Journal of Chromatography, 267 (1983) 183–189
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 15,932

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF CHLOROPHENOLS IN CARDBOARD FOOD CONTAINERS AND RELATED MATERIALS

SHA SHANG-ZHI
Shanghai Light Industries Research Institute, Shanghai (China)
and
G. STANLEY*
CSIRO Division of Food Research, P.O. Box 52, North Ryde, NSW 2113 (Australia)
(Received April 18th, 1983)

SUMMARY

A technique has been developed for the extraction of a clean concentrate of chlorophenols from cardboard food containers and adhesives of the type used in their manufacture. A simple, reversed-phase, isocratic high-performance liquid chromatographic (HPLC) system employing an optimised mobile phase permitted the separation of nineteen different phenols. The use of a carefully selected internal standard permitted the estimation of (sub) parts per million levels of pentachlorophenol, 2,3,4,6-tetrachlorophenol and 2,4,6-trichlorophenol in several samples of adhesives and various sections of cardboard food containers. Pentachlorophenol (50 ng) and 2,4,6-trichlorophenol (20 ng) gave significant HPLC peaks, readily permitting their detection in parts per billion from 100-g samples.

INTRODUCTION

Chlorophenols and their salts have been manufactured and used extensively as fungicides and bacteriocides in industry throughout the world. Some chlorophenols are also formed by the reaction of chlorinated (municipal) water supplies with phenols in the environment, and also by the degradation of chlorinated pesticides[1]. Food contaminated with chlorophenols has an objectionable disinfectant-like taste, and these compounds are detectable at the ppb level[2]. Furthermore, many moulds and bacteria are capable of methylating chlorophenols to produce chloroanisoles with characteristic mouldy or musty tastes detectable at even lower concentrations[3]. Recent complaints of disinfectant taints and mouldy or musty flavours in foods packaged in cardboard cartons necessitated the development of techniques for the analysis of chlorophenols in cardboard and in the starch and polyvinyl acetate (PVA) adhesives used in carton manufacture.

Gas chromatography (GC) with electron-capture (ECD) or mass spectrometric detection provides a very sensitive and selective means of analysing chlorinated com-

0021-9673/83/$03.00

pounds. The chlorophenols, however, are highly polar and the minute amounts frequently encountered in food analysis are easily lost by adsorption in the GC column unless special precautions are taken[4,5]. Derivatization of the chlorophenols prevents such adsorption at the expense of an extra and undesirable step, frequently employing very toxic chemicals. Recent developments in high-performance liquid chromatography (HPLC) offer a versatile mode of analysis for underivatized chlorophenols[1,6–9]. A reversed-phase isocratic system appears to be well suited to the analysis of these compounds. The apparatus for isocratic elution is simpler, less expensive and more suited to routine analysis than that required for the gradient system, and the time saved with a gradient elution is frequently lost whilst re-equilibrating the column and stationary phase between successive analyses. A C_{18} column and a methanol–acetonitrile–water mobile phase was found to separate phenol, seventeen chlorophenol congeners and 3-nitrophenol in less than 30 min.

To ensure reliable results and long column life a very thorough sample clean-up procedure is required. It should have a high degree of specificity for the chlorophenols, it should concentrate the sample and should be flexible, permitting a wide range of sample types to be handled. Extraction of the chlorophenols by steam distillation from an acidified solution[10,11], combined with solvent washing (whilst alkaline) to remove unwanted neutral compounds and concentration by Sep-Pak cartridge filtration[12,13], was found to meet these requirements.

The HPLC detector should be both sensitive and specific. It has been shown[6] that UV detection at 280 nm is more specific for the chlorophenols than the commonly used 254 nm or the more sensitive 220–230 nm.

The internal standard used to quantify the chlorophenol analysis should be chosen carefully. It must have similar properties to the chlorophenols so that it will follow them through the entire extraction and clean-up procedure and it must run as a single peak in the chromatogram, free from interfering compounds. We found that 2,4,6-trichloro-3,5-xylenol and 2,4,6-tribromophenol met these requirements in most instances.

EXPERIMENTAL

Equipment and chemicals

The HPLC equipment consisted of a Kortec K35 twin-piston pump, a Rheodyne 7125 injection valve with a 20-μl sample loop, a BDH LiChrosorb RP-18 column (250 × 4 mm, particle size 10 μm), a Dupont 860 UV detector (280 nm) and a Leeds and Northrup XL 680 recorder (10 mV F.S.D.). The preferred mobile phase was methanol–acetonitrile–water (58:7:35), pH 3.5, and the flow-rate was 1.1 ml/min. Unichrom HPLC-grade solvents and analytical-reagent quality reagents from Unilab were used. Chlorophenol standards were obtained from Fluka and the internal standards (2,4,6-trichloro-3,5-xylenol and 2,4,6-tribromophenol) were laboratory-synthesised and purified. Four separate standard solutions were prepared containing 2,4,6-trichlorophenol (2,4,6-TCP) ($4.04 \cdot 10^{-4}$ g/ml), 2,3,4,6-tetrachlorophenol (2,3,4,6-TeCP) ($7.50 \cdot 10^{-4}$ g/ml), pentachlorophenol (PCP) ($8.50 \cdot 10^{-4}$ g/ml) and 2,4,6-trichloro-3,5-xylenol internal standard ($8.58 \cdot 10^{-4}$ g/ml) in methanol. Sep-Pak C_{18} cartridges (Waters Assoc.) were washed with methanol (2 ml) and water (20 ml) before use.

Extraction and clean-up procedure

Cardboard boxes were torn into small pieces (by hand) and 100 g were weighed into a distillation flask. Distilled water (1.5 l) was added and the mixture was acidified (pH 2) with 2 *M* sulphuric acid and allowed to stand until the cardboard started to soften (about 1 h). The mixture was distilled and 500 ml of distillate were collected. For the analysis of starch and PVA adhesives a 10-g sample was used, 150 ml water were added and 50 ml of distillate were collected. The distillate containing the chlorophenols was made alkaline (pH $\geqslant$ 10) with sodium hydroxide solution and the internal standard solution (20 μl) was added. The (now) alkaline distillate was washed with 70 ml, then 50 ml of *n*-pentane to remove neutral compounds, concentrated to 50–80 ml in a rotary evaporator at 75°C, acidified to pH 2–3 with 2 *M* sulphuric acid and passed through a Sep-Pak C_{18} cartridge. The cartridge was washed with distilled water (20 ml) and the chlorophenols were eluted with methanol (3 ml). The chlorophenols were finally concentrated by making the methanolic solution alkaline with sodium hydroxide solution, evaporating almost to dryness and then adding acetic acid–isopropanol (1:1) to a final volume of 100 μl. Twenty μl of this solution were used per HPLC injection.

Calibration

Aliquots of 10–60 μl of each of the chlorophenol standard solutions were added separately to 1.5 l of distilled water, the internal standard solution (20 μl) was added and the mixture was distilled and the recovered chlorophenols were worked up and chromatographed as described above. From each chromatogram the ratio of the peak heights for the chlorophenols to the peak height for the internal standard was measured and plotted against the weight of chlorophenol to give the calibration curve in Fig. 1. A complete blank showed no interference from the reagents or the water used for the analysis.

Recovery test and limit of detection

Chlorophenol-free filter-paper (150 g) was impregnated with known weights of 2,4,6-TCP (8 μg), 2,3,4,6-TeCP (15 μg) and PCP (17 μg) and analysed. The results of eight replicate analyses showed a mean recovery of 91% (standard deviation S.D. $\pm$ 10%) for 2,4,6-TCP, 95% (S.D. $\pm$ 7%) for 2,3,4,6-TeCP and 96% (S.D. $\pm$ 8%) for

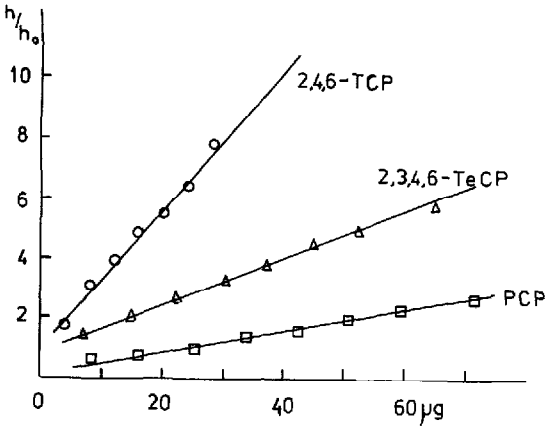

Fig. 1. Calibration. h and h_0 are peak heights for the chlorophenols and the internal standard respectively.

PCP. The limit of detection varies for the different chlorophenol congeners and is also dependent on the UV wavelength used for measurement and on the peak sharpness. With a clean sample we could detect 20 ng of 2,4,6-TCP or 50 ng of PCP per HPLC injection of 20 μl at 280 nm, thus permitting detection well into the ppb range. Higher sensitivity could be obtained at 220 nm with considerable loss in specificity.

RESULTS AND DISCUSSION

The chlorophenols are weak acids with pK_a values ranging from 9.8 for 4-chlorophenol to 4.9 for PCP[14]. To chromatograph the chlorophenols satisfactorily using a reversed-phase HPLC system, the pH of the mobile phase should be adjusted with acid so that the difference pK_a − pH exceeds 2 (ref. 15). At pH values close to pK_a, peak broadening and varying retention times are observed, particularly with PCP. Fig. 2 shows the effect of pH on the relative retention times of five chlorophenols, and indicates that the pH of the mobile phase should be less than 4 to ensure stable retention times.

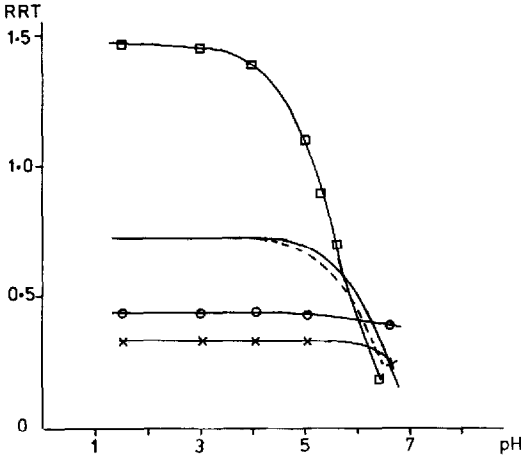

Fig. 2. Effect of pH on the relative retention time (RRT) of chlorophenols; ——, 2,3,4,6-TeCP; – – –, 2,3,5,6-TeCP; □—□, PCP; ○—○, 2,4,6-TCP; ×—×, 2,3,6-TCP.

TABLE I

EFFECT OF MOBILE PHASE COMPOSITION ON RESOLUTION AND ANALYSIS TIME

No.	*Composition (%)*			*Resolution**	*Analysis** time (min)*
	Water	*Methanol*	*Acetonitrile*		
1	30	70		1.60	19
2	35	65		1.64	30
3	35	60	5	1.74	28
4	35	58	7	1.84	25

* Measured using peaks for 2,3,4,6-TeCP and 2,4,6-tribromophenol.

** Time to PCP peak.

Obviously the separation of chlorophenols in a reversed-phase HPLC system is not only dependent on pH, but also on the mobile phase composition. Simple methanol–water mixtures containing more than 70% methanol gave incomplete resolution of the chlorophenols; diluting the mixture below 70% improved the resolution slightly at the expense of greatly increased analysis time, peak broadening and loss of sensitivity. Acetonitrile–water–acetic acid mixtures[16] and acetonitrile–methanol–phosphate buffer[1] have been used for the reversed-phase separation of the chlorophenols. We found that replacement of some of the methanol with acetonitrile (up to 7% of the mobile phase) gave a considerable improvement in resolution without sacrificing analysis time, as shown in Table I. Fig. 3 shows the separation of phenol, 4-nitrophenol, 2,4,6-trichloro-3,5-xylenol and sixteen chlorophenols using mobile phase 4, *i.e.*, methanol–acetonitrile–water (58:7:35) at pH 3.5. Under these conditions 2,3,4,6-TeCP and 2,3,5,6-TeCP do not separate; however, by adjusting the pH of mobile phase 4 to 5.6, it is possible, as suggested by Fig. 2, to obtain near baseline resolution of this pair of congeners from a simple mixture of reference compounds, as shown in Fig. 4.

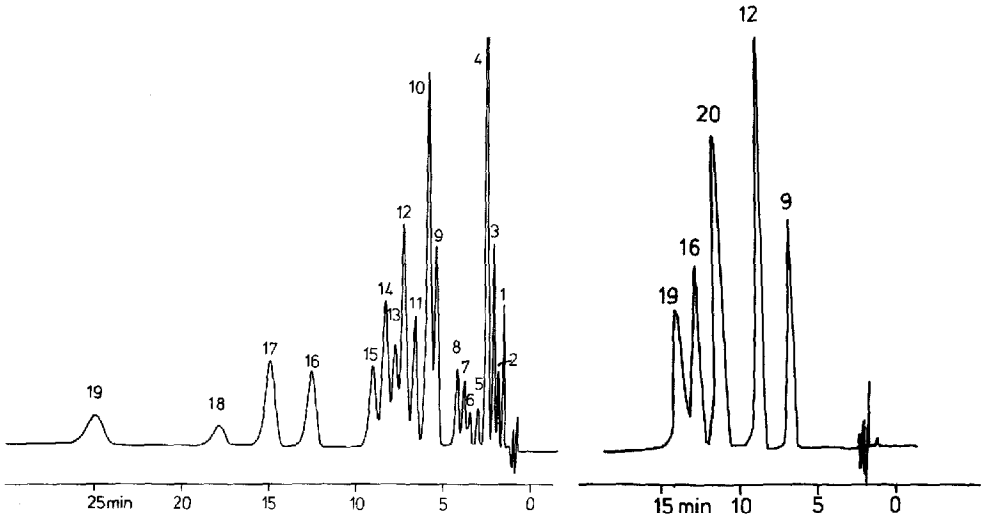

Fig. 3. Standard test mixtures. For HPLC conditions refer to text. Peaks: 1 = phenol; 2 = 4-nitrophenol; 3 = 2-chlorophenol; 4 = 4-chlorophenol; 5 = 2,6-dichlorophenol (DCP); 6 = 2,3-DCP; 7 = 2,5-DCP; 8 = 2,4-DCP; 9 = 2,3,6-TCP; 10 = 3,5-DCP; 11 = 2,3,4-TCP; 12 = 2,4,6-TCP; 13 = 2,4,5-TCP; 14 = 2,3,5-TCP; 15 = 3,4,5-TCP; 16 = 2,3,4,6-TeCP; 17 = 2,3,4,5-TeCP; 18 = 2,4,6-trichloro-3,5-xylenol; 19 = PCP.

Fig. 4. Separation of 2,3,4,6-TeCP (16) and 2,3,5,6-TeCP (20) at pH 5.6.

The sides, lid, bottom and liner of a cardboard container suspected of contaminating the food content were analysed separately for chlorophenols. Fig. 5 is a typical chromatogram for the analysis using mobile phase 4 and the results for the assay of 2,4,6-TCP, 2,3,4,6-TeCP and PCP are listed in Table II.

Peak assignment for the three compounds assayed was verified by collecting the HPLC separated compounds and analysing them by GC–ECD as their acetates. The high level of chlorophenols found in the container lid was in agreement with the relatively high level of the off-flavour in the top layer of the food.

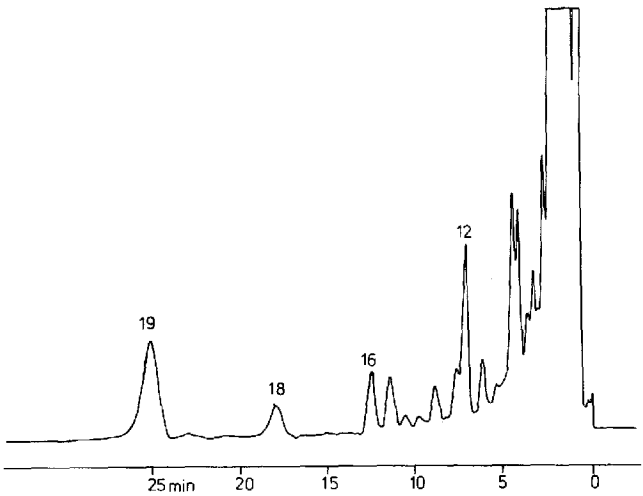

Fig. 5. A typical chromatogram for the analysis of a cardboard food container. HPLC conditions as in Fig. 4 and text.

TABLE II

ANALYSIS OF CARDBOARD FOOD CONTAINER

Part of box	*2,4,6-TCP (ppm)*	*2,3,4,6-TeCP (ppm)*	*PCP (ppm)*
Lid	0.68	0.55	0.60
Bottom	0.10	0.18	0.75
Sides	0.12	0.18	0.81
Liner	0.13	0.14	0.72

PVA and starch adhesives used in the manufacture and sealing of the containers are a likely source of chlorophenols which could have been added to the adhesives as fungicides or bacteriocides. Seven samples of starch adhesive and three of PVA were analysed for chlorophenols. Five of the starch samples contained significant quantities of 2,3,4,6-TeCP and PCP, one starch sample contained exceptionally high levels of 2,3,4,6-TeCP and PCP, and one had no detectable chlorophenols (Table III). No chlorophenols were detected in the three PVA samples examined.

The presence of 2,3,4,6-TeCP and PCP in samples of cardboard and adhesives indicates an industrial source for these compounds rather than a natural or accidental source such as the chlorination of environmentally occurring phenol by chlorinated (municipal) water supplies, which would only yield mono-, di- and trichlorophenols.

CONCLUSIONS

A successful procedure for obtaining a clean, concentrated sample of chlorophenols from cardboard food containers and related adhesives has been developed. The HPLC conditions for a simple isocratic separation of the chlorophenols have

TABLE III

ANALYSIS OF STARCH ADHESIVES

ND = Not detected.

Sample no.	*2,3,4,6-TeCP (ppm)*	*PCP (ppm)*
1	30–50	>80
2	ND	ND
3	0.017	0.2
4	2.7	4.2
5	0.04	0.04
6	0.02	0.02
7	0.41	0.02

been examined and the importance of careful control of the pH and composition of the mobile phase have been illustrated. Careful choice of internal standards has permitted the quantitation of 2,4,6-TCP, 2,3,4,6-TeCP and PCP at the parts per billion level. High levels of chlorophenols, particularly 2,3,4,6-TeCP and PCP, were found in samples of cardboard and starch adhesive, indicating an industrial source for this contamination.

ADDENDUM

While this manuscript was in preparation a related HPLC analysis of urine was published[17] showing a separation of four chlorophenols, including 2,3,4,5-TeCP and 2,3,4,6-TeCP, using a methanol–water gradient-elution system in which the pH of the mobile phase was slightly increased by the addition of ammonium carbonate. The degree of separation obtained is similar to our findings and in agreement with our observation on the effect of pH on the resolution of this pair of compounds (Fig. 3).

REFERENCES

1 E. M. Lores, T. R. Edgerton and R. F. Moseman, *J. Chromatogr. Sci.*, 19 (1981) 466.
2 F. Dietz and J. Traud, *Gas-Wasserfach, Wasser/Abwasser*, 119 (1978) 318.
3 N. M. Griffiths, *Chem. Senses Flavor*, 1 (1974) 187.
4 T. E. Edgerton and R. F. Moseman, *J. Chromatogr. Sci.*, 18 (1980) 25.
5 M. A. White and K. R. Parsley, *Biomed. Mass Spectrom.*, 6 (1979) 570.
6 K. Ugland, E. Lundanes, T. Greibrokk and A. Bjørseth, *J. Chromatogr.*, 213 (1981) 83.
7 H. A. McLeod and G. Laver, *J. Chromatogr.*, 244 (1982) 385.
8 H. C. Smit, T. T. Lub and W. J. Vloon, *Anal. Chim. Acta*, 122 (1980) 267.
9 K. Ogan and E. Katz, *Second Chemical Congress of the North American Continent, Division of Analytical Chemistry, Las Vegas, August 24–29, 1980*, Paper No. 035.
10 A. Stark, *J. Agr. Food. Chem.*, 17 (1969) 871.
11 M. G. Gee, D. G. Land and D. Robinson, *J. Sci. Food Agr.*, 25 (1974) 829.
12 L. Renberg and K. Lindström, *J. Chromatogr.*, 214 (1981) 327.
13 D. E. Mundy and A. F. Machin, *J. Chromatogr.*, 216 (1981) 229.
14 *Kirk-Othmer Encyclopedia of Chemical Technology*, Vol. 5, Wiley, New York, 3rd ed., 1979, p. 864.
15 W. Butte, C. Fooken, R. Klussmann and D. Schuller, *J. Chromatogr.*, 214 (1981) 59.
16 C. R. Daniels and E. P. Swan, *J. Chromatogr. Sci.*, 17 (1979) 628.
17 K. Pekari and A. Aitio, *J. Chromatogr.*, 232 (1982) 129.