquid chromatography[7-9] or capillary gas chromatography. Gas chromatography (GC) was often preferred, offering unrivalled high resolution and easy coupling with sensitive and selective detectors.[10-16] HPLC detection was reported to be prone to interference from matrix compounds, such as humic substances naturally occurring in environmental samples.[9] Recently, multicomponent profiling analysis by high resolution capillary GC and GC/MS has been widely used in systematic screening to detect new and unexpected compounds and also to determine changes in the ratios of different compounds. A number of GC methods have been developed to separate small groups of target phenols[16] and phenol profiling analysis.[11-14, 25-26]

In the literature, gas chromatography/mass spectrometry (GC/MS) has been preferentially employed for multicomponent profiling analyses because of its inherent high resolving power, high sensitivity and positive peak confirmation.[17-24] However, due to the low volatility of some compounds containing a hydroxyl group, derivatization steps aimed at increasing the assay ability of the 11 phenolic EDCs were required to improve the technique before applying it to environmental analysis. To overcome these problems, cyanomethylation, acetylation, benzylation, silylation, and isobutoxycarbonylation have been employed.[17-24] Acetylation and benzylation, however, require phenols to be reacted as phenolate anions in alkaline solution, and were thus not suitable for phenols such as pentachlorophenol that are prone to oxidative degradation at pH above 8. In our previous work on the isoBOC reaction of structurally diverse phenols, a two-phase isoBOC reaction in acidic aqueous solution (pH 2) followed by a shift to pH 8, with subsequent solid-phase extraction (SPE) using Chromosorb P, was found to be efficient for the recovery of phenols. The resulting *O*-isoBOC phenols were isolated and were directly analyzed by GC/MS. However, in spite of these reported methods for the derivatization of phenols, a need remains for a simple and fast technique which allows the derivatization of phenols relevant to environmental water quality analysis, including alkylphenols, chlorophenols, and bisphenol A.[18] Moreover, the electron ionization impact (EI) mass spectra of the derivatives should be characteristic, in order to allow trace-level determination of individual phenols in environmental samples by GC/MS detection.

The purpose of the study was to develop an accurate and reproducible multicomponent profiling analysis to detect trace amounts of alkylphenols (4-*t*-butylphenol, 4-*n*-butylphenol, 4-*n*-pentylphenol, 4-*n*-hexylphenol, 4-*t*-octylphenol, 4-*n*-hepthyphenol, nonylphenol and 4-*n*-octylphenol), chlorophenols (2, 4-dichlorophenol, pentachlorophenol), and the most important bisphenol A in five types of wastewater influent samples. Therefore, I have developed a simple and rapid sample preparation method for GC/MS-SIM followed by two work-up methods for comparison: isoBOC derivatization and TBDMS derivatization in five types of wastewater samples.

2. Materials and Methods

2.1. Selection of Sampling Sites

The study was focused on five types of wastewater influents. The five types of wastewater influents, from factories producing ham, paper, dyes, petrochemicals, and livestock were collected in South Korea. The samples were collected in 4 L amber glass bottles with glass plugs and cooled to 4°C. Analyses were performed within 2 days after sampling.

2.2. Materials and Standard Solution

The nine phenolic EDCs standards, phenanthrene-d_{10}, and bisphenol-d_{16} were purchased from Sigma-Aldrich (Milwaukee, WI, USA); 4-*n*-hexylphenol was purchased from TCI (Tokyo, Japan); and 4-*n*-heptylphenol from Acros (Belgium). *N*-methyl-*N*-(*tert*-butyl-dimethylsilyl)-trifluoroacetamide (MTBSTFA) was obtained from Pierce (Rockford, IL, USA) and isobutylchloroformate (isoBCF) from Acros (Belgium). Triethylamine (TEA), sulfuric acid, and anhydrous sodium sulfate were obtained from Junsei (Tokyo, Japan). Acetonitrile, methanol, and *n*-hexane were purchased from J.T. Baker Analytical (Phillipsbug, NJ, USA). All other chemicals were of analytical grade and used as received. AMBERLITE XAD-4 (20-60 mesh) was purchased from Sigma (St. Louis, MO, USA). A luer-tipped glass tube (10 mm I.D.) packed with XAD-4 (500 mg) was washed successively with dichloromethane, *n*-hexane, ethylacetate, acetonitrile, and deionized water followed by activation in pH 2 water prior to being used as a solid-phase extraction (SPE) tube. The reference water (Water for chromatography) was purchased from Merck (Germany); pH 2 water was acidified to pH 2 with H_2SO_4.

Each stock solution of the phenols was made up at 1 mg/mL in acetonitrile and stored frozen. Working solutions were made by combining aliquots of each stock solution and diluting with acetonitrile. These were stored in a refrigerator. Two separate internal standard (I.S.) solutions were prepared by dissolving phenanthrene-d_{10}, and bisphenol A-d_{16} at 0.1 mg/mL and 0.05 mg/mL in acetonitrile, respectively.

2.3. Solid-Phase Extraction

The collected wastewater influents formed a very fine emulsion, which was very tedious to filter and easily clogged the SPE cartridge. Therefore, 20 mL of each wastewater influent was diluted 10-fold with reference water. Next, after acidification (adjustment to pH 2 by addition of sulfuric acid) the diluted wastewater influents were cleaned up with *n*-hexane (3 × 80 mL). The residual diluted wastewater influent was used for the determination of the 11 phenolic EDCs. The diluted 200 mL samples were spiked with bisphenol A-d_{16} as I.S. at 10 ng/mL, and then passed through a pre-activated 500 mg of the XAD-4 tube, using a solid-phase extractor (IST, UK). The wastewater samples were eluted at a flow rate of 6 mL/min. The column was eluted with hexane (40 mL) and the eluate was discarded. Next, phenolic EDCs were eluted twice with 5 mL of acetonitrile, allowing the solvent to react with the adsorbent for 5 min before elution. The eluate was divided into 5 mL aliquots to pool the two fractions from the column first and then collected in TEA (50 uL), respectively. The solvents were then evapo-

rated to 50 uL of acetonitrile phase in a concentrator (N_2 steam, 60°C).

2.4. O-isobutoxycarbonylation for GC/MS

A 50 uL aliquot of the eluate obtained from SPE, with 20 uL of added TEA was derivatized at 100°C using 20 uL of isoBCF. The derivatization was carried out in Reacti-vials (Pierce) with Teflon-lined sampler caps, placed in a heating module for 1 h. The solution containing the derivatives was added to 1 ug of phenanthrene-d_{10}. All the samples were individually prepared in triplicate and directly examined by GC/MS-SIM.

2.5. O-tert-butyldimethylsilylation for GC/MS

A 50 uL of the eluate obtained from SPE was derivatized at 100°C using 40 uL of MTBSTFA. The derivatization was performed as described above (section O-isobutoxycarbonylation for GC/MS). All the samples were individually prepared in triplicate and directly examined by GC/MS-SIM.

2.6. Determination by GC/MS (SIM)

To obtain mass spectra, a Hewlett-Packard HP 6890 Plus gas chromatograph with a DB-5MS (SE-54 bonded phase) capillary column (30 m × 0.25 mm I.D., 0.25 um film thickness), interfaced to an HP 5973 mass selective detector (70 eV, electron impact mode) and on-line to an HP G1701 DA MS Chemstation was used. The 1.0 uL volume samples were injected in splitless mode with a purge delay time of 0.7 min. The oven temperature was initially 60°C for 1 min and then raised to 280°C at 10°C/min, and held for 20 min. The injector and interface temperatures were 270 and 280°C, respectively (Table 1). The GC/MS measurements were performed by monitoring the ion mode. The quantitation ions for SIM are shown in Table 2. The solvent delay time was set to 5 min. For selected ion monitoring (SIM), two characteristic ions were selected for each compound and scanned using corresponding time windows, with dwell times of the range 150 ms per ion. The insert liner was exchanged after a maximum of 50 injections.

2.7. Recoveries and Blank Samples

For the determination of recoveries, reference water (Water for chromatography) was spiked with a stock solution containing the individual 11 phenolic EDCs in acetonitrile. The resulting concentrations were 100 pg/mL for alkylphenols, chlorophenols and bisphenol A, respectively. The 1 L blank sample of reference water were only spiked with the recovery standards alkylphenols, chlorophenols, bisphenol A and bisphenol A-d_{16} and analyzed. Also, the 1 L real samples of five types wastewater were spiked with the recovery standards 100 ng/mL for bisphenol A-d_{16} and analyzed.

2.8. Calculations and Quantitation

Stock solution containing all analytes at accurately defined concentrations was made in acetonitrile by dilution. All the quantitative calculations for the recoveries and linearity tests were based on the peak area ratios relative to the I.S. The SIM response curves used for quantitation were generated from derivatized phenol standards at five concentrations ranging from 50 to 4,000 pg/uL. Least-squares regression analysis was performed on the measured peak area ratios against increasing weight ratios of phenols to I.S., in order to test the linearity of the whole procedure and to plot calibration curves.

2.9. Limits of Quantitation (LOQ)

The LOQ for the GC/MS-SIM method was evaluated by spiking three replicates of reference water samples with alkylphenols, chlorophenols, and bisphenol A at a concentration estimated to yield a signal-to-noise ratio of 3-5.

Table 1. Operating Conditions for GC/MS Analysis

Parameter		Conditions	
Column		DB-5MS (30 m x 0.25 mm I.D., 0.25 um film thickness)	
Carrier gas		He	
GC/MS Temp. Information			
Injection Temp.		260°C	
Interface Temp.		200°C	
Ion Source Temp.		250°C	
Initial Temp.		60°C	
Initial Time		1.00 min	
Ramp			
#	Rate (°C/min)	Temp. (°C)	Time (min)
1	10.00	280	20.00
Purge Delay Time		1.00 min	
Injection Volume		1 uL	
Solvent Delay Time		5 min	
Ion Dwell Time		150 ms	

3. Results

3.1. GC/MS Characteristic of the 11 Phenolic EDCs

The 11 phenolic EDCs were analyzed on two derivatization methods of different reaction mechanism, the isoBOC derivatization and the TBDMS derivatization. The GC/MS-SIM chromatogram in Fig. 1 demonstrates the separation of a derivatized standard mixture containing 11 phenolic EDCs from spiked reference water samples. The simultaneous detection and identification of 11 phenolic EDCs in a single analysis has become increasingly important for accurate environmental monitoring. In this study, 11 phenolic EDCs in various wastewater influent samples were cleaned up with *n*-hexane, and then underwent SPE with XAD-4 and subsequent conversion to isoBOC derivatives or TBDMS derivatives for sensitivity analysis with GC/ MS-SIM.

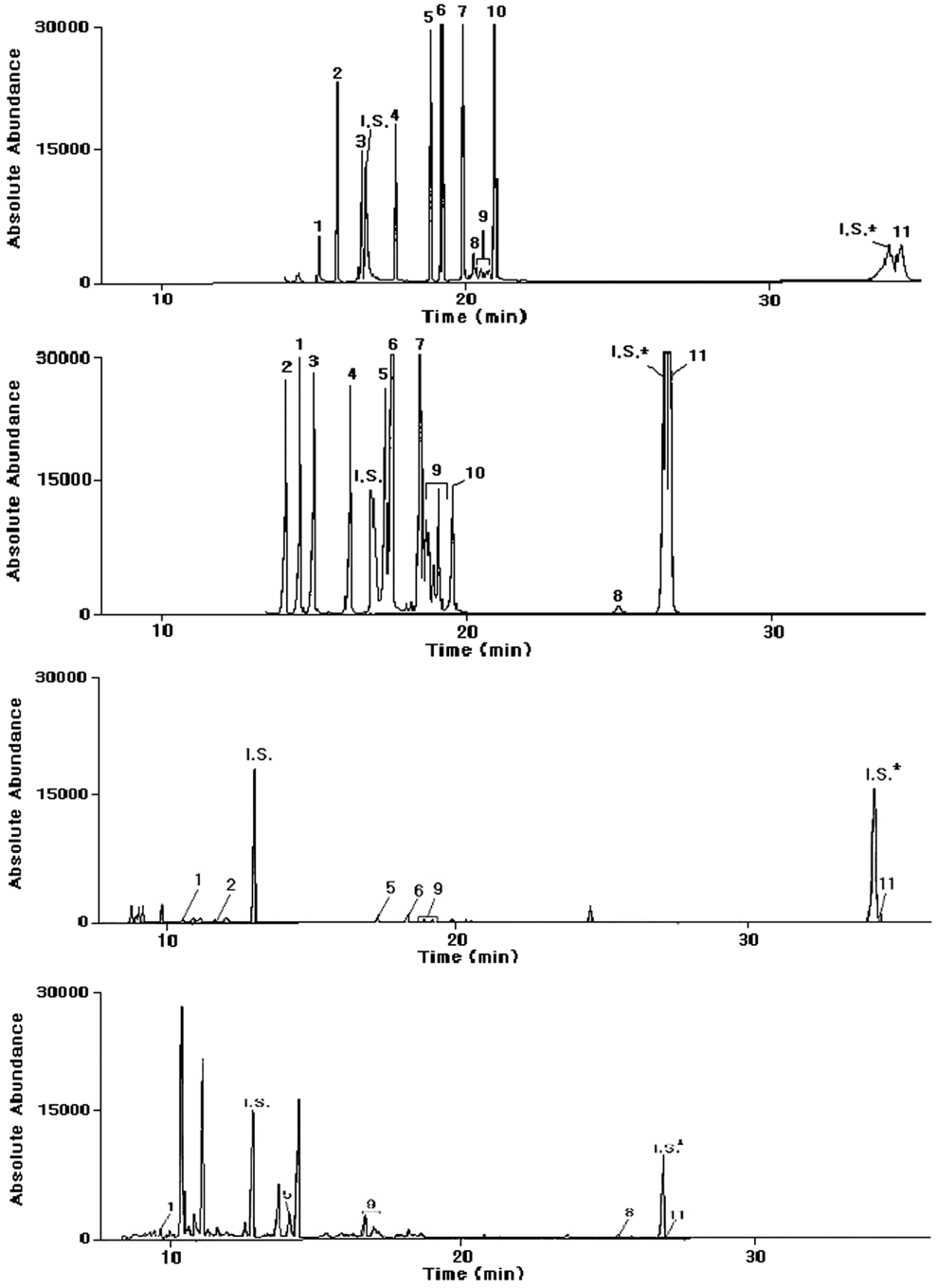

Fig. 1. SIM chromatograms obtained from spiked reference water at 3 ppb of 11 phenolic EDCs after isoBOC derivatization of phenols (1 st trace), after TBDMS derivatization of phenols (2 nd trace), and from the wastewater influent sample of the petrochemical plant after isoBOC derivatization of phenols (3 rd trace), and after TBDMS derivatization of phenols (4 th trace). Peaks: peak 1, 2, 4-dichlorophenol; peak 2, 4-*t*-butylphenol; peak 3, 4-*n*-butylphenol; I.S., phenanthrene-d_{10}; peak 4, 4-*n*-pentylphenol; peak 5, 4-*n*-hexylphenol; peak 6, 4-*t*-octylphenol; peak 7, 4-*n*-heptylphenol, peak 8, pentachlorophenol; peak 9, nonylphenol; peak 10, 4-*n*-octylphenol; I.S.*, bisphenol A-d_{16}; 11, bisphenol A.

3.2. O-isoBOC Derivatization

Upon the isoBOC reaction, all phenolic hydroxyl groups of the phenolic EDCs were converted to their corresponding isoBOC groups, yielding a single derivative for each phenolic EDCs compound studied. Under the present GC conditions, the separation of the 11 phenolic EDCs into their isoBOC derivatives was completed within 35 min, as shown in Fig. 1. The SIM response of the pentachlorophenol derivative (peak 8) was considerably lower than that of the other phenol derivatives. In most monohydroxybenzenes, $[M-100]^+$ rearrangement ions formed by the loss of one isoBOC function constitute the base peaks. In the isoBOC derivative of *n*-alkylphenols, there were only a few fragments of moderate intensity in addition to the base peak at m/z 107. The ion at m/z 107 arose from the additional loss of the alkyl group. Also, in the isoBOC derivative of *tert*-alkylphenols, there were only a few fragments of moderate intensity in addition to the base peak, at m/z 135. The ion at m/z 135 resulted from the successive α-cleavage of CH_3 or C_5H_{19} from the major fragment $[M-100]^+$.

3.3. O-TBDMS Derivatization

On the whole, all the 11 phenolic EDCs included in our study have been silylated successfully, by applying derivatization with MTBSTFA. A major advantage of the derivatization using MTBSTFA, compared to other silylating reagents, was the considerable stability of the TBDMS ethers toward moisture. This stability of the derivative to hydrolysis can be explained by the steric hindrance caused by the large *tert*-butyl moiety, which protects the silicon-oxygen bond from a hydrolytic attack by water molecules. The resulting TBDMS derivatives produced very characteristic mass spectra with electron impact-mass spectrometry (EI-MS). In mass spectra, the molecular ion was very weak, but the spectra were dominated by base peaks formed by the loss of the *tert*-butyl moiety $[M-57]^+$. In the case of the TBDMS ether of 2, 4-dichlorophenol, the base peak was appended by the corresponding chlorine isotope peaks exhibiting a characteristic chlorine cluster. Furthermore, the TBDMS ether of 2, 4-dichlorophenol exhibited ions at m/z 93 and 95 resulting from a dimethylsilyl chloride fragment, which was the result of a rearrangement reaction. Other fragments resulting from the successive loss of chlorine atoms from the base peak exhibited only weak intensities in the EI mass spectrum. In almost all EI mass spectra, the ion $[M-57]^+$ resulting from the cleavage of the *tert*-butyl moiety (M^+-$C(CH_3)_3$), or the ion $[M-15]^+$ resulting from the cleavage of the methyl moiety ($(CH_3)_2$-CH_3) from the molecule, was the base peak.

3.4. Selected Ion Monitoring (SIM)

Since GC/MS in the full-scan mode does not often provide the sensitivity necessary in trace-level analysis, SIM was applied as a routine method to achieve lower detection limits. The target-compound analyses included only a selection of relevant compounds. As an example, the detection of all phenols classified as EDC by the Japan Environmental Health and Safety Division, Environmental Health Department, Environment Agency with appropriate retention time-window setting in a single GC run will be presented. Two characteristic ions from the mass spectrum for each compound were selected and recorded in corresponding retention time windows. For all isoBOC and TBDMS derivatives, the ions $[M-100]^+$, $[M-57]^+$ and one other indicative ions were recorded as qualifiers (Table 2).

3.5. Recovery and Blanks of Analytical Protocol

The analytical performance of the method was evaluated through estimation of the efficiency, linearity, repeatability, and sensitivity of the method. For reference water, recoveries were determined by adding known and appropriate volumes of the working standard solution to previously analyzed aqueous samples. Three recovery experiments were performed for reference water samples. The results of these experiments are summarized in Table 2. Analyte recoveries were greater than 86% in reference water, but TBDMS derivatives were matrix-dependent in only case of pentachlorophenol. Because of TBDMS derivatization reacted with alcoholic hydroxyl groups and phenolic hydroxyl groups in wastewater influent matrix, but isoBOC derivatization could get good recovery results because did not reacted with alcoholic hydroxyl groups. Therefore, wastewater influents samples were thought that isoBOC derivatization method had been more effective than TBDMS derivatization method to apply to a lot of matrix samples.

The relative standard deviation (RSD) for replicate recovery analyses was in the range of 2.6-10.3%. The combined methods of SPE and isoBOC derivatization or TBDMS derivatization were examined to test the linear relation between detector response (expressed as peak area ratio) and amounts of the 11 phenolic EDCs. The isoBOC and TBDMS derivatizations linear responses were obtained for the 11 phenolic EDCs in range of 50-400 ng, with correlation coefficients varying by 0.9717-0.9995 and 0.9842-0.9980, for their quantitative measurement in unknown samples. In Fig. 1, such a SIM chromatogram, matrix from the TBDMS derivatization reaction interfered with the analyte detection, but the isoBOC derivatization reaction was not performed because the matrix became virtually transparent to GC/MS by applying SIM in spiked reference water at 3 ppb of 11 phenolic EDCs.

3.6. Limits of Quantitation (LOQ)

A list of LOQs for isoBOC derivatization and TBDMS derivatization was given in Table 2. The LOQ was 0.001-0.050 ng/mL and 0.003-0.050 ng/mL for alkylphenols, chlorophenols, and bisphenol A derivatized with isoBOC and TBDMS, respectively.

3.7. Application to Five Types of Wastewater Influent Samples

Fig. 1 shows the GC/MS-SIM chromatogram of a typical petrochemical plant wastewater influent sample. Five types of

wastewater influent samples containing factories producing ham, paper, dyes, petrochemicals, and livestock were analyzed by the proposed method. The Five types of wastewater influent samples, representing the major source of hormonally active compounds for the aquatic environment, contain the highest concentrations of all analyzed samples (see Table 3). Each type of wastewater influent matrix considered analyte recoveries determined by the peak area ratio relative to bisphenol A-d_{16} vs. phenanthrene-d_{10} ratio values. In Table 3, the results of wastewater influent samples are summarized. The 11 phenolic EDCs were almost unexceptionally in the lowest nanogram per milliliter range. The isoBOC derivatization was occurred matrix peak at pentachlorophenol peak position and TBDMS derivatization was occurred matrix peak at 4-*t*-octylphenol peak posi-

Table 2. Relative Retention Time, Precursor, Product Ion used in GC/MS Analysis for the Detection of the Selected 11 Phenolic EDCs and Their Respective Extracted Recoveries with XAD-4, and Limit of Quantitation

Compound	Relative retention time (RRT)[a]		Precursor ion (*m/z*)		Product ion (*m/z*)		XAD-4 recovery (%)[b]		Limit of quantitation (ppt)[c]	
	isoBOC	TBDMS	isoBOC	TBDMS	isoBOC	TBDMS	isoBOC (% rsd)	TBDMS (% rsd)	isoBOC	TBDMS
2,4-Dichlorophenol	0.910	0.875	162	219	164	221	93.8 (6.8)	97.6 (10.3)	6	25
4-*t*-Butylphenol	0.946	0.847	135	151	150	264	87.6 (2.6)	142.7 (7.5)	1	50
4-*n*-Butylphenol	0.992	0.901	107	207	150	264	92.2 (8.3)	102.4 (3.8)	2	13
Phenanthrene-d_{10}	1.000	1.000	188	188	188	188	-	-	-	-
4-*n*-Pentylphenol	1.058	0.970	107	221	164	278	88.6 (2.8)	104.0 (3.2)	1	3
4-*n*-Hexylphenol	1.123	1.039	107	235	178	292	101.3 (4.7)	107.5 (2.8)	1	3
4-*t*-Octylphenol	1.146	1.052	135	249	235	320	86.6 (3.9)	110.4 (5.3)	1	13
4-*n*-Heptylphenol	1.185	1.103	107	249	192	306	88.0 (5.7)	116.0 (4.7)	1	25
Pentachlorophenol	1.198	1.482	266	357	268	358	95.0 (7.8)	M[d]	6	25
Nonylphenol	1.191	1.097	149, 135	263, 249	320	334	95.1 (8.2)	119.4 (2.6)	50	50
	1.203	1.109	149, 107	263, 249	320	334				
	1.208	1.117	149, 107	263, 249	320	334				
	1.214	1.120	149, 135	263, 249	320	334				
	1.230	1.129	149, 107	263, 249	320	334				
	1.235	1.138	149, 107	263, 249	320	334				
4-*n*-Octylphenol	1.243	1.165	107	263	206	320	105.2 (5.2)	97.8 (5.5)	1	3
Bisphenol A-d_{16}	1.857	1.533	224	453	242	470	-	-	-	-
Bisphenol A	1.870	1.540	213	441	228	456	93.0 (4.1)	107.1 (5.4)	2	3

[a]Relative retention time (RRT): RT of analyte / RT of pheanathrene-d_{10}
[b]A reference water spiked with alkylphenols, chlorophenols, and bisphenol A (0.10 ng/mL)
[b,c]I.S.: pheanathrene-d_{10}
[d]M: matrix peak

Table 3. Selected Analysis Present in Wastewater Influent Samples and Recovery

Compound	isoBOC[a]						TBDMS[b]					
	Ref.[c]	Ham	Paper	Dye	Petro.	Stock	Ref.	Ham	Paper	Dye	Petro.	Stock
2,4-Dichlorophenol	ND[d]	ND	4.5[e]	4.1	2.7	ND	ND	ND	5.8	3.5	2.1	ND
4-*t*-Butylphenol	ND	ND	ND	ND	0.2	15.4	ND	ND	ND	ND	ND	12.4
4-*n*-Butylphenol	ND	ND	14.2	2.8	ND	ND	ND	ND	15.5	2.2	ND	ND
4-*n*-Pentylphenol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-*n*-Hexylphenol	ND	ND	ND	ND	0.3	ND	ND	ND	ND	ND	0.4	ND
4-*t*-Octylphenol	ND	0.8	0.9	8.3	0.9	7.2	M[f]	M	M	M	M	M
4-*n*-Heptylphenol	ND	ND	ND	ND	ND	8.2	ND	ND	ND	ND	ND	7.3
Pentachlorophenol	ND	ND	M	M	M	M	ND	ND	13.7	2.4	0.8	0.6
Nonylphenol	ND	1.7	1.9	0.8	2.4	ND	1.1	2.8	2.4	1.3	3.6	1.1
4-n-Octylphenol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bisphenol A	ND	1.8	99.6	5.4	1.9	2.2	ND	1.1	147.4	7.7	1.3	1.8
Recovery (%)[g]	92.6	90.2	94.8	114.9	85.3	126.7	101.7	100.5	90.9	114.3	85.5	86.9

[a, b]I.S.: phenanthrene-d_{10}
[a, b]A aqueous sample spiked with bisphenol A-d_{16} (0.10 ug/mL)
[c]Ref.: reference water sample
[d]ND: not detected
[e]Value: ng/mL
[f]M: matrix peak
[g]I.S.: bisphenol A-d_{16}

tion by alcoholic hydroxyl groups containing matrix substances in wastewater influent samples. Therefore, isoBOC derivatization and TBDMS derivatization was cross-checked profiling analysis to detect trace amounts of alkylphenols (4-*t*-butylphenol, 4-*n*-butylphenol, 4-*n*-pentylphenol, 4-*n*-hexylphenol, 4-*t*-octylphenol, 4-*n*-hepthyphenol, nonylphenol and 4-*n*-octylphenol), chlorophenols (2, 4-dichlorophenol, pentachlorophenol), and the most important bisphenol A in five types of wastewater influent samples. Concentration values obtained for five selected wastewater influents after isoBOC derivatization and TBDMS derivatization were in the range of 0.2-99.6 ng/mL and 0.4-147.4 ng/mL, respectively. The experimental results showed that the 11 phenolic EDCs recoveries were 85.3-126.7% (isoBOC) and 85.5-114.3% (TBDMS), respectively.

4. Discussion

In this study, a very sensitive method for the determination of 11 phenolic EDCs were used to determine endocrine-disrupting chemicals in five types of wastewater influents, from factories producing ham, paper, dyes, petrochemicals, and livestock were collected in South Korea. The isoBOC and TBDMS derivatization methods allowed rapid screening for the 11 phenolic EDCs when applied to environmental aqueous samples spiked with phenolic EDCs. The resulting isoBOC and TBDMS derivatives had characteristic EI-MS spectra. The simple derivatization procedure was well suited to the trace-level determination of the 11 phenolic EDCs, with limits of quantitation of 0.001-0.050 ng/mL (isoBOC derivatization) and 0.003-0.050 ng/mL (TBDMS derivatization) for gas chromatography with EI-MS detection in the SIM mode.

This study was to developed an accurate and reproducible multicomponent profiling analysis to detect trace amounts of alkylphenols (4-*t*-butylphenol, 4-butylphenol, 4-pentylphenol, 4-hexylphenol, 4-*t*-octylphenol, 4-Hepthyphenol, nonylphenol and 4-octylphenol), chlorophenols (2, 4-dichlorophenol, pentachlorophenol), and the most important bisphenol A in five types of wastewater influent samples. When these methods were applied to five selected wastewater influent samples, for isoBOC derivatization and TBDMS derivatization in the range of 0.2-99.6 ng/mL and 0.4-147.4 ng/mL, and the each variation value of concentration detected were about ±20%. According to Benjamin *et el.* and Holger *et el*., among wastewater, concentrations of phenols were determined by 381-5,320 ng/L and 6-135 ng/L. But, two papers can not distinguished occurrence special quality by kind of each pollution source of industry wastewater, and hard to determined correct value of phenols in each pollution source of industry wastewater that was produced by difference of dilution factor by difference of pollution source and sampling points.

5. Conclusions

The proposed method allowed the multivariate determination of 11 phenolic EDCs in wastewater influent samples. The method developed offers good precision and accuracy without the need of standard additions or previous computational estimation of the shape of the spectrum of the interference components.

An extension of the present method for the rapid profiling and screening of wastewater influent samples and environmental matrix samples for toxic 11 phenolic EDCs and their quantitative measurements is in progress.

Acknowledgements

The authors thank the Department of Ministry of Commerce, Industry and Energy and Sangju National University, for supporting this research.

References

1. Japan Environment Agency. Strategic Programs on Environmental, *JEA*, 68-77 (1998).
2. Lagana, A., Bacaloni, A., Leva, I. D., Faberi, A., Fago, G., and Marine, A., "Analytic-al methodologies for determining the occurrence of endocrine disrupting chemicals in sewage treatment plants and natural waters," *Analytica Chimica Acta.*, **501**, 79-88 (2004).
3. Braun, P., Moeder, M., Schrader, St., Popp, P., Kuschk, P., and Engewald, W., "Trace analysis of technical nonylphenol, bisphenol A and 17α-ethinylestradiol in wastewater using solid-phase microextraction and gas chromatography-mass spectrometry," *J. Chromatogr. A*, **988**, 41-51 (2003).
4. Holger M. K. and Karlheinz B., "Determination of endorcrine-disrupting phenolic compounds and estrogens in surface and drinking water by HRGC-(NCI)-MS in the picogram per liter range," *Environ. Sci. Technol.*, **35**, 3201-3206 (2001).
5. Benjamin L. L. T, Darry W. H., Jochen F. M., Frederic D. L. L., Louis A. T., and Heather F. C., "Comprehensive study of endocrine disrupting compounds using grab and passive sampling at selected wastewater treatment plants in South East Queensland, Australia," *Australia. Environ. Inter*, **3**, 654-669 (2007).
6. Espejo, R., Valter, K., Simona, M., Janin, Y., and Arrizabalaga, P., "Determination of nineteen 4-alkylphenol endocri-ne disrupters in Geneva municipal sewage wastewater," *J. Chromatogr. A*, **976**, 335-343 (2002).
7. Pocurull, E., Calull, M., Marce, R. M., and Borrull, F., "Determination of phenolic compounds at low μg 1-1 levels by various solid-phase extractions followed by liquid chromatography and diode-array detection," *J. Chromatogr. A*, **719**, 105-112 (1996).
8. Pocurull, E., Sanchez, G., Borrull, F., and Marce, R. M., "Automated on-line trace enrichment and determination of phenolic compounds in environmental waters by high-performance liquid chromatography," *J. Chromatogr. A*, **696**, 31-39 (1995).
9. Landzettel, W. J., Hargis, K. J., Caboot, J. B., Adkins, K. L., Strein, T. G., Veening, H., and Becker, H. D. "High-perfor-mance liquid chromatographic separation and detection of phenols using 2-(9-anthrylethyl) chloroformate as a

fluorophoric derivatizing reagent," *J. Chromatogr. A*, **718**, 45-51 (1995).
10. Herterrich, R., "Gas chromatographic determination of nitrophenols in atmospheric liquid water and airborne particulates," *J. Chromatogr.*, **549**, 313-324 (1991).
11. Weber, L., "Gas chromatographic determination of urinary phenol conjugates after acid hydrolysis/extractive acetylation," *J. Chromatogr.*, **574**, 349-351 (1992).
12. Ballesteros, E., Gallego, M., and Yalcarcel, M., "Automatic gas chromatographic determination of N-methylcarbamates in milk with electron capture detection," *Anal. Chem.*, **65**, 1773-1778 (1993).
13. Kumar, A., and Panwar, A., "Gas chromatographic separation of isomeric aminophenols, aniline, phenol, benzoquinone and azobenzene on a HP-1 capillary column," *Mikrochim. Acta*, **111**, 177-182 (1993).
14. Cooper, J. F., Tourte, J., and Gros, P., "Determination of pentachlorophenol residues in wine and corks by solvent extraction methodology and specific gas chromatography detection," *Chromatographia*, **38**, 147-150 (1994).
15. Pendergrass, S. M., "An Alternative Method for the Analysis of Phenol and o-, m-, and p-Cresol by Capillary GC/FID," *Am. Ind. Hyg. Assoc. J.*, **55**, 1051-1054 (1994).
16. Turnes, M. I., Rodriguez, I., Mejuto, M. C., and Cela, R. "Determination of chlorophenols in drinking water samples at the subnanogram per millilitre level by gas chromatography with atomic emission detection," *J. Chromatogr. A*, **683**, 21-29 (1994).
17. Kim, H., Hong, J. K., Kim Y. H., and Kim, K. R. "Combined isobutoxycarbonylation and tert-butyldimethylsilylation for the GC/MS-SIM detection of alkylphenols, chlorophenols and bisphenol A in Mackerel samples," *Archives of Pharmacal Research*, **26**, 697-705 (2003).
18. Kim K. R., and Kim, H., "Gas chromatographic profiling and screening for phenols as isobutoxycarbonyl derivatives in aqueous samples," *J. Chromatogr. A*, **866**, 87-96 (2000).
19. Heberer, T., and Stan, H. J., "Detection of more than 50 substituted phenols as their t-butyldimethylsilyl derivatives using gas chromatography-mass spectrometry," *Anal, Chimical Acta*, **341**, 21-34 (1997).
20. Boyd, J., "Indentification and quantification of mono-, di- and trihydroxybenzenes (phenols) at trace concentrations in seawater by aqueous acetylation and gas chromatographic-mass spectrometric analysis," *J. Chromatogr.*, **662**, 281-292 (1994).
21. Shin, H. S., Park, C. H., Park, S. J., and Pyo, H. S., "Sensitive determination of bisphenol A in environmental water by gas chromatography with nitrogen–phosphorus detection after cyanomethylation," *J. Chromatogr. A*, **912**, 119-125 (2001).
22. Boyd, Th. J., "Indentification and quantification of mono-, di- and trihydroxybenzenes (phenols) at trace concentrations in seawater by aqueous acetylation and gas chromatographic-mass spectrometric analysis," *J. Chromatogr.*, **662**, 281-292 (1994).
23. Cline, R. E., Todd, G. D., Ashley, D. L., Grainger, J., McCraw, J. M., Alley C.C., and Hill, R. H., "Gas chromatographic and spectral properties of pentafluorobenzyl derivatives of 2,4-dichlorophenoxyacetic acid and phenolic pesticides and metabolites," *J. Chromatogr. Sci.*, **28**, 167-174 (1990).
24. Tulp, M. T. M., and Hutzinger, O., "Use of ethyl ethers, deuteriomethyl ethers and cyclic n-butylboronates of hydroxychlorobiphenyls in identification of metabolites of polychlorinated biphenyls," *J. Chromatogr.*, **139**, 51-59 (1977).
25. Blackburn M. A., and Waldock M. J., "Concentration of alkylphenols in rivers and estuaries in England and Wales," *Wat. Res.*, **29**(7), 623-1629 (1995).
26. Leusch F. D. L., Chapman H. F., Heuval M. R., Tan B. L. L., Gooneratne S. R., Tremblay L. A., Gooneratne S. R., and Tremblay L. A., "Bioassay-derived androgenic and estrogenic activity in municipal sewage in Australia and New Zealand," *Eco. Environ. Safety*, **65**, 403-411 (2006).