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Gas chromatographic profiling and screening for phenols as isobutoxycarbonyl derivatives in aqueous samples

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Abstract

An efficient method is described for the simultaneous determination of phenol and 49 substituted phenols present in aqueous samples. The method is based on the extractive two-phase isobutoxycarbonyl (isoBOC) derivatization with subsequent solid-phase extraction (SPE) for the direct analysis by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS). Phenolic hydroxyl groups in acidic aqueous solutions were allowed to react with isobutyl chloroformate present in the dichloromethane phase containing triethylamine. The resulting isoBOC derivatives were then recovered by SPE using Chromosorb P in normal-phase partition mode, followed by direct GC and GC–MS analysis. Using this combined procedure, linear detector responses were obtained in the concentration range of 0.5–8 $\mu g\,ml^{-1}$, with correlation coefficients varying from 0.925 to 0.999 for most of the phenols studied except for 2,4-dinitorphenol (0.789). The temperature-programmed retention index (I) sets as measured on DB-5 and DB-17 dual-capillary columns of different polarity were characteristic of each isoBOC phenol derivative and thus, useful in the screening for isomeric phenols by I matching only. The mass spectral patterns, exhibiting characteristic $[M-100]^+$, $[M-200]^+$ and $[M-300]^+$ ions for the mono-, di- and trihydroxybezenes, respectively with common ions at m/z 57, facilitated their rapid structural confirmation. The present method allowed rapid screening for phenols when applied to water samples spiked with phenols.

Keywords: Derivatization, GC; Isobutoxycarbonyl derivatization; Retention indices; Phenols

1. Introduction

Currently the phenols, a group of ubiquitous compounds in environmental samples mainly because of their heavy uses in the chemical industries, constitute an important class of ground water contaminants [1–4]. The phenolic micropollutants generally include chloro-, bromo-, nitro-, and alkylphenols. Some of theses are either known or suspected endocrine disrupters. With the growing concern about the water quality, trace-level analysis of these phenols has become important in recent years.

The simultaneous detection and identification of a wide range of phenols in a single analysis is now commonly encountered problems in the environmental screening works as well as in the controlling of their overuses. In the literature, high-resolution capillary gas chromatography (GC) combined with mass spectrometry (MS) has been preferentially employed for the multicomponent profiling analyses in screening works because of its inherent high resolving

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power, high sensitivity and positive peak confirmation as well [2,4–21]. It is now well established that GC alone can provide rapid identification of each separated peaks based on the retention index (*I*) matching with reference values measured on capillary columns of different polarity. However, only the GC retention times or retention ratios that are dependent on experimental conditions have been used for the confirming purposes of phenol peaks [5,7,21].

The accurate GC analysis of phenols requires one or more appropriate derivatization procedures to block active protons in phenolic hydroxyl and other polar groups, and mainly, acetylation [2,5,6,12,17,19], benzylation [7,11,15,16], benzoylation [10,13], alkylation [14,20] and silylation [18,21] have been employed. The preparation of volatile and less polar, yet stable derivatives directly in the aqueous solutions by a single-step procedure is most desirable, especially for the polar di- and trihydroxybezenes which are not readily extracted from aqueous samples [6,19]. The aqueous acetylation and benzoylation are the closest approach to this goal. However, these reactions require phenols to be reacted as phenolate anions in the alkaline solutions, thus not suitable for phenols such as catechol and hydroquinone that are prone to oxidative degradation at pH above 8. As a different approach in the literature [22], simple chloro- and alkylphenols were reacted with isobutyl chloroformate (isoBCF) for the conversion to their corresponding isobutoxycarbonyl (isoBOC) derivatives in the alkaline solutions. This direct isoBOC reaction procedure at a fixed alkaline pH is, however, not applicable to the labile phenols either. Therefore, an aqueous derivatization which takes place under the neutral or acidic conditions is required for the simultaneous screening for a wide range of phenols including oxidatively labile phenols.

In our previous work on the isoBOC reaction of structurally diverse amines [23], the two-phase isoBOC reaction with a pH shift was found to be useful for the recovery of amines containing labile phenolic functions such as catecholamines. Phenolic hydroxyl groups of catecholamines in phosphate buffer at pH 7.5 were first allowed to react with isoBCF present in the dichloromethane, followed by increasing pH of the aqueous phase to 12.0, for the reaction of basic amino functions. The resulting isoBOC derivatives were recovered by solid-phase extraction (SPE) using Chromosorb P in normal-phase partition mode for the analysis by dual-capillary column GC. The accurate measurements of retention index (*I*) values on the dual columns of different polarity allowed positive peak identification through computer library matching based on the two reference *I* sets. With the two-phase procedure, isoBOC reaction, requiring smaller amount of isoBCF, was found to be more efficient and rapid.

As the first step toward systematic phenolic profiling and screening works for the environmental monitoring, the present study was undertaken to examine the optimum conditions of simultaneous isoBOC reaction of phenol and 49 various substituted phenols including labile hydroquinone and catechol. The method was based on the extractive two-phase isoBOC reaction in acidic aqueous solutions. The resulting isoBOC derivatives were then subjected to the SPE with subsequent dual-column GC profiling analysis as described previously [23]. The structures of the isoBOC derivatives that are new to the literature were confirmed by GC–MS.

2. Experimental

2.1. Materials

The 50 phenolic standards examined for this study and isobutyl chloroformate (isoBCF) were purchased from Sigma-Aldrich (Milwaukee, WI, USA), Sigma (St. Louis, MO, USA) and other vendors. Toluene and triethylamine (TEA) were obtained from Junsei (Tokyo, Japan) and *n*-hydrocarbon standards (C_{10}–$C_{32,}$ even numbers only) from Polyscience (Niles, IL, USA). Isooctane, acetonitrile, methanol and dichloromethane of spectroanalyzed grade were purchased from Fisher Scientific (Fair Lawn, NJ, USA) and diethyl ether from Oriental Chemical Industry (Seoul, South Korea). All other chemicals were of analytical grade and used as received. Chromosorb P (80–100 mesh) was purchased from Supelco (Bellefonte, PA, USA). A luer-tipped glass tube (12 mm I.D.) packed with Chromosorb P (2.0 g) was washed successively with 0.01 *M* sodium hydroxide, methanol, acetone, and dichloromethane, followed by

activation (150°C, 3 h) prior to being used as an (SPE) tube.

2.2. Phenolic stock and internal standard solutions

Each standard stock solution of phenols was made up at 10 μg μl^{-1} in acetonitrile and stored in a freezer. Working solutions were made by combining aliquots of each stock solution and diluting to appropriate concentrations with acetonitrile and stored in a refrigerator. *n*-Docosane used as internal standard was prepared by dissolving at 2 μg μl^{-1} in isooctane.

2.3. Isobutoxycarbonylation and solid-phase extraction

Phenolic aqueous standard solutions were prepared by diluting aliquots of the phenol working solutions to the desired concentrations (0.5–8 μg ml^{-1}) with water (pH 2) and *n*-docosane was added at 2.0 μg ml^{-1}. After saturation of the phenolic aqueous solutions (1.0 ml) with sodium chloride in screw-capped glass vials, two-phase isoBOC reaction was performed by vortex mixing (10 min) with dichloromethane containing isoBCF (5 μl) and TEA (20 μl). Subsequently, the pH of aqueous layer was adjusted to 8 with sodium hydroxide (5 *M*) and was subjected to SPE as described elsewhere [23]. Briefly, the whole reaction mixture including the solvent phase was loaded onto a preactivated Chromosorb P tube, using a solid-phase extractor (Supelco, Bellefonte, PA, USA). The aqueous phase was allowed to advance homogeneously until about 80% of the tube was moist. Next, isoBOC derivatives of phenols were eluted with dichloromethane (3 ml) and the eluate was collected in toluene (30 μl) as keeper. Most of dichloromethane was removed by evaporation (gentle stream of nitrogen, 60°C). Samples for linearity tests [flame ionization detection (FID) responses] for the 50 phenols were prepared using phenolic aqueous standard solutions (0.5, 3.0, 5.5, and 8.0 μg ml^{-1} each) spiked with fixed amount (2 μg ml^{-1}) of *n*-docosane (I.S.). Phenols that are not resolved on HP-5 were separately processed for the linearity test. Inter-day assay tests were performed to water samples (pH 2) spiked with 10 phenols at three different concentrations (0.5, 2 and 4 μg ml^{-1} each) and I.S. at 2 μg ml^{-1}. To test the effects of TEA and its amount on the recoveries of phenols, isoBOC reaction was carried out with four different amounts of TEA (0, 10, 20, or 30 μl). Water samples (pH 2) spiked with 10 phenols (3 μg ml^{-1} each) and I.S. (2 μg ml^{-1}) were used for this test. Samples were analyzed in a day for linearity tests and on every other day for inter-day assay tests. All samples were individually prepared in triplicate and directly examined by GC and GC–MS.

2.4. Sample preparation

Aliquots (5, 10, or 100 ml) of acidic water (pH 2) were fortified with 10 phenols (1 μg each) and I.S. (2 μg). Each water sample was placed into a separatory funnel containing dichloromethane (2 ml for 5 and 10 ml of water samples but 10 ml for 100 ml of water sample), isoBCF (5 μl) and TEA (20 μl), followed by shaking for 10 min to perform two-phase extractive isoBOC reaction. The lower organic solvent layer was dried over magnesium sulfate and toluene (30 μl) was added as keeper. Most of dichloromethane was removed by evaporation (gentle stream of nitrogen, 60°C) for the direct analysis by GC.

2.5. Gas chromatography and gas chromatography–mass spectrometry

GC analyses for optimization of the procedure were performed by a DONAM 6200 model gas chromatograph (Donam Industry, Seoul, Korea) equipped with a spit/splitless capillary inlet system and a FID system interfaced to a *dsCHROM*^{PLUS} data acquisition system. The injector and detector temperatures were 260 and 280°C, respectively. Samples (ca. 1.0 μl) were injected in the splitless mode (purge delay time of 0.7 min) and analyzed on an HP-5 (SE-54 bonded phase) capillary column (30 m×0.32 mm I.D., 0.25 μm film thickness; Hewlett-Packard, Avondale, PA, USA). The oven temperature was held at 80°C for 0.7 min, then programmed to 150°C at a rate of 30°C min^{-1}, and then to 280°C at a rate of 5°C min^{-1}. The inlet pressure of nitrogen was set to 28 kPa.

GC analyses for the retention index measurements

were performed with a Hewlett-Packard HP model 5890A series II gas chromatograph (Hewlett-Packard) equipped with a split/splitless capillary inlet system and two FID system. The inlet pressure of helium was set to 137.9 kPa. Samples (1.0 μl) injected into a dual-capillary column system made of DB-5 (SE-54 bonded phase) and DB-17 (OV-17 bonded phase) fused-silica capillary columns (J. and W. Scientific, Rancho Corodova, CA, USA; dimension 30 m×0.25 mm I.D., 0.25 μm film thickness) were analyzed under the identical operating conditions as described above. The two FID signals were processed simultaneously in dual-channel mode by the HP 3365 Chem software program. A standard solution of *n*-hydrocarbons (C_{10}–C_{32} even numbers only) in isooctane was injected as the external references for *I* measurements. Temperature-programmed *I* values were then computed via built-in retention index program by linear interpolation between the retention times of adjacent hydrocarbon standards. For peak identification by computer *I* matching, a database of reference *I* library using *I* sets of 50 phenolic standards as isoBOC derivatives measured on the dual columns was built in the GC computer system.

The mass spectra of phenols as isoBOC derivatives were obtained using an HP 5890A series II gas chromatograph installed with an Ultra-2 (SE-54 bonded phase) capillary column (25 m×0.20 mm I.D., 0.11 μm film thickness), interfaced to an HP 5970B mass selective detector (70 eV, electron impact mode) and on-line to an HP 59940A MS Chemstation. The inlet pressure of helium was set to 85 kPa. Samples were introduced in the split injection mode (10:1) and the oven temperature was initially 120°C for 2 min and then raised to 280°C at 5°C min^{-1}. The injector and interface temperatures were 260 and 280°C, respectively. The mass range scanned was 50–650 *u* at a rate of 0.99 scan s^{-1}.

2.6. Calculations

The peak area ratios relative to I.S. were used for all the quantitative calculations of the recoveries and linearity tests. 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 linearity of the whole procedure and to plot calibration curves for the quantitative measurements of phenols.

3. Results and discussion

The simultaneous detection and identification of all diverse phenolic compounds in a single analysis become increasingly important for the accurate environmental monitoring. In this study, two-phase extractive isoBOC reaction in acidic aqueous solutions was examined using dichloromethane as the organic solvent phase containing isoBCF as the derivatizing reagent and TEA as the acid scavenger, with subsequent SPE using Chromosorb P as the adsorbent. And the temperature-programmed *I* set of each isoBOC phenol derivative measured on DB-5 and DB-17 dual-capillary columns of different polarity were tested whether they are useful in the screening for unknown phenols by *I* matching.

3.1. GC and GC–MS characteristics of phenols as isoBOC derivatives

Upon the simultaneous extractive isoBOC reaction, all phenolic hydroxyl groups of 50 phenols were converted to their corresponding isoBOC groups, yielding a single derivative for each phenolic compound studied. Under the present GC condition, the separation of 50 phenols as their isoBOC derivatives on dual-capillary columns was completed within 30 min as shown in Fig. 1. Each phenol displayed a single peak with good peak shape. The FID responses of the dinitrophenol derivatives (peaks 41, 42 and 43) were considerably lower than other phenol derivatives. Six sets of two peaks and two sets of three peaks were unresolved on DB-5 column of low polarity, while nine sets of two peaks and three sets of three peaks were not resolved on DB-17 column of intermediate polarity. However, unresolved peaks on DB-5 column were well resolved on DB-17 column and vice versa with the exception of 3-chlorophenol (peak 8) and 2,5-dimethylphenol (peak 9), which were not resolved on both columns. The dual-capillary column system together provided a complete separation for the rest of peaks, achieving excellent resolutions between the positional isomers. Moreover, it was noticed that the elution orders of

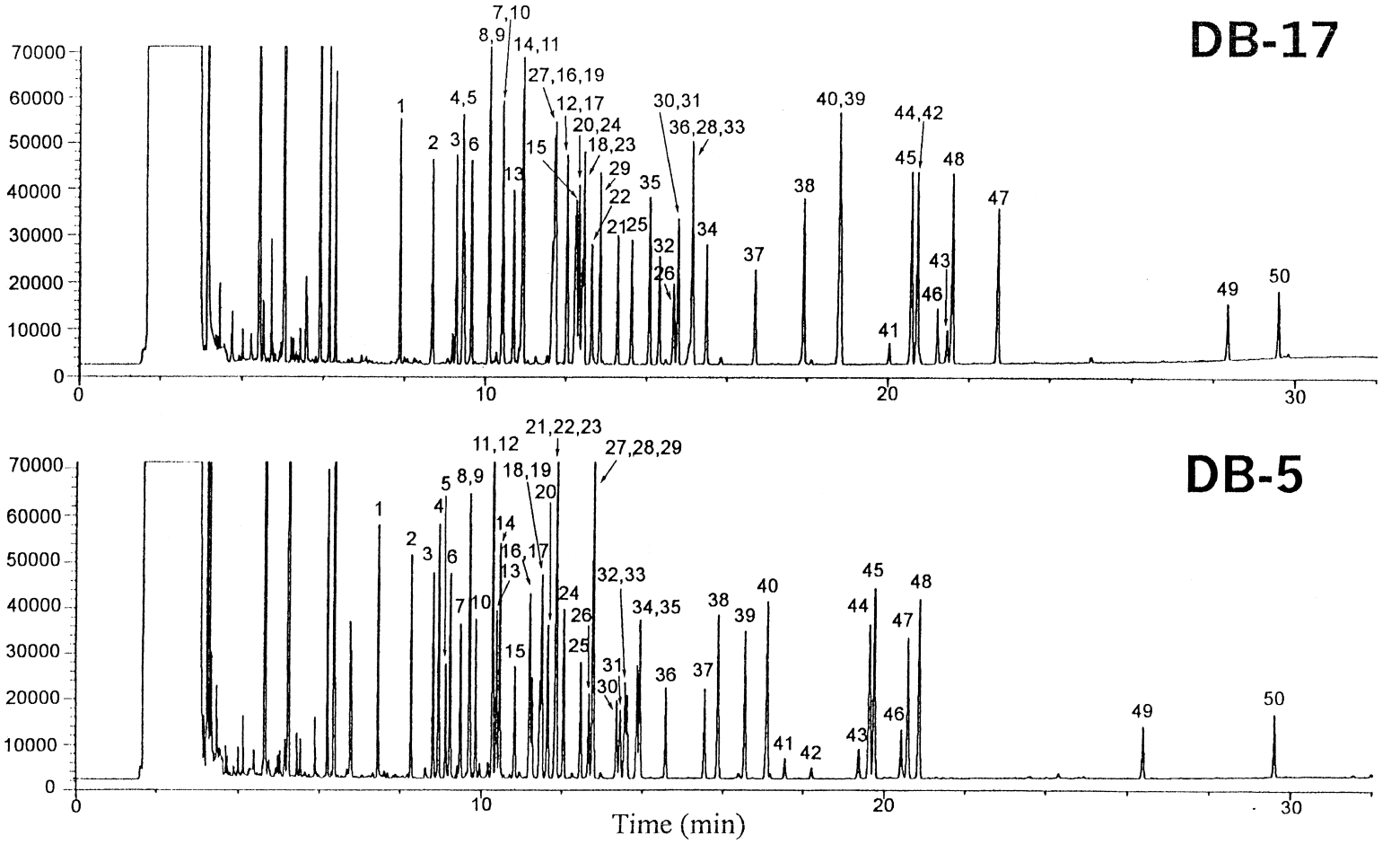

Fig. 1. Dual chromatograms of phenols as their isoBOC derivatives separated on DB-5 and DB-17 (both 30 m×0.25 mm I.D., 0.25 μm film thickness) dual-capillary column system. The inlet pressure of helium was set to 137.9 kPa. Samples (ca. 1.0 μl) injected in splitless mode. The oven temperature was held at 80°C for 0.7 min, then programmed to 150°C at a rate of 30°C min^{-1}, and then to 280°C at a rate of 5°C min^{-1}. The inlet pressure of helium was set to 137.9 kPa. Peaks: 1=phenol; 2=*o*-cresol; 3=*m*-cresol; 4=*p*-cresol; 5=2,6-dimethylphenol; 6=2-ethylphenol; 7=2-chlorophenol; 8=3-chlorophenol; 9=2,5-dimethylphenol; 10=4-chlorophenol; 11=2,3-dimethylphenol; 12=2-methoxyphenol; 13=2-*n*-propylphenol; 14=4-ethylphenol; 15=2-bromophenol; 16=2,3,6-trimethylphenol; 17=3-bromophenol; 18=4-bromophenol; 19=4-isopropylphenol; 20=4-chloro-*m*-cresol; 21=3,5-dichlorophenol; 22=2,4-dichlorophenol; 23=2,3,5-trimethylphenol; 24=4-*n*-propylphenol; 25=2,3-dichlorophenol; 26=2-iodophenol; 27=3,4-dichlorophenol; 28=4-*tert*.-butylphenol; 29=2-nitrophenol; 30=3-iodophenol; 31=3-nitrophenol; 32=2,4,6-trichlorophenol; 33=4-iodophenol; 34=4-nitrophenol; 35=4-*n*-butylphenol; 36=2,4,5-trichlorophenol; 37=2,3,4-trichlorophenol; 38=1-naphthol; 39=2-phenylphenol; 40=catechol; 41=2,5-dinitrophenol; 42=2,4-dinitrophenol; 43=4,6-dinitro-*o*-cresol; 44=hydroquinone; 45=2-methylresorcinol; 46=pentachlorophenol; 47=4-phenylphenol; 48=orcinol; 49=pyrogallol; 50=phloroglucinol.

most of the phenols on the two columns were very different. The temperature-programmed *I* set was thus characteristic of each phenol (Table 1) and useful as a cross-check for each phenolic compound by simple *I* matching. This *I* matching was very useful especially for the discrimination among the positional isomers since their mass spectral patterns are mostly indistinguishable.

The isoBOC phenol derivatives were subjected to GC–MS analysis and their electron-impact MS data are summarized in Table 1. The molecular ion peaks of most phenol derivatives were either unobservable or very weak (less than 10%). 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 the preferential cleavages of bonds between phenolic oxygen and carbon of the isoBOC group occurred with migration of hydrogen atoms from the isoBOC groups to phenolic oxygens, yielding rearrangement ions that are identical to the molecular ions of corresponding underivatized phenols. In most monohydroxybenzenes, $[M-100]^+$ rearrangement ions formed by the loss of one isoBOC function constitute the base peaks. When the substituted alkyl chain is C_2 or longer as in 2- and 4-ethylphenols, 2-*n*- and 4-*n*-propylphenols, 4-isopropylphenol, 4-*tert*.-butylphenol and 4-*n*-butylphenol, the most intense peaks were derived by the further benzylic cleavage from the $[M-100]^+$. In most of nitrophenols, peaks at m/z 57 were the most abundant and the molecular ion peaks were missing. The dihydroxybenzenes such as catechol, hydroquinone, 2-methylresorcinol and orcinol cleaved analogously to give the base peaks corresponding to rearrangement $[M-200]^+$ ions formed by the elimination of two isoBOC groups. The structures of trihydroxybenzens, pyrogallol and phloroglucinol were readily confirmed by the presence of characteristic pair of intense isobutyl ion at m/z 57 and the most intense $[M-300]^+$ ion peaks (derived by the loss of three isoBOC groups). As expected, their molecular ions were undetectable and $[M-200]^+$ ion peaks were weak.

3.2. IsoBOC derivatization combined with solid-phase extraction

In this study, the optimal conditions of the two-phase extractive isoBOC reaction method was examined for the simultaneous derivatization of 50 phenols, which differ in their acidities and volatilities. The subsequent SPE procedure requires the use of low boiling organic solvent [23] and a number of preliminary experiments were thus performed for the choice of a suitable solvent. Like the previous works on amines [23], dense dichloromethane was chosen as the optimal solvent phase and 5 μl volume of isoBCF reagent as the optimal amount for the isoBOC reaction of phenols in the concentration range of 0.5–8 $\mu g\ ml^{-1}$. The use of excess amount of isoBCF was avoided. In preliminary experiments, saturation of the acidified aqueous phase with sodium chloride was found to be effective for phenols in their unionized stable forms to transfer into dichloromethane phase where they were reacted with isoBCF. The isoBOC reaction proceeded rapidly to completion at room temperature by vortex mixing for 10 min. The addition of TEA into the solvent phase was prerequisite for the removal of hydrochloric acid liberated during the reaction. The effects of TEA and its amount on the yields of derivatives for 10 phenols were tested. As shown in Table 2, without TEA addition, most of the phenols were recovered much lower compared to the yields with 10 μl of TEA. When the amount of TEA was increased to 20 μl, the overall yields of all the isoBOC derivatives increased accordingly. However, with further increase in TEA amount to 30 μl, no improvements in yields but unknown interfering peaks were noted. Moreover, unknown peaks formed from the reagents, which were confirmed by sample blank runs, disturbed the GC separation of several phenols. Therefore, the optimal amount of the acid scavenger was set to 20 μl.

After the reaction, the pH of the aqueous phase was adjusted to 8.0 to exclude unreacted phenols and other acidic interference. The derivatives were recovered from the aqueous and solvent phases by SPE in normal-phase partition mode using hydrophilic Chromosorb P as the adsorbent and dichloromethane as the eluent. Compared with the laborious solvent extraction [5–7,10,13,17,20], the SPE method permitted efficient and rapid extraction of the isoBOC phenols in dried forms within two steps.

The combined method of isoBOC reaction and SPE, under the selected optimal conditions, was examined to test the linear relation between detector

Table 1
Gas chromatographic and mass spectral data of phenols as isobutoxycarbonyl derivatives of phenols

No.	Phenolic compound	GC I[a] data set		Mass spectral data[b]							
		DB-5	DB-17	$[M]^+$	$[M-100]^+$	$[M-200]^+$	$[M-300]^+$	Other ions			
1	Phenol	1388	1617	194 (1)	94 (100)			57 (23)	65 (11)	77 (19)	
2	*o*-Cresol	1446	1676	208 (1)	108 (100)			57 (27)	91 (14)	77 (13)	107 (17)
3	*m*-Cresol	1484	1719	208 (1)	108 (100)			57 (22)	91 (13)	77 (10)	107 (20)
4	*p*-Cresol	1492	1731	208 (2)	108 (100)			57 (18)	91 (11)	77 (11)	107 (23)
5	2,6-Dimethylphenol	1504	1733	222 (6)	122 (100)			57 (35)	91 (14)	77 (22)	107 (22)
6	2-Ethylphenol	1512	1745	222 (3)	122 (99)			57 (46)	107 (100)	77 (22)	
7	2-Chlorophenol	1529	1804	228 (0)	128 (100)			57 (36)	75 (11)	111 (15)	130 (33)
8	3-Chlorophenol	1546	1780	228 (1)	128 (100)			57 (56)	111 (16)	130 (30)	75 (15)
9	2,5-Dimethylphenol	1546	1780	222 (4)	122 (100)			57 (26)	91 (11)	107 (28)	77 (16)
10	4-Chlorophenol	1555	1804	228 (2)	128 (100)			57 (37)	75 (12)	130 (31)	111 (14)
11	2,3-Dimethylphenol	1584	1836	222 (3)	122 (100)			57 (28)	77 (21)	91 (11)	107 (35)
12	2-Methoxyphenol	1586	1903	224 (2)	124 (100)			57 (21)	77 (16)	109 (43)	
13	2-*n*-Propylphenol	1591	1821	236 (2)	136 (51)			57 (34)	77 (10)	91 (18)	107 (100)
14	4-Ethylphenol	1598	1833	222 (2)	122 (56)			57 (23)	77 (17)	107 (100)	
15	2-Bromophenol	1618	1915	272 (0)	172 (100)			57 (74)	63 (28)	174 (89)	65 (19)
16	2,3,6-Trimethylphenol	1639	1882	236 (7)	136 (100)			57 (34)	91 (24)	121 (38)	
17	3-Bromophenol	1643	1903	272 (2)	172 (100)			57 (76)	65 (15)	109 (45)	
18	4-Bromophenol	1652	1927	272 (2)	172 (100)			57 (57)	155 (12)		
19	4-Isopropylphenol	1656	1884	236 (2)	136 (22)			57 (18)	121 (100)		
20	4-Chloro-*m*-cresol	1665	1917	242 (2)	142 (100)			57 (41)	77 (22)	107 (30)	125 (9)
21	3,5-Dichlorophenol	1674	1942	262 (0)	162 (100)			57 (68)	63 (14)	164 (63)	
22	2,4-Dichlorophenol	1674	1977	262 (0)	162 (100)			57 (83)	145 (13)	164 (63)	
23	2,3,5-Trimethylphenol	1676	1928	236 (8)	136 (100)			57 (42)	91 (20)	121 (47)	
24	4-*n*-Propylphenol	1686	1919	236 (2)	136 (30)			57 (17)	107 (100)		
25	2,3-Dichlorophenol	1710	2002	262 (0)	162 (100)			57 (92)	63 (14)	164 (64)	73 (12)
26	2-Iodophenol	1720	2063	320 (4)	220 (100)			57 (28)	203 (9)		
27	3,4-Dichlorophenol	1722	1876	262 (0)	162 (100)			57 (68)	63 (12)		
28	2-Nitrophenol	1726	2089	239 (0)	139 (100)			57 (93)	56 (58)	63 (25)	122 (24)
29	4-*tert*.-Butylphenol	1727	1952	250 (2)	150 (11)			57 (21)	135 (100)		
30	3-Iodophenol	1760	2068	320 (8)	220 (100)			57 (45)	76 (17)	93 (7)	
31	3-Nitrophenol	1765	2068	239 (0)	139 (27)			57 (100)	76 (15)	56 (25)	93 (10)
32	2,4,6-Trichlorophenol	1771	2041	296 (0)	196 (100)			57 (87)	179 (13)	97 (14)	198 (94)
33	4-Iodophenol	1775	2090	320 (9)	220 (100)			57 (35)	76 (15)		
34	4-Nitrophenol	1790	2110	239 (0)	139 (25)			57 (100)	109 (13)	56 (25)	63 (16)
35	4-*n*-Butylphenol	1794	2026	250 (1)	150 (28)			57 (18)	107 (100)		
36	2,4,5-Trichlorophenol	1827	2087	296 (0)	196 (80)			57 (100)	179 (15)	97 (13)	198 (69)
37	2,3,4-Trichlorophenol	1878	2176	296 (0)	196 (100)			57 (97)	181 (14)		
38	1-Naphthol	1895	2248	244 (8)	144 (100)			57 (20)	115 (28)	56 (42)	
39	2-Phenylphenol	1931	2306	270 (5)	170 (100)			57 (20)	115 (13)	169 (33)	
40	Catechol	1961	2298	310 (0)	210 (1)	110 (100)		57 (55)			
41	2,5-Dinitrophenol	1984	2375	284 (0)	184 (0)			57 (100)	56 (13)	63 (14)	
42	2,4-Dinitrophenol	2021	2419	284 (0)	184 (0)			57 (100)	56 (33)	63 (11)	
43	4,6-Dinitro-*o*-cresol	2084	2463	298 (0)	198 (8)			57 (100)		181 (8)	
44	Hydroquinone	2099	2411	310 (0)		110 (100)		57 (35)			
45	2-Methylresorcinol	2105	2403	324 (1)	224 (3)	124 (100)		57 (57)			
46	Pentachlorophenol	2137	2448	364 (0)	264 (40)			57 (100)	249 (14)	266 (56)	268 (40)
47	4-Phenylphenol	2148	2541	270 (6)	170 (100)			57 (14)	115 (13)	141 (10)	171 (13)
48	Orcinol	2166	2464	324 (0)	224 (3)	124 (100)		57 (37)			
49	Pyrogallol	2486	2908	426 (0)	326 (0)	226 (2)	126 (100)	57 (56)			
50	Phloroglucinol	2692	2994	426 (0)	326 (1)	226 (3)	126 (100)	57 (54)			

[a] Retention index (I) values on DB-5 and DB-17 (30 m×0.25 mm I.D., 0.25 μm film thickness) capillary columns programmed from 60°C (0.7 min) then programmed to 150°C at a rate of 30°C min^{-1}, and then to 280°C at a rate of 5°C min^{-1}.

[b] m/z values, with relative abundances of ions (%) in parentheses.

Table 2
Effects of triethylamine and its amount on the yield of isobutoxycarbonylation[a]

No.	Phenolic compound	Peak area ratio (mean ±SD)[b]			
		Amount of TEA (μl)			
		0[c]	10	20	30
4	*p*-Cresol	0.81 ±0.31	3.11 ±0.57	3.34 ±0.55	2.95 ±0.77
10	4-Chlorophenol	0.70 ±0.26	1.98 ±0.37	2.14 ±0.35	1.91 ±0.46
11	2,3-Dimethylphenol	0.55 ±0.19	2.75 ±0.49	2.93 ±0.50	2.64 ±0.64
13	2-Propylphenol	1.10 ±0.34	2.38 ±0.40	2.54 ±0.44	2.31 ±0.55
18	4-Bromophenol	0.53 ±0.20	1.27 ±0.22	1.40 ±0.23	1.20 ±0.25
23	2,3,5-Trimethylphenol	0.44 ±0.13	2.73 ±0.48	2.99 ±0.46	2.60 ±0.54
26	2-Iodophenol	0.58 ±0.17	1.13 ±0.18	1.24 ±0.19	1.07 ±0.21
29	2-Nitrophenol	0.51 ±0.11	1.57 ±0.25	1.69 ±0.27	1.48 ±0.28
32	2,4,6-Trichlorophenol	1.39 ±0.41	1.40 ±0.22	1.55 ±0.20	1.34 ±0.23
39	2-Phenylphenol	1.84 ±0.38	2.00 ±0.22	2.19 ±0.26	1.94 ±0.25

[a] Aliquots (1.0 ml) of water samples (pH 2) spiked with phenols (3 μg each) and *n*-docosane as I.S. (2 μg) were subjected to two-phase extractive isoBOC reaction at different amount of TEA, followed by SPE as described in the text.
[b] Peak area ratio relative to I.S. and standard deviation for triplicate runs.
[c] Direct isoBOC reaction without TEA.

Table 3
Linear regression analysis of relative response against relative weights of phenols as their isobutoxycarbonyl derivatives

No.	Phenolic compound	Regression line[a]			No.	Phenolic compound	Regression line[a]		
		m	*b*	*r*			*m*	*b*	*r*
1	Phenol	1.58	0.38	0.956	26	2-Iodophenol	0.33	0.03	0.999
2	*o*-Cresol	1.33	0.34	0.976	27	3,4-Dichlorophenol	0.63	0.07	0.999
3	*m*-Cresol	1.58	0.40	0.954	28	4-*tert*.-Buthylphenol	1.31	0.19	0.985
4	*p*-Cresol	0.90	0.08	0.999	29	2-Nitrophenol	0.47	0.04	0.999
5	2,6-Dimethylphenol	1.12	0.16	0.984	30	3-Iodophenol	0.57	0.09	0.991
6	2-Ethylphenol	0.95	0.14	0.999	31	3-Nitrophenol	0.44	0.11	0.989
7	2-Chlorophenol	0.14	0.23	0.999	32	2,4,6-Trichlorophenol	0.41	0.02	0.999
8	3-Chlorophenol	0.41	0.31	0.934	33	4-Iodophenol	0.94	0.23	0.968
9	2,5-Dimethylphenol	0.40	0.47	0.939	34	4-Nitrophenol	0.15	0.05	0.903
10	4-Chlorophenol	0.60	0.08	0.999	35	4-*n*-Butylphenol	0.24	0.46	0.923
11	2,3-Dimethylphenol	0.88	0.16	0.999	36	2,4,5-Trichlorophenol	0.63	0.11	0.972
12	2-Methoxyphenol	1.16	0.32	0.962	37	2,3,4-Trichlorophenol	0.45	0.04	0.999
13	2-Propylphenol	0.67	0.02	0.999	38	1-Naphthol	0.77	0.06	0.999
14	4-Ethylphenol	1.82	0.39	0.958	39	2-Phenylphenol	0.60	0.06	0.999
15	2-Bromophenol	0.85	0.03	0.988	40	Catechol	1.11	0.02	0.999
16	2,3,6-Trimethylphenol	1.26	0.31	0.984	41	2,5-Dinitrophenol	0.11	0.02	0.994
17	3-Bromophenol	0.01	0.18	0.993	42	2,4-Dinitrophenol	0.08	0.29	0.789
18	4-Bromophenol	0.37	0.01	0.999	43	4,6-Dinitro-*o*-cresol	0.24	0.15	0.925
19	4-Isopropylphenol	0.79	0.22	0.963	44	Hydroquinone	0.73	0.04	0.997
20	4-Chloro-*m*-cresol	1.76	0.36	0.960	45	2-Methylresorcinol	0.78	0.00	0.999
21	3,5-Dichlorophenol	0.68	0.09	0.999	46	Pentachlorophenol	0.22	0.08	0.991
22	2,4-Dichlorophenol	0.84	0.21	0.982	47	4-Phenylphenol	0.63	0.06	0.997
23	2,3,5-Trimethylphenol	0.80	0.06	0.999	48	Orcinol	0.77	0.00	0.998
24	4-*n*-Propylphenol	1.57	0.42	0.982	49	Pyrogallol	0.43	0.04	0.989
25	2,3-Dichlorophenol	1.24	0.34	0.962	50	Phloroglucinol	0.20	0.00	0.969

[a] m=Slope=relative mass response=mean peak area ratio of phenol×mass of I.S./mass of phenol; b=y-intercept; r=correlation coefficient.

Table 4
Inter-day assay tests with water spiked with known amounts of phenols[a]

No.	Phenolic compound	Area ratio (mean±SD)[b]		
		0.5 μg	2 μg	4 μg
4	*p*-Cresol	0.64±0.13	1.71±0.45	4.64±0.08
10	4-Chlorophenol	0.59±0.25	1.12±0.30	3.06±0.04
11	2,3-Dimethylphenol	0.82±0.09	1.71±0.53	4.39±0.34
13	2-Propylphenol	0.57±0.13	1.35±0.32	3.66±0.05
18	4-Bromophenol	0.36±0.15	0.69±0.18	1.99±0.09
23	2,3,5-Trimethylphenol	0.63±0.14	1.47±0.37	4.07±0.08
26	2-Iodophenol	0.30±0.13	0.63±0.18	1.76±0.06
29	2-Nitrophenol	0.29±0.08	0.87±0.34	2.39±0.18
32	2,4,6-Trichlorophenol	0.40±0.13	0.81±0.20	2.25±0.09
39	2-Phenylphenol	0.49±0.08	1.18±0.31	3.16±0.03

[a] Aliquots (1.0 ml) of water samples (pH 2) spiked with phenols (0.5, 2 and 4 μg each) and I.S. (2 μg) were subjected to isoBOC reaction followed by SPE as described in the text.
[b] Peak area ratio relative to I.S. and standard deviation for triplicate runs.

responses (expressed as peak area ratio) and increasing amounts of phenols. As listed in Table 3, linear responses were obtained for most phenols in the range of 0.5–8 μg ml^{-1} with correlation coefficients varying from 0.925 to 0.999, except for 2,4-dinitrophenol (0.789). The linearities of the two combined steps for GC separation of most phenols appear to be satisfactory for their quantitative measurements in unknown samples. Likewise the inter-day assay tests (Table 4) performed toward to the 10 phenols indicated that the overall reproducibility and linearity of the method is satisfactory for the quantitative measurements of phenols. And the derivatives of most phenols except for nitrophenols, once extracted into dichloromethane, were found to be stable for at least a week when stored in the refrigerator.

3.3. Effect of dilution with water on the recoveries of phenols

When applied to different volumes (5, 10 or 100 ml) of water samples spiked with known amounts (1

Table 5
Effect of dilution with water on the yield of isobutoxycarbonylation[a]

No.	Phenolic compound	% Recovery (mean ±SD)[b]		
		Volume of water		
		5 ml	10 ml	100 ml
4	*p*-Cresol	110.9±1.7	94.2±1.3	58.5±7.0
10	4-Chlorophenol	105.6±2.0	99.5±1.8	84.7±2.8
11	2,3-Dimethylphenol	115.5±3.1	103.8±11.5	41.6±41.1
13	2-Propylphenol	106.1±2.5	104.1±3.8	90.6±27.5
18	4-Bromophenol	109.7±2.1	107.8±1.1	79.4±12.7
23	2,3,5-Trimethylphenol	109.8±1.8	106.6±2.3	86.9±17.5
26	2-Iodophenol	107.9±2.1	107.2±1.2	93.6±17.4
29	2-Nitrophenol	108.1±1.7	102.2±8.2	72.6±3.0
32	2,4,6-Trichlorophenol	107.7±1.7	110.5±6.3	116.6±13.2
39	2-Phenylphenol	111.9±1.3	111.2±8.0	124.2±8.8

[a] Aliquots (5, 10, or 100 ml) of acidic water (pH 2) were fortified with 10 phenols (1 μg each) and I.S. (2 μg). Each water sample was placed into a separatory funnel containing dichloromethane (1 ml for 5 and 10 ml of water samples but 10 ml for 100 ml of water sample), isoBCF (5 μl) and TEA (20 μl), followed by shaking for 10 min to perform two-phase extractive isoBOC reaction.
[b] Standard deviation for triplicate runs.

μg each) of 10 phenols, simultaneous screening and accurate confirmation of each spiked phenol could be achieved by comparison of *I* sets with the reference values in the home-built *I* library as described elsewhere [23]. Each phenolic compound was confirmed by GC–MS. The *I* library will continue to be expanded to include other phenols for the identification of unknown phenols in the screening work.

The overall recovery of each phenol and its precision were found to be decreased with dilution (Table 5). This was probably due to the fact that the two-phase extractive isoBOC reaction was not efficient when shaking large volume of samples with reagents contained in a separatory funnel compared to vortex mixing of smaller volume in screw-capped vials. This result indicates that preconcentration of trace phenols present in large volume (≥10 ml) of water samples is required prior to the extractive isoBOC reaction.

4. Conclusions

A significant advantage of the present extractive two-phase isoBOC derivatization is that upon extraction from acidic water phase, labile phenolic hydroxyl groups in their stable free forms are reacted instantly with minimal amount of isoBCF in the dichloromethane phase using TEA as the proton scavenger. Following the isoBOC reaction, the subsequent SPE in normal-phase partition mode using hydrophilic Chromosorb P as the adsorbent and dichloromethane as the eluent allowed efficient and rapid extraction of the resulting isoBOC phenols from the reaction mixture in two steps. Dual columns differing in their polarities together provide complete separation of most of phenols examined with characteristic *I* sets that can be used for routine phenol screening work. Moreover, the mass spectral patterns, exhibiting characteristic $[M-100]^+$, $[M-200]^+$ and $[M-300]^+$ ions for the mono-, di- and trihydroxybezenes, respectively with common ions at m/z 57, facilitated their rapid structural confirmation. The characteristic mass spectral patterns of phenols studied as isoBOC derivatives might be useful for their identification in aqueous samples. An extension of the present method for the rapid profiling and screening of wastewater and various environmental samples for toxic phenols and their quantitative measurements is in progress.

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