Journal of Chromatography, 350 (1985) 407–416
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 168

DETERMINATION OF PHENOLS IN WATER AND WASTEWATER BY POST-COLUMN REACTION DETECTION HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

F. P. BIGLEY and R. L. GROB*
Villanova University, Villanova, PA 19085 (U.S.A.)
(First received May 2nd, 1985; revised manuscript received September 5th, 1985)

SUMMARY

The analysis of phenols using high-performance liquid chromatography and post-column reaction detection has been studied. The system design utilizes the reaction of ten of the phenols on the U.S. Environmental Priority Pollutant List with 4-aminoantipyrine and potassium ferricyanide. The chromophores that are produced can be quantified at either 509 nm or 470 nm depending upon the structure of the eluting phenol.

The separation procedure uses a linear gradient consisting of 0.2% phosphoric acid and methanol. The phosphoric acid concentration is varied from 90% to 20% in 20 min providing for resolution of each of the ten phenols.

The concentration of each of the reagents and the pH used for the post-column reaction have been studied in order to optimize the post-column quantification.

In addition two methods for concentrating the phenols in water and wastewater have been examined. The recovery and repeatability of these are presented. A series of influent and effluent samples from a wastewater treatment plant have been assayed using the method presented. The results of this study are discussed.

INTRODUCTION

Phenols are generated by a number of processes[1], including the petroleum industry[2], the pulp and paper industry[3], and in the syntheses of plastics and pharmaceuticals[4]. Chlorinated phenols have been used as insecticides, antiseptics and disinfectants, and have been found in drinking water following chlorination[5]. As a result of the toxicity of these compounds, the U.S. Environmental Protection Agency (USEPA) has included eleven phenols among the list of compounds on the Priority Pollatant list[6,7].

Several methods for the quantification of phenols have been reported. Lamparski and Nestrick[8] have reported the gas chromatographic (GC) analysis of phenol and substituted phenols following the production of the heptafluorobutyryl derivatives in a benzene extract. Hoshika and Muto[9] have reported the separation and quantification of eight phenols by GC after conversion to their corresponding bromo-

0021-9673/85/$03.30

phenols. Coutts *et al.*[10] formed the acetate esters of six phenols before extraction with methylene chloride from water and analysis by GC. These were shown to be quantitatively extracted.

Realini[4] was able to separate the phenols on the priority pollutant list using high-performance liquid chromatography (HPLC). Preconcentration was accomplished by extracting the phenols into methylene chloride using tetrabutylammonium chloride as an ion pairing agent. The liquid chromatographic separation of chlorophenols has been reported by McLeod and Laver[11] using reversed-phase HPLC (RP-HPLC). The mobile phases used in their work covered the pH range from 7.4 to 12.0. Their procedures provide for the separation of thirteen of the nineteen congeners of the chlorophenols by an isocratic procedure. Additionally, Shoup and Mayer reported the detection of trace phenols in the ppt* range using liquid chromatography–electrochemistry[12].

In addition to the separation procedures, Norwitz and co-workers[13,14] have extensively studied the reaction of phenols with 4-aminoantipyrine and potassium ferricyanide. This same reaction was used as a pre-column derivatization procedure in order to enhance the extraction of the more polar phenols from water and to increase the sensitivity of the method[15].

Werkhoven-Gowie *et al.*[16] have reported an enrichment factor of >300 using a precolumn concentration procedure with a divinylbenzene–styrene copolymeric sorbent to remove chlorophenols from water. Following post-column photochemical dichlorination, the fluorescent products formed were detected.

In this study, the use of 4-aminoantipyrine has been investigated in the post-column reaction of ten priority pollutant phenols following reversed-phase liquid chromatography. Two procedures using solid phase extraction to concentrate the phenols studied in this work from water and wastewater have been investigated.

EXPERIMENTAL

Reagents and equipment

The phenol standards were purchased from Chem Services (West Chester, PA, U.S.A.), the potassium ferricyanide from Merck (Rahway, NJ, U.S.A.), and the 4-aminoantipyrine from Aldrich (Milwaukee, WI). The methanol and phosphoric acid were HPLC grade and purchased from Fisher Scientific (Fair Lawn, NJ, U.S.A.) as was the reagent grade sodium chloride and ammonium acetate. The 0.1 *M* ammonium acetate buffer was prepared by adding 7.70 g of this reagent to approximately 900 ml of distilled water. The solution pH was adjusted with ammonium hydroxide.

Stock standards (1000 ppm) were prepared by accurately weighing 100 mg of each of the phenol standards into separate 100-ml volumetric flasks and diluting to volume with methanol. Working standards were prepared by diluting 1 ml of each of these standards to 100 ml with methanol just prior to use (10 ppm). These were further diluted with water as needed. The separation was performed using a Zorbax ODS column from DuPont (Wilmington, DE, U.S.A.). Pre-column concentration studies were conducted with a cyclohexyl bonded phase packing in 1.0 g Bond Elut columns from Analytichem International (Harbor City, CA, U.S.A.) and were from

* Throughout this article the American trillion (10^{12}) is meant.

lot number 09662. The samples were prepared using the Bond Elut vacuum manifold from the same company.

The chromatographic separations were carried out on either a Hewlett-Packard Model 1084B liquid chromatograph (Palo Alto, CA, U.S.A.) or a Perkin Elmer Series 4 liquid chromatograph (Norwalk, CT, U.S.A.) equipped with a Hewlett-Packard Model 1040A diode array spectrophotometer. The post-column reaction was performed by pumping potassium ferricyanate solution with a Kratos (West Wood, NJ, U.S.A.). Spectro Flow 400 pump and the 4-aminoantipyrine reagent with a DuPont Model 870 pump that was connected to a "low pulse" pulse dampener from Scientific Systems (State College, PA, U.S.A.). Integration was done either with the integrator on the Hewlett-Packard 1084B or with two Hewlett-Packard Model 3392A integrators attached to the Model 1040A spectrophotometer.

Analytical methodology

The ten phenols were separated on a Zorbax ODS column (DuPont) with 0.2% phosphoric acid and methanol. A linear gradient from 10% to 80% methanol was used over 20 min. The column was held at 50°C throughout the analysis. Following the separation, the initial conditions were established in 5 min and the column was re-equilibrated for an additional 10 min. As shown in Fig. 1, the resolution of each

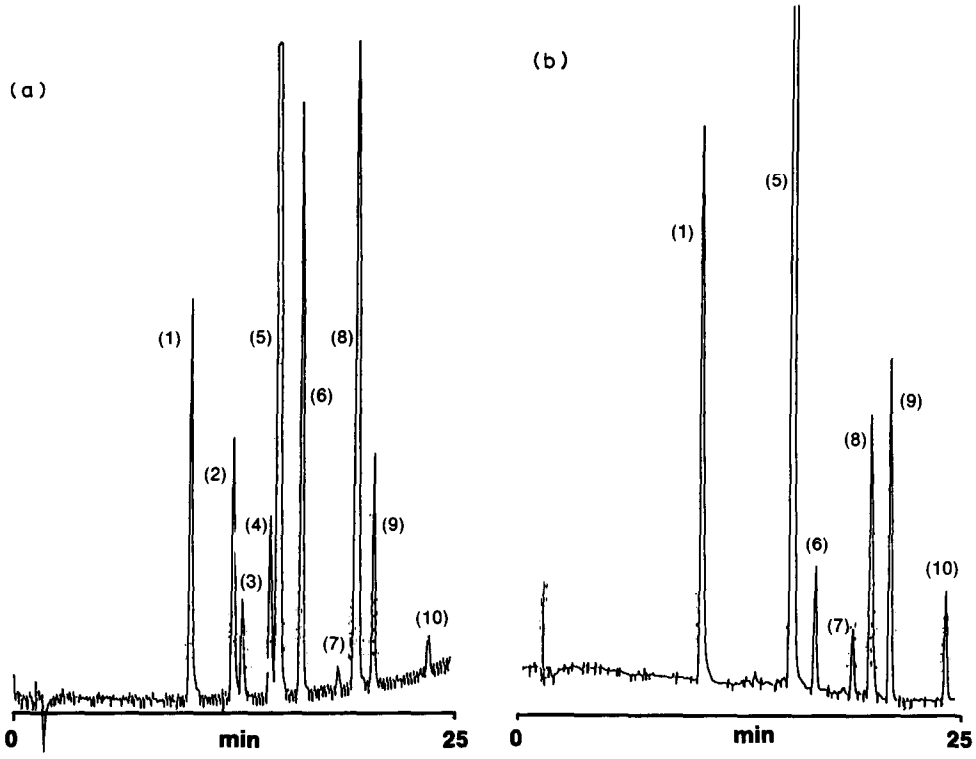


Fig. 1. HPLC Separation of the phenols studied using post-column reaction. (a) Detection at 470 nm. (b) Detection at 509 nm. Peak identification 1 = phenol; 2 = *p*-nitrophenol; 3 = 2,4-dinitrophenol; 4 = *o*-chlorophenol; 5 = *o*-nitrophenol; 6 = 2,4-dimethylphenol; 7 = 4-chloro-*m*-cresol; 8 = 4,6-dinitro-*o*-cresol; 9 = 2,4-dichlorophenol; 10 = 2,4,6-trichlorophenol.

TABLE I

SUMMARY OF DATA OBTAINED FOR EACH OF THE PHENOLS STUDIED DURING METHOD DEVELOPMENT

Compound	*k'*	*Relative peak height vs. phenol at 10 ppm*		*R.S.D. 10 ppm (%)*		*Correlation coefficient*		*Lowest detectable limits (ppm)*		*Analytical wavelength*
		470 nm	*509 nm*	*470 nm*	*509 nm*	*470 nm*	*509 nm*	*470 nm*	*509 nm*	
Phenol	4.07	1.00	1.23	14.80	6.16	0.999	1.000	2.50	0.50	509
p-Nitrophenol	5.15	0.84	–	6.90	–	0.998	–	3.54	–	470
2,4-Dinitrophenol	6.24	0.36	–	8.25	–	0.997	–	2.20	–	470
o-Chlorophenol	6.58	0.63	–	8.99	–	0.999	–	5.02	–	470
o-Nitrophenol	7.22	6.98	10.87	1.55	1.56	0.997	0.992	0.62	0.08	509
2,4-Dimethylphenol	7.98	1.91	0.44	1.66	4.89	1.000	0.9999	1.92	2.44	470
4-Chloro-*m*-cresol	8.67	0.10	0.24	18.39	9.61	0.994	0.988	11.28	5.12	509
4,6-Dinitro-*o*-cresol	8.76	3.04	0.75	2.22	4.36	0.999	0.999	1.32	1.06	470
2,4-Dichlorophenol	9.21	0.71	0.98	6.42	3.54	1.000	1.000	4.04	1.02	509
2,4,6-Trichlorophenol	10.78	0.21	0.36	24.95	7.02	0.990	0.999	8.34	3.42	509

of the phenols is satisfactory. The resolution factor was calculated and is shown for each phenol in Table I.

Water treatment samples

Samples (1.5 l) of influent and effluent water were collected at a municipal water treatment plant that feeds a stream that is stocked with trout each spring by the Pennsylvania Department of Fish and Game.

These samples were taken at approximately the same time each day for a period of seven days. They were acidified and mixed with copper(II) sulphate in order to halt bacterial growth and preserve the phenols in solution[17]. All were refrigerated at approximately 4°C until they were analyzed.

Post-column reaction and detection

The column eluant was first mixed at a tee connector (0.01 in. I.D.) from Valco, (Houston, TX, U.S.A.) with potassium ferricyanide and then at another tee, with 4-aminoantipyrine. Since these reactants absorb light at the wavelengths that were monitored, pump pulsations produced baseline noise that reduced the sensitivity of the procedure. All attempts to utilize piston pumps without proper pulse dampening were unsuccessful since the pump stroke obscured the response of the eluted compounds. For this reason, the reactants were pumped with the well dampened pumps listed above in the equipment section. The area results for each of the phenols studied were normalized against the response of phenol at 470 nm and are reported in Table I. In this form it is easy to see the extent of the reaction with each of the phenols and also to see the benefit of quantification at both wavelengths.

The separation is adequate for the resolution of the compounds of interest, however, it was found to be important to reduce the risk of band spreading as much as possible. For this reason, all transfer tubing throughout the post-column reactor is 0.005 in. I.D. In addition, the reaction is run inside the oven chamber without any time delay coils or bed reactors. Once the reagents were added to the stream, the sample reached the detector within about three seconds.

RESULTS AND DISCUSSION

The linearity of the combined chromatographic and colorimetric procedures were studied with standard solutions prepared with distilled water. The concentrations of each of the ten phenols was varied from 3.33 ppm to 50 ppm.

Initial attempts at changing the slope of the gradient or the initial and final conditions led to the merging of *o*-chlorophenol and nitrophenol as well as 4,6-dinitro-*o*-cresol and 4-chloro--cresol. Similarly when the separation was attempted at room temperature, *p*-nitrophenol, 2,4-dinitrophenol, *o*-chloro- and *o*-nitrophenol merged.

The associated correlation coefficients clearly show a linear response for all of the compounds in this study. The repeatability of the procedure was determined at concentrations of 10 ppm for each compound. The results are shown in Table I.

The data for the linearity and repeatability studies clearly points to the need to monitor both wavelengths. This is particularly so when the lowest detectable limits were determined. This was defined as the point where the peak height is at least twice

the noise level of the baseline. These values are also included in Table I.

The reaction produced chromophores that absorb light at 470 nm and 509 nm. As shown in Table I, all of the compounds do not absorb light to the same extent at the two wavelengths and therefore, both were monitored. When the diode array spectrophotometer was used, this was done simultaneously. When the Hewlett-Packard Model 1084B liquid chromatograph was used, the sample was injected twice so that data could be collected at each wavelength. This allowed for increased sensitivity for certain phenols and in general increased the selectivity of the procedure.

It is interesting to note that the use of both wavelengths also provides the analyst with additional selectivity since the ratio of the peak heights or areas at both wavelengths must be identical to those of the standards if the compound is properly identified. In addition, as the data show, the sensitivity of the assay is greatly enhanced for many of the compounds at either of the wavelengths.

The concentration of 4-aminoantipyrine was studied at a flowrate of 1.0 ml/min. This data was generated while keeping the concentration of potassium ferricyanide constant at 150 mg/100 ml of acetate buffer (pH = 9.00). The reaction was not enhanced by the addition of more than 100 mg/100 ml of this reagent. For this reason, the concentration was set at 150 mg/100 ml for the remainder of the work.

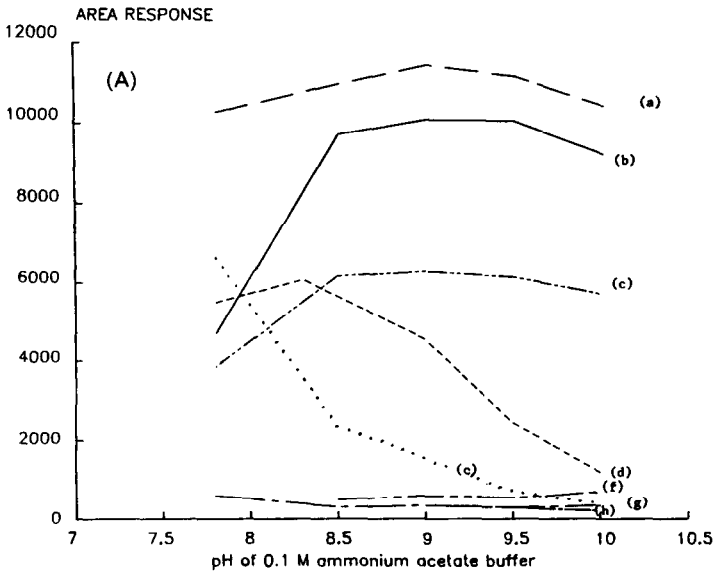
The effect of changing the concentration of potassium ferricyanide, pumped at 0.5 ml/min, on the reaction was similarly studied. After an initial rise in the reaction efficiency, the data show a slight increase in the response for each of the phenols studied as the reducing agent is increased. However, after the concentration of the reagent was increased beyond 175 mg/100 ml of buffer, the base line noise also began to increase. For this reason, a working concentration of 150 mg/100 ml of acetate buffer was established.

The optimum pH for the reaction was studied using all of the phenols used in this work. As evident in Fig. 2, no pH can be chosen that will produce the maximum response from all of the compounds studied. In order to maximize the response from the compounds that are eluted later in the chromatogram, a pH of 9.00 was used. This allowed for the determination of all of the compounds when both wavelengths were monitored.

Sample concentration of the phenols was attempted using three procedures. The first, extraction into chloroform, evaporation and reconstitution with methanol failed, probably due to the vapor pressure of the phenols and the slow transport across the phase barrier. Direct injection of the organic phase onto the reversed-phase column was attempted but this solvent eluted about midway through the chromatogram and made it impossible to quantify many of the compounds. The third approach was to use the solid phase extracting columns noted in the equipment section. Using the procedure recommended by the manufacturer, phenol, *p*-nitrophenol and 2,4-dinitrophenol were not retained.

Two modification procedures to this third approach were attempted with these columns. Initially, 100 ml of a standard containing approximately 100 ppb* of each of the phenols in distilled water was mixed with 1 ml of 1.0 *M* tetrabutylammonium hydroxide. A cyclohexyl bonded phase concentrating column was conditioned by passing 25 ml of methanol through the column at about 5 ml/min. Next, 50 ml of

* Throughout this article the American billion (10^9) is meant.



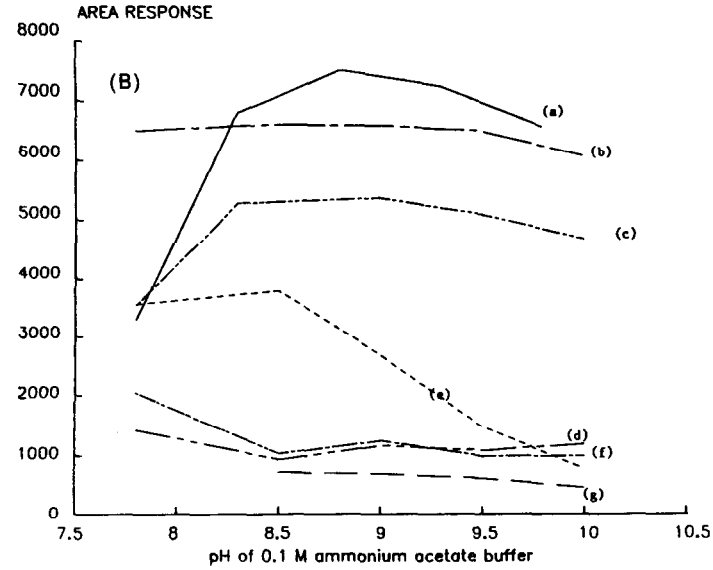


Fig. 2. Optimization of pH. (A) Detection at 509 nm of (a) *o*-chlorophenol, (b) phenol, (c) 4-chloro-*m*-cresol, (d) 2,4-dichlorophenol, (e) 2,4,6-trichlorophenol, (f) 2,4-dimethylphenol, (g) *o*-nitrophenol and (h) 4,6-dinitro-*o*-cresol. (B) Detection at 470 nm of (a) phenol, (b) *o*-chlorophenol, (c) 4-chloro-*m*-cresol, (d) *o*-nitrophenol, (e) 2,4-dichlorophenol, (f) 4,6-dinitro-*o*-cresol and (g) *p*-nitrophenol.

0.01 *M* tetrabutylammonium hydroxide was passed through the column at the same flow-rate. Finally, the sample was applied to the column. The vacuum was adjusted during the application of the sample so that the flow-rate was approximately 5 ml/min. Air was drawn through the column for about 5 min to remove any remaining

TABLE II

RECOVERY AND REPEATABILITY STUDIES OF 100 ppb OF EACH PHENOL WITH SOLID PHASE EXTRACTION

Compound	*Sodium chloride method*		*Tetrabutylammonium hydroxide method*	
	Recovery (%)	*R.S.D. (%)*	*Recovery (%)*	*R.S.D. (%)*
Phenol	52.5	2.61	17.1	9.17
p-Nitrophenol	64.5	7.88	57.2	9.87
2,4-Dinitrophenol	44.2	7.02	78.3	9.93
o-Chlorophenol	56.5	8.75	79.1	3.74
o-Nitrophenol	74.0	1.81	83.2	7.85
2,4-Dimethylphenol	55.3	9.16	53.0	10.06
4-Chloro-*m*-cresol	64.9	7.82	72.8	4.22
4,6-Dinitro-*o*-cresol	66.8	5.30	85.6	6.48
2,4-Dichlorophenol	63.6	8.32	79.1	3.81
2,4,6-Trichlorophenol	40.6	16.07	72.9	4.66

liquid, and the column was then eluted with 2 ml of methanol. The recovery and repeatability of this procedure for each of the phenols are shown in Table II.

A modification of the procedure recommended by the manufacturer was used to further study the extraction of these same samples. In this study, 100 ml of the sample was mixed with 40 g of sodium chloride and then adjusted between pH 1.0 and 1.5 with 1.0 *M* hydrochloric acid. The samples were placed on a shaker for 2 h so that as much of the salt as possible would dissolve. The concentrator columns were conditioned by passing through 25 ml of methanol at about 5 ml/min. Immediately after the methanol was through the column, 50 ml of a saturated solution of sodium chloride was applied to the column to remove the methanol and set up the proper ionic strength. The samples were then applied at about 5 ml/min (all of the salt did not dissolve while shaking the samples). After decanting as much of the solution as possible into the column, the remaining slurry was washed with three 5-ml portions of saturated sodium chloride solution which was added to the column. No deliterious effects were observed when salt crystals were inadvertently added to

TABLE III

QUANTIFICATION OF PHENOLS IN INFLUENT SAMPLES FROM A WASTEWATER TREATMENT PLANT

Sampling day	*Phenol (ppb)*	*4,6-Dinitrophenol (ppb)*	*o-Nitrophenol (ppb)*
Monday	67	14	2
Tuesday	54	2	3
Wednesday	43	1	4
Thursday	52	1	2
Friday	55	1	1
Saturday	30	–	–
Sunday	–	–	–

the head of the concentrator columns. The columns were dried by drawing air through them for about 5 min, and the samples were eluted with 2.0 ml of methanol. The results of the recovery and repeatability studies are included in Table II.

In both cases, the samples had to be centrifuged following elution from the column. In the first case to remove precipitated tetrabutylammonium chloride while in the second to remove crystals of sodium chloride. The injection volume for both sets of samples was 200 μl.

Although both procedures recover the phenols, the tetrabutylammonium hydroxide procedure only recovered about 30% as much phenol as the sodium chloride method. The ion-pairing agent, however, did generally provide for greater recovery for seven of the ten compounds studied. For general survey analysis, the sodium chloride procedure would be recommended, because of phenol. However, for analysis of any of the other phenols, the ion-pairing procedure would be preferred as it is less time consuming and produces more sensitive results.

The wastewater and treated water samples were quantified using the procedure discussed above with the sodium chloride based concentration procedure. Samples and standards (at 100 ppb) were extracted by the solid phase process. Six of the seven samples contained phenol at levels ranging from 43 to 67 ppb. In addition, five of the samples (taken from Monday to Friday) contained between 6 and 14 ppb of 4,6-dinitro-*o*-cresol. *o*-Nitrophenol was found in these same five samples at levels ranging from 2.3 to 3.9 ppb (see Table III). As expected, the post-column derivatization procedure provided for facil quantification of these samples.

Of particular interest is the fact that although several phenols were detected in the influent samples, none were detected at the exit of the treatment plant. This data clearly shows that the treatment plant is able to reduce the concentration of these pollutants from the wastewater before it enters the stream.

CONCLUSION

The procedure has been shown to be highly selective for the determination of the ten phenols studied. The sensitivity of the method has been shown to be enhanced by the use of preconcentration techniques. Using these procedures in tandum has allowed for the detection and quantification of these phenols in water and wastewater.

REFERENCES

1 R. L. Grob (Editor), *Analysis of the Environment*, Marcel Dekker, New York, 2nd ed., 1983.
2 R. B. Baird, L. G. Caromone and R. L. Jenkins, *Bull. Environ. Contam. Toxicol.*, 17 (1977) 764.
3 K. Tsuji, *J. Chromatogr.*, 158 (1978) 337.
4 P. A. Realini, *J. Chromatogr. Sci.*, 19 (1981) 124.
5 M. Deinzer, R. Schaumburg and E. Klein, *Environ. Health Perspects.*, 24 (1978) 209.
6 Environmental Protection Agency, *Toxic Substance Control Act*, USEPA, Washington, DC, 1979.
7 *Federal Register*, 44 (1979) 69464–69575.
8 L. L. Lamparski and T. J. Nestrick, *J. Chromatogr.*, 156 (1978) 143.
9 Y. Hoshika and G. Muto, *J. Chromatogr.*, 179 (1979) 105.
10 R. T. Coutts, E. E. Hargesheimer and F. M. Psautto, *J. Chromatogr.*, 179 (1979) 291.
11 H. A. McLeod and G. Laver, *J. Chromatogr.*, 244 (1982) 385.
12 R. E. Shoup and G. S. Mayer, *Anal. Chem.*, 54 (1982) 1164.

13 G. Norwitz and P. N. Keliher, *Anal. Chim. Acta*, 144 (1982) 273.
14 G. Norwitz, A. H. Bardsley and P. N. Keliher, *Anal. Chim. Acta*, 128 (1981) 251.
15 J. Gasparic, D. Svobodova and A. Matysova, *J. Chromatogr.*, 88 (1974) 364.
16 C. E. Werkhoven-Goewie, W. M. Boon, A. J. J. Praat, R. W. Frei, U. A. T. Brinkman and C. J. Little, *Chromatographia*, 16 (1982) 53.
17 *Standard Methods for the Examination of Water and Wastewater*, American Public Health Association, Washington, DC, 13th ed., 1971, p. 501.