Journal of Chromatography, 302 (1984) 159–166
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 411

SEPARATION OF POLYCHLORINATED BIPHENYLS (AROCLOR 1254) BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

H. J. ISSAQ*, J. KLOSE and G. M. MUSCHIK
Program Resources, Incorporated, NCI-Frederick Cancer Research Facility, Frederick, MD 21701 (U.S.A.)

SUMMARY

The separation of Aroclor 1254 on various reversed-phase columns was investigated. The results show that the cyano- and phenyl-bonded columns performed poorly, but C_{18} bonded columns gave better results. It was also found that the absorption wavelength at which the effluent is monitored has great effect on the detection of these isomers. The optimum conditions for the separation and detection are: C_{18}, 5μm; reversed-phase column, 20 cm × 4.6 mm I.D.; acetonitrile–water (60:40); flow-rate 1.5 ml/min; detection at 210 nm or 205 nm.

INTRODUCTION

Polychlorinated biphenyls (PCBs) are a class of chlorinated aromatic compounds which found wide industrial use due to their chemical and physical properties. As a result, it is now known that PCBs are widespread in the environment and accumulate in the food chain. It is also known that they are toxic to animals and man. The mechanism of the toxicity is not known, and renewed interest has increased in recent years in the separation of the individual PCB isomers in order to understand the mechanism of action of chlorinated biphenyls. PCBs are usually analyzed by gas–liquid chromatography (GLC) with an electron-capture detector[1,3]. The electron-capture detector response for PCB isomers shows great variation, which would result in erroneous quantitative measurements. However, Klimisch and Ingebrigston[4] showed that liquid chromatography (LC) can be used for the quantification of PCBs. Dong and DiCesare[5] showed that high speed can be attained in the analysis of PCB 1248 when a gradient of 65% to 100% acetonitrile in 7 min is used. Kaminsky and Fasco[6] used reversed-phase high-performance liquid chromatography (HPLC) to separate the isomers of Aroclor 1221, 1016 and 1254 with gradient elution and C_{18} packed columns, monitored at 254 nm. Our study deals with the separation of Aroclor 1254 on various reversed-phase columns by means of isocratic elution. The effect of column temperature on the separation and of absorption wavelength on the detection of individual isomers of Aroclor 1254 were studied.

0021-9673/84/$03.00

EXPERIMENTAL

Aroclor 1254 was obtained from Monsanto (St. Louis, MO, U.S.A.) and was used without fractionation or purification. The acetonitrile used was glass-distilled (Burdick & Jackson, Muskegon, MI, U.S.A.). Water was glass distilled, deionized, filtered through a 0.22-μm membrane filter (Millipore, Bedford, MA, U.S.A.), and degassed before use.

A liquid chromatograph, Model 1090 (Hewlett-Packard, Avondale, PA, U.S.A.) equipped with a variable-wavelength detector, a column oven, variable automatic injector, automatic sampler, and HP-85 personal computer, and a 200 × 4.6 mm I.D. column prepacked with 5μm spherical C_{18} particles (Hewlett-Packard), were used. The results were printed on an HP-3390A reporting integrator. Also, a Waters Assoc. (Milford, MA, U.S.A.) HPLC system, equipped with a Schoeffel (Schoeffel Instrument GmbH, F.R.G.) 770 6M monochromator and SF 770 spectro flow monitor, and U6K injector (Waters Assoc.) was used. The columns were packed with different reversed-phase materials and different size particles. These included: Hewlett-Packard, spherical, 5μm, C_{18} particles, 20 cm × 4.6 mm; Waters Assoc. μBondapak C_{18} particles, 10 μm, 30 cm × 3.9 mm; Clear Sil-ODS, spherical, C_{18} particles, 10 μm, 25 cm × 4.0 mm (R. E. Gourly, Laurel, MD, U.S.A.) Vydac, C_{18}, 10 μm, 30 cm × 3.9 mm (The separations group, Hespera, CA, U.S.A.). A Spherosorb cyano-bonded column, 5 μm, 30 cm × 3.9 mm (Phase Separations, Norwalk, CT, U.S.A.) and Spherosorb phenyl-bonded column, 5 μm, 30 cm × 3.9 mm (Phase Separations). The last three columns were slurry-packed by R. E. Gourley, by the upward technique at 8000 p.s.i. with a pneumatic pump.

Aroclor 1254 was dissolved in acetonitrile to give a concentration of 5 μg/μl. Experimental details are given in the figure legends.

RESULTS AND DISCUSSION

The separation of Aroclor 1254 into its individual isomers by HPLC did not result in the resolution of all the 65 isomers present[7]. Gradient elution with acetonitrile–water seems to be the preferred HPLC procedure[5,6]. In the present study the use of isocratic elution from various columns of different manufacturers was investigated in order to achieve the best separation possible. Figs. 1–6 show the separation of Aroclor 1254 by acetonitrile–water (60:40) at room temperature on various types of column. It is clear that the μBondapak C_{18} column performed better than the others (Fig. 6), except the HP C_{18} column, which gave better overall separation (Fig. 5). Although the total volume is virtually the same for the Waters and HP columns, while the particle size is different (5 μm for HP, 10 μm for μBondapak), the results are different.

The effect of the absorption wavelength on the detection was investigated, since detection at 254 nm[6], at 210 nm[5], and at 205 nm[8] has been reported. It was found that the 254 nm absorption wavelength is the least sensitive for the detection of Aroclor 1254 isomers. Figs. 6–9 show the chromatograms of Aroclor 1254, monitored at 254 nm (Fig. 7), 234 nm (Fig. 8), 210 nm (Fig. 6) and 205 nm (Fig. 9). The detection limit increases ten-fold or more for certain isomers when monitored at 210 nm or 205 nm instead of 254 nm. For example, at 254 nm the isomers that are eluted between

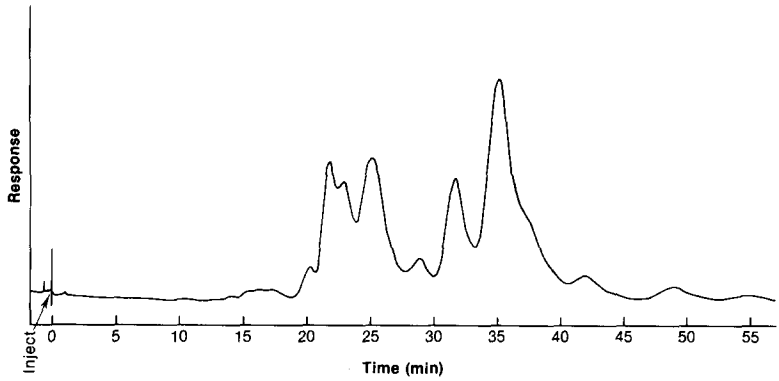

Fig. 1. HPLC separation of Aroclor 1254 (5 μg/μl) on a Clear Sil-ODS column (250 × 4 mm I.D.) using acetonitrile–water (60:40) at a flow-rate of 1.5 ml/min, monitored at 210 nm. Injection volume, 2 μl.

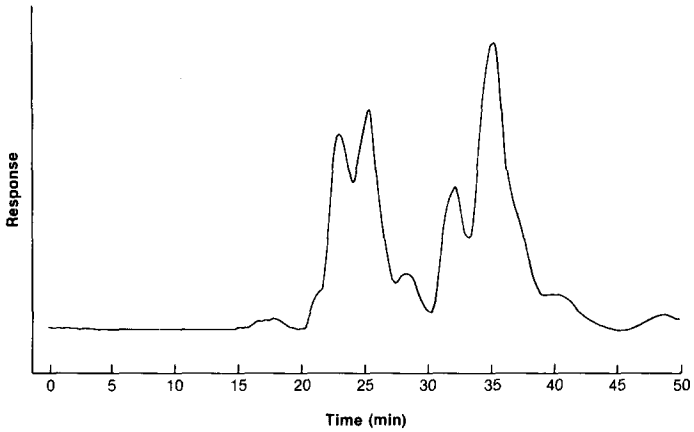

Fig. 2. Conditions as in Fig. 1, except that a Vydac C_{18} column (300 × 3.9 mm I.D.) was used at a mobile-phase flow-rate of 1 ml/min.

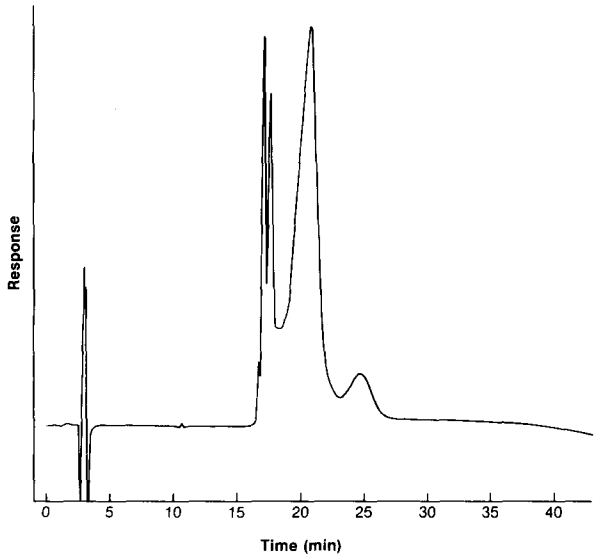

Fig. 3. Conditions as in Fig. 2, except that a cyano-bonded column was used.

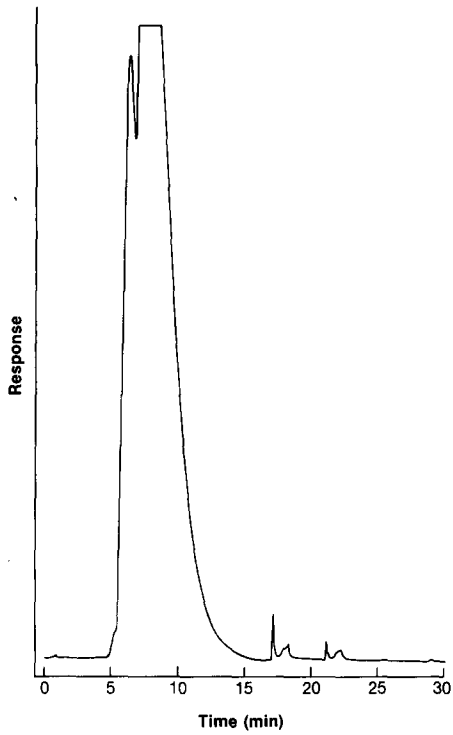

Fig. 4. Conditions as in Fig. 2, except that a phenyl-bonded column was used.

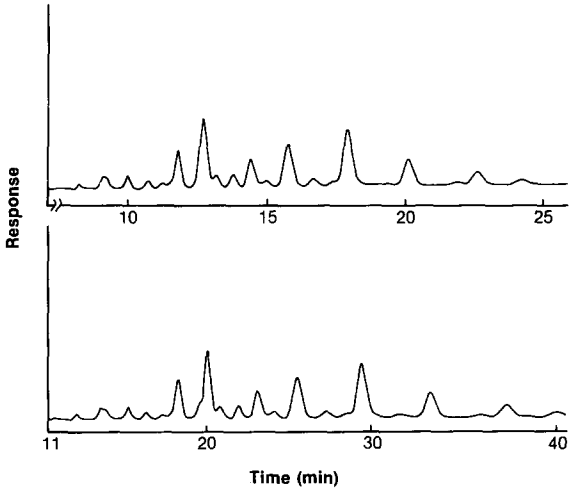

Fig. 5. Conditions as in Fig. 1, except that a 5 μm, C_{18} bonded Hewlett-Packard column was used, and 0.2 μl (1 μg) was injected.

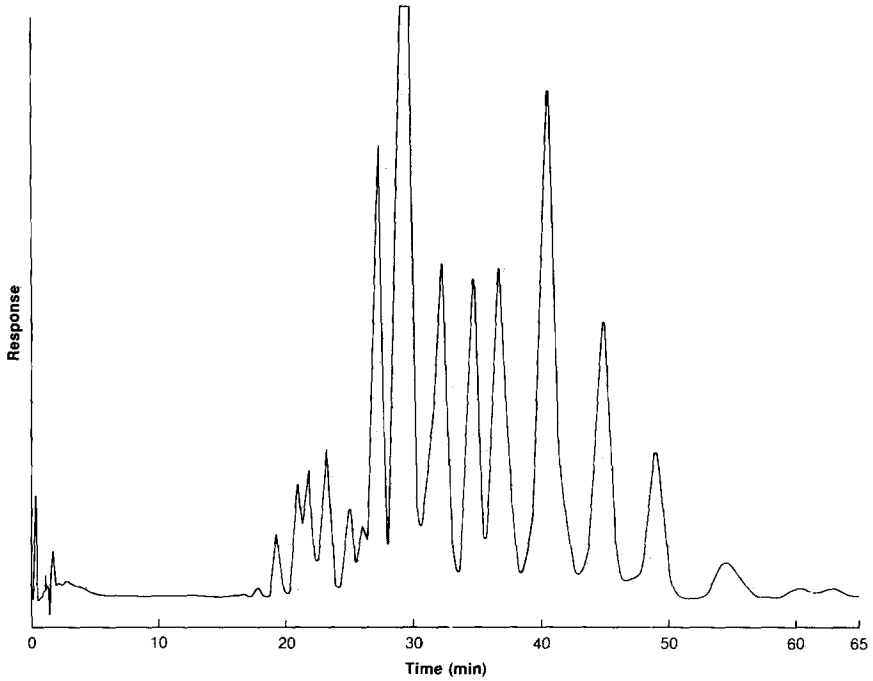

Fig. 6. Conditions as in Fig. 1, except that a μBondapak C_{18} column was used.

18 and 26 min (Fig. 7) were not detected, while at 234 (Fig. 8) some of them are detected and at 210 and 205 nm (Figs. 6 and 9) they are clearly detected. The same is true for the isomers eluted between 31 and 36 mins and between 45 and 55 min. Therefore, the recommended detection wavelength for Aroclor 1254 should be 205–210 nm.

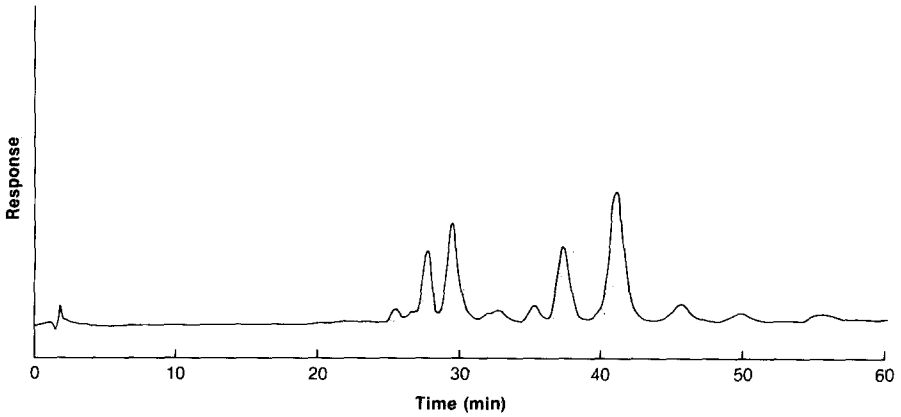

Fig. 7. Conditions as in Fig. 6, except that detection was monitored at 254 nm.

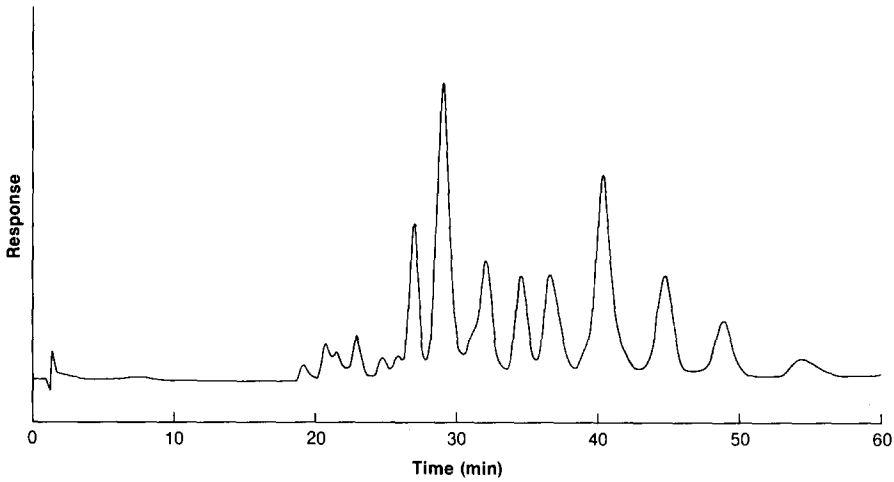

Fig. 8. Conditions as in Fig. 6, except that detection was monitored at 234 nm.

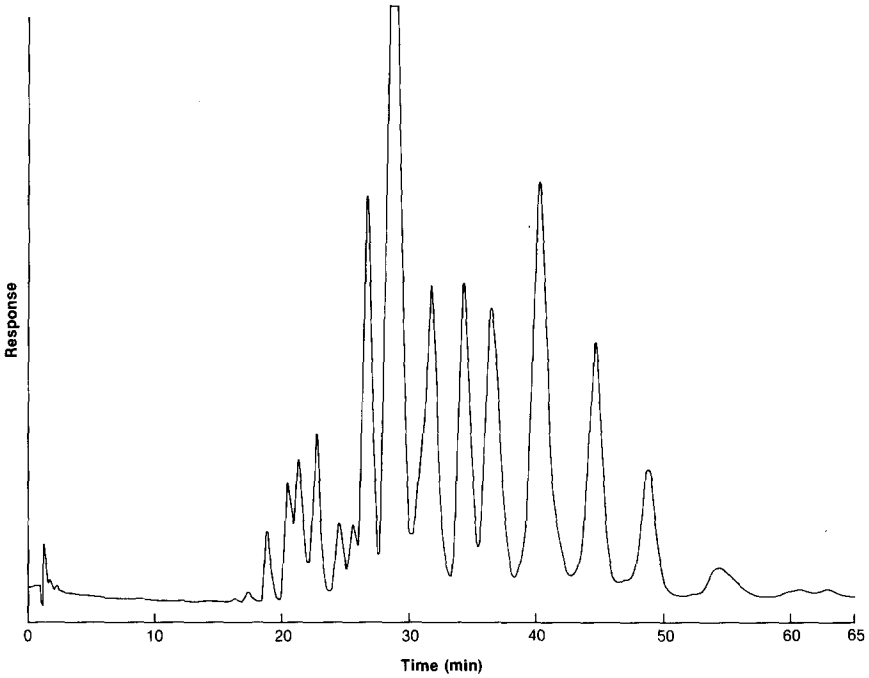

Fig. 9. Conditions as in Fig. 6, except that detection was monitored at 205 nm.

The effect of temperature on the separation and mobile phase composition was investigated. The results (Fig. 10) show that better resolution and sensitivity of the Aroclor 1254 isomers are achieved at 50°C than at 30°C, and the analysis time is shorter. Also, Fig. 10 shows that although acetonitrile–water (70:30) was used at

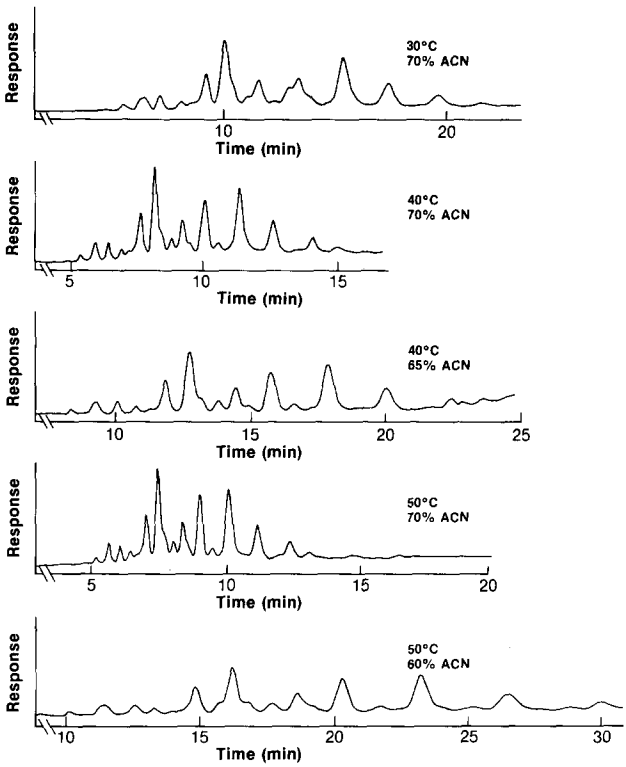

Fig. 10. Effect of temperature on the separation of Aroblor 1254 on a 5 μm, C_{18} column with variable organic solvent concentrations, as specified; 0.2 μl (1 μg) of Aroclor 1254 was injected. ACN = acetonitrile.

30°C, the chromatographer can decrease the percentage of organic solvent in the mobile phase as the temperature of the column increases, and still achieve comparable results, *e.g.* acetonitrile–water (60:40) at 50°C.

In a previous study[9] it was observed that short columns, 3 cm and 5 cm, packed with 3 μm and 5 μm particles were not adequate for the separation of Aroclor 1254 isomers.

CONCLUSION

The separation of the Aroclor 1254 isomers by HPLC is governed by the column used, the temperature of the column and the mobile phase. The optimum conditions found are: a C_{18} reversed-phase 5 μm Hewlett-Packard 20 cm × 4.6 mm I.D. column and an isocratic mobile phase of acetonitrile–water (60:40) at a detection wavelength of 205–210 nm.

ACKNOWLEDGEMENTS

The authors thank Kimberly Lindsey for technical support. Research sponsored by the National Cancer Institute, DHHS, under Contract No. N01-CO-23910

with Program Resources, Inc. The content of this publication do not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

REFERENCES

1 D. Sissons and D. Welti, *J. Chromatogr.*, 60 (1971) 15.
2 P. W. Albro, J. K. Haseman, T. A. Clemmer and B. J. Corbett, *J. Chromatogr.*, 136 (1977) 147.
3 E. Schulte and L. Acher, *Z. Anal. Chem.*, 268 (1974) 260.
4 H. M. Klimisch and D. N. Ingebrigston, *Anal. Chem.*, 52 (1980) 1675.
5 M. W. Dong and J. L. DiCesare, *J. Chromatogr. Sci.*, 20 (1982) 517.
6 L. S. Kaminsky and M. J. Fasco, *J. Chromatogr.*, 155 (1978) 363.
7 S. N. Chesler, W. E. May, P. A. White, R. M. Parris and F. R. Guenther, *National Bureau of Standards Special Publication, 584*, Washington D.C., 1980, pp. 295–297.
8 H. P. M. van Vliet, T. C. Bootsman, R. W. Frei and U. A. T. Brinkman, *J. Chromatogr.*, 185 (1979) 483.
9 H. J. Issaq, *J. Liquid Chromatogr.*, 7 (1984) 883.