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Note

Separation of chlorinated phenols by reversed-phase high-performance liquid chromatography at an alkaline pH

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Recently there has been interest in the high-performance liquid chromatographic (HPLC) separation of the nineteen congeners (Table I) of the chlorophenols. Eugland *et al.*[1] have successfully resolved a mixture of eighteen chlorophenols and phenol on a C_{18} column using a 30-min linear gradient with a mobile solvent at pH 4.0. Smit *et al.*[2] separated eleven congeners using a reversed-phase system at pH 3, while Ogan and Katz[3] separated fifteen using a reversed-phase system at pH 2.15. Lores *et al.*[4] separated ten mono- and dichlorophenols using a mobile solvent of phosphate buffer, methanol and acetonitrile at pH 4 and the other nine with phosphate buffer and acetonitrile only. Another acid pH reversed-phase system in which separation of four chlorophenols was demonstrated has been described[5]. Mundy and Machin[6] reported the separation of pentachlorophenol (P_5CP) and the three tetrachlorophenols on a porous silica column with a mobile solvent of methanol and water. Ivanov and Magee[7] using a normal phase system separated eight of the nineteen congeners, and two other systems[8,9] for pentachlorophenol only, have been reported.

TABLE I

LIST OF CHLOROPHENOL CONGENERS

	Monochlorophenols, three isomers
	1. 2-
	2. 3-
	3. 4-
Dichlorophenols, six isomers	*Trichlorophenols, six isomers*
4. 2,3-	10. 2,3,4-
5. 2,4-	11. 2,3,5-
6. 2,5-	12. 2,3,6-
7. 2,6-	13. 2,4,5-
8. 3,4-	14. 2,4,6-
9. 3,5-	15. 3,4,5-
Tetrachlorophenols, three isomers	*Pentachlorophenol, one isomer*
16. 2,3,4,5-	19. 2,3,4,5,6-
17. 2,3,4,6-	
18. 2,3,5,6-	

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TABLE II

RETENTION TIMES RELATIVE TO PENTACHLOROPHENOL FOR CHLOROPHENOL CONGENERS AT SEVERAL pH VALUES AND TIME TO ELUTE ALL NINETEEN COMPOUNDS

RRT P_5CP	*pH and chlorophenol congener*					
	7.4	*8*	*9*	*10*	*11*	*12*
0.20					2-	2-
0.21						3-
0.22					2,6-	4-
0.23					2,3-	2,6-
0.25				2,6-	2,5-	2,3-
0.26						2,4-
						3,5-
0.27					2,4-; 3,5-	2,5-
0.28			2,6-		3-	3,4-
0.30					3,4-	
0.31				2,5-; 3,5-		
0.32				2,3-	2,3,6-	2,3,6-
0.33					2,4,6-	2,4,6-
0.35			2,3,6-		2,3,4-	2,3,4-
0.36				2,3,6-	4-	
0.37					2,4,5-	2,4,5-
0.38		2,3,6-	2,4,6-	2,4,6-	2,3,5-	2,3,5-
0.40				2,4-	3,4,5-	3,4,5-
0.42				2,3,4-		
0.43				2-		
0.44				2,4,5-		
0.45				2,3,5-		
0.46		3,4-				
0.48		2,4,6-				
0.49	2,3,6-					
0.51			2,3,5-			
0.53					2,3,4,6-	
0.55			2,3,4,6-	2,3,4,6-		2,3,4,6-
0.56		2,3,4,6-				
0.57			2,3,5,6		2,3,5,6-	
0.58	2,3,5,6-	2,3,5,6-				2,3,5,6-
0.60	2,3,4,6-	2,6-	2,4,5-		2,3,4,5-	2,3,4,5-
0.61				2,3,5,6-		
0.62			2,5-	2,3,4,5-		
0.64			3,5-			
0.66				3,4,5-		
0.67			2,3,4-			
0.68	2,4,6-		2,3,4,5-			
0.72			2,3-			
0.85				3,4-		
0.92				3-		
1.00	2,6-; P_5CP	P_5CP	2,4-; P_5CP	P_5CP	P_5CP	P_5CP
1.10			2-			
1.16		2,3,5-				
1.20		2,3,4,5-				
1.30				4-		
1.47	2-					
1.65		2-				

TABLE II *(continued)*

RRT P_5CP	*pH and chlorophenol congener*					
	7.4	*8*	*9*	*10*	*11*	*12*
1.68		2,4,5-				
1.73	3-					
1.82	2,3,4,5-					
1.87	2,3,5-					
1.89	4-					
1.90			3,4,5-			
1.92		3,5-				
1.98		2,5-				
2.12		2,3,4-	3-			
2.15		4-				
2.24		2,3-				
2.30		3-				
2.50			4-			
2.55	2,3-					
2.57	3,5-					
2.59	2,5-					
2.67	2,4,5-					
2.89		2,4-	3,4-			
3.25	2,3,4-					
3.32	2,4-					
4.30	3,4-					
6.32		3,4,5-				
7.80	3,4,5-					
Elution time (min)	73	55	17.2	6.9	6.1	5.8

In the course of evaluating the methodology for chlorophenol residues in biological samples we investigated the potential of the Hamilton PRP-1 column, operating in a reversed-phase mode at an alkaline pH. This report details the characteristic separation patterns obtained for the nineteen congeners over a pH range of 7.4–12 and the optimum conditions for separating thirteen of the nineteen congeners under isocratic conditions.

EXPERIMENTAL

Reagents

Acetonitrile (HPLC grade), trisodium and sodium dihydrogen phosphate of reagent grade quality were purchased from local laboratory supply houses. Chlorophenol standards of reagent purity were purchased from Supelco (Bellefonte, PA, U.S.A.) except for 2,3,4,6-tetrachlorophenol. The latter compound was purchased from Pfaltz and Bauer (Stamford, CT, U.S.A.).

Stock and working solutions

Stock solutions were prepared by dissolving accurately weighed portions (20.65–28.40 mg) of each chlorophenol congener in acetonitrile separately and diluting to 25 ml. Serial dilutions of working solutions were made by diluting a 25-μl aliquot of each separately to 10 ml with mobile solvent of the appropriate pH.

Solutions of 0.1 *N* Na_3PO_4 and 0.1 *N* NaH_2PO_4 were prepared using deionized

water and portions combined to give separate solutions of pH 7.4, 8, 9, 9.2, 10, 11 and 12. The mobile solvent systems were prepared by combining 2.5 parts of the appropriate buffer solution with a part acetonitrile, v/v.

Apparatus

A Waters Assoc. (Milford, MA, U.S.A.) Model 6000A pump, a Valco syringe 10-μl loop injector, a Waters Assoc. Model 440 fixed-wavelength detector (254 nm) and a Westronica Model MT22 paper strip recorder driven at 15 in./h were assembled in a normal configuration. All readings were made at 254 nm using the 0.02 absorbance scale.

Column preparation

A Hamilton PRP-1 column purchased from Chromatographic Specialities (Brockville, Canada) was connected into the HPLC system. This 150 × 4.1 mm column was said to have more than 22,000 theoretical plates per metre at pH 12. Mobile solvents were degassed and the system allowed to equilibrate (45–50 min) at ambient temperature using a flow-rate of 1.2 ml/min.

Procedure

The retention time and response of each compound was determined by co-injecting separately an appropriate volume (7–10 μl) of the dilute stock solution of

TABLE III

RESPONSE OF EACH CONGENER AT VARIOUS pH LEVELS OF MOBILE SOLVENT

Congener	*pH and response* in cm/ng*					
	7.4	*8*	*9*	*10*	*11*	*12*
2	0.05	0.1	0.3	0.7	1.5	1.4
3	0.02	0.03	0.1	0.5	1.3	2.2
4	0.02	0.6	0.2	0.7	2.7	5.3
2,3	0.05	0.1	0.6	1.5	1.4	2.0
2,4	0.07	0.3	0.6	2.2	2.6	3.6
2,5	0.08	0.2	0.8	1.5	2.1	1.9
2,6	0.02	0.4	1.1	1.3	1.4	1.8
3,4	0.03	0.3	0.4	1.1	2.4	4.5
3,5	0.06	0.2	0.8	1.3	1.7	2.6
2,3,4	0.1	0.3	1.0	1.9	2.0	3.0
2,3,5	0.1	0.3	0.8	1.4	1.2	1.9
2,3,6	0.4	0.6	1.0	1.3	1.1	1.8
2,4,5	0.1	0.4	0.9	1.8	1.7	2.3
2,4,6	0.5	0.9	1.8	2.0	2.0	2.0
3,4,5	0.03	0.08	0.4	1.3	1.9	3.3
2,3,4,5	0.2	0.4	1.0	1.3	1.3	2.1
2,3,4,6	0.4	0.5	0.7	1.0	1.1	1.1
2,3,5,6	0.4	0.5	0.9	0.8	0.9	1.7
P_5CP	0.3	0.4	0.7	0.9	0.8	1.4

* Noise level equivalent to 1 mm peak to peak.

each congener with an aliquot of pentachlorophenol standard solution. The retention times relative to pentachlorophenol, RRT, were calculated for observations made at pH 7.4, 8.0, 9.0, 10.0, 11.0 and 12.0.

RESULTS AND DISCUSSION

Table II lists the retention times of the chlorophenols and times for complete elution of all nineteen congeners. At pH 7.4, 73 min were required for all to elute. As the pH increased complete elution time was drastically reduced to 55 min at pH 8.0, 17.2 min at pH 9.0, 6.9 min at pH 10.0 and for pH 11 and 12 it became relatively constant at 6.1 and 5.8 min respectively.

At pH 7.4, thirteen of the congeners eluted after pentachlorophenol, with the 2,6 congener co-eluting with the penta compound. At pH 8.0, twelve congeners followed the penta, at pH 9.0, four with the 2,4-dichlorophenol co-eluting) and at pH 10.0, one only. At pH 11.0 and 12.0 all congeners eluted before the pentachlorophenol.

The sequence of elution of each isomer in a homologous series with respect to each other usually followed a regular pattern. Deviations occurred when the retention times of two or more isomers were similar and within the error of measurement. Operating at ambient temperature and making individual observations over extended time periods could have contributed to these differences.

Limits of detection, LOD, were estimated for each congener at the different pH values by measuring peak heights and relating to the nanograms of compounds injected. These data are summarized in Table III as cm/ng. The noise level experienced never exceeded 1 mm as measured peak to peak on baseline. On the basis of a signal to noise ratio of 2.5:1, any observation of 0.25 cm/ng would be considered detectable. Referring to the data in Table III, all compounds exceeded this criteria at pH 10 and above, most (seventeen), at pH 9, thirteen at pH 8 and only five at pH 7.4.

An interesting comparison is the extent of change in RRT for each congener between pH 7.4 and 12.0, *i.e.*, ΔRRT = RRT (pH 7.4) − RRT (pH 12.0). These data were calculated and summarized in Table IV. Note that the difference is minimal for 2,3,5,6 and 2,3,4,6 but rapidly increases through the series with the 3,4,5 congener

TABLE IV

CHLOROPHENOL CONGENERS LISTED IN ORDER OF INCREASING ΔRRT_{P_5CP} OVER pH RANGE OF 7.4–12.0

ΔRRT	*Congener*	*ΔRRT*	*Congener*
0	P_5CP	1.52	3
0.05	2,3,5,6	1.67	4
0.09	2,3,4,6	2.30	2,4,5
0.17	2,3.6	2.31	3,5
0.22	2,3,4,5	2.34	2,3; 2,5
0.36	2,4,6	2.93	2,3,4
0.80	2,6	3.06	2,4
1.27	2	4.02	3,4
1.50	2,3.5	7.40	3,4,5

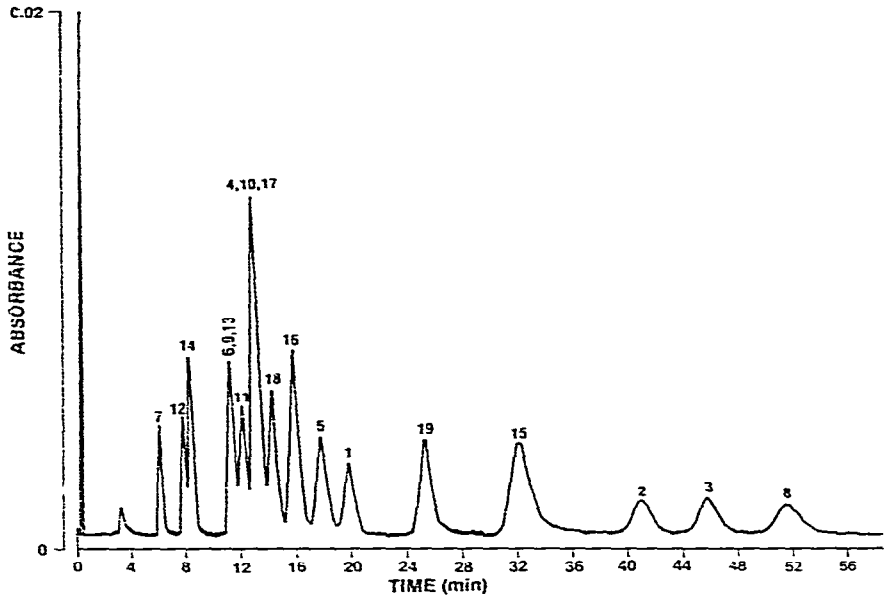

Fig. 1. Characteristic separation pattern of the nineteen chlorophenol congeners by two PRP-1 columns in series, mobile solvent pH 9.2, flow-rate 0.7 ml/min. Numbers on peaks correspond to listing in Table I.

demonstrating the greatest change. This same relative order also occurs with chlorophenol pK values for P_5CP, 2,3,5,6-, 2,3,6-, 2,6- and the dissociation constants of P_5CP, 2,3,5,6, 2,3,4,6- and 2,3,4,5-chlorophenol. However the remaining pK and dissociation constant values of the other chlorophenol congeners do not correlate with the ΔRRT pattern values. At this time there is insufficient data to rationalize these phenomena.

The separation of thirteen congeners from a mixture of nineteen is illustrated in Fig. 1. The numbers associated with each peak correspond to the congeners listed in Table I. Conditions, under which this separation was achieved were: two PRP-1 columns in series at a flow-rate of 0.7 ml/min, mobile solvent at pH 9.2 and ambient temperature. The time for elution of all congeners was 52 min.

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