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DETERMINATION OF CHLORINATED PHENOLS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

The separation of mono-, di-, tri- and tetrachlorinated phenols and pentachlorophenol has been investigated by high-performance liquid chromatography using three different systems, adsorption chromatography on silica and reversed-phase chromatography on a polar bonded phase (aminoalkyl) and a non-polar bonded phase (octadecyl). A satisfactory group separation as well as a satisfactory separation of isomers could not be obtained by adsorption chromatography on silica. A reasonable separation of isomers was obtained with amino columns, but the group separation was not satisfactory. The octadecyl columns were superior with reference both to separation of groups and of isomers within these groups as well as to stability and speed of analysis. A mixture of nineteen different phenols was resolved on a C_{18} column with a 30-min linear gradient of 56–80% methanol and 44–20% of 0.02 *M* KH_2PO_4 (pH 4.0).

INTRODUCTION

Chlorinated phenols (CPs) have been used extensively as insecticides, fungicides, antiseptics and disinfectants. The annual amount of pentachlorophenol used in the U.S.A. alone has been reported to exceed 40,000 tons[1]. An unknown amount of CPs is also released to the environment by chlorination of drinking water containing phenols from various industrial sources. Effluents from the pulp and paper industry are known to contain toxic as well as mutagenic components[2]. Some of these compounds appear in effluents from chlorine bleacheries and have been identified as chlorinated phenols, guaiacols, catechols and trihydroxybenzenes, as well as dehydroabietic and unsaturated carboxylic acids[3–8].

Recent determinations of CPs have mainly been based on gas chromatography, with a wide variety of derivatives and detectors. Since CPs are soluble in aqueous as well as in organic solvents, high-performance liquid chromatography (HPLC) can be employed as a complementary analytical tool. Adsorption chromatography on

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silica would also seem appropriate, bearing in mind the possible existence of structural isomers. Other possibilities are aminoalkylated silica, based on the presence of the acidic phenol group, and reversed-phase chromatography on non-polar supports, based on the solubility of CPs. Procedures for the determination of only pentachlorophenol and one tetrachlorophenol isomer had been published[9,10] until a recent report of the reversed-phase separation of eleven CPs[11].

In this investigation, adsorption chromatography on silica, reversed-phase/ion exchange on aminopropyl silica and reversed-phase chromatography on octadecylsilica (ODS) have been compared. A sample from a pulp and paper plant has also been studied.

EXPERIMENTAL

Apparatus

A DuPont 830 liquid chromatograph equipped with a Rheodyne Model 7120 syringe loading sample injector and a DuPont 837 spectrophotometric detector was used in most experiments. The gradient elution was performed with a combination of Waters Model 6000A pumps, Model 660 solvent flow programmer, Model U6K valve-loop injector and Model 440 UV-detector at 280 nm.

Columns

Silica columns (250 × 4.6 mm) were slurry packed in methanol with LiChrosorb Si 60 (5 μm) from E. Merck (Darmstadt, G.F.R.). The amino column (200 × 4.6 mm), purchased from Chrompack (Middelburg, The Netherlands) was packed with Nucleosil 5 NH_2 from Macherey, Nagel & Co. (Düren, G.F.R.). The ODS columns (230 × 4.6 mm) used for isocratic elution were slurry packed in methanol either with Spherisorb S-5-W ODS (5 μm) from Phase Separations (Queensferry, Great Britain) or with ODS-Hypersil (5 μm) from Shandon Southern Products (Runcorn, Great Britain).

Chemicals

Phenol (pure), *o*-chlorophenol (pure), *m*-chlorophenol (pure), *p*-chlorophenol (pure), 2,4-dichlorophenol (pract) and 3,4-dichlorophenol (pure) were purchased from Koch-Light Labs. (Colnbrook, Great Britain), 2,3-dichlorophenol, 2,5-dichlorophenol, 2,6-dichlorophenol, 3,5-dichlorophenol, 2,3,6-trichlorophenol, 2,4,5-trichlorophenol, 2,4,6-trichlorophenol and 2,3,4,6-tetrachlorophenol from Norsk Hydro (Porsgrunn, Norway) and 2,3,4-trichlorophenol (pure), 3,4,5-trichlorophenol (pure), 2,3,4,5-tetrachlorophenol (pure), 2,3,5,6-tetrachlorophenol (pure) and pentachlorophenol (pure) from Fluka (Buchs, Switzerland). 2,3,5-Trichlorophenol was not available. The water was distilled twice. Methanol (p.a.), hexane (Uvasol grade), dichloromethane (p.a.), acetic acid (p.a.), cyclohexane (distilled technical grade), sodium acetate (p.a.), potassium dihydrogenphosphate (p.a.), dipotassium hydrogenphosphate (p.a.) and orthophosphoric acid were obtained from Merck. Trifluoroacetic acid (pure) was purchased from Koch-Light and methanol (HPLC-grade S) from Rathburn (Walkerburn, Great Britain). 1 *M* solutions of the salts were filtered through 0.45-μm Millipore solvent filters and stored at 4°C. Prior to use, all the HPLC solvents were degassed ultrasonically for 1 h.

Pulp-bleaching effluent sample

The sample was part of 5 l of the effluent after the first chlorination step at a Kraft pulp mill at Hurum (Norway) which had been processed as follows. After adjusting the pH from 2 to 10 with NaOH, the aqueous solution was extracted with cyclohexane (200 ml), acidified to pH 2 with H_2SO_4 and subsequently extracted with butyl acetate (173 ml). The butyl acetate phase was evaporated and the residue was dissolved in the initial mobile phase of the gradient.

RESULTS AND DISCUSSION

Detection

Solutions of the chlorinated phenols in methanol had absorbance maxima at approximately 220 nm and at 250–320 nm, depending on the structure (Fig. 1). The high wavelength region was chosen since there was less interference in actual samples. The possible use of fluorescence detection was examined by scanning the excitation from 250 to 400 nm. With the exception of phenol itself and a weak emission from the monochlorinated phenols, no fluorescence of CPs was found.

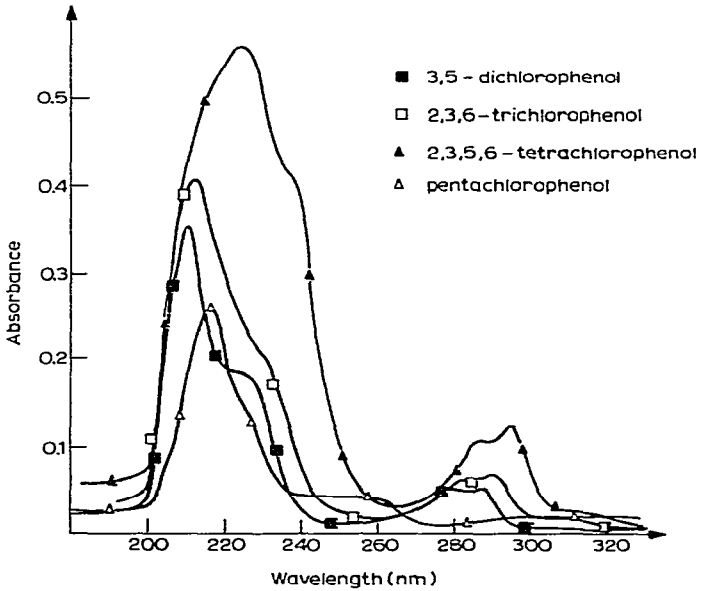

Fig. 1. UV absorption spectra of chlorinated phenols in methanol.

Chromatography

Silica. Mixtures of hexane and dichloromethane were used as mobile phase. The samples were dissolved in dichloromethane due to their poor solubility in hexane. Generally the resolution was poor, but improved significantly when 1% methanol was added as an adsorbent modifier, in addition to 80% hexane and 19% dichloromethane. The positional isomers of mono- and di-CPs were baseline resolved, 2,3,6- and 2,4,6-trichlorophenol were not separated and tetrachlorophenols were poorly resolved. The strongly retained broad peaks of the highly substituted CPs were better

resolved by adding trifluoroacetic acid (0.01 *M*) to the mobile phase. Even when the majority of the positional isomers could be resolved, a group separation of mono-, di-, tri- and tetra-CPs could not be obtained. Thus, in a mixture of eighteen CPs, several peaks overlapped. Even with methanol as adsorbent modifier, a constant activity of the silica was difficult to obtain, resulting in poor reproducibility of retention.

Aminopropylsilica. Depending on the mobile phase, aminopropylsilica may act as a normal phase support, a semi-reversed-phase support or as a weak anion exchanger. Since the pK_a values of CPs range from 4.7 to 9.9 (Table I), strong coulombic forces would be expected and generally a pK_a-dependent retention was observed.

TABLE I

ACID DISSOCIATION CONSTANTS OF CHLORINATED PHENOLS

Number	*Compound*	pK_a
1	Phenol	9.92
2	*o*-Chlorophenol	8.52
3	*p*-Chlorophenol	9.37
4	*m*-Chlorophenol	8.97
5	2,6-Dichlorophenol	6.78
6	2,3-Dichlorophenol	7.71
7	2,5-Dichlorophenol	7.51
8	2,4-Dichlorophenol	7.90
9	3,4-Dichlorophenol	8.62
10	3,5-Dichlorophenol	8.25
11	2,3,6-Trichlorophenol	5.80
12	2,3,4-Trichlorophenol	6.97
13	2,4,6-Trichlorophenol	5.99
14	2,4,5-Trichlorophenol	6.72
15	3,4,5-Trichlorophenol	7.55
16	2,3,5,6-Tetrachlorophenol	5.03
17	2,3,4,6-Tetrachlorophenol	5.22
18	2,3,4,5-Tetrachlorophenol	5.64
19	Pentachlorophenol	4.74

The positional isomers of di-CP were resolved with 0.008 *M* sodium acetate + 0.002 *M* acetic acid in methanol (Fig. 2), and the isomers of tri-CP with 0.01 *M* acetic acid in methanol (Fig. 3). The higher substituted CPs were too strongly retained with these solvents, but with 0.1 *M* acetic acid in methanol–water (70:30) the tetra-CPs were separated (Fig. 4) and penta-CP was eluted after 30 ml. With this mobile phase the lower CPs were poorly separated. A gradient of 0.01 *M* sodium acetate to 0.1 *M* acetic acid in 70% methanol did not resolve the majority of the CPs examined. In addition, the time (1 h) needed for column equilibration between two gradient runs was too high to be useful for high speed analysis.

ODS. Positional isomers of mono- (Fig. 5), di- (Fig. 6), tri- (Fig. 7) and tetra-CPs (Fig. 8) were separated with methanol–water (55:45). The highly dissociated CPs gave broad peaks in this solvent (Figs. 7 and 8). In order to obtain a complete separation of the nineteen phenols examined, the ionization was suppressed by the addition of an acid. Gradient elution with a phosphate buffer (pH 4.0) gave complete

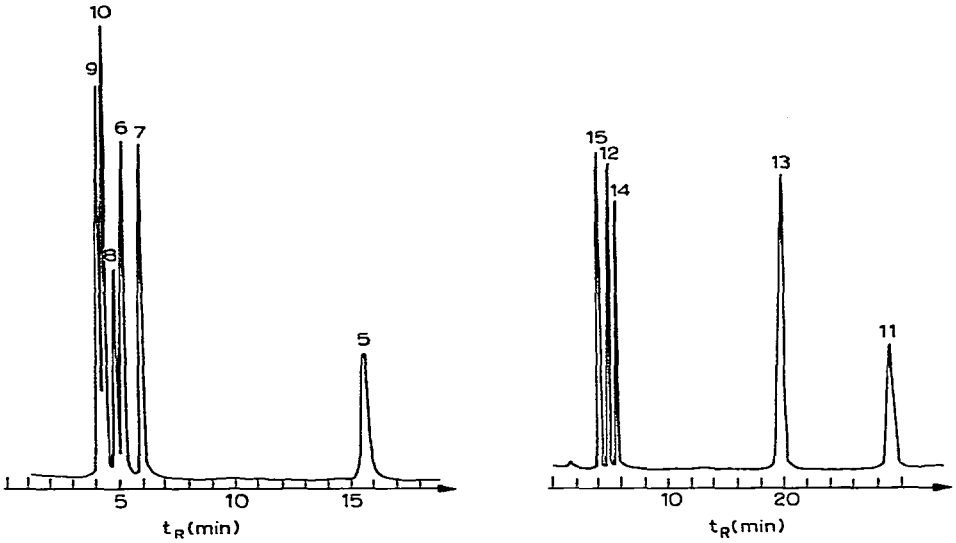

Fig. 2. Separation of dichlorophenols (numbered as in Table I) on a Nucleosil amino column using 0.008 *M* sodium acetate–0.002 *M* acetic acid in methanol as mobile phase, flow-rate 1.0 ml/min. Detection at 275 nm.

Fig. 3. Separation of trichlorophenols (numbered as in Table I) on a Nucleosil amino column using 0.01 *M* acetic acid in methanol as mobile phase, flow-rate 1.0 ml/min. Detection at 275 nm.

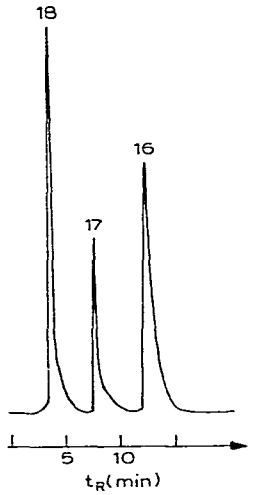

Fig. 4. Separation of tetrachlorophenols (numbered as in Table I) on a Nucleosil amino column using 0.1 *M* acetic acid in methanol–water (70:30) as mobile phase, flow-rate 110 ml/min. Detection at 290 nm.

separation of all components (Fig. 9). At pH 5.0, only sixteen components were resolved. At pH 3.0, 2,3,5,6- and 2,3,4,6-tetra-CP were eluted as one peak. The retention increased with increasing degree of chlorination, and at constant chlorination increased with increasing pK_a. At constant chlorination and little difference in pK_a, the retention decreased with increasing molecular dipole moment.

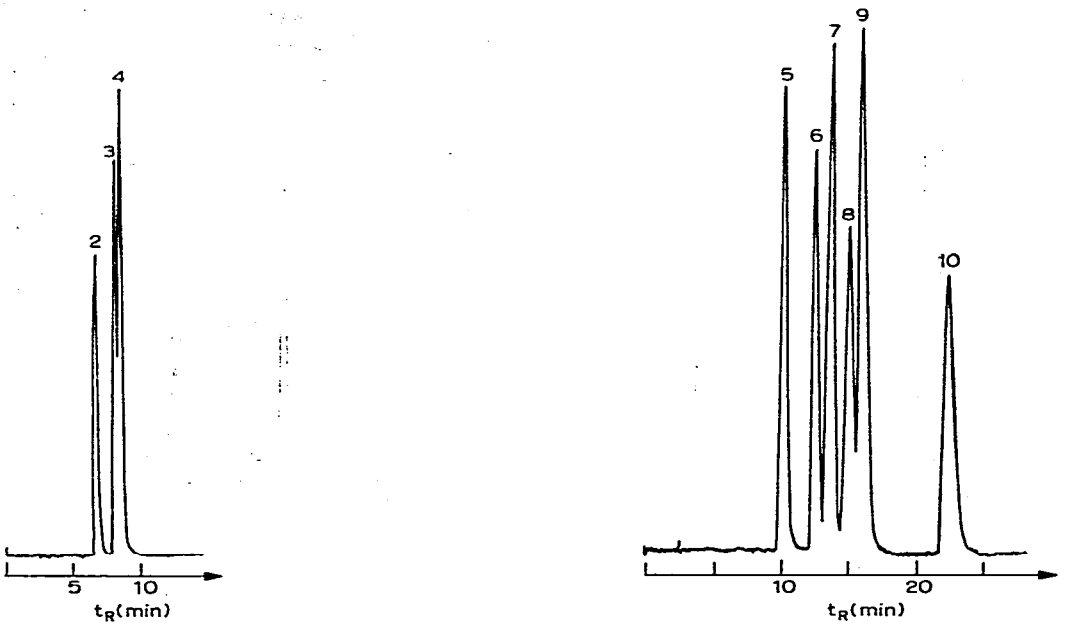

Fig. 5. Separation of monochlorophenols (numbered as in Table I) on a Spherisorb ODS column using methanol–water (55:45) as mobile phase, flow-rate 1.0 ml/min. Detection at 285 nm.

Fig. 6. Separation of dichlorophenols on a Spherisorb ODS column. Details as in Fig. 5.

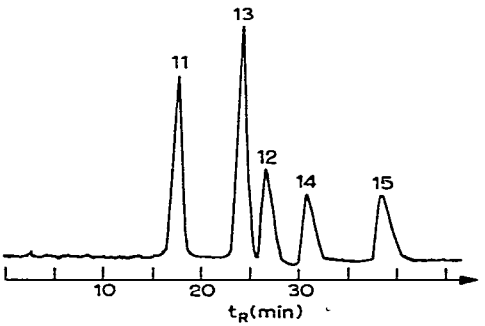

Fig. 7. Separation of trichlorophenols on a Spherisorb ODS column. Details as in Fig. 5.

Pulp-bleaching effluent sample

The usefulness of the gradient elution on C-18 columns with samples of high complexity was tested for a sample from a pulp-bleaching effluent. Owing to the large amount of interfering components, the gradient conditions had to be adjusted to suit the sample, as shown in Fig. 10. Of the chlorinated phenols studied, none was identified in measurable quantities. This means that the concentrations in the effluent stream did not exceed 10 ppm of mono-CP and 100 ppm of the highest chlorinated phenols (due to the lower sensitivity at 280 nm).

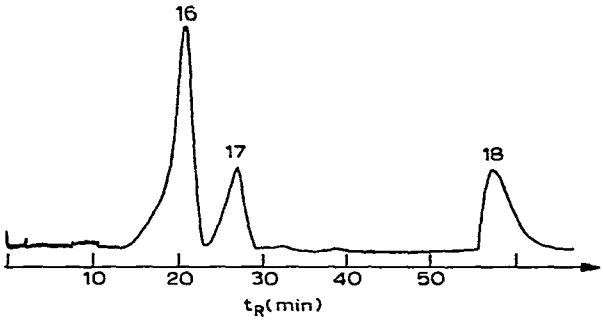

Fig. 8. Separation of tetrachlorophenols on a Spherisorb ODS column. Mobile phase flow-rate 1.1 ml/min. Other details as in Fig. 5.

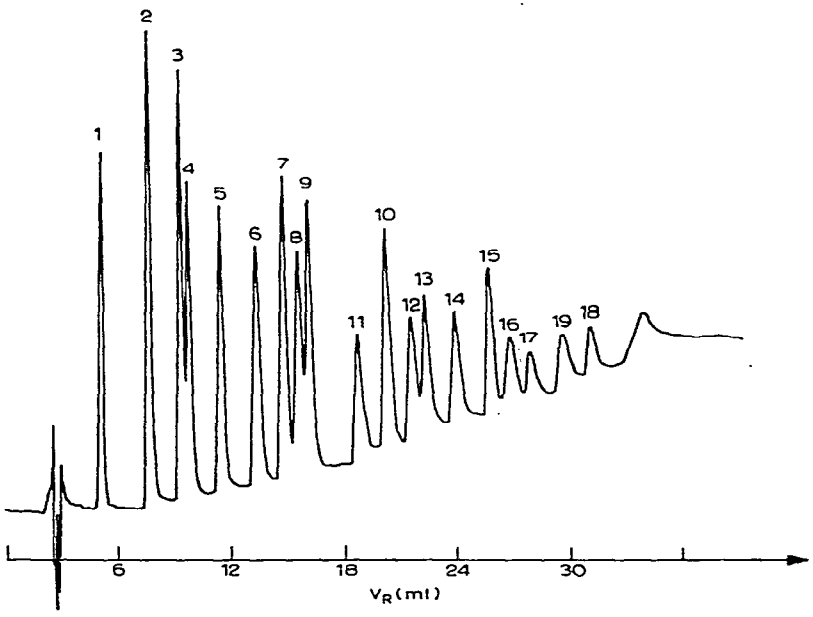

Fig. 9. Separation of chlorinated phenols and phenol (numbered as in Table I) on an Hypersil ODS column using a 30-min linear gradient from methanol–0.02 *M* KH_2PO_4, pH 4.0 (56:44 to 80:20), as mobile phase, flow-rate 1.0 ml/min. Detection at 280 nm.

CONCLUSION

The comparison between the three different HPLC systems showed that reversed-phase chromatography on C_{18} columns was superior for the analysis of chlorinated phenols. This conclusion was based on the ability to separate positional isomers, to separate according to chlorination rate and to separate a multi-component mixture, as well as requirements for stability and speed of analysis. The analysis of the extract from a pulp-bleaching effluent stream demonstrated that, even in such highly complex samples, concentrations of 10–100 ppm of approximately eighteen chlorinated phenols could be determined in one gradient run. With samples allowing UV detection at 220–230 nm, the sensitivity could be increased, especially for the highly substituted CPs.

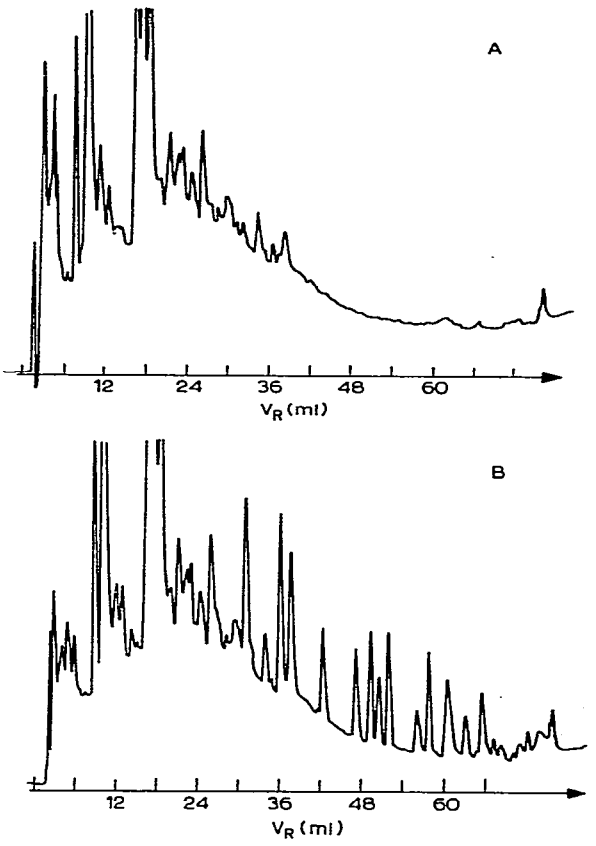

Fig. 10. Chromatogram of pulp-bleaching effluent sample on an Hypersil ODS column using a 75-min linear gradient, methanol–0.02 *M* KH_2PO_4, pH 4.0 (20:80 to 80:20), as mobile phase, flow-rate 1.0 ml/min. Detection at 280 nm. Samples: A, effluent; B, effluent + a mixture of phenol and eighteen chlorinated phenols.

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