Direct Acetylation and Determination of Chlorophenols in Aqueous Samples by Gas Chromatography Coupled with an Electron-Capture Detector

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A method was developed that offers a rapid, simple and accurate technique for the determination of chlorophenols at trace levels in aqueous samples with very limited volumes of organic solvents. These compounds were acetylated, then preliminarily extracted with *n*-hexane. The enriched chlorophenols were directly analyzed using gas chromatography with an electron-capture detector. The detection limits were in the range of 0.001–0.005 mg/L, except for 2-chlorophenol, which was always above 0.013 mg/L. Relative standard deviation for the spiked water samples ranged from 2.2 to 6.1%, while relative recoveries were in the range of 67.1 to 101.3%.

Introduction

Chlorophenols are a source of ecological anxiety because they are toxic and potentially carcinogenic at concentrations of only a few µg/L (1). Their wide distribution in industry and daily usage as wood preservative agents, pesticides, disinfectants, explosives and petrochemicals leads to pollution in the environment (2). However, purifying drinking water with chlorine may produce chlorophenols, because chlorine reacts to form chlorophenols when in contact with the dissolved phenolic compounds (3).

Liquid chromatography (LC) and gas chromatography (GC) are the best techniques to determine chlorophenols (CPs) at ultra-trace levels. Sample preparation using a selective solid-phase extractor (SPE) for the chlorinated phenols has improved the detection limits of the methods, because it enriches the ultra-trace amounts to a detectable concentration (4, 5). Analysis by GC with an electron-capture detector (ECD) is an even better choice for routine monitoring of these compounds.

In GC, pre-column derivatization is routinely employed when necessary to enhance thermal stability, increase volatility and improve separation (6). Hydrophobicity significantly increases with the increase in solute molar volume and octanol–water partition coefficient (7). Different derivatization procedures have been suggested, but alkylation and acetylation are the easiest techniques with the highest conversion yields (8). Acetylations with acetic anhydride using different catalysts have been reported in the presence of sulfamic acid (8), anhydrous nickel chloride (9), *p*-toluenesulfonamide complexes (10), tin oxide (11), melamine trisulfonic acid (12), silica sulfate (13), lithium chloride (14) and other catalysts. Nevertheless, because of what is known as the ortho-effect, CP isomers possess considerably different octanol–water partition constants (K_{ow}), depending on whether the chlorine atom is substituted at the ortho-, meta- or para-position (15). The ortho-effect has a major consequence on the solubility and chemical activity of a molecule. Chlorophenyl acetates (CPAs) exhibited higher retention index values on low-polarity GC capillary columns than the corresponding CPs (16).

The official method of the Environmental Protection Agency (EPA), which is based on liquid–liquid extraction (LLE), derivatization and measurement by GC–ECD has given limits of detection between 0.58 and 2.2 µg/L for the 11 priority pollutant phenols (17). CPs and other phenolics were pre-concentrated from aqueous samples using SPE cartridges and determined as trimethylsilyl derivatives by GC–mass spectrometry (MS); the detection limits were 0.01–0.25 µg/L (18).

In this work, a rapid and simple method was developed that is capable of resolving a wide range of chlorinated phenolic compounds at concentration levels of few µg/L. The efficient procedure combines simple acetylation by acetic anhydride without the use of a catalyst, followed by LLE with *n*-hexane and determination using GC–ECD. The method is convenient for the routine determination and monitoring of CPs in different water sources.

Experimental

Apparatus

The analysis of CPs was performed using GC (Agilent Technologies 7890A GC-system) equipped with a nickel-63 ECD. The separating column was from J&W Scientific DB-5 (60 m i.d. × 0.32 mm × film 1 µm). The injector was from Agilent Technologies (7683B series). Injection was accomplished in a pulsed, splitless mode at 300°C. Analysis was performed with an initial column temperature of 60°C for 1 min, followed by heating to 300°C at 20°C/min and held for 8 min. ECD was held at 320°C. Nitrogen gas was used as the makeup gas for the ECD, the purge flow was 60 mL/min for 45 s, and gas saver flow was 20 mL/min for 2 min. Helium gas was employed as carrier gas at a constant flow rate of 1 mL/min.

Materials

The standard materials for chlorinated phenols [2,4-dichlorophenol (2,4-DCP), 2,4,6-trichlorophenol (2,4,6-TCP), 2,3,4,5-
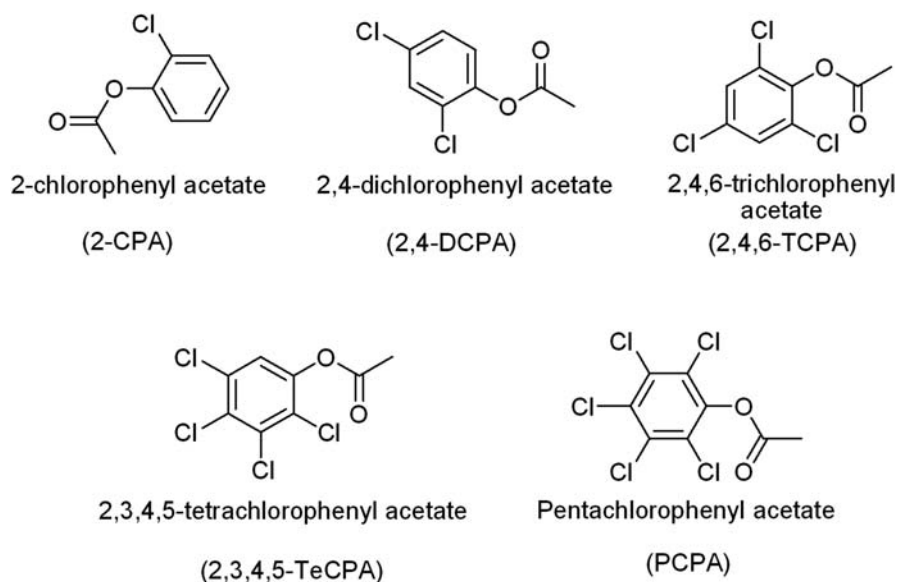
Figure 1. Structures and abbreviations of the produced acetylated chlorophenol derivatives.

tetrachlorophenol (2,3,4,5-TeCP) and pentachlorophenol (PCP)] were supplied by Supelco. 2-Chlorophenol (2-CP) was supplied by Aldrich Chemie (Steinheim, Germany). Glacial acetic acid (100%), acetone, acetic anhydride and *n*-hexane were from BDH Chemical Co. and sodium hydroxide was from Merck (Darmstadt, Germany). Test solutions were prepared at different concentrations by spiking appropriate amounts of stock solutions of these compounds in deionized distilled water, which was prepared by a Milli-Q system (Bedford, MA).

Preparation of standard solutions

A set of five stock solutions at a concentration of 20 mg/L were prepared for each CP standard by accurately weighing 0.002 g of pure material and dissolving it in acetone in a 100-mL volumetric flask. From these solutions, a composite stock standard of 3 mg/L concentration was prepared by transferring exactly 15 mL of each individual stock solution to a 100-mL volumetric flask and diluting to volume with acetone. The resulting composite standard solution was further diluted to obtain a new set of standard composite solutions at concentrations of 0.1, 0.5, 1, 1.5 and 2 mg/L. The prepared standard composite solutions were then esterified by acetic anhydride in the presence of sodium hydroxide, and standard calibration curves were established for the derivatized CPs. Recovery data were obtained for aqueous samples spiked with known concentrations of CPs after being subjected to acetylation and extraction.

Table I

Acidity, Solubility, Octanol–Water Partition Coefficient and Degree of Ionization as a Function of pH for Chlorophenols

Chlorophenol	Acidity (pKa)	Solubility in water (mg/L) at 20–25°C	Log K_{ow}	DOI*
2CP	8.4	28,500	2.16	0.7153
24DCP	7.9	4,500	2.98	0.4427
246TCP	6.4	800	3.56	0.0245
2345TeCP	6.35	200	4.46	0.0219
PCP	4.8	20	5.01	0.0006

*Degree of ionization (fraction of neutral species) as a function of pH.

Derivatization and extraction procedure

In a 10-mL screw cap round bottom test tube, the acidity of a 5-mL water sample containing the previously mentioned standard concentrations of CPs was adjusted to a pH of 9 by adding NaOH solution. Then, after the addition of 0.5 mL of acetone with an excess of 100 μL of acetic anhydride and 0.5 mL of *n*-hexane, the resulting solution was shaken vigorously for 2 min, followed by centrifugation for 3 min at 5,000 rpm to collect the acetylated product, as shown in Equation 1. A 1-μL portion of the organic layer was withdrawn using a micro-syringe and injected directly into the GC–ECD system.

The preceding reaction is similar to the Schotten-Baumann technique for acetylation of hydroxyl compounds by acid chloride, in which the base, usually sodium hydroxide or

$$(1)$$
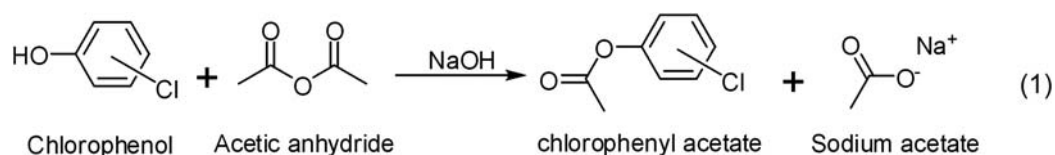
pyridine, plays a dominant role in the reaction as a catalyst, producing phenoxides and neutralizing the liberated acid (19).

Water samples

The applicability of the optimized method to real water samples was also tested. For this purpose, rain and lake water were used. Rain water samples were collected from Serdang city, and lake water samples were from a small lake in The National University of Malaysia (UKM).

Results and Discussion

Acetylation of the chlorophenols was carried out to reduce the polarity and increase the volatility inside the GC column

(Figure 1). The formation of phenolate in solution requires an alkaline pH because the pKa value of 2-CP is 8.3 (see Table I) (20–22). The optimum pH for acetylation has been obtained at $pH = 9 \pm 1$, depending on the pKa for each chlorophenol. pH values outside this range have resulted in poor derivatization; a pH above 10 increases the rate of hydrolysis of acetic anhydride over acetylation reaction, whereas a pH value lower than 8 is not enough to initiate acetylation reaction.

A comparison of the Fourier transform-infrared (FT-IR) spectra for the CPs confirmed the formation of the acetylated derivatives. Characteristic wave-numbers of the bulk samples exhibited distinguishable peaks in the positions of the Ar-Cl group at 786 and 725 cm (23), while the C = C benzene ring stretching was present at 1,538 and 1,544 cm in the aromatic region. The diagnostic band of the free hydroxyl group of
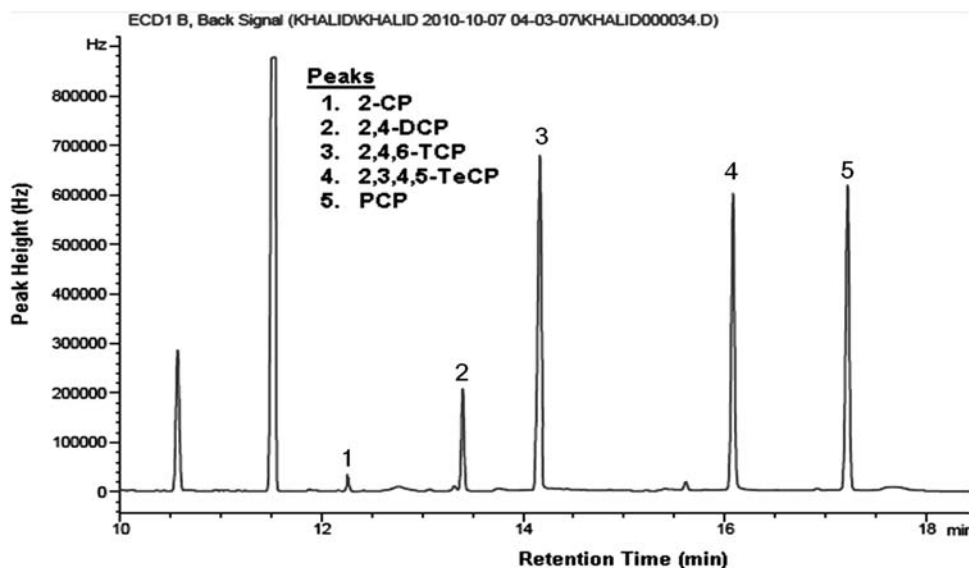
Figure 2. GC–ECD chromatogram representing the separation of a standard mixture of five acetylated chlorophenols in *n*-hexane at a concentration of 2 ppm each using a DB-5, 60-m column length.
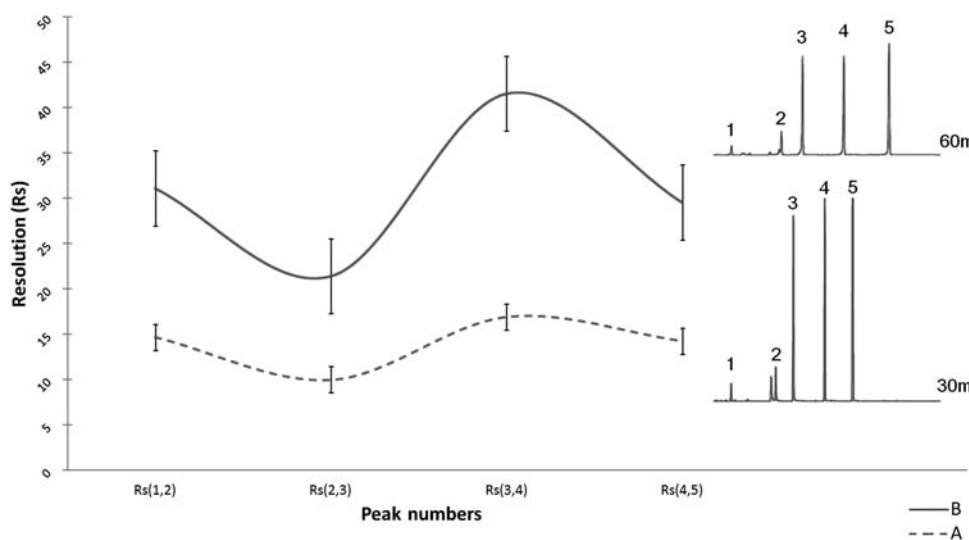
Figure 3. Peak resolution as a function of the separation efficiency of: 30-m long GC column (A); 60-m long GC column (B); Rs(1,2) resolution between peak 1 and 2; Rs(2,3) resolution between peak 2 and 3; Rs(3,4) resolution between peak 3 and 4; Rs(4,5) resolution between peak 4 and 5.

phenols that appears at 3,413 cm (24, 25) disappeared after derivatization. The development of the peak in the carbonyl region at 1,730 and 1,695 cm indicated the formation of an ester C = O bond (26).

The acetylation process requires the addition of acetic anhydride in excess to ensure a high yield of chlorophenol esterification. There was no significant effect of the reaction time on the derivatization yield. A period of 5 min was enough to ensure completion of the acetylation process. An additional 10 min was applied to ensure that the hydrolysis of all the excess acetic anhydride left over from the acetylation process did not interfere with the analysis results.

The extraction of acetylated CPs by *n*-hexane was improved using three successive extractions, instead of using only a one-batch extraction, followed by centrifugation at 5,000 rpm to separate the two phases.

Gas chromatography

The extracted samples of the esterified chlorophenols were subjected to GC−ECD analysis using a DB-5 column. The separation of the compounds was remarkably improved when a 60-m long column was used, as presented in Figure 2. The peak resolutions (Rs) were investigated as a function of the separation efficiency when comparing the 30 and 60-m long columns (Figure 3).

The calibration curves were established by preparation of five different standard concentrations for each compound,

Table II

Analytical Parameters of Distilled, Rain and UKM Lake, Water Samples Spiked with 0.5 ppm CPs, then Acetylated and Subjected to GC−ECD

| Chlorophenol	MDL*	Recovery (%)			Linearity equation	Linearity (R^2)	RSD (%)
		Standard	Rain	UKM lake			
2CP	0.013	67.1	65.3	61	$y = 23260x - 6700$	0.9995	6.1
24DCP	0.005	81	79.1	77.9	$y = 106446x - 3970.3$	0.9998	5.7
246TCP	0.001	97	98.3	99.7	$y = 344650x - 5096.8$	0.9983	2.7
2345TeCP	0.003	89.3	89	89.1	$y = 307089x - 16303$	0.9972	3.5
PCP	0.001	101.3	99.9	95.7	$y = 312682x - 14998$	0.9979	2.2

*Method detection limit.

ranging from 0.01 to 2 mg/L. The method shows good linearity in which the correlation coefficients (R^2) were between 0.9972 and 0.9998 for all the chlorophenols. The yield was calculated as a function of the peak areas. The recovery values were obtained by dissolving known amounts of chlorinated phenols in deionized distilled water followed by the processes of derivatization, extraction and analysis using GC−ECD. Relative recoveries were in the range of 67.1 to 101.3%, in which the symmetrical compounds (246TCP and PCP) had the highest recoveries. Relative standard deviation for the spiked water samples was in the range of 2.2 to 6.1%. The detection limits of the method were 0.001−0.005 mg/L, except for 2-CP, which was never less than 0.013 mg/L (Table II). The limit of detection of this method was significantly more satisfactory than other common methods of detection of chlorophenols such as high-performance liquid chromatography−diode array detection (HPLC−DAD) (27), HPLC−ultraviolet detection (4) and GC−flame ionization detection (5).

The intramolecular hydrogen bonding between the adjacent hydroxyl and chlorine functional groups reduces the ability of 2-CP for esterification; hence the higher detection limit, especially for the syn-conformer, which is more stable than the anti-isomer (28, 29).

Other than the ortho-effect, which reduces the chemical activity of 2-CP (15), the high degree of ionization as a function of pH, and the high charge distribution on the structure of the 2-CP molecule, especially after applying the highest-occupied molecular orbital surface, as predicted by the ArgusLab software modeling (ArgusLab 4.0.1., Mark A. Thompson, Planaria Software, Seattle, WA), indicate a hydrophilic characteristic of the mono-chlorophenol molecule, as shown in Figure 4. The acetic anhydride as a derivatizing agent is expected to be more present in the organic layer, which produces poor derivatization. All these parameters contribute to the low yield of esterification for mono-chlorophenol, which is attributed to the low response of the GC−ECD signal, as clearly indicated in peak number 1 in Figure 2.

Application to real samples

The collected water samples were filtrated, spiked and acetylated, and CPs were determined using GC−ECD, as detailed
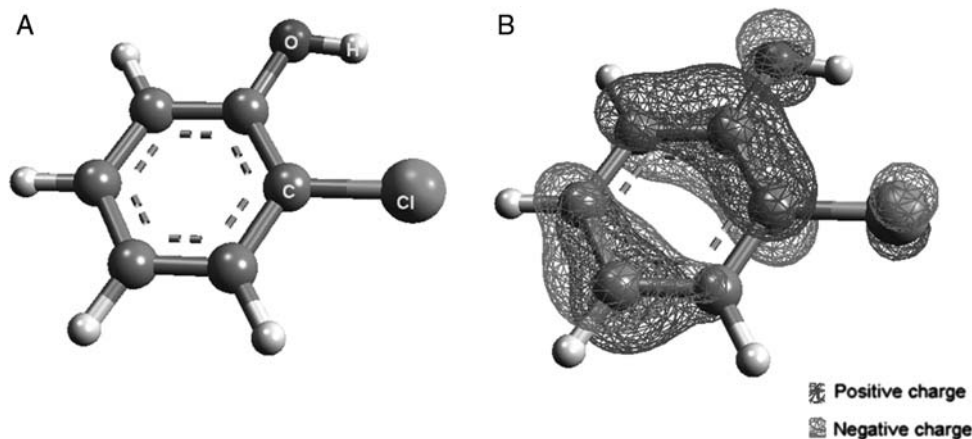
Figure 4. Representation of the high charge distribution on the molecule of 2-CP: (A) 2-CP structure, (B) 2-CP structure after applying the highest occupied molecular orbital surface (HOMO surface).

earlier. Table II illustrates the recoveries obtained for spiked solutions of standard, rain and lake water samples by GC−ECD after acetylation.

The developed method was successfully applied for the analysis of waters with complex matrices such as rain and lake water samples. Application of the method to rain and lake water samples demonstrated the absence of CPs in the analyzed samples.

Conclusions

A simple new approach for the determination of chlorinated phenols in aqueous samples has been developed in this work. The method is simple to operate, with good repeatability and sensitivity, uses small sample volumes and can be applied for the examination of all chlorophenols in different water sources, such as drinking water, wastewater or any other environmental water samples.

Acknowledgments

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