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C_{18} REVERSED-PHASE TRACE ENRICHMENT OF CHLORINATED PHENOLS, GUAIACOLS AND CATECHOLS IN WATER

LARS RENBERG*
National Swedish Environment Protection Board, Special Analytical Laboratory, Wallenberg Laboratory, University of Stockholm, S-106 91 Stockholm (Sweden)
and
KRISTER LINDSTRÖM
Swedish Forest Products Research Laboratory, Box 5604, S-114 86 Stockholm (Sweden)
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SUMMARY

Reversed-phase adsorption with octadecyl-modified silica gel was found to give quantitative recoveries of di-, tri-, tetra- and pentachlorophenol, tri- and tetrachloroguaiacol and tri- and tetrachlorocatechol from water. After desorption with acetone the chlorophenolic compounds were determined as their acetyl derivatives, using quartz capillary column gas chromatography. Different aqueous acetylation methods were compared, the highest yields being obtained with potassium carbonate solution. The procedure described has been applied to natural and industrial waste waters.

INTRODUCTION

Chlorinated phenolic compounds enter the environment from a variety of sources, including both accidental and non-accidental discharges from industrial activities. Pentachlorophenol, together with 2,3,4,6-tetra- and 2,4,6-trichlorophenol, are widely used as pesticides, mainly as fungicides for wood protection. Tri- and tetrachloroguaiacols and catechols have been identified in effluents from pulp and paper industries, where they are formed during bleaching[1–3].

The interest in chlorophenolic as environmental contaminants is due mainly to the high toxicity of chlorophenols[4–6], guaiacols and catechols[7–10]. Also, bioaccumulation properties have been established for chlorophenols[11–13] and chloroguaiacols[9,14].

The release of chlorophenolics through waste waters into water recipients has created a need for monitoring these substances in industrial effluents and natural waters. Sensitive and selective methods based on gas chromatography have therefore been developed.

Many methods have been used successfully for the isolation and concentration of chlorophenolic compounds from water samples. Usually, liquid–liquid extractions

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have been applied for the determination of pentachlorophenol[15–20] and for the determination of chlorinated guaiacols and catechols[3,9]. The main problem with liquid–liquid extraction methods, when applied to waste and natural waters, is the formation of emulsions which tend to complicate the phase separation. Also, the partition coefficients for relatively water-soluble substances, *e.g.*, chlorinated catechols, are sometimes unfavourable and may result in inadequate extraction efficiencies.

Other methods that have been employed for the isolation of chlorophenols from water samples are ion-exchange techniques[21–24] and reversed-phase adsorption on cross-linked polystyrene resins[25], although the latter technique in certain instances has been less successful[26].

Reversed-phase chemically bonded stationary phases offer an attractive alternative for the isolation and concentration of organic substances. The development of such stationary phases for high-performance liquid chromatography (HPLC) has also resulted in commercially available cartridges packed with octadecyl-modified silica gel. Such cartridges have been applied successfully to the isolation of chlorinated hydrocarbons[27] and benzidine and related compounds[28].

In this work, the possibility of applying C_{18} cartridges to the trace enrichment of chlorophenolics has been investigated. After the isolation, chlorinated phenols, guaiacols and catechols are converted into their acetyl derivatives and analysed by gas chromatography. The method has been applied to industrial waste waters and natural water.

EXPERIMENTAL

Reagents and materials

Methanol, acetone, sulphuric acid, 0.1 *M* potassium carbonate solution, *n*-hexane, acetic anhydride, sodium sulphate (anhydrous), ascorbic acid, Sep-Pak C_{18} cartridges (9 × 9 mm I.D.) (Waters Assoc., Milford, MA, U.S.A.) and a 10-ml Hamilton gas-tight 1010 W syringe with a T-tube connection (Waters Assoc.) were used. All reagents and materials were of analytical-reagent grade and tested in blank procedures. Reference compounds were synthesized as described earlier[9,29,30]. 2,6-Dibromophenol (Fluka, Buchs, Switzerland) was used as an internal standard.

Trace enrichment step

Before use, a C_{18} cartridge is activated by passing methanol (2 ml) through it, followed by distilled water (5 ml). The water sample (100 ml or less) is transferred into a glass beaker and, if chlorocatechols are to be included in the analysis, ascorbic acid (100–200 mg) is added to the water sample to prevent auto-oxidation. After acidification to pH < 5 by dropwise addition of sulphuric acid, the water sample is passed through the cartridge at a flow-rate of 30–40 ml/min with the aid of a glass syringe. The cartridge is disconnected and eluted in the reverse direction with acetone, and 4 ml of eluate are collected. If desired, the acetone extract is gently concentrated at room temperature to approximately 1 ml using a gentle stream of nitrogen.

Derivatization step

Before the gas chromatographic analyses, the chlorophenolics are converted

into their acetate derivatives, mainly according to the procedure described by Chau and Coburn[18] and Voss *et al.*[31]. The acetone extract (1 ml) is transferred into a test-tube containing 0.1 *M* potassium carbonate (3 ml). After the addition of *n*-hexane (1–2 ml) containing internal standard and acetic anhydride (50 μl), the test-tube is immediately shaken for 1 min. After centrifugation the *n*-hexane extract is transferred into another test-tube and a few crystals of sodium sulphate are added.

Gas chromatographic determination

The *n*-hexane extracts were injected into a Varian 3700 gas chromatograph with a ^{63}Ni electron-capture detector. The capillary quartz column (25 m × 0.2 mm I.D., SE-30 methylsilicone) was maintained at 200°C and the injector and detector temperatures were 250°C. Helium was used as the carrier gas (0.6 ml/min) and argon–methane (95:5) as the make-up gas (30 ml/min). The injector splitting ratio was usually 1:20.

Recovery experiments

Natural water (100 ml, pH 8.3) taken from the Baltic Sea was poured into a glass beaker and an ethanolic solution (10 μl) containing the substances listed in Table I was added. Concentration levels of 0.1–1 μg per litre of water were thus obtained. The sample was then stirred and analysed as described. The beaker was rinsed with acetone (10 ml) to dissolve compounds that might be partially adsorbed on the glass walls. The acetone extract was then gently concentrated to 1 ml. After acetylation, the extract was injected into the gas chromatograph.

TABLE I

COMPARATIVE STUDY OF SOME ACETYLATION PROCEDURES

The yields are reported relative to those obtained by the procedure described here. The results are expressed as mean values of triplicate determinations.

Compound	*Relative yield*		
	0.1 M potassium carbonate buffer[18]	*0.1 M borax buffer*[16]	*1.25 M sodium hydroxide buffer*[33]
2,4-Dichlorophenol	0.98	1.0	0.94
2,4,6-Trichlorophenol	0.98	0.97	0.98
2,3,4,6-Tetrachlorophenol	0.97	0.76	1.1
Pentachlorophenol	1.0	0.58	1.1
4,5,6-Trichloroguaiacol	1.0	0.89	0.89
Tetrachloroguaiacol	0.97	0.67	0.84
3,4,5-Trichlorocatechol	0.95	0.13	0.12
Tetrachlorocatechol	0.80	0.10	0.20

RESULTS AND DISCUSSION

Trace enrichment and recoveries

In trace enrichment techniques, based on column chromatography, a maxi-

mum sample volume is mainly determined by the retention volume for each individual component. No attempt was made, however, to determine the elution volumes of the substances studied. For practical reasons a 100-fold concentration of the water sample was assumed to be sufficient for most purposes, and recovery experiments were therefore carried out using 100-ml water samples. In a series of five such experiments, the recoveries for all substances indicated in Table I exceed 90%. No adsorption on the glass wall occurred and no breakthrough on the C_{18} cartridge was noticed. These observations indicated that the chlorophenols were quantitatively trapped on the cartridge. Fig. 1 illustrates the device used for the enrichment technique. The enrichment can be carried out rapidly at the sampling location and the cartridge sent to the laboratory for further processing.

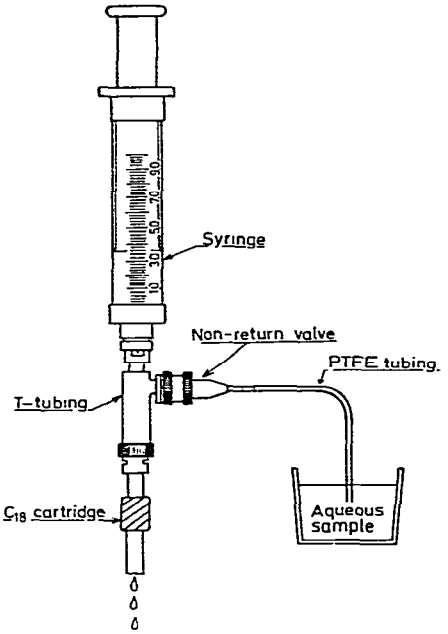

Fig. 1. Enrichment of an aqueous sample on a C_{18} cartridge by means of a syringe.

In the desorption step, acetone was chosen as elution solvent as no interference from acetone was noticed in the subsequent acetylation step. To minimize the volume of the elution solvent required, the cartridge was eluted in the reverse direction. All substances were desorbed quantitatively with 4 ml of acetone.

Additional recovery experiments with tri- and tetrachloroguaiacol and tetra- and pentachlorophenol, using 1000 ml of water, resulted in recoveries of 85% and higher. The strong retention of these compounds probably corresponds to their relatively strong hydrophobicities in comparison with chlorocatechols.

In order to prevent auto-oxidation of the chlorocatechols into the corresponding chloro-*o*-benzoquinones, which may be caused by dissolved oxygen, a small amount of ascorbic acid (which serves as a weak reducing agent) was added to the water samples. Without this treatment, the recoveries of the chlorocatechols varied irregularly. Similar observations with tetrachlorinated hydroquinone and catechols, present

as metabolites in urine samples, were reported by Edgerton *et al.*[32], who employed sodium disulphite as the reducing agent.

If other factors, *e.g.*, sample matrix, are suspected to influence the recovery, it is recommended that two cartridges be coupled in series during the adsorption step, and then eluted separately. If no breakthrough occurs into the second column the adsorption can be assumed to be quantitative.

Acetylation

The most popular derivatization procedures employed for the gas chromatographic determination of chlorophenols are alkylation with diazoalkanes[3,11–15,22] and acetylation with acetic anhydride[9,16,18,26,31,33]. The latter procedure was preferred for this study as acetylation was found to be rapid and non-contaminating with no hazardous reagents involved. Although not always recognized, acetylation of phenols in alkaline aqueous solutions was first described by Chattaway[34]. This procedure has been applied to residue analyses of chlorinated phenols[16,26,31,33], chloroguaiacols[9,31] and chlorocatechols[31]. When evaluating the different acetylation procedures described in the literature, we found that the yield varied considerably. The results are summarized in Table I. The procedure used by Voss *et al.*[31] seemed to be the most satisfactory.

The choice of aqueous phase, together with the anhydride to buffer molar ratio, was found to be most critical. This is probably due to differences in reaction rates between the acylation of the phenolate ion and the hydrolysis of the anhydride, *e.g.*, if the pH is too high in the aqueous phase the acetic anhydride will be destroyed before the acetylation process is completed.

On the other hand, a lower pH (at pH > pK_a) corresponds to lower concentrations of the reactive phenolate ions and thus the reaction rate is considerably reduced, leading to much lower yields. The decrease in pH, as a function of time, of an aqueous potassium carbonate solution mixed with acetic anhydride is shown in Fig. 2. As the pH changes from about 10 to 7 within a few seconds, the acetylation reaction must be completed within that time. It is therefore important to have the

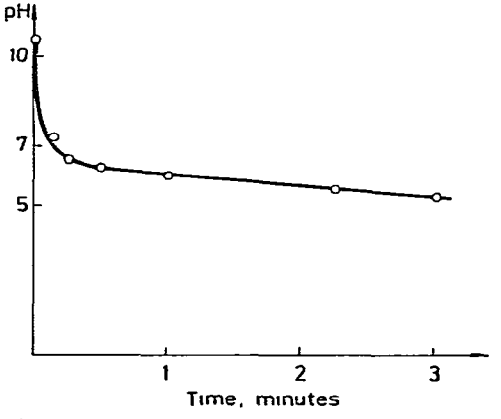

Fig. 2. Decrease of pH with time mixing 0.1 *M* potassium carbonate solution (15 ml) and acetic anhydride (125 µl).

TABLE II

THE RELATIVE MEAN VALUES OF THE ELECTRON-CAPTURE DETECTOR RESPONSES, RETENTION TIMES AND STANDARD DEVIATIONS OF SIX REPLICATE DETERMINATIONS OF CHLOROPHENOLIC COMPOUNDS IN SPIKED AQUEOUS SOLUTIONS

Acetylated compound	*Relative response factor*	*Relative retention time*	*Relative standard deviation (%)*
2,4-Dichlorophenol	10.1	0.79	8
2,4,6-Trichlorophenol	2.67	0.91	5
2,6-Dibromophenol (internal standard)	1.00	1.00	–
4,5-Dichloroguaiacol	13.5	1.16	7
2,3,4,6-Tetrachlorophenol	1.47	1.29	5
3,4,5-Trichloroguaiacol	1.27	1.52	3
4,5,6-Trichloroguaiacol	1.35	1.60	4
3,4,5-Trichlorocatechol	1.52	1.90	7
Pentachlorophenol	0.74	1.97	7
Tetrachloroguaiacol	0.83	2.08	2
Tetrachlorocatechol	1.33	2.60	10

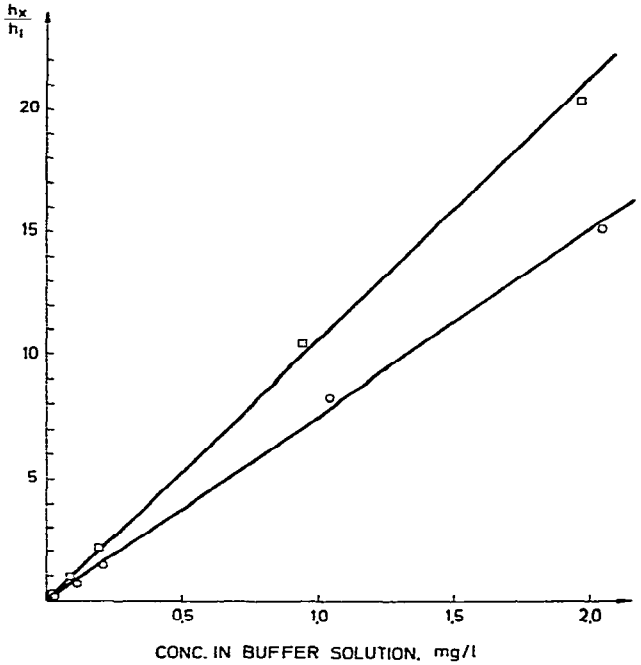

Fig. 3. Formation of acetyl derivatives of 3,4,5-trichlorocatechol (○) and tetrachlorocatechol (□) at various concentrations in waters. h_x and h_i are the peak heights of the chlorocatechols and the internal standard, respectively.

optimal anhydride to buffer molar ratio, particularly when derivatization of polyhydroxy groups in the benzene ring is carried out.

Of all the substances studied, the acetylation of chlorocatechols seems to be the most critical. Commonly used catalysts for acylation reactions, *e.g.*, pyridine, were found to reduce the yield considerably. In Table I, results for the evaluation of different acetylation methods are summarized.

Gas chromatography

To decrease the analytical time required and increase the resolution, ordinary glass capillary columns were chosen for the gas chromatographic separation. The precision of the gas chromatographic analysis was very low, however, probably owing to partial and irregular degradation of the relatively alkali-labile ester derivatives on the surface of the glass column. On replacing the glass capillary columns with quartz capillary columns the precision increased considerably. The relative mean detector response, retention time and standard deviation from six replicate determinations of chlorophenolic compounds in aqueous solutions are given in Table II.

The linearity of the response for each compound was also investigated. As an example, the relationship between the concentration and the peak-height ratio for the chlorocatechols and internal standard (0.1 mg/l) are shown in Fig. 3. Similar results were obtained for the other chlorophenolic compounds.

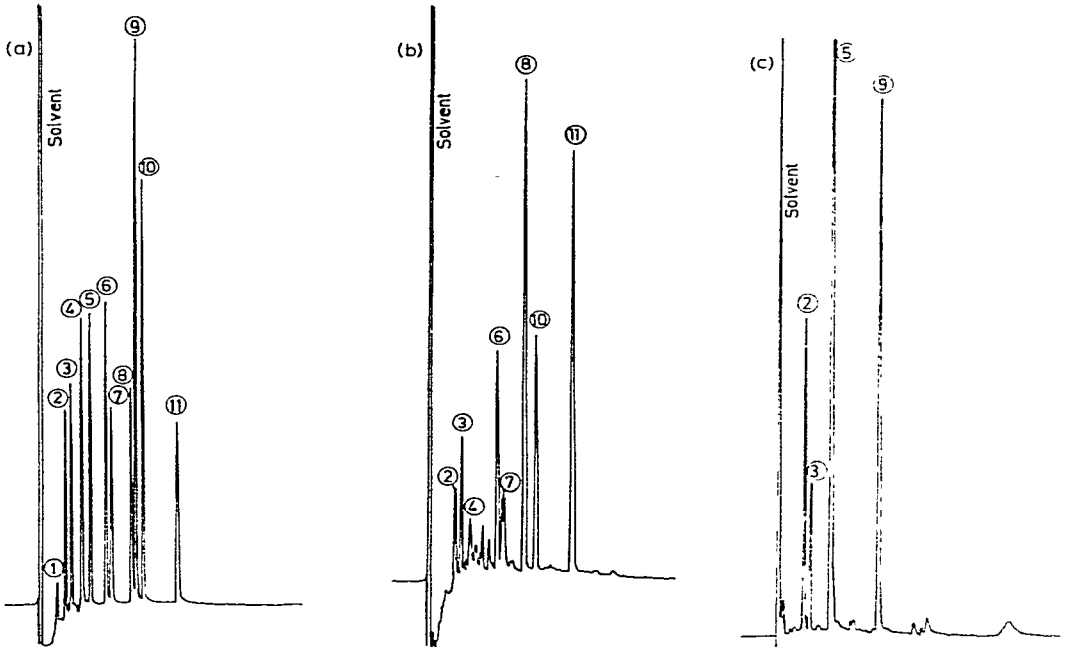

Fig. 4. Gas chromatograms of acetylated chlorophenolic compounds obtained with electron-capture detection for different samples. (a) Standard mixture: 1 = 2,4-dichlorophenol (0.10 μg/ml); 2 = 2,4,6-trichlorophenol (0.12 μg/ml); 3 = 2,6-dibromophenol (internal standard) (0.054 μg/ml); 4 = 4,5-dichloroguaiacol (1.1 μg/ml); 5 = 2,4,5,6-tetrachlorophenol (0.11 μg/ml); 6 = 3,4,5-trichloroguaiacol (0.12 μg/ml); 7 = 4,5,6-trichloroguaiacol (0.084 μg/ml); 8 = 3,4,5-trichlorocatechol (0.11 μg/ml); 9 = pentachlorophenol (0.14 μg/ml); 10 = tetrachloroguaiacol (0.13 μg/ml); 11 = tetrachlorocatechol (0.10 μg/ml). (b) Effluent at the outflow of an aerated lagoon at a kraft pulp bleach plant. The levels are 8.0, 31, 6.1, 1.1, 18, 4.0 and 14 μg per litre of water, corresponding to peaks 2, 4, 6, 7, 8, 10 and 11 in (a). (c) Lake water, downstream of a saw mill, taken 1 week after an accidental discharge of a chlorophenolic wood-protecting formulation. The levels are 0.5, 1.8 and 0.3 μg per litre of water, corresponding to peaks 2, 5 and 9 in (a).

Applications

The method described has been applied to several types of water samples, including industrial waste waters and natural waters. The chromatogram in Fig. 4b shows the presence of chlorinated guaiacols and catechols in the effluent of an aerated lagoon at a Swedish kraft pulp bleachery. The chromatogram in Fig. 4c originates from a lake-water sample taken downstream of a saw mill 1 week after an accidental discharge of a wood protection formulation containing tri-, tetra- and pentachlorophenol.

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