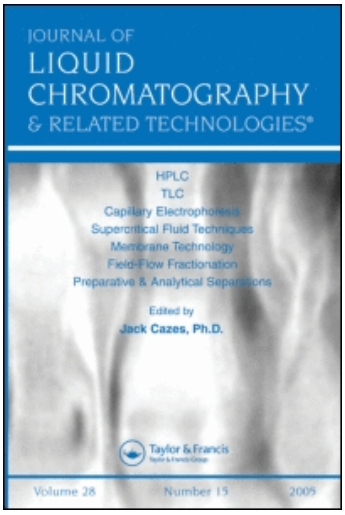

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Separation of Positional Isomers of Chlorophenols by Reverse Phase HPLC

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SEPARATION OF POSITIONAL ISOMERS OF CHLOROPHENOLS BY REVERSE PHASE HPLC

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ABSTRACT

With RP-HPLC, an optimum gradient elution was achieved for separation of 15 chlorophenol positional isomers. Recoveries were satisfactory. The lowest detection limit is 5×10^{-10} g. This method is suitable for determining these substances in environmental samples. The relationship between Capacity Factor (k'), Total Surface Area (TSA), and Molecular Connectivity Index (^1x, $^1x^v$) of chlorophenol congeners was studied, which is reported here for the first time.

INTRODUCTION

Chlorophenols are widely used as intermediates in chemical synthesis of various compounds, and as broad-spectrum biocides and preservatives for leather, glue and some textiles. Thus, chlorophenol congeners have made them ubiquitous contaminants of soil and water. Chlorophenols are highly toxic to man and aquatic organisms; even at very low concentration (less than 1 ng/L) phenols affect the taste and odor of water and fish.[1] In addition, they are also resistant to degradation and tend to bioaccumulate. Therefore, chlorophenols are of great concern in the aquatic environment, and it is necessary to develop a

method for determinating these compounds. Both HPLC-UV and HPLC-EC have succeeded in analyzing chlorophenols. EC detection is more sensitive and selective,[2-4] but it is not suitable for determining all chlorophenols with gradient elution with high sensitivity. This limits the application of EC detection for complete separation of all chlorophenol positional isomers at one injection. UV detection offers the possibility to separate chlorophenols, with a gradient technique, with good resolution, in a short run time. Although several authors reported that they attained a complete separation of chlorophenols using RP-HPLC with gradient elution, there are still some problems with their chromatograms for analysis of real water samples.[3,5,6]

The aims of the present study are (1) to achieve an optimum gradient elution for separating 15 positional isomers of chlorophenol; (2) to develop an optimized method for the determination trace amounts of chlorophenols in water samples; (3) to investigate the relationship between k', TSA and Molecular Connectivity Index.

EXPERIMENTAL

Materials and Reagents

o-Chlorophenol, m-chlorophenol, p-chlorophenol, 2,3-dichlorophenol, 2,5-dichlorophenol, 2,4-dichlorophenol, 3,4-dichlorophenol, 3,5-dichlorophenol, 2,3,6-trichlorophenol, 2,3,4-trichlorophenol, 2,3,5-trichlorophenol, 2,3,4,6-tetrachlorophenol, pentachlorophenol were obtained from Aldrich Chem. Co., USA. Methanol (HPLC grade), analytical grade glacial acetic acid, butyl acetate and cyclohexane were obtained from Tianjin, China. Water was obtained from a Milli-Q system (Millipore, Bedford, MA, USA). C_{18} Sep-Pak cartridges (Waters, Milford, MA, USA) were used as solid phase columns.

Instrumentation

The HPLC system (Model 244, Waters) consisted of two LC pumps (Model 510), an injection valve (Model U6K), a UV detector (Model 481), a temperature control system, an automatic gradient controller (Model 680) and a data module (Model 730) for monitoring outputs. Chlorophenols were separated on an analytical reverse phase column which was a 3.9mm i.d. X 150mm Delta Pak C_{18} 300-Å column (Waters). Samples were eluted with a gradient elution program. The effluent was monitored using the UV detector at an analytical wavelength of 280 nm. The column temperature was 35°C.

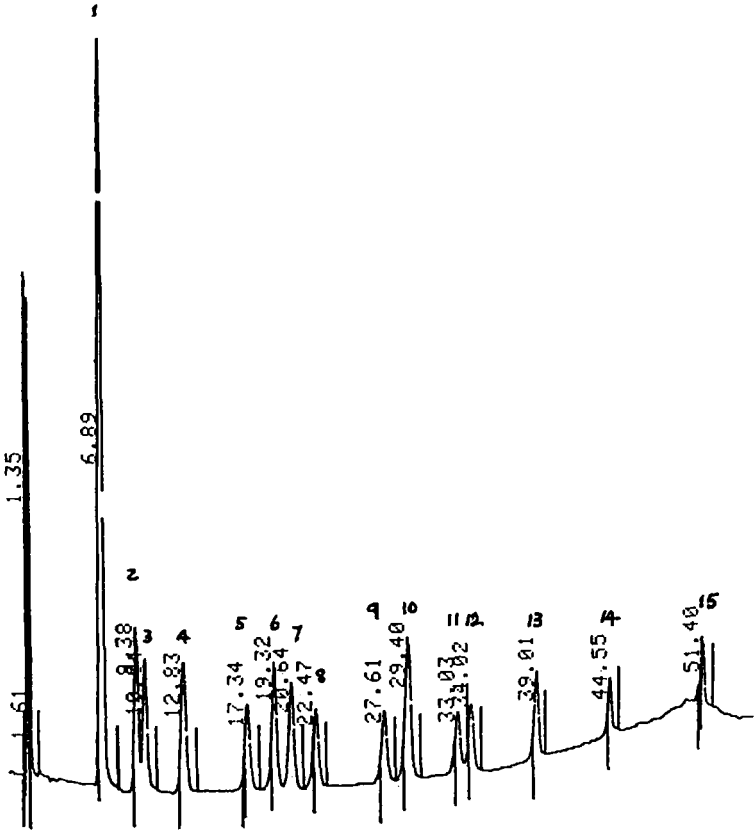

Figure 1. Gradient elution separation of 15 chlorophenols on a Delta C_{18} column using a 50 min linear gradient program as stated in the text. 1: 2-CP; 2: 4-CP; 3: 3-CP; 4: 2,6-DCP; 5: 2,3-DCP; 6: 2,5-DCP; 7: 2,4-DCP; 8: 3,4-DCP; 9: 2,3,6-Tri-CP; 10: 3,5-DCP; 11: 2,3,4-Tri-CP; 12: 2,4,6-Tri-CP; 13: 2,3,5-Tri-CP; 14: 2,3,4,6-Tetra-CP; 15: Penta-CP.

Mobile Phase

Mobile phase A was prepared by mixing 35% methanol and 65% buffer (0.01M sodium acetate + 0.002 M sodium EDTA, pH 4.0). Mobile Phase B was 100% methanol. Prior to use, all the mobile phases were filtered and degassed ultrasonically.

Chlorophenol congeners were weighed accurately and dissolved in an appropriate volume of methanol to prepare a stock solution containing 10^3 μg/mL, and kept at 4°C in the dark. This stock solution was further diluted to obtain working standard solution with a concentration of 10 μg/mL. Using appropriate volumes of this working standard solution, solutions were prepared containing 25, 50, 75, 100, 125, 150 ng/mL of chlorophenol congener mixture, which was used to propare a standard calibration curve.

Sample Preparation

3L water sample was filtered with 0.45 μm filter; 1M NaOH was used to adjust the pH to 11, and subsequently was extracted with 50 mL cyclohexane. The separated organic phase was discarded. The pH of the aqueous phase was adjusted to 3-4 with glacial acetic acid, then the water phase was passed through a C_{18} Sep-Pek for enrichment. Finally, 2 mL of 75% methanol was used to elute chlorophenols from C_{18} cartridge, and the eluate was collected for analysis. Injection volume was 20 μL.

RESULTS AND DISCUSSION

The optimized linear gradient program was as follows:

	Flow Rate (ml/min)	A%	B%
Initial	1.00	100	0
30 min	1.00	75	25
45 min	1.00	46	54
55 min	1.00	46	54

According to these conditions, we obtained a complete separation of 15 positional isomers of chlorophenol. (Figure 1).

The influence of temperature on the chromatographic capacity factor (k') was investigated. Results are shown in Table 1. The capacity factor (k') of chlorophenol isomers decreased with increasing temperature, except for the 2,4,6- and 2,3,4- congeners. It was found that, for temperatures up to 45°C, these two peaks were eluted as a single peak and the resolution was completely

Table 1

Capacity Factor (k') of Chlorophenols at Various Temperatures

No.	Compound	Temperature, °C 25	35	45	γ*
1	2-CP	3.05	2.43	1.95	0.9989
2	4-CP	3.89	3.21	2.57	0.9996
3	3-CP	4.06	3.38	2.73	0.9999
4	2,,6-DCP	4.89	4.16	3.46	0.9999
5	2,3-DCP	5.98	5.17	4.28	0.9985
6	2,5-DCP	6.43	5.60	4.68	0.9984
7	2,4-DCP	6.76	5.90	4.97	0.9987
8	3,4-DCP	7.25	6.32	5.30	0.9985
9	2,3,6-Tri-CP	8.32	7.40	6.38	0.9984
10	3,5-DCP	8.78	7.79	6.68	0.9982
11	2,3,4-Tri-CP	9.03	8.32	7.52	0.9981
12	2,4,6-Tri-CP	9.88	8.87	7.52	0.9939
13	2,3,5-Tri-CP	10.73	9.71	8.57	0.9982
14	2,3,4,6-Tetra-CP	12.30	11.30	10.17	0.9980
15	Penta-CP	14.67	13.65	12.51	0.9982

* Regression Correlation Coefficient.

lost. Therefore, in our experiments, considering the resolution and analysis time, a column temperature of 35°C was selected.

The effect of pH on capacity factor has been reported by Alarcon.[7] In our experiments, the result is consistent with that of Alarcon. Over the range of pH 3.5 - 5, the differences of k' values for the fifteen chlorophenols are significant and a desirable resolution of these positional isomers can be achieved. Thus pH = 4 was selected for this study.

The LDL of fifteen chlorophenol congeners was from 0.5 ng to 5 ng. Within the linear response range of the detector, using 15 μL standard mixture of concentration at 5 ppm, the solution was analyzed six times. The relative standard deviation (RSD) was in the range from 1% to 5% (Table 2).

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Table 2

LDL Precision and Recovery

No.	Compound	LDL(ng)*	RSD(%)	Recovery (X±SD)%** 20μg/L	50 μg/L
1	2-CP	0.5	1	42.5±1.2	51.1±6.5
2	4-CP	2.0	2	50.5±3.3	82.2±5.0
3	3-CP	2.5	2	64.0±4.3	87.1±4.8
4	2,6-DCP	2.5	2	91.6±13.9	89.9±7.6
5	2,3-DCP	5.0	3	95.6±11.7	97.1±5.3
6	2,5-DCP	2.0	2	87.7±12.9	99.3±5.0
7	2,4-DCP	5.0	3	92.1±16.1	97.2±4.4
8	3,4-DCP	5.0	3	97.7±12.3	102.1±3.0
9	2,3,6-TCP	5.0	2	104.4±9.3	98.0±4.0
10	3,5-DCP	2.0	3	91.7±11.4	99.9±4.9
11	2,3,4-TCP	5.0	3	98.8±11.7	101.8±3.6
12	2,4,6-TCP	5.0	3	107.6±16.9	99.8±3.5
13	2,3,5-TCP	5.0	4	95.5±10.7	97.7±4.6
14	2,3,4,6-Tetra-CP	5.0	4	98.2±14.0	96.3±4.5
15	PCP	5.0	5	101.4±10.2	96.5±3.7

* LDL = Lowest Detection Limit evaluated as the amount of chlorophenol that gives a signal two-times greater than the noise level.

** Average of six extractions.

The recoveries are quantitative and greater than 90% for all chlorophenol congeners except for mono-chlorophenol isomers (Table 2). This is because mono-congeners have lower retention on the C_{18} column than other chlorophenols. Thus their recoveries are in the range 42 to 87%, which are increased with higher concentration. This may be due to loss by evaporation during analysis. Therefore the methods proposed is only suitable for quantitative analysis of chlorophenols except for monochlorophenols.

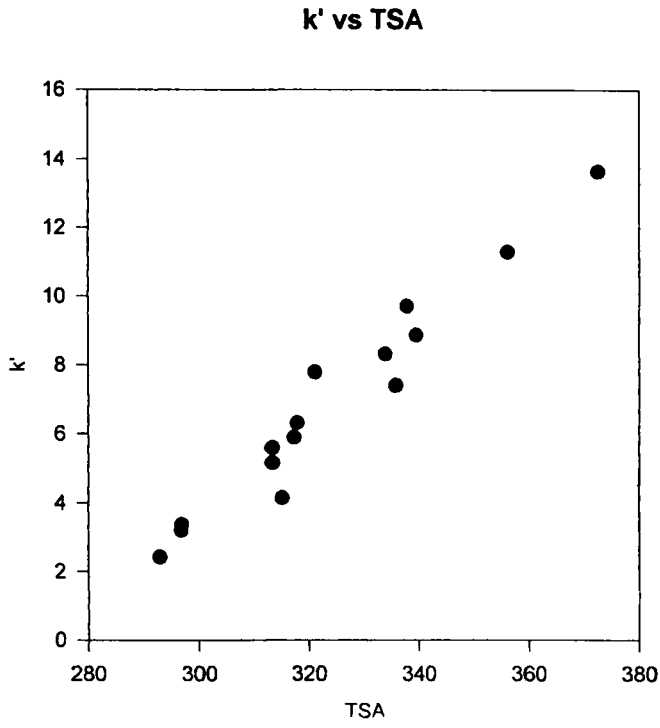

Figure 2. Capacity factor (k') vs total surface area (TSA).

equations are y = 277.1x + 6.82 (r = 0.9767) and y = 2.421 X + 0.1432 (r = 0.9592). There is also good correlation between k' and $^1X^V$ (Y = 2.154x + 0.1655, r = 0.9587). From this observation, it can be predicted that a peak emerged on the chromatogram identifies which chlorophenol congener. When four positional isomers, including 2,6-, 3,5-, 2,3,6- 2,3,5-congeners, are not taken account, the correlation coefficients between k' and those two parameter (TSA and ^1X) reach 0.9983 and 0.9936, respectively. This reveals that some other structural variables other than TSA and ^1X influence the chromatographic behavior of these four isomers. This phenomenon has to be further investigated.

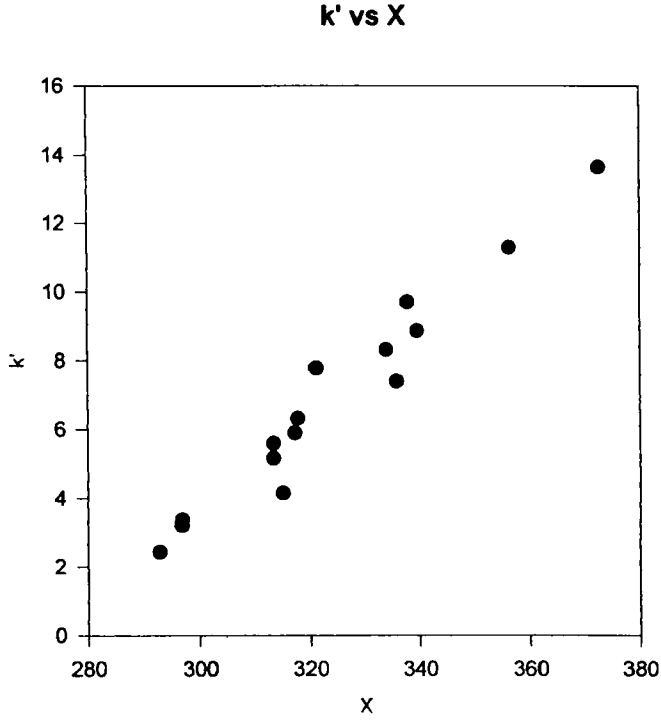

Figure 3. Capacity factor (k') vs molecular connectivity index (^{1}X).

APPLICATION

Four water samples were collected for analysis of chlorophenols. Using the method described previously, mono-, di-, and penta-chlorophenols were determined in these four kinds of water samples (Table 3); addition of standards identified the peaks which eluted, and standard calibration curves were prepared for quantitative analyses. For example, one real water sample analysis is illustrated in Figure 4. Chlorophenols were found in the tap water. This could be due to sterilization with chlorine. Tap water samples were taken early in the morning or in the evening. At these times there was an irritant odor in the tap water. Pentachlorophenol was detected in rain water collected near the main teaching building of Nankai University. This suggests that the the atmosphere above Nankai University was contaminated with pentachlorophenol from a factory nearby, becauce pentachlorophenol can exit as air-particulates in the local atmosphere. Chlorophenols occur in well water.

Table 3

Determination of Chlorophenols in Real Water Samples

Sampling Date	Location	Sample	Concentration of Chlorophenols (ppb)*,** PCP	2-CP	2,3-DCP	2,4-DCP	3,5-DCP
5/19/94	Lab at Nankai Univ.	Tap Water	0.40	--	--	0.10	--
11/9/93	Tianjin Paper Mill	Pulp-Bleach Effluent	1.28	--	4.55	--	2.61
5/12/94	Near Main Teaching Bldg. of Nankai Univ.	Rain Water	1.01	--	--	--	--
5/17/94	Tianjin Pesticide Factory	Well Water	1.35	0.05	--	--	--

* Average of two determinations
** -- Not detected

This results, possibly, from contamination by chlorophenol pollutants which were produced during generation processes. These pollutants were drained out with waste water, then permeated through underground soil, eventually leading to the contamination of the well water. Chlorophenol contaminants were also detected in the pulp-bleaching effluent from the paper mill.

This method has the advantages of simplicity, good recovery and replication. In addition, in real sample analysis with complex matrices, it can give a complete separation chromatogram for quantitative analysis .

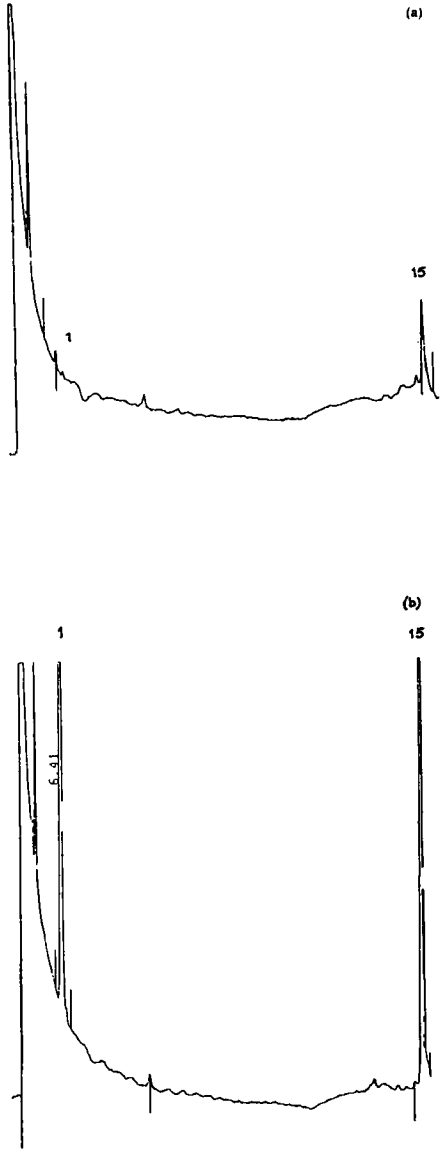

Figure 4. Chromatogram of well water sample on a Delta C_{18} column using a 50 minute linear gradient program as described in the text; (a) Real sample; (b) Real sample + standards. For peak identification, see Table 1.

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