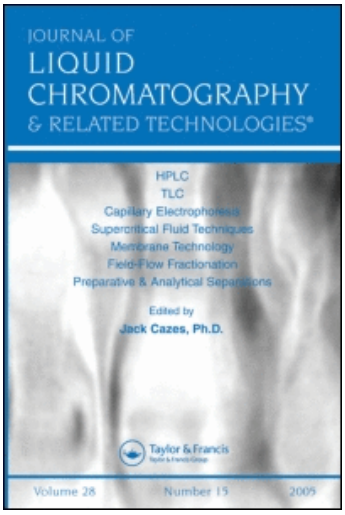

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Separation of Chlorinated Phenols by Isocratic High-Performance Liquid Chromatography on Reverse Phase Column

J. Nair[a]; K. M. Munir[a]; S. V. Bhide[a]
[a] Carcinogenesis Division, Cancer Research Institute Tata Memorial Centre, Bombay, India

To cite this Article Nair, J. , Munir, K. M. and Bhide, S. V.(1983) 'Separation of Chlorinated Phenols by Isocratic High-Performance Liquid Chromatography on Reverse Phase Column', Journal of Liquid Chromatography & Related Technologies, 6: 14, 2829 — 2837
To link to this Article: DOI: 10.1080/01483918308064950
URL: http://dx.doi.org/10.1080/01483918308064950

JOURNAL OF LIQUID CHROMATOGRAPHY, 6(14), 2829-2837 (1983)

SEPARATION OF CHLORINATED PHENOLS BY ISOCRATIC HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY ON REVERSE PHASE COLUMN

J. Nair, K.M. Munir and S.V. Bhide
Carcinogenesis Division
Cancer Research Institute
Tata Memorial Centre
Parel, Bombay-400 012
India.

ABSTRACT

A method is described for isocratic high-performance liquid chromatographic separation of chlorinated phenols using methanol : phosphate buffer - pH 7.20 (50:50) solvent system. 13 out of 15 congeners of chlorophenols and phenol have been separated in 32 minutes at a flow rate of 0.8 ml per minute. The system is found to be useful for the separation of chlorophenols extracted from mouse liver fed with hexachlorocyclohexane.

INTRODUCTION

Hexachlorocyclohexane (HCH) is a widely used pesticide in developing countries and is reported to be carcinogenic in mice by many laboratories (1 - 3). We in our laboratory were interested in finding out the profile of the chlorophenols which are possible metabolites of HCH in mouse liver - the target tissue. Among the various separation techniques, high-performance liquid chromatography (HPLC) with reverse phase column is found to be very effective for

 0148-3919/83/0614-2829$3.50/0

the separation of chlorophenols in recent years. McLeod and Laver (4) have reported an isocratic system for the separation of 13 out of 19 congeners of chlorophenols using two reverse phase columns and acetonitrile : phosphate buffer - pH 9.2. Smit et al. (5) have separated 11 congeners of chlorophenols using tetrahydrofuran : perchloric acid - pH 3.0 solvent system. Ugland et al. (6) have successfully resolved 18 congeners of chlorophenols using a 30 minute linear gradient from methanol : phosphate buffer - pH 4.0 (56 : 44 to 80 : 20) as mobile phase. Lores et al. (7) separated several chlorophenols using methanol : acetonitrile : phosphate buffer - pH 4.0 (40:14:46) and acetonitrile : phosphate buffer (50:50).

The present communication reports an isocratic HPLC separation of chlorophenols using a solvent system consisting of methanol : phosphate buffer - pH 7.20 (50:50). Separation of 13 congeners of chlorophenols out of 15 and phenol is achieved by this system with a single C_{18} reverse phase column. The system has been successfully used to separate chlorophenols extracted from mouse liver fed with HCH. The clean up procedure of the chlorophenols from the liver and the chromatographic parameters like retention time, response factor are discussed.

MATERIALS AND METHODS

HPLC was carried out using a Waters Associates (Milford, Massachusettes, U.S.A.) HPLC system fitted with a model 6000 A solvent delivery system, μ Bondapak C_{18} reverse phase column (30 cm X 0.29 cm), U6K universal injector and a model 440 absorbance

detector fitted with 254 nm filter. Chromatograms were recorded on Omniscribe (Houston Instruments, Austin, Texas) strip-chart recorder.

The chlorophenols were obtained from Aldrich Chemical Co. and were purified before use, if necessary. Stock solutions were prepared by dissolving 4-10 mg of the compounds in 5 ml of methanol. The stock solutions were diluted in methanol singly or in mixture so as to get desired standard concentrations.

Distilled E.Merck (India) G.R. grade methanol and triple distilled water from all glass apparatus were used for preparing solvent system.

Solvent System : Two stock solutions of 50 mM dipotassium hydrogen phosphate and 50 mM of potassium dihydrogen phosphate were prepared from AnalaR grade reagent and stored at 4 °C. The stock solutions were mixed and diluted appropriately to make a buffer of 0.5 mM strength and pH 7.20 ± 0.02. Running solvent was prepared by mixing methanol and phosphate buffer (50:50) after filtering through appropriate Millipore filters.

Sample Preparation : Livers were collected from male Swiss mice (8-week-old) fed with a diet containing 500 ppm HCH continuously for 2 months. Livers were minced and the chlorophenols were extracted from it by alkaline and acid hydrolysis followed by distillation and extraction in toluene as described by Sackmauerova-veningerova et al. (8). Instead of 15 ml, 3 X 50 ml of toluene was used. The combined toluene extract was then concentrated to dryness using rotary vacuum evaporator at 60 °C. The residue was taken in 2 ml

benzene and cleaned up using Sephadex QAE Q-25-120 anion exchanger (Sigma) as described by Renberg (9). The final benzene solution was concentrated to dryness in a stream of nitrogen at 40 °C. The residue was then taken in 0.5 ml of running solvent and filtered before injection.

Procedure : C_{18} μ Bondapak column was first washed with methanol for 15 minutes and then with running solvent for 60 minutes at a rate of 2 ml per minute. The flow rate was then adjusted to 0.8 ml per minute. Suitable aliquots of standard mixture and the samples were injected (1-5 μl) by Hamilton syringe.

RESULTS AND DISCUSSION

Fig. 1 shows the separation of standard chlorophenols from a mixture using the present system. 13 out of 15 congeners of chlorophenols and phenol can be well separated. At a flow rate of 0.8 ml per minute 32 minutes were required to elute all chlorophenols under the present experimental condition. 2-chlorophenol eluted with 2,3,4,5-tetrachlorophenol while 2,4-dichlorophenol eluted with 2,3,4-trichlorophenol. The column required a minimum of 60 minutes washing for getting constant retention time of the chlorophenols.

The pH of the running solvent was very critical in the elution pattern of the chlorophenols. Change in pH caused change in the retention time and co-elution of several congeners. We have tried the separation with methanol : water (50:50) and methanol : phosphate buffer (50:50) ranging from pH 6 - 8. We found that pH 7.20 $\pm$ 0.02 was the optimum pH at which maximum separation of 13 congeners of chlorophenols and phenol was obtained. Methanol : water and methanol : phosphate buffer - pH $<$ 6 caused

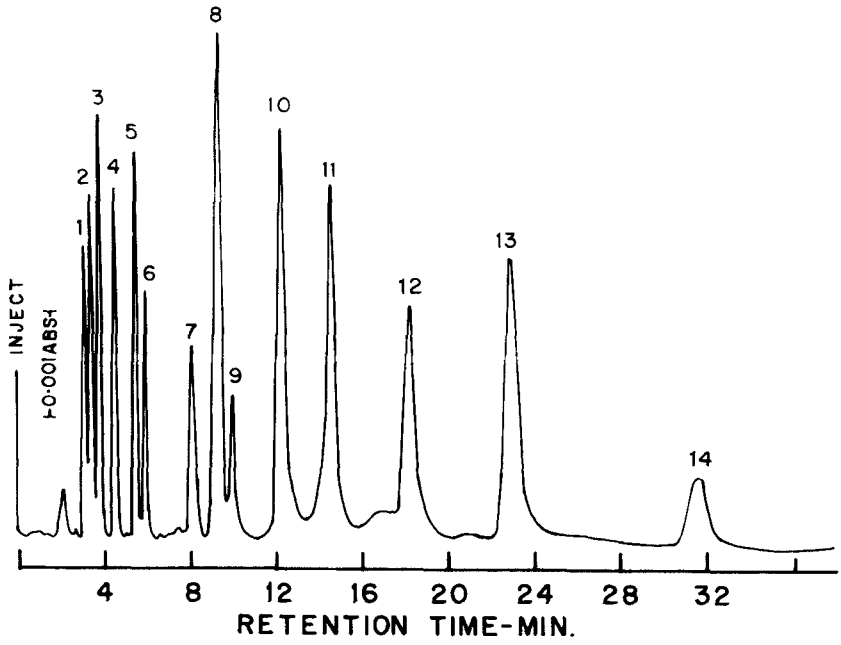

FIGURE 1. HPLC profile of the Chlorophenols and Phenol (Refer Table 1 for the Congeners of Chlorophenols).

poor resolution of the later eluting congeners. Although separation of chlorophenols have been reported using solvent with pH > 8 (4,10) we have not tried solvent system with higher pH as it might damage the reverse phase.

The retention time, response factor (peak area/ concentration) and the minimum detectable amounts of chlorophenols at 0.01 AUFS (254 nm) are given in Table 1. Pentachlorophenol has the maximum response factor followed by tetrachlorophenols, trichlorophenols and dichlorophenols. Phenol and monochlorophenols have the least response factor. It can be noted that ng of chlorophenols can be detected at 0.01 AUFS.

Table 1. Retention Time, Response Factor and Minimum Detectable Amounts of Chlorophenols at 0.01 AUFS (254 nm)

Peak No.	Phenols	Retention Time, Min.	Response Factor *	Minimum Detectable amount, ng.
1.	2,3,5,6-Tetrachloro-	3.00	72.85	5.0
2.	2,3,6-Trichloro -	3.26	67.40	5.0
3.	Pentachloro-	3.66	86.65	4.0
4.	2,4,6-Trichloro-	4.34	58.19	9.0
5.	2,6-Dichloro -	5.34	23.10	16.0
6.	Phenol	5.82	7.14	35.0
7.	2-Chloro-and	8.00	4.70	40.0
	2,3,4,5-Tetrachloro-	8.00	77.65	7.0
8.	2,3,5-Trichloro-	9.08	37.90	20.0
9.	4-Chloro-	9.90	4.72	45.0
10.	2,4,5-Trichloro-	12.08	46.76	9.0
11.	2,4-Dichloro-and	13.40	14.30	25.0
	2,3,4-Trichloro-	13.40	43.65	9.0
12.	3,4-Dichloro-	17.08	7.19	45.0
13.	3,5-Dichloro-	22.74	11.54	40.0
14.	3,4,5-Trichloro-	31.40	30.42	25.0

* Peak area $cm^2/mg \times 10^{-3}$, calculated from three concentrations.

Fig. 2 shows the HPLC profile of the chlorophenols extracted from mouse liver fed with HCH. It can be seen that 2,6-dichlorophenol was the major chlorophenol in the sample. Apart from this, 2,3,5,6-, 2,3,4,5-tetrachlorophenols, 2,3,6-, 2,4,6-, 2,3,5-trichlorophenols and pentachlorophenol were present to a lesser extent. Alkaline and acid hydrolysis

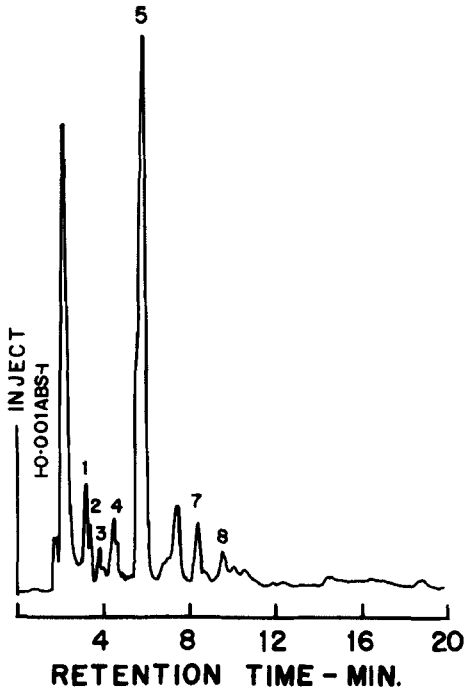

FIGURE 2. HPLC Profile of the Chlorophenols Extracted from HCH Treated Mouse Liver (Refer Table 1 for the Congeners of Chlorophenols).

followed by distillation and solvent extraction is reported to give good recovery of the chlorophenols from the biological samples (8). However, we found that some unknown compounds interfered with separation of chlorophenol if the samples were injected directly without QAE Sephadex ion exchange clean-up.

The present system is useful for separating most of the congeners of chlorophenols from a mixture. It can be used for estimating chlorophenols in biological samples where they are present as degraded products

of chlorinated pesticides. The method may further be useful for the quantitation of chlorophenols in environmental samples.

ACKNOWLEDGEMENT

The authors are thankful to the Government of India for the award of a Fellowship to one of them (K.M.M.).

REFERENCES

1. Nigam, S.K., Bhatt, D.K., Karnik, A.B., Thakore, K.N., Aravinda Babu, K., Lakkad, B.C., Kashyap, S.K. and Chaterjee, S.K., Experimental Studies on Insecticides Commonly Used in India, J.Cancer Res. Clin. Oncol., 99, 143, 1981.
2. Ito, N., Hanahochi, M., Sugimara, S., Shirai, T., Fukushima, S. and Nagasaki, H., Reversibility and Irreversibility of Liver Tumour in Mice Induced by γ-Isomer of 1,2,3,4,5,6- Hexachlorocyclohexane, Cancer Res., 36, 2227, 1976.
3. Nagasaki, H., Marugami, M., Tomi, S., Mega, T. and Ito, N., Development of Hepatoma in Mice Treated with Benzenehexachloride, Gann, 62, 431, 1971.
4. McLeod, H.A. and Laver, G., Separation of Chlorinated Phenols by Reverse Phase High-Performance Liquid Chromatography at an Alkaline pH, J. Chromatogr., 244, 385, 1982.
5. Smit, H.C., Lub, T.T. and Vloon, W.J., Application of Correlation High-Performance Liquid Chromatography to the Reverse Phase Separation of Traces Chlorinated Phenols, Anal. Chim. Acta, 122, 267, 1980.
6. Ugland, K., Lundanes, E., Greibrokk, T. and Bjorseth, A., Determination of Chlorinated Phenols by High-Performance Liquid Chromatography, J. Chromatogr., 213, 83, 1981.
7. Lores, E.M., Edgerton, T.R. and Moseman, R.F., Method of Confirmation of Chlorophenols in Human Urine by LC with an Electrochemical Detector, J. Chromatogr. Sci., 19, 466, 1981.

8. Sackmauerova-Veningerova, M., Uhnak, J., Szokolay, A. and Kocan, A., Identification of Chlorinated Phenols as Degradation Products of Chlorinated Pesticides in Biological Materials, J. Chromatogr., 205, 194, 1981.

9. Renberg, L., Ion-exchange Technique for the Determination of Chlorinated Phenols and Phenoxy Acids in Organic Tissue, Soil and Water, Anal. Chem., 46, 459, 1974.

10. Lee, D.P., Reversed - Phase HPLC from pH 1 to 13, J. Chromatogr. Sci., 20, 203, 1982.