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Determination of chlorophenols at the sub-ppb level in tap water using derivatization, solid-phase extraction and gas chromatography with plasma atomic emission detection

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Abstract

A method for the determination of eight chlorophenols in tap water is proposed, involving the direct acetylation of the chlorophenols in water samples with acetic anhydride in the presence of K_2CO_3 , extraction of the acetylated derivatives by passage through a graphitized carbon cartridge, elution of the cartridge and measurement of the chlorophenol derivatives by means of a microwave-induced plasma atomic emission detector coupled to a gas chromatograph. The quantitiveness of the derivatization process and analyte losses through preconcentration and evaporation were investigated. The limits of quantification obtained permit the application of the proposed method to the analysis of natural and tap water samples.

1. Introduction

Phenols are frequent environmental pollutants that originate as by-products of the carbon and oil industry [1] and as wastes from phenolic resin, paint, pharmaceutical formulation and pesticide manufacturing processes [2].

The US Environmental Protection Agency (EPA) has compiled a list of eleven phenol compounds considered priority pollutants [3]; among them, chlorophenols are the most toxic and carcinogenic. Also, European Community (EC) legislation has set a maximum allowable phenol concentration of 0.5 ng/ml in tap water.

Current instrumental techniques do not afford

the direct determination of phenols at the legally established levels in municipal waters. In fact, they entail the preconcentration of samples by liquid–liquid extraction with an organic solvent [4,5] or solid–liquid extraction with various types of resin [6], C_{18} [7,8] or graphitized carbon [9–11].

Chromatography remains the analytical technique of choice for the determination of phenols on the grounds of its high sensitivity and resolving power. However, because of their high polarity, phenols tend to give broad, tailed peaks, the effect increasing as the column ages [12]. One way of circumventing these shortcomings is to derivatize phenols to less polar compounds in order to obtain more favourable chromatographic peaks [13]. Of the wide variety

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of derivatizing reagents used for this purpose [14], acetylating agents have been employed to the greatest extent [15,16]. Phenols can be acetylated in the original aqueous medium or following extraction from it [17, 18].

A combination of derivatization and a suitable preconcentration procedure with a highly selective detection system after chromatographic separation should allow the ready determination of these species at the internationally allowed levels without the need to process large amounts of samples.

This paper reports a method for the determination of chlorophenols in waters that involves direct acetylation in the sample using alkaline acetic anhydride, followed by solid–liquid extraction of the derivatives by passage through graphitized carbon and detection by gas chromatography–microwave-induced plasma atomic emission spectroscopy (GC–MIP–AES). This coupled technique is highly selective for monitoring some elements included among the analytes of interest not present in the other substances that make up the matrix [19–21]. Specifically, the chromatograms obtained by monitoring the emission line for chlorine are much better resolved than those provided by electron-capture (ECD) or flame ionization detection [22].

2. Experimental

2.1. Reagents

Methanol, *n*-hexane, potassium carbonate and acetic anhydride of the highest available purity were supplied by Merck (Darmstadt, Germany). Tetramethylammonium hydroxide (TMAOH) was purchased from Aldrich (Milwaukee, WI, USA).

Water samples were preconcentrated by the use of Supelclean ENVI-Carb SP cartridges containing 0.25 or 0.50 g of graphitized carbon black that were obtained from Supelco (Bellefonte, PA, USA).

The standards used, viz., 2-chlorophenol (2CP), 4-chloro-3-methylphenol (4C3MP), 2,4-dichlorophenol (24DCP), 2,4,6-trichlorophenol

(246TCP), 2,3,6-trichlorophenol (236TCP), 2,4,5-trichlorophenol (245TCP), 2,3,5,6-tetrachlorophenol (2356TCP) and pentachlorophenol (PCP), were purchased from Aldrich or Merck. They were employed to prepare individual stock standard solutions containing 4.0 mg/ml of each compound in methanol that were stored refrigerated at 4°C in the dark. These solutions were used to make working standard solutions by appropriate dilution.

2.2. Apparatus

An HP 5890 Series II chromatograph from Hewlett-Packard (Avondale, PA, USA) furnished with an HP 7673 split/splitless autoinjector and coupled to an HP 5921A microwave-induced plasma atomic emission detector was used. The whole assembly was controlled by an HP 35920A Chemstation. A 50 m × 0.33 mm I.D. BP-5 methylphenylsilicone capillary column of 1- μ m film thickness supplied by Scientific Glass Engineering (Ringwood, Victoria, Australia) and 99.999% helium as carrier gas were also employed. The optimum settings for the determination of the analytes are given in Table 1.

Another identical chromatograph was also used in conjunction with an electron capture detector. An SE-54 column (30 m × 0.25 mm I.D., 0.25- μ m film thickness) was used (Alltech, Lancashire, UK) with nitrogen as the carrier and make-up gas. The optimum settings for the separation of chlorophenols on this column are given in Table 2; underivatized 236TCP and 245TCP could not be resolved.

2.3. Acetylation of standards

The acetylation procedure used was a modification of that reported by Renberg and Lindstrom [7]. A volume of 1 ml of a methanol solution containing the phenols studied in the concentration range 0.2–10.0 μ g/ml was mixed with 2 ml of 5% K₂CO₃ and 2 ml of *n*-hexane containing 200 μ l of acetic anhydride. The mixture was shaken for 1 min and the organic phase allowed to separate. The aqueous phase was then extracted with a further 1 ml of *n*-hexane

Table 1
CG–AES operating conditions used for the separation of chlorophenols

<i>GC parameters</i>	
Injection port	Split/splitless
Purge time on	200 s
Injection port temperature	250°C
Injected volume	2 μ l
Column head pressure	135 kPa
Split flow	6.4 ml/min
Oven programme:	
Initial temperature	80°C
Rate	15°C/min
Temperature	180°C
Time	7 min
Rate	20°C/min
Temperature	260°C
Time	10 min
<i>AES parameters</i>	
Transfer line temperature	260°C
Cavity block temperature	260°C
Wavelength	Chlorine, 480.192 nm Carbon, 495.724 nm
Helium make-up flow	44.4 ml/min
Ferrule purge	28 ml/min
Spectrometer purge flow	2 l/min N ₂
Solvent vent beginning	2.5 min
Solvent vent end	7.2 min
Reagent gas	Oxygen

Table 2
CG–ECD operating conditions used for the separation of chlorophenols

<i>GC parameters</i>	
Injection port	Split/splitless
Purge time on	120 s
Injection port temperature	250°C
Injected volume	2 μ l
Column head pressure	120 kPa
Split flow	59 ml/min
Column flow	1.1 ml/min
Oven programme:	
Initial temperature	40°C
Initial time	2 min
Rate	10°C/min
Temperature	190°C
Rate	20°C/min
Temperature	260°C
Time	10 min

(with no derivatizing reagent added). The two *n*-hexane phases were collected and mixed, dried over anhydrous sodium sulphate and injected into the GC–AES system. By derivatizing various concentrations of phenols, calibration graphs were constructed as plots of the concentration of each phenol against the peak height of its acetylated derivative.

2.4. Sample preparation

A known volume of Milli-Q-purified water or prefiltered tap water (up to 2 l for 0.5-g cartridges) was spiked with chlorophenol standards in methanol (spiked samples) and the pH was adjusted to 11–11.5 with K₂CO₃. Acetic anhydride (5 ml per litre of water sample) was added and the mixture stirred mechanically for 15 min. A volume of methanol equivalent to ca. 2% of the water sample volume was then added and the mixture was passed through a pre-conditioned graphitized carbon cartridge at a flow rate of 40 ml/min. The cartridge was pre-conditioned by washing with 5 ml of methanol and activated with 5 ml of Milli-Q-purified water at pH 2–3 [23]. When the sample had passed through, the cartridge was dried with a stream of nitrogen for 20 min and turned upside down for elution with 3 ml of *n*-hexane containing 1% TMAOH. The final extract was concentrated to 0.5 ml under a stream of nitrogen at 55 kPa at room temperature using a Turbo Vap II workstation from Zymark (Hopkinton, MA, USA). Finally, a volume of 2 μ l of the concentrated extract was injected into the GC–MIP–AES system.

3. Results and discussion

3.1. Optimization of the derivatization process

Notwithstanding the wide use of phenol acetylation for quantitative purposes in gas chromatography for many years [14], a literature search revealed a lack of information on the extent and quantitiveness of this derivatization procedure. These two factors are essential for

validating any chromatographic derivatization process. Consequently, we investigated it by using underivatized chlorophenol standards as described in Section 2.3. Injection of the derivatized standards into the AES detector revealed no trace of underivatized chlorophenols or changes in peak heights or areas with the amount of potassium carbonate or acetic anhydride used, so we concluded that the acetylation reaction proceeded to completion.

Even so, we injected these standards, which were presumably derivatized completely, into a chromatograph equipped with an electron-capture detector. ECD is not sensitive enough to detect 2CP or 4C3MP at the working concentrations used; on the other hand, it is more sensitive than AES to the other chlorophenols studied, which it can detect in very small amounts. The use of 0.5% K_2CO_3 with ECD was found to result in the presence of significant amounts of underivatized chlorophenols in the final *n*-hexane phase (Fig. 1). The proportion of underivatized analyte was assessed at various concentrations (except for monochlorophenols) and was found to be in the region of 10%.

In principle, an increase in the amount of derivatizing agent used (acetic anhydride) should result in a decrease in the proportion of un-

derivatized chlorophenols, but this was not the case. On the other hand, the use of 5% K_2CO_3 ensured virtually complete derivatization (Fig. 2), but the peak areas for the derivatives remained essentially the same. This can be ascribed to the acetylation reaction producing acetic acid, part of which may be transferred to the organic phase (*n*-hexane) and facilitate the presence of underivatized phenols in it. If the aqueous medium used was alkaline enough, the excess base (K_2CO_3) would neutralize any acid transferred to the organic phase; in the absence of protons, no underivatized chlorophenols could therefore possibly exist. In fact, if the *n*-hexane phase containing underivatized chlorophenols was back-extracted with a slightly alkaline aqueous phase (e.g., 0.5% K_2CO_3) and subsequently injected into the GC–ECD system, no underivatized chlorophenols were detected. Therefore, the chlorophenols were derivatized in a reproducible manner to an extent of ca. 90% that was scarcely affected by the derivatization conditions.

3.2. Cartridge elution

We initially used *n*-hexane to elute the chlorophenol acetates retained in the carbon cartridges. A volume of 4 ml of this solvent was found to elute a 0.25-g cartridge thoroughly,

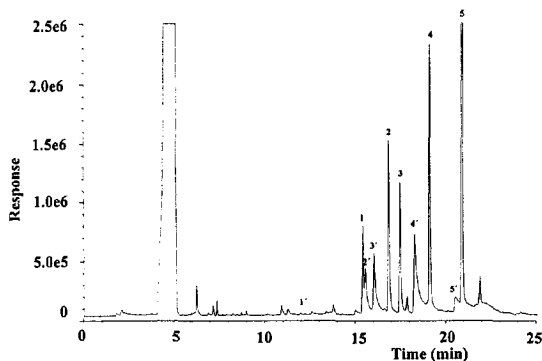


Fig. 1. ECD chromatogram for a standard of chlorophenols derivatized with acetic anhydride containing 0.5% K_2CO_3 . Peaks: 1 = 24DCP; 2 = 246TCP; 3 = 236TCP; 4 = 2356TCP; 5 = PCP (all as acetyl derivatives); 1' = 24DCP; 2' = 246TCP; 3' = 236TCP; 4' = 2356TCP; 5' = PCP (all as underivatized phenols).

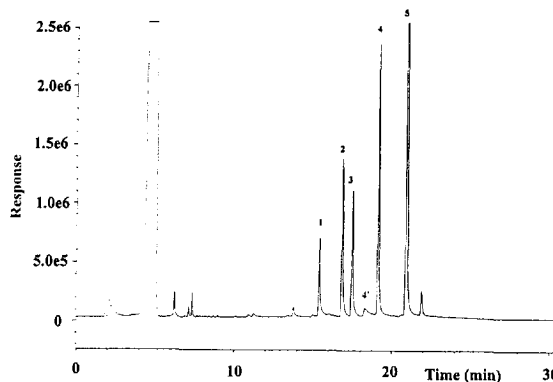


Fig. 2. ECD chromatogram for a standard of chlorophenols derivatized with acetic anhydride containing 0.5% K_2CO_3 . Peaks as in Fig. 1.

except for PCP, which could not be eluted even with larger *n*-hexane volumes (8–10 ml). The absence of PCP acetate from the cartridge eluates was due to its strong retention by graphitized carbon, as confirmed by extracting into *n*-hexane samples that were derivatized before and after passage through the cartridge.

The problem was therefore to find a solvent phase capable of eluting PCP acetate retained in the cartridge. After several attempts, we found that 8 ml of *n*-hexane containing 1% TMAOH extracted all the derivatives from the cartridge. Also, if the cartridge was turned upside down prior to elution, 3 ml sufficed to elute 0.25- and 0.50-g cartridges thoroughly. This decreased extractant volume requirement can be ascribed to the high affinity of PCP acetate for carbon and its being retained at the top of the cartridge; consequently, turning the cartridge upside down should expedite its elution [24].

3.3. Analyte losses through concentration

The extracts eluted from the cartridge were concentrated to 0.5 ml by using a stream of nitrogen at room temperature. Based on our previous experience [22], the process can give rise to major analyte losses, so we evaluated the recovery obtained by concentrating 8 or 3 ml of a derivatized chlorophenol solution to which *n*-hexane containing 1% TMAOH had been added (in this way, the solutions were similar to those

eluted from the cartridges). Table 3 summarizes the results obtained. As can be seen, evaporation losses increased with increasing sample volume. Therefore, as small an eluent volume as possible should be used, and turning the cartridges upside down after loading was the most effective way of accomplishing this.

3.4. Limits of quantification

Table 4 gives the limits of quantification (LOQ, $S/N = 10$) obtained for the chlorophenols studied under the operating conditions shown in Table 1. The results were obtained by calculating the average noise for a series of chromatograms and were experimentally confirmed by injecting standards of derivatized chlorophenols at the stated concentration levels. The LOQs for the water samples were calculated by dividing such values into the maximum concentration factors obtained with the carbon cartridges (1000:0.5 and 2000:0.5 for the 0.25- and 0.5-g cartridges, respectively), which were also experimentally confirmed.

3.5. Recovery from carbon cartridges

We carried out extraction experiments by using various volumes of water containing different chlorophenol concentrations and both 0.25- and 0.50-g cartridges. The chromatograms obtained allowed the highly selective detection of

Table 3
Preconcentration losses under a stream of nitrogen at room temperature

Compound	Concentration ($\mu\text{g/ml}$)	Volume (ml)			
		8		3	
		Recovery (%)	S.D. (%)	Recovery (%)	S.D. (%)
2CP	1.06	101.3	9.5	96.1	3.4
4C3MP	1.07	103.7	10.7	98.8	3.9
24DCP	1.07	94.8	8.5	95.9	3.4
246TCP	0.54	84.8	12.6	93.7	3.2
236TCP	0.54	101.4	10.5	95.5	5.4
245TCP	0.54	85.4	15.9	96.5	4.0
2356TCP	1.07	62.5	11.4	85.9	3.6
PCP	2.21	58.8	8.1	83.0	3.9

Table 4
Limits of quantification ($S/N = 10$) obtained with the GC–MIP–AES system

Compound	LOQ in standards ($\mu\text{g/ml}$)	LOQ in water samples (ng/ml)	
		0.25-g cartridge	0.50-g cartridge
2CP	0.53	0.26	0.13
4C3MP	0.70	0.35	0.18
24DCP	0.45	0.22	0.11
246TCP	0.22	0.11	0.05
236TCP	0.20	0.09	0.05
245TCP	0.30	0.15	0.08
2356TCP	0.25	0.13	0.06
PCP	0.33	0.17	0.08

all the chlorophenols studied, even in tap water samples.

Fig. 3 shows the results for a tap water sample that was spiked with the chlorophenols and Fig. 4 for its unspiked counterpart. The latter was found to contain traces of 246TCP, even though the peak was not strong enough to reach the LOQ, so its concentration was calculated to be below 0.09 ng/ml.

With the 0.25-g cartridges (Table 5), the chlorophenol recovery was independent of the sample volume (up to 1000 ml) and phenol concentration used. Above 1500 ml, analyte

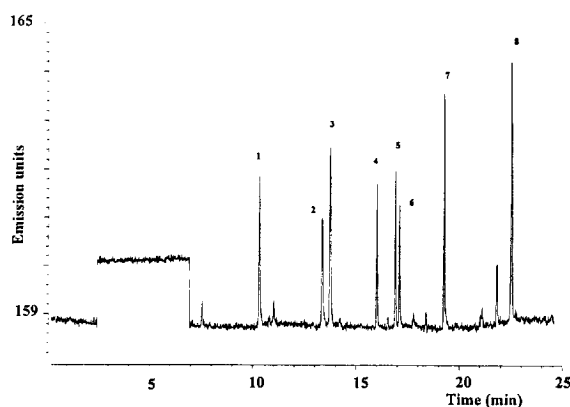


Fig. 3. Chromatogram for the chlorine emission line at 479 nm obtained for 1 l of tap water spiked with chlorophenols. Peaks: 1 = 0.6 ng/ml 2CP; 2 = 0.6 ng/ml 4C3MP; 3 = 0.6 ng/ml 24DCP; 4 = 0.3 ng/ml 246TCP; 5 = 0.3 ng/ml 236TCP; 6 = 0.3 ng/ml 245TCP; 7 = 0.6 ng/ml 2356TCP; 8 = 1.0 ng/ml PCP (all as acetyl derivatives).

losses became significant, particularly those of 2CP and PCP, which were even more marked at 2000 ml, where the recoveries for all other chlorophenols were still acceptable. It should be noted that the 2CP and PCP losses reflected in Table 5 come from different origins. In the case of 2CP, cartridge breakthrough appears with increasing sample volume. In contrast, for PCP, no breakthrough was observed because its extremely high retention on graphitized carbon black. Losses arise from incomplete elution of the retained PCP in the final sample preparation stages. Hence PCP losses could be avoided, in principle, by increasing the solvent volume.

Sample volumes of up to 2000 ml resulted in favourable recoveries with the 0.50-g cartridges (Table 6). Note that in this case no losses for

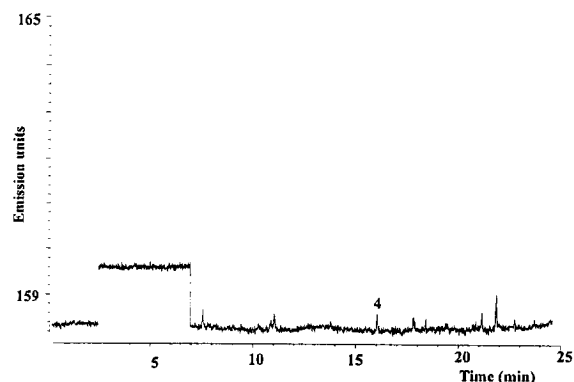


Fig. 4. Chromatogram for the chlorine emission line at 479 nm obtained for 1 l of unspiked tap water. Peak 4 as in Fig. 3.

Table 5
Analyte recoveries obtained with various sample volumes and chlorophenol concentrations using 0.25-g cartridges

Compound	Sample volume (ml)								
	200			500			1000		
	Concentration (ng/ml)	Recovery (%)	S.D. (%)	Concentration (ng/ml)	Recovery (%)	S.D. (%)	Concentration (ng/ml)	Recovery (%)	S.D. (%)
2CP	3.19	105.5	9.3	1.28	115.2	4.5	0.64	109.7	3.9
4C3MP	3.22	97.4	9.0	1.29	104.6	5.2	0.64	101.3	6.9
24DCP	3.21	82.8	7.1	1.28	88.7	2.6	0.64	86.0	4.5
246TCP	1.62	83.5	8.1	0.65	92.7	4.1	0.32	85.2	5.6
236TCP	1.61	84.1	8.2	0.64	90.8	3.7	0.32	86.3	5.2
245TCP	1.62	84.5	10.8	0.65	88.1	6.7	0.32	84.0	8.8
2356TCP	3.21	84.4	10.3	1.28	82.9	3.5	0.64	81.0	7.5
PCP	4.96	70.3	9.3	1.99	71.2	6.2	0.99	74.0	4.9

Compound	Sample volume (ml)								
	1000			1500			2000		
	Concentration (ng/ml)	Recovery (%)	S.D. (%)	Concentration (ng/ml)	Recovery (%)	S.D. (%)	Concentration (ng/ml)	Recovery (%)	S.D. (%)
2CP	0.32	108.0	5.0	0.42	67.6	14.3	0.32	57.5	15.7
4C3MP	0.32	96.4	9.0	0.43	88.1	11.7	0.32	76.6	12.5
24DCP	0.32	84.7	2.4	0.43	87.3	12.9	0.32	91.0	13.8
246TCP	0.16	85.4	4.4	0.22	84.9	10.1	0.16	85.1	16.9
236TCP	0.16	84.5	2.3	0.21	79.9	10.3	0.16	80.3	14.3
245TCP	0.16	89.2	6.1	0.22	97.3	13.2	0.16	90.9	6.3
2356TCP	0.32	79.0	4.8	0.43	79.0	11.1	0.32	74.5	8.9
PCP	0.49	87.6	3.6	0.66	67.6	6.81	0.49	59.4	5.0

Table 6
Analyte recoveries obtained with various sample volumes and chlorophenol concentrations using 0.50-g cartridges

Compound	Sample volume (ml)					
	2000			3000		
	Concentration (ng/ml)	Recovery (%)	S.D. (%)	Concentration (ng/ml)	Recovery (%)	S.D. (%)
2CP	0.32	91.2	12.7	0.21	48.6	8.5
4C3MP	0.32	94.1	12.1	0.21	70.2	5.7
24DCP	0.32	90.6	10.0	0.21	73.3	4.2
246TCP	0.16	88.5	10.6	0.11	71.8	9.8
236TCP	0.16	87.1	9.8	0.11	69.9	9.6
245TCP	0.16	99.0	11.9	0.11	76.9	6.9
2356TCP	0.32	85.4	10.6	0.21	67.8	4.2
PCP	0.49	84.6	8.22	0.33	66.8	7.7

PCP occur on processing sample volumes of 2000 ml. This can be explained by the physical dimensions of the 0.25- and 0.5-g cartridges, which have identical height but proportionally different cross-sections. Thus, PCP is adsorbed in a narrower band in 0.5-g cartridges, which makes its elution more effective with a fixed solvent volume. However, above 3000 ml, analyte losses were appreciable for all the compounds studied. Therefore, the maximum allowable sample volumes for preconcentration were 1000 and 2000 ml for the 0.25- and 0.50-g cartridges, respectively.

The lowest recoveries were always obtained for 2345TCP and PCP, which were the analytes lost to the greatest extent in concentrating extracts under a stream of nitrogen.

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