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Combined Isobutoxycarbonylation and *tert*-Butyldimethylsilylation for the GC/MS-SIM Detection of Alkylphenols, Chlorophenols and Bisphenol A in Mackerel Samples

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The alkylphenols, chlorophenols, and bisphenol A were determined by gas chromatography/mass spectrometry-selected ion monitoring (GC/MS-SIM) followed by two work-up methods for comparison: isobutoxycarbonyl (isoBOC) derivatization and *tert*-butyldimethylsilyl (TBDMS) derivatization. Eleven endocrine disrupting chemicals (EDCs) of phenols in biological samples were extracted with acetonitrile and then the acetonitrile layer underwent freezing filtration 60°C for 2 hours. Solid-phase extraction (SPE) was used with XAD-4 and subsequent conversion to isoBOC or TBDMS derivatives for sensitivity analysis with the GC/MS-SIM mode. For isoBOC derivatization and TBDMS derivatization the recoveries were 92.3~150.6% and 93.8~108.3%, the method detection limits (MDLs) of bisphenol A for SIM were 0.062 μg/kg and 0.010 μg/kg, and the SIM responses were linear with the correlation coefficient varying by 0.9755~0.9981 and 0.9908~0.9996, respectively. When these methods were applied to mackerel samples, the concentrations of the 11 phenol EDCs were below the MDL.

Key words: Endocrine disrupting chemicals, Phenol, isoBOC, TBDMS, Freezing filtration, Method detection limits, GC/MS-SIM

INTRODUCTION

Environmental contaminants affect a wide variety of biological events in many species. Alkylphenols, chlorophenols and bisphenol A are typical environmental contaminants that exert adverse estrogen-relation effects. Although their anti-estrogenic actions are well described, alkylphenols, chlorophenols and bisphenol A can also induce endometriosis and estrogen-dependant tumors, implying possible estrogenic effects.

Concern has recently increased about the endocrine disrupting effects of these compounds. Due to their toxicological potentials and ubiquitous environmental occurrence, 11 phenols were classified as "endocrine disrupting chemical" (EDC) by the Japan Environmental Health and Safety Division, Environmental Health Department, Environment Agency. In Japan, the maximum admissible concentration of phenol EDCs in a biological sample is 1 μg/kg(nonylphenol 10 μg/kg) for individual content (Japan Environment Agency, 1998).

Surveys of alkylphenols, chlorophenols and bisphenol A in water samples of rivers, sewage effluents and estuaries have been widely carried out in many countries (Ahel *et al.*, 1994; Isobe *et al.*, 1998). However, few reports (Ekelund *et al.*, 1990; Ahel *et al.*, 1993; Wahlberg *et al.*, 1990) have been published for the analysis of these compounds in marine or freshwater biological samples. Especially, the fat content of the edible part of the mackerel, which consists of saturated and unsaturated fat, is 10.4 g/100 g.

The standard method (SPEED 98) consists of solvent extraction in a separated funnel, and then Silicagel clean up for environmental biological samples. The extracts are analyzed by gas chromatography/mass spectrometry-selected ion monitoring (GC/MS-SIM). However, these methods are only capable of simultaneously analyzing a few phenols, especially when environmental samples are analyzed, and the sample preparation is tedious and

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time consuming. The simultaneous detection and identification of 11 phenol EDCs in a single analysis is now a commonly encountered problem in environmental screening as well as in the controlling of their overuse.

Many analytical approaches have been used for the trace-level analysis of phenols, mainly using high performance liquid chromatography (HPLC) (Ruana *et al.*, 1993; Achii *et al.*, 1995; Pocurull *et al.*, 1995) or capillary gas chromatography (GC) for the analysis of phenols. GC has often been preferred, as it offers unrivalled high resolution and easy coupling with sensitive and selective detectors. HPLC detection was reported to be prone to interference from matrix compounds, such as humic substances naturally occurring in environmental samples (Pocurull *et al.*, 1995).

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 (Boyd, 1994; Lamprecht *et al.*, 1994) and phenol profiling analysis (Achii *et al.*, 1995; Lee *et al.*, 1983; Heberer *et al.*, 1997, Kim *et al.*, 2000). In the literature, high-resolution capillary GC/MS-SIM has been preferentially employed for multicomponent profiling analysis in screening because of its inherent high resolving power, high sensitivity and positive peak confirmation as well (Heberer *et al.*, 1997).

In general, phenols were amenable to GC without derivatization (Buchholz *et al.*, 1994; Wennrich *et al.*, 1995; EPA method 625, 1976). But at lower concentrations, peak tailing and discrimination in the injector or capillary column might occur (Buchholz *et al.*, 1994), especially when environmental samples were analyzed. To overcome these problems, acetylation, benzylation, benzoylation, alkylation, and silylation have been employed (Boyd *et al.*, 1994; Cline *et al.*, 1990; Street *et al.*, 1975; Tulp *et al.*, 1977; Herterrich, 1991; Kim *et al.*, 2000).

In our previous work on the isobutoxycarbonyl (isoBOC) reaction of structurally diverse phenols (Kim *et al.*, 2000), two-phase isoBOC reaction in an 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 underwent direct analysis 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 in environmental analysis, including alkylphenols, chlorophenols and bisphenol A.

Moreover the electron ionization/impact (EI) mass spectra of the derivatives should be characteristic to allow trace level determination of individual phenols in environmental samples by GC/MS detection. Therefore, we have developed a simple and rapid sample preparation method for GC/MS-SIM followed by two work-up methods for comparison; the isoBOC derivatization method and the TBDMS derivatization method in mackerel samples.

MATERIALS AND METHODS

Materials

The 9 phenol standards, phenanthrene-d_{10}, and bisphenol-d_{16} were purchased from Sigma-Aldrich (Milwaukee, WI, USA), 4-*n*-hexylphenol from TCI (Tokyo, Japan) and 4-*n*-heptylphenol from Acros (Belgium). *N*-methyl-*N*-(*tert*-butyldimethylsilyl)-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 Analyzed (Phillipsburg, NJ, USA). All other chemicals were of analytical grade and were 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 (0.5 g) was washed successively with acetonitrile, methanol and deionized water followed by activation in pH 2 water prior to being used as an SPE tube. The pH 2 water was acidified to pH 2 with H_2SO_4.

Phenol and internal standard solutions

Each phenol stock solution was made up at 1 $\mu g/\mu L^{-1}$ in acetonitrile and stored frozen.

Working solutions were made by combining aliquots of each stock solution, diluting with acetonitrile and storing 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 $\mu g/\mu L^{-1}$ and 0.05 $\mu g/\mu L^{-1}$ in acetonitrile, respectively.

Sample preparation

The biological samples of 40 g were spiked with the 11 phenol EDCs at 0.15 $\mu g/g^{-1}$ each and with bisphenol A-d_{16} as I.S. at 0.05 $\mu g/g^{-1}$. The biological samples was ultrasonicated twice with 100 mL of acetonitrile after adding 20 g of anhydrous sodium sulfate. The combined acetonitrile layer was refrigerated at -60°C for 2 h and then the organic layer was filtered. The acetonitrile layer was collected in reacti-vial with TEA (100 μL) by rotary-vacuum evaporator to dryness at 40°C. The residue was dissolved in 10 mL of pH 2 water and passed through a preactivated XAD-4 tube, using the SPE (IST, UK). The column was eluted with hexane (20 mL) and then the eluate was discarded. Next, the 11 phenol EDCs were

eluted twice with 3 mL of acetonitrile allowing the solvent to react with the adsorbent for 5 min before elution. The eluate was divided into 3 mL aliquots and collected in TEA (50 μL). Most of the acetonitrile was removed by evaporation (N_2 steam, 60°C).

O-Isobutoxycarbonylation

Twenty microliter of TEA was added to 50 μL of the eluate obtained from SPE, and the mixture was derivatized at 100°C using 20 μL of isoBCF. The derivatization was carried out in reacti-vials with Teflon-lined sampler caps, which were placed in a heating module for 1 h. To the solution containing the derivatives was added 1 μg of phenanthrene-d_{10}. All the samples were individually prepared in seven replicates and were directly examined by GC/MS-SIM (Fig. 1).

O-*tert*-Butyldimethylsilylation

The derivatization of 50 μL of the eluate obtained from SPE added to 40 μL of MTBSTFA, proceeded at 100°C in reacti-vials with Teflon-lined sampler caps which were placed on a heating module for 1 h. To the solution containing the derivatives was added 1 μg of phenanthrene-d_{10}. All the samples were individually prepared in seven replicates and were directly examined by GC/MS-SIM (Fig. 1).

Gas chromatography/mass spectrometry

To obtain mass spectra, a TRACE-GC 2000 gas chromatograph with a DB-5 (SE-54 bonded phase) capillary column (30 m×0.25 mm I.D., 0.25 μm film thickness), interfaced to a FINIGAN POLARIS/GCQ Plus ion trap mass selective detector (70 eV, electron impact mode) was run on-line with an Xcaliber program. Samples (ca. 1.0 μL) were injected in splitless mode with a purge delay time of 0.7 min. The oven temperature was initially set at 60°C for 1 min, 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.

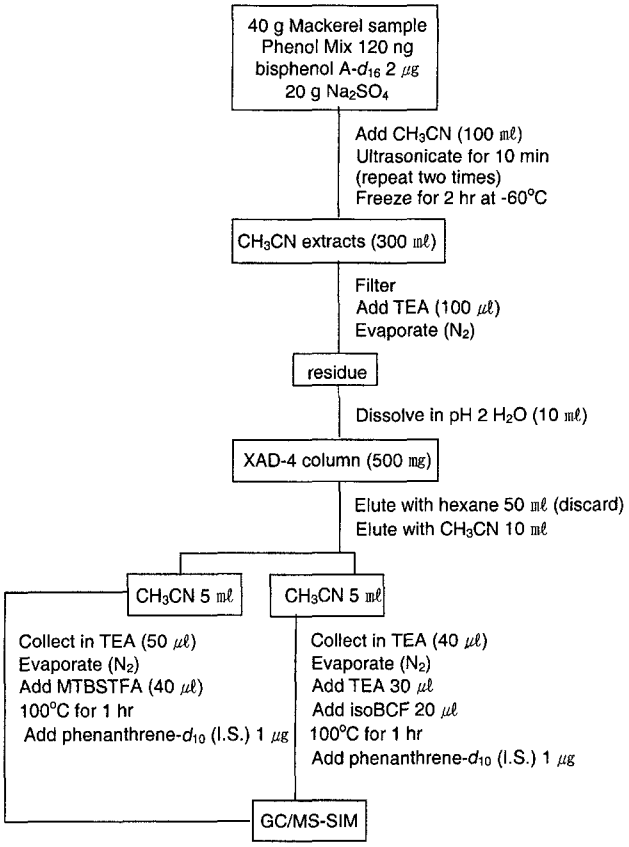

Fig. 1. Schematic flow diagram for sample preparation of alkylphenols, bisphenol A and chlorophenols in mackerel samples.

Table I. Selected ion groups for phenols in SIM mode

Compound	Time window (min)		Selected ion (m/z)		Dwell time (ms per ion)
	isoBOC	TBDMS	isoBOC	TBDMS	isoBOC, TBDMS
2,4-Dichlorophenol	14.00-18.10	13.50-15.50	164, 162	221, 219	150
4-*t*-Butylphenol	14.00-18.10	13.50-15.50	135, 107	264, 151	150
4-*n*-Butylphenol	14.00-18.10	13.50-15.50	150, 107	264, 225	150
Phenanthrene-d_{10}	14.00-18.10	15.50-18.00	188	188	150
4-*n*-Pentylphenol	18.10-19.40	15.50-18.00	164, 107	278, 239	150
4-*n*-Hexylphenol	18.10-19.40	15.50-18.00	178, 107	292, 253	150
4-*t*-Octylphenol	18.10-19.40	15.50-18.00	235, 135	320, 249	150
4-*n*-Heptylphenol	19.40-22.00	18.00-20.00	192, 107	306, 267	150
Pentachlorophenol	19.40-22.00	24.00-25.50	268, 266	375, 357	150
Nonylphenol	19.40-22.00	18.00-20.00	320, 220,	334, 277,	150
4-*n*-Octylphenol	19.40-22.00	18.00-20.00	149, 107	267, 249	150
			206, 107	320, 281	150
Bisphenol A-d_{16}	22.00-34.00	25.50-34.00	242, 224	470, 453	150
Bisphenol A	22.00-34.00	25.50-34.00	228, 213	456, 441	150

Mass spectrometric measurements were performed with EI at 70 eV. The GC-MS measurement was performed by monitoring the ion mode. The quantitation ions for SIM are shown in Table I. The time for solvent delay was set to 5 min. One microliter volume samples were injected using hot-splitless injection, with the split closed for 0.7 min. For 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. Insert liners were exchanged after a maximum of 50 injections.

Calculations

All the quantitative calculations for the recoveries and linearity tests were based on the peak area ratios relative to I.S. The SIM response curves used for quantitation were generated from derivatized phenols standards at five concentration levels ranging from 50 to 4,000 pg/μL. 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.

Method detection limit

Method detection limits (MDLs) were calculated from replicate (n = 7) injections at the 0.5 ng/g (isoBOC) and 0.25 ng/g (TBDMS) level, using the recommended EPA protocol. MDL for the GC/MS-SIM method was evaluated by spiking seven replicates of the biological samples with bisphenol A analyses at a concentration five times greater than the estimated MDL. After extraction, derivatization, GC/MS-SIM analysis, and quantification, the standard deviations of the seven replicates for bisphenol A were calculated.

MDL was obtained by, MDL = $t_{(n\text{-}1,\ 1\text{-}\alpha=0.99)}S$,
where $t_{(n\text{-}1,\ 1\text{-}\alpha=0.99)}$ is the Students' value appropriate for a 99% confidence level and S is the standard deviation estimate with n-1 degrees of freedom.

RESULTS

The simultaneous detection and identification of 11 phenol EDCs in a single analysis has become increasingly important for accurate environmental monitoring. In this study, 11 phenol EDCs in biological samples were extracted with acetonitrile, froze for 2 h at 60°C, and then underwent SPE with XAD-4 and subsequent conversion to isoBOC derivatives or TBDMS derivatives for sensitivity analysis with GC/MS-SIM.

GC/MS characteristics of the 11 phenol EDCs

IsoBOC derivatization

Upon the isoBOC reaction, all phenolic hydroxyl groups of the phenols were converted to their corresponding isoBOC groups, yielding a single derivative for each phenolic compound studied. Under the present GC conditions, the separation of the 11 phenol EDCs into their isoBOC derivatives was completed within 35 min, as shown in Fig. 2. The SIM response of the pentachlorophenol derivative (peak 9) was considerably lower than that of the other phenol derivatives.

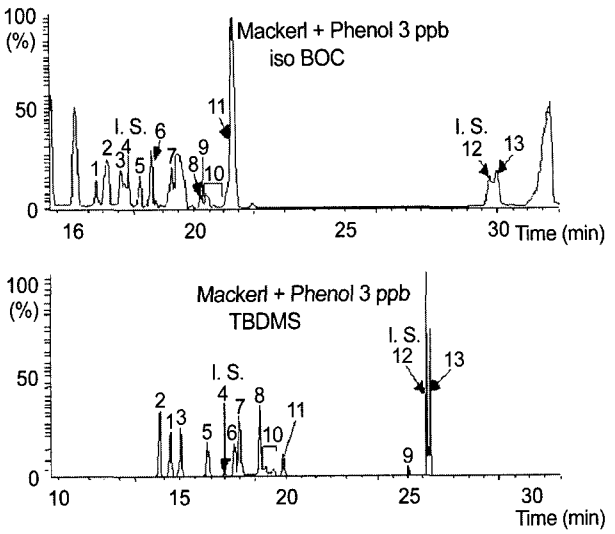

Fig 2. SIM chromatograms obtained from spiked mackerel samples at 3 ppb of alkylphenols, chlorophenols and bisphenol A after isoBOC derivatization (upper trace), and after TBDMS derivatization (lower trace), separated on a DB-5 (30 m×0.25 mm I.D., 0.25 film thickness) capillary column system. Peaks; 1=2,4-dichlorophenol; 2=*t*-butylphenol; 3=*n*-butylphenol; 4=pentachlorophenol; I.S.=phenanthrene-d_{10}; 5=*n*-pentylphenol; 6=*n*-hexylphenol; 7=*t*-octylphenol; 8=*n*-heptylphenol; 9=nonylphenol; 10=*n*-octylphenol; I.S.= bisphenol A-d_{16}; 11=bisphenol A.

The isoBOC phenol derivatives were subjected to GC/MS analysis and their electron-impact MS data are summarized in Table II. The molecular ion peaks of most phenol derivatives were either unobservable or very weak (less than 10%) (Fig. 3). Interestingly, the overall mass spectral patterns were fairly similar to those of underivatized intact phenols, except for the presence of intense isobutyl ion at m/z 57. This indicates that the preferential cleavage of bonds between phenolic oxygen and carbon of the isoBOC group occurred with migration of hydrogen atoms from the isoBOC group to phenolic oxygen, yielding a rearrangement ion that is identical to the molecular ion of the corresponding underivatized phenol.

In most monohydroxybenzenes, $[M\text{-}100]^+$ rearrangement ions formed by the loss of one isoBOC function constitute the base peaks. In the mass spectrum of 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 mass spectrum of the isoBOC

Table II. Relative retention times (RRT) and mass spectral characteristic ions of phenol isobutoxycarbonyl derivatives

compound	RT	RRT	$[M]^+$	$[M-100]^+$	Other characteristic ions, m/z (%)
2,4-Dichlorophenol	15.29	0.910	262 (0)	162 (100)	166 (11) 164 (65)
4-*t*-Butylphenol	15.90	0.946	250 (4)	150 (28)	135 (100) 107 (15)
4-*n*-Butylphenol	16.67	0.992	250 (4)	150 (46)	107 (100)
Phenanthrene-d_{10}	16.80	1.000	188 (100)		
4-*n*-Pentylphenol	17.78	1.058	264 (3)	164 (46)	107 (100)
4-*n*-Hexylphenol	18.86	1.123	278 (1)	178 (53)	107 (100)
4-*t*-Octylphenol	19.26	1.146	306 (2)	206 (1)	235 (37) 135 (100) 107 (13)
4-*n*-Heptylphenol	19.90	1.185	292 (3)	192 (57)	107 (100)
Pentachlorophenol	20.12	1.198	364 (0)	264 (62)	268 (64) 266 (100) 270 (20)
Nonylphenol	20.01	1.191	320 (1)	220 (3)	121 (100) 107 (36) 263 (30)
	20.21	1.203	320 (4)	220 (9)	149 (100) 121 (60) 135 (86)
	20.30	1.208	320 (1)	220 (6)	135 (100) 191 (15) 107 (10)
	20.40	1.214	320 (3)	220 (7)	121 (100) 107 (68) 177 (90)
	20.66	1.230	320 (2)	220 (7)	149 (100) 191 (61) 107 (67)
4-*n*-Octylphenol	20.88	1.243	306 (2)	206 (65)	107 (100)
Bisphenol A-d_{16}	31.20	1.857	442 (2)	342 (5)	242 (15) 224 (100)
Bisphenol A	31.42	1.870	428 (1)	328 (6)	228 (17) 213 (100)

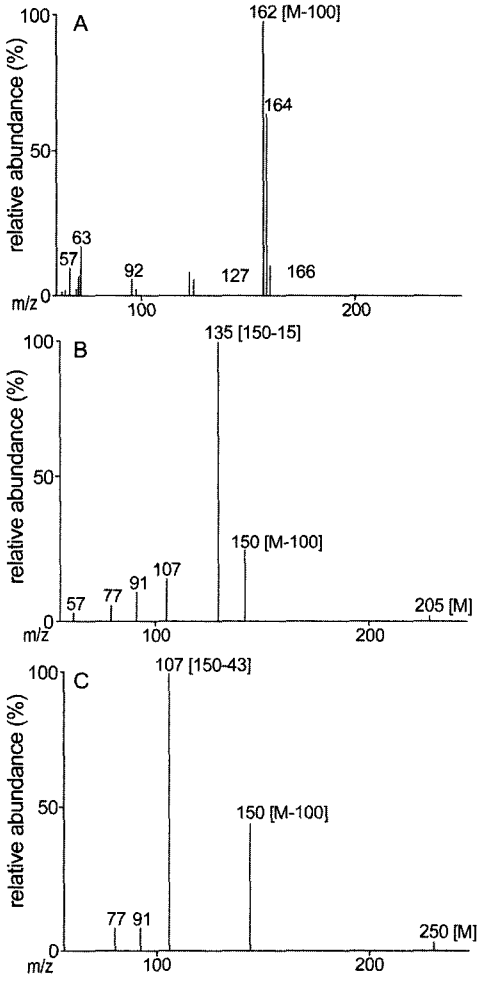

Fig. 3. EI mass spectra of isoBOC derivatized phenols. (A) 2,4-dichlorophenol-*O*-isoBOC, (B) *t*-butylphenol-*O*-isoBOC, (C) *n*-butylphenol-*O*-isoBOC.

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 $[M-100]^+$. As well as the base peak, there were several other characteristic fragments in the mass spectra of the isobutoxycarbonated phenols, as depicted in Table III.

TBDMS derivatization

On the whole, all the 11 phenol 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, is 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), as demonstrated with the TBDMS ethers of 2,4-dichlorophenol that are shown in Fig. 4. 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

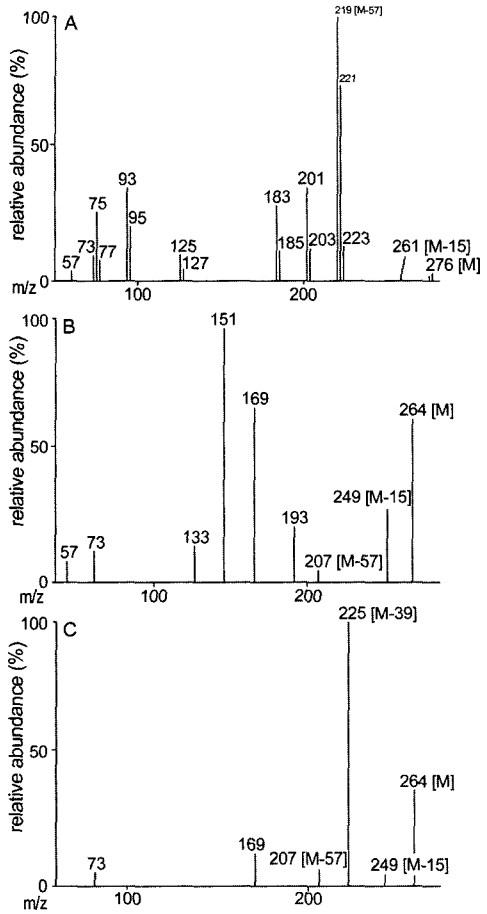

Fig. 4. EI mass spectra of TBDMS derivatized phenols. (A) 2,4-dichlorophenol-OTBDMS, (B) *t*-butylphenol-OTBDMS, (C) *n*-butylphenol-OTBDMS.

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.

Table III. compiles the most important masses and their corresponding relative abundances in the EI mass spectra of the TBDMS derivatives of all 11 phenol EDCs that were investigated in our study. 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. As well as the base peak, there were several other characteristic fragments in the mass spectra of the silylated phenols, as depicted in Table III.

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 I). In Fig. 2, such an SIM chromato-

Table III. Relative retention times (RRT) and mass spectral characteristic ions of phenol *t*-butyldimethylsilyl derivatives

Compound	RT	RRT	$[M]^+$	$[M-15]^+$	$[M-57]^+$	Other characteristic ions, m/z (%)
2,4-Dichlorophenol	14.71	0.875	276 (3)	261 (1)	219 (100)	221 (74) 201 (36) 183 (28)
4-*t*-Butylphenol	14.24	0.847	264 (62)	249 (27)	207 (4)	193 (20) 169 (66) 151 (100)
4-*n*-Butylphenol	15.16	0.901	264 (36)	249 (2)	207 (7)	225 (100) 208 (10) 169 (12)
Phenanthrene-d_{10}	16.82	1.000	188 (100)			
4-*n*-Pentylphenol	16.32	0.970	278 (40)	263 (2)	263 (2)	221 (9) 239 (100) 169 (20)
4-*n*-Hexylphenol	17.47	1.039	292 (37)	277 (1)	235 (6)	253 (100) 183 (11) 165 (28)
4-*t*-Octylphenol	17.70	1.052	320 (7)	305 (1)	263 (1)	249 (100) 73 (12)
4-*n*-Heptylphenol	18.55	1.103	306 (43)	291 (3)	291 (3)	249 (7) 267 (100) 165 (41)
Pentachlorophenol	24.93	1.482	378 (1)	363 (0)	321 (1)	376 (29) 375 (100) 357 (77)
Nonylphenol	18.45	1.097	334 (8)	319 (0)	277 (96)	306 (51) 267 (100) 235 (75)
	18.66	1.109	334 (18)	319 (0)	277 (3)	305 (37) 263 (53) 249 (100)
	18.73	1.114	334 (23)	319 (2)	277 (5)	305 (33) 263 (74) 249 (100)
	18.78	1.117	334 (15)	319 (2)	277 (10)	249 (100) 73 (14)
	18.84	1.120	334 (9)	319 (1)	277 (100)	305 (18) 291 (29) 235 (49)
	18.99	1.129	334 (2)	319 (6)	277 (100)	235 (34) 221 (26)
	19.14	1.138	334 (5)	319 (0)	277 (1)	305 (15) 263 (20) 249 (100)
4-*n*-Octylphenol	19.59	1.165	320 (55)	305 (3)	263 (8)	281 (100) 264 (21) 165 (74)
Bisphenol A-d_{16}	25.79	1.533	470 (37)	455 (4)	413 (0)	453 (100) 217 (39)
Bisphenol A	25.91	1.540	456 (32)	441 (100)	399 (0)	207 (35)

Relative retention times (RRT): RT of analyte / RT of phenanthrene-d_{10}

gram of a derivatized standard mixture containing the 11 phenol EDCs is presented. By-products from the isoBOC derivatization reaction interfered with the analyte detection, but the TBDMS derivatization reaction was not performed because the by-products were virtually transparent to GC/MS by applying SIM.

Effect of freezing filtration and solvent

Various solvent systems such as methanol, dichloromethane and acetonitrile were tested for the elution of the 11 phenol EDCs from XAD-4 adsorbent. The extraction proceeded rapidly to completion by ultrasonication, and then excess amount of lipids were removed by freezing filtration at -60°C for 2 h. The final 50 μL of eluate obtained from SPE was derivatized by MTBSTFA. The recovery of the combined freezing filtration and SPE method was not sufficient in the methanol solvent, because the silyl reagent was cleavage by the hydroxyl OH group in methanol during derivatization. However, the recovery results with acetonitrile ranged between 93.8 and 108.2% for the 11 phenol EDCs studied. Acetonitrile showed a higher recovery result than methanol or dichloromethane. The effect of the solvents on extraction of the phenol EDCs from the mackerel samples is shown in Fig. 5. Based on the recovery test, acetonitrile was chosen as the most adequate solvent for complete extraction of phenols from XAD-4 adsorbents.

Recovery, Precision and Method Detection Limits

A spike and recovery study was performed to determine the efficiency and reproducibility for the target analyte. Three mackerel sample replicates were spiked with all target analytes, extracted and analyzed. The spiked samples for GC/MS-SIM analysis were derivatized prior to analysis. The experimental results showed that the 11 phenol EDCs had average recoveries ranging from 70.1~150.6% (isoBOC) and 93.8~108.3% (TBDMS). The relative standard deviation (RSD) for replicate recovery analyses ranged from 3.9~34.4% (isoBOC) and 0.5~9.3% (TBDMS) (Table IV).

The combined method of SPE and isoBOC or TBDMS derivatization was examined to test the linear relation between detector response (expressed as peak area ratio) and phenol amounts. As listed in Table V, linear responses were obtained for the 11 phenol EDCs in the range of 5~400 ng with correlation coefficients varying from 0.9755~0.9981 (isoBOC) and 0.9908~0.9996 (TBDMS) (Table VI). The linearity of the two combined derivatizations for GC/MS-SIM separation of the 11 phenol EDCs appeared to be satisfactory for their quantitative measurements in unknown samples.

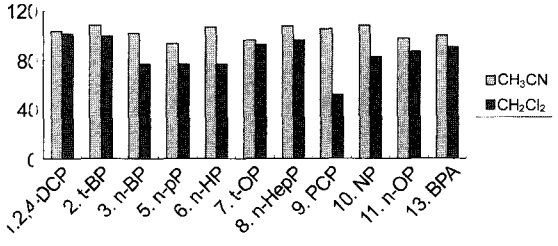

Fig. 5. Effect of eluting solvents on recovery rate.

Table IV. Recoveries of alkylphenols, chlorophenols, and bisphenol A extracted from biological samples using GC/MS-SIM mode

Compound	Recovery (%)	
	isoBOC[a]	TBDMS[b]
2,4-Dichlorophenol	144.4±11.19	103.2±4.51
4-*t*-Butylphenol	147.7±12.57	108.2±0.46
4-*n*-Butylphenol	137.0± 8.13	101.9±7.16
4-*n*-Pentylphenol	150.6±25.04	93.8±7.09
4-*n*-Hexylphenol	103.2± 6.30	106.8±6.90
4-*t*-Octylphenol	124.9±19.17	97.0±0.91
4-*n*-Heptylphenol	92.3± 3.85	107.8±7.39
Pentachlorophenol	122.6±29.47	105.5±9.29
Nonylphenol	127.0±34.44	108.3±5.77
4-*n*-Octylphenol	70.1± 6.99	97.8±1.57
Bisphenol A	128.1±11.87	100.5±1.28

[a,b] A mackerel sample spiked with bisphenol A (3 μg/20 g)
[a, b] I.S. : phenanthrene-d_{10}

MDL represents the lowest concentration level at which, in seven parallel analyses, bisphenol A could definitively be seen with a signal-to-noise ratio of 2.5:1. MDL was 0.062 μg/kg (isoBOC derivatization) and 0.010 μg/kg (TBDMS derivatization) for bisphenol A. An example for the performance of the method applied to biological samples containing a high load of matrix is presented in Fig. 2, showing the SIM-chromatogram of a spiked mackerel sample. This showed that chromatograms can be evaluated easily, along with the identification and quantitation of the origin of the sample and its matrix content.

DISCUSSION

The 11 phenol EDCs in mackerel samples were extracted with acetonitrile, and then underwent SPE with XAD-4 and subsequent conversion to isoBOC or TBDMS derivatives for sensitivity analysis with the SIM mode. The isoBOC derivatization and TBDMS derivatization methods allowed rapid screening for the 11 phenol EDCs when applied to environmental samples spiked with phenols. The resulting isoBOC derivatives and TBDMS derivatives produced characteristic mass spectra by applying EI-MS.

The simple derivatization procedure was well suited for the trace-level determination of bisphenol A with MDLs of

Table V. Linear regression analysis of relative response against relative weights of phenols as isoBOC & TBDMS derivatives

Compound	isoBOC			TBDMS		
	Regression line[a]		Correlation coefficient	Regression line		Correlation coefficient
	m	b	r	m	b	r
2,4-Dichlorophenol	0.0004	-0.0082	0.9943	0.0014	0.0099	0.9963
4-*t*-Butylphenol	0.0016	-0.0164	0.9979	0.0011	0.0576	0.9928
4-*n*-Butylphenol	0.0015	-0.0340	0.9909	0.0023	0.0246	0.9991
4-*n*-Pentylphenol	0.0013	-0.0134	0.9949	0.0021	0.0235	0.9996
4-*n*-Hexylphenol	0.0013	0.0463	0.9902	0.0021	0.0263	0.9995
4-*t*-Octylphenol	0.0015	0.0508	0.9900	0.0059	1.0293	0.9978
4-*n*-Heptylphenol	0.0012	0.0479	0.9981	0.0034	0.0441	0.9962
Pentachlorophenol	0.0001	0.0026	0.9958	0.0007	0.0198	0.9925
Nonylphenol	0.0010	0.0609	0.9931	0.0006	0.0308	0.9986
4-*n*-Octylphenol	0.0013	0.0289	0.9755	0.0019	0.0468	0.9908
Bisphenol A	0.0011	0.0124	0.9968	0.0085	0.139	0.9958

[a] m=Slope; relative mass response=mean peak area ratio of phenol x mass of I.S./mass of phenol; b=y-intercept. Calibration range; 5 ~ 400 ng

0.062 μg/kg (isoBOC derivatization) and 0.010 μg/kg (TBDMS derivatization), by applying GC with EI-MS detection in the SIM mode. An extension of the present method, for the rapid profiling and screening of biological samples and various fat samples for toxic phenols and their quantitative measurements, is in progress.

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