Journal of Chromatography, 211 (1981) 135–143
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,723

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF CARBARYL AND 1-NAPHTHOL AT RESIDUE LEVELS IN VARIOUS WATER SOURCES BY DIRECT INJECTION AND TRACE ENRICHMENT

R. J. BUSHWAY
Department of Food Science, University of Maine, Orono, ME 04469 (U.S.A.)
(First received December 16th, 1980; revised manuscript received February 9th, 1981)

SUMMARY

A high-performance liquid chromatographic method is described for determining carbaryl and its hydrolysis product, 1-naphthol, in water at residue levels by direct injection and trace enrichment. Water from three sources, public water supply, stream and the ocean, was analyzed for carbaryl and 1-naphthol at concentrations as low as 3.78 and 10 ppb*, respectively, without a clean-up, concentration or derivatization step. Carbaryl was detected at 0.1 ppb and 1-naphthol at 0.5 ppb by employing a concentration step involving a C_{18} Sep-Pak cartridge. The coefficients of variation for all determinations ranged from 2.5 to 10.7%. Fourteen other widely used pesticides —carbofuran, methomyl, thiram, azinphos-methyl, benomyl, monuron, diuron, propham, chlorpropham, pentachlorophenol, the oxygen analogue of azinphos-methyl, pentachloronitrobenzene, simazine and atrazine— were chromatographed using this system. Pentachloronitrobenzene, diuron and thiram interfered with the determination of 1-naphthol, while atrazine co-chromatographed with carbaryl.

INTRODUCTION

Carbaryl (1-naphthyl methylcarbamate) is a broad-spectrum insecticide that is used extensively because of its effectiveness and low acute mammalian toxicity (the oral LD_{50} to rats is 560 mg/kg). However, recent studies have indicated that carbaryl may be a viral enhancer[1] and a teratogen[2]. Because of this new information about chronic toxicity and because of its wide use near water supplies and soil, a rapid, accurate and sensitive method is needed to determine carbaryl and its degradation product, 1-naphthol, in water.

Numerous spectrophotometric[3–7], gas chromatographic[8–29] and liquid chromatographic methods[30–49] have been developed to analyze carbaryl and/or 1-naphthol, but these procedures require at least one of the following lengthy steps: derivatization, concentration and/or clean-up before the analysis can be performed. A

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

0021-9673/81/0000–0000/$02.50

recent high-performance liquid chromatographic (HPLC) method[50] involving an electrochemical detector shows promise for determining low concentrations of several carbamate pesticides, but not carbaryl or 1-naphthol, in water without performing any of these time-consuming steps.

The method described here involves a rapid, accurate and sensitive HPLC procedure for the determination of carbaryl and 1-naphthol in water at low ppb concentrations by direct injection of the sample. For amounts lower than those determined by direct analysis, a concentration step involving a C_{18} Sep-Pak cartridge was developed.

EXPERIMENTAL

Solvents and pesticides

Acetonitrile and water were of HPLC grade and were purchased from Fisher Scientific (Pittsburgh, PA, U.S.A.). Carbaryl with a purity of 99.9% was used as received from Union Carbide (South Charleston, WV, U.S.A.), but 1-naphthol, purchased from Fisher Scientific, was recrystallized twice from ACS-grade chloroform (Fisher Scientific). All other pesticides were obtained from the Environmental Protection Agency (Research Triangle Park, NC, U.S.A.) with purities ranging from 98 to 99.9%. All standards were dissolved in ACS-grade methanol, and HPLC-grade acetonitrile was used to elute carbaryl and 1-naphthol from the C_{18} Sep-Pak cartridge (Waters Assoc., Milford, MA, U.S.A.); ACS-grade methanol and HPLC-grade water were employed to activate the Sep-Pak.

Water samples

Water samples were of three types —drinking, stream and salt water. The drinking water was obtained from the laboratory, the stream water was collected in Orono (ME, U.S.A.) and the salt water from the Atlantic Ocean in Sullivan (ME, U.S.A.).

Standard preparation

Stock solutions of carbaryl were prepared in methanol at 0.1 mg/ml for the trace-enrichment study and at 0.75 mg/ml for direct-analysis experiments. A stock solution of 0.2 mg/ml of 1-naphthol in methanol was used for both studies. All other pesticide stock solutions were prepared by appropriate dilutions with methanol.

Apparatus

The HPLC system incorporated a Waters Assoc. 6000A pump, a U6K injector, and a differential refractometer, together with a Schoeffel (Westwood, NJ, U.S.A.) variable-wavelength UV detector and a Houston Instruments (Austin, TX, U.S.A.) dual-pen recorder. The column (30 cm × 4 mm I.D.) was of μBondapak C_{18} (Waters Assoc.; particle size 10 μm). Operating conditions were: mobile phase, acetonitrile–water (43:57); flow-rate, 1 ml/min; column temperature, ambient; wavelength, 222 nm; attenuation, 0.04 a.u.f.s.; and chart speed, 0.4 in./min.

For trace-enrichment studies, a Fluid Metering (Oyster Bay, NY, U.S.A.) pump (Model RP-SY) was employed. Connections at the outlet and inlet ends of the pump were made with PTFE tubing with a frit (pore size 10 μm) at the inlet and a

Waters Assoc. C_{18} Sep-Pak cartridge at the outlet. The flow-rate was set at 22.2 ml/min.

Analytical procedure

Direct analysis. Water samples as received and spiked were filtered (2 ml) through a 0.45-μm Millipore aqueous filter (Waters Assoc.) and injected (230 μl) directly into the HPLC system. The injection volume varied with concentration; water samples containing 3.78 to 20 ppb of carbaryl and 1-naphthol were injected at a volume of 230 μl, while 23-μl portions of samples spiked at 150 to 200 ppb were injected.

Trace enrichment. Water samples (1000 ml) were spiked with 99 ng of carbaryl and passed through an activated C_{18} Sep-Pak cartridge using the Fluid Metering pump. The Sep-Pak was activated by pre-wetting the cartridge with 4 ml of methanol followed by 5 ml of water. To elute the carbaryl and 1-naphthol adsorbed on the packing, 2 ml of acetonitrile was passed through the cartridge; a 50-μl aliquot was injected into the HPLC system. When ocean water was analyzed by trace enrichment, it was necessary to remove the salt water trapped in the Sep-Pak by passing 4 ml of HPLC-grade water through the cartridge before the acetonitrile elution step.

RESULTS AND DISCUSSION

Table I lists the results from three types of water samples that were spiked with carbaryl and 1-naphthol at concentrations from 3.78 to 207 ppb. All samples were chromatographed without any clean-up, concentration or derivatizing step. Such a procedure is not only rapid and simple, but also is quite precise, with coefficients of variation ranging from 2.48 to 7.78% for six consecutive injections of each type of

TABLE I

DIRECT ANALYSIS OF CARBARYL AND 1-NAPHTHOL BY HPLC

Each value represents the mean of six samples analyzed.

Water Source	*Carbaryl*			*1-Naphthol*			*Blank for both*
	Level spiked (ppb)	*Peak height (cm)*	*C.V. (%)*	*Level spiked (ppb)*	*Peak height (cm)*	*C.V. (%)*	
Salt	3.78	0.48	4.58	10.0	0.66	5.92	0
Salt	7.55	0.87	4.25	20.5	1.49	2.95	0
Salt	15.10	1.64	2.50	207.0	1.38	3.99	0
Salt	151.00	1.62	3.09	–	–	–	0
Stream	3.78	0.44	4.55	10.0	0.67	5.46	0
Stream	7.55	1.12	7.50	20.5	1.26	4.68	0
Stream	15.10	1.96	7.63	207.0	1.33	3.98	0
Stream	151.00	2.26	2.48	–	–	–	0
Drinking	3.78	0.54	7.78	10.0	0.56	5.17	0
Drinking	7.55	1.22	3.52	20.5	1.18	4.07	0
Drinking	15.10	2.11	5.21	207.0	1.15	2.96	0
Drinking	151.00	2.08	4.76	–	–	–	0

water at each spiking level. The majority of these coefficients are less than 5% (Table I). It should be noted that different types of water containing the same concentration of carbaryl or 1-naphthol do not have the same peak height. For example salt, stream and drinking water spiked with carbaryl at a concentration of 3.78 ppb yielded carbaryl peak heights of 0.48, 0.44 and 0.54 cm, respectively. This was probably due to the water matrix and/or variation in such HPLC conditions as the composition of the mobile phase from day to day. In order to obtain the most accurate results, one should make the working standard for each compound in a water matrix as near as possible in composition to the sample being analyzed; also, the standard should be prepared each day. Although the lowest concentrations detected in this study by direct injection of water was 3.78 ppb for carbaryl and 10.0 ppb for 1-naphthol, it should be possible to detect 1.5 ppb of carbaryl and 3.5 ppb of 1-naphthol, provided that the sample matrix is free of co-chromatographing compounds, by injecting 460

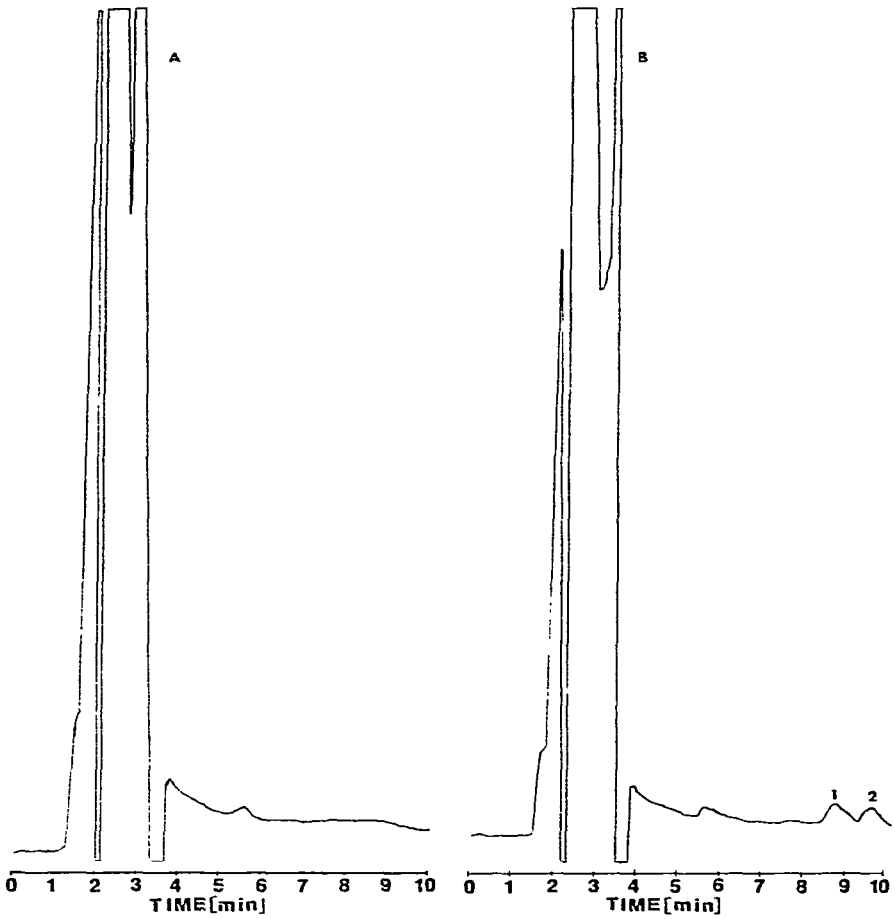

Fig. 1. Chromatogram of (A) salt water and (B) salt water spiked with 3.78 ppb of carbaryl and 10.0 ppb of 1-naphthol: direct injection. Solvent system, acetonitrile–water (43:57); flow-rate, 1 ml/min; detector sensitivity. 0.04 a.u.f.s.; wavelength, 222 nm; chart speed, 0.4 in./min; amount injected, 230 μl. Peaks: 1 = carbaryl; 2 = 1-naphthol.

μl and setting the detector sensitivity to 0.02 a.u.f.s. A typical chromatogram of a spiked salt-water sample and its blank is shown in Fig. 1. There are no interfering peaks, as can be determined from the blank, and even though peak heights are small at these low concentrations, the precision is not adversely affected (Table I).

Analysis time is lengthy, 8–11 min before both compounds are eluted, but in many instances this time is needed to prevent interference. A shorter analysis time can be attained,when possible, by increasing the acetonitrile concentration by 10–15%.

Several other pesticides and metabolic products were chromatographed using the same conditions as above; their retention times are listed in Table II. Of these fourteen compounds, only atrazine would interfere with the analysis of carbaryl, and thiram, diuron and pentachloronitrobenzene would co-chromatograph with 1-naphthol.

TABLE II

NAMES OF PESTICIDES AND METABOLIC PRODUCTS USED

Pesticide or metabolic product	*Chemical name*	*Retention time*	
		cm	*min*
Carbaryl	1-Naphthyl methylcarbamate	8.60	8.74
1-Naphthol	1-Hydroxynaphthalene	9.75	9.91
Methomyl	(1-Methylthioethylidene) amino methylcarbamate	3.80	3.86
Thiram	Bis(dimethylthiocarbamyl)disulfide	9.65	9.80
Azinphos-methyl	O,O-Dimethyl S-[4-oxo-1,2,3-benzotriazin-3(4H)-ylmethyl] phosphorodithioate	16.55	16.81
Oxygen analogue of azinphos-methyl	O,O-Dimethyl O-[4-oxo-1,2,3-benzotriazin-3(4H)-yl methyl] phosphorodithioate	5.45	5.54
Benomyl	Methyl (1-butylcarbamoylbenzimidazol-2-yl)carbamate	4.35	4.42
Monuron	3-(*p*-Chlorophenyl)-1,1-dimethylurea	11.31	11.49
Diuron	3-(3,4-Dichlorophenyl)-1,1-dimethylurea	9.65	9.80
Propham	Isopropyl carbanilate	11.75	11.94
Chlorpropham	Isopropyl-*m*-chlorocarbanilate	21.40	21.74
PCP	Pentachlorophenol	4.75	4.83
PCNB	Pentachloronitrobenzene	9.85	10.01
Simazine	2-Chloro-4,6-bis(ethylamino)-*s*-triazine	6.55	6.65
Atrazine	2-Chloro-4-ethylamino-6-isopropylamino-*s*-triazine	9.00	9.14
Carbofuran	2,3-Dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate	7.45	7.57

In order to be able to detect and quantify carbaryl and 1-naphthol in water at concentrations lower than those possible by direct injection, trace enrichment using a C_{18} Sep-Pak cartridge was developed; the results are given in Table III. Water samples were spiked with carbaryl at 0.099 ppb and 1-naphthol at 0.516 ppb. Recoveries ranged from 94 to 107%, with coefficients of variation from 4.72 to 10.77%. Typical chromatograms of the trace enrichment of carbaryl and 1-naphthol are shown in Figs. 2 and 3.

Unlike the direct-analysis samples, carbaryl and 1-naphthol were trapped separately in the trace-enrichment procedure. This was because the acid added to the water in order to determine 1-naphthol caused many more compounds to be adsorbed on the Sep-Pak, and these compounds interfered with analysis for carbaryl

TABLE III

C_{18} SEP-PAK ENRICHMENT OF CARBARYL AND 1-NAPHTHOL

Each value represents the mean of five samples analyzed.

Carbaryl				*1-Naphthol*			*Blank for both*
Water source	*Level spiked (ppb)*	*Recovery (%)*	*C.V. (%)*	*Level spiked (ppb)*	*Recovery (%)*	*C.V. (%)*	
Drinking	0.099	–	–	0.516	94.1	6.24	0
Stream	0.099	98.9	5.41	0.516	94.8	4.72	0
Salt	0.099	107	10.77	–	–	–	0

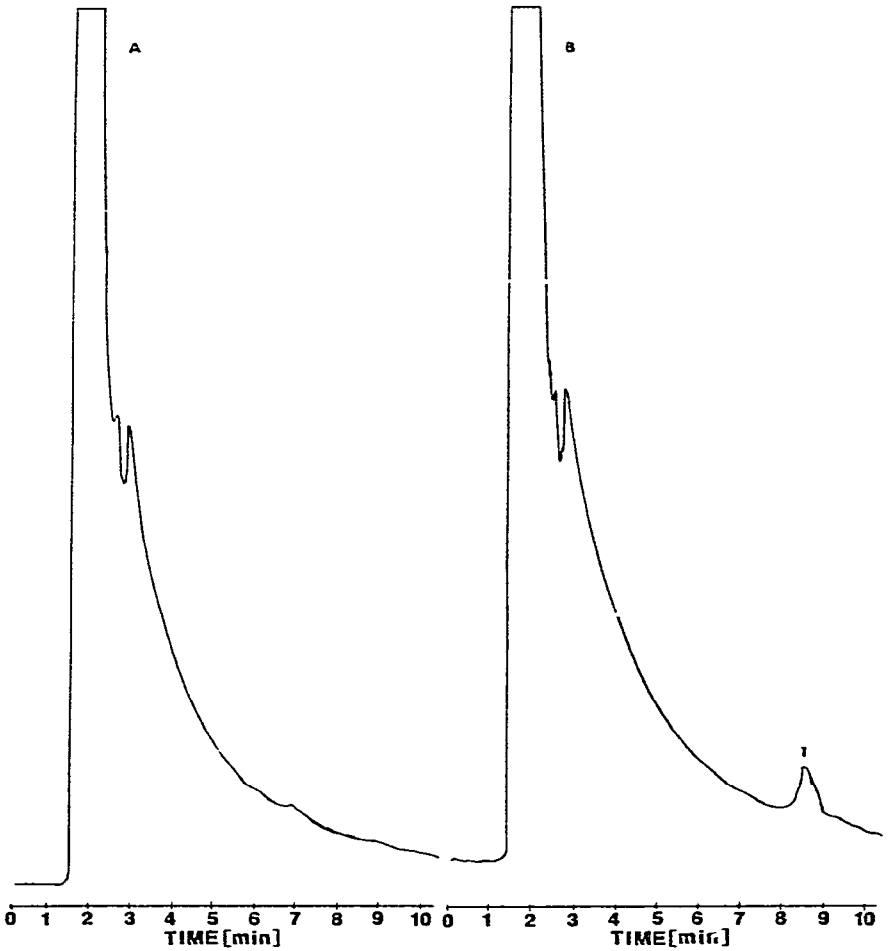

Fig. 2. Chromatogram of (A) stream water and (B) stream water spiked with 0.099 ppb of carbaryl: trace enrichment with C_{18} Sep-Pak. Amount injected, 50 μl; other conditions as in Fig. 1. Peak: 1 = carbaryl.

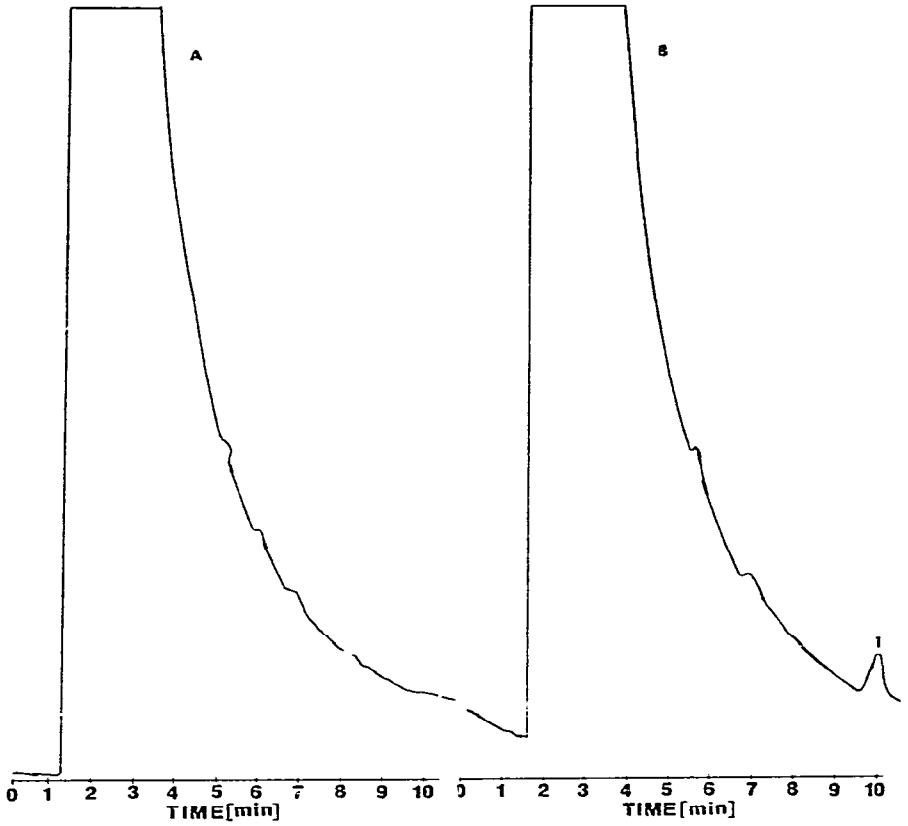

Fig. 3. Chromatogram of (A) stream water and (B) stream water spiked with 0.516 ppb of 1-naphthol; trace enrichment with C_{18} Sep-Pak. Conditions as in Fig. 2. Peak: 1 = 1-naphthol.

(but not that for 1-naphthol). Further, the capacity of 1-naphthol on these C_{18} cartridges was between 1.2 and 2.6 μg (recovery of 1.2 μg of 1-naphthol was 100%, whereas that of 2.6 μg was 86.7%). The break-through amount for carbaryl was never determined, since even for 4.5 μg of carbaryl the recovery was still near 100%. Thus, carbaryl at concentrations from 0.1 to 4.5 ppb could be determined by passing 1 l of water through a Sep-Pak cartridge. However, 1-naphthol at concentrations from 0.5 to 10 ppb was not as easy to determine, since, at the 0.5-ppb level, 500 ml of water could be used, but at 10 ppb only 100 ml of water could be employed (capacity factor).

A study was conducted to determine how many times a Sep-Pak cartridge could be re-used. Under our conditions, each cartridge could be used three times; obviously, the ability to re-use the cartridge would have to be determined for each water source analyzed.

The detector response is linear from 0.1 to 151 ppb for carbaryl and from 0.5 to 207 ppb for 1-naphthol. However, when performing trace-enrichment determinations, the standard curve must be determined by using standards that are dissolved in 30–40 ml of HPLC-grade water and passed through a Sep-Pak cartridge, followed

by elution with 2 ml of acetonitrile. If the standards are not prepared in this manner, the concentrations of carbaryl and 1-naphthol in the unknowns will be incorrect, since the standards passed through the cartridge gave slightly higher peaks than those not passed through; this was not due to contamination, since the blank samples were clean.

A comparison between the C_{18} trace-enrichment technique and the methylene chloride extraction technique was done for carbaryl. Five salt-water samples were spiked at 0.087 ppb and extracted with methylene chloride; the mean recovery was 47.1%, with a coefficient of variation of 41.7%. When compared with the trace-enrichment results (Table III), one can see that extraction with methylene chloride at this level is not good. Also, there were many late eluting peaks from the methylene chloride extracts, which interfered with other injections. This was not observed in the trace-enrichment studies.

CONCLUSIONS

The large extinction coefficients for carbaryl and 1-naphthol at 222 nm and the relatively pure simple matrix of water, permits the determination of these two compounds in water at low ppb levels by directly injecting the sample into an HPLC system. The method could also be used to analyze for other carbamate pesticides due to their high extinction coefficients between 206 and 222 nm. This procedure is much faster and simpler than previously published methods for analyzing carbaryl and 1-naphthol in water; it also offers the advantage of not having to work with extracting solvents like methylene chloride. Lower concentrations of these compounds in water can be determined by trace enrichment.

REFERENCES

1 L. H. Abrahamsen and M. A. Jerkofsky, *Appl. Environ. Microb.*, in press.
2 J. Seifert and J. E. Casida, *Biochem. Pharmacol.*, 27 (1978) 2611.
3 R. Miskus, H. T. Gordon and D. A. George, *J. Agr. Food Chem.*, 7 (1959) 613.
4 D. P. Johnson, F. E. Critchfield and B. W. Arthur, *J. Agr. Food Chem.*, 11 (1963) 77.
5 E. E. Vonessch and M. H. C. K. Riveros, *J. Ass. Offic. Anal. Chem.*, 54 (1971) 128.
6 J. R. Rangaswamy and S. K. Majunder, *J. Ass. Offic. Anal. Chem.*, 57 (1974) 592.
7 S. K. Handa and A. K. Dikshit, *Analyst (London)*, 104 (1979) 1185.
8 J. W. Rolls and A. Cortes, *J. Gas Chromatogr.*, 2 (1964) 132.
9 W. H. Gutenmann and D. J. Lisk, *J. Agr. Food Chem.*, 13 (1965) 48.
10 L. I. Butler and L. M. McDonough, *J. Agr. Food Chem.*, 16 (1968) 403.
11 E. D. Holden, W. M. Jones and M. Beroza, *J. Agr. Food Chem.*, 17 (1969) 56.
12 R. F. Cook, R. P. Stanovick and C. C. Cassil, *J. Agr. Food Chem.*, 17 (1969) 277.
13 M. Riva and A. Carisano, *J. Chromatogr.*, 42 (1969) 464.
14 R. L. Tilden and C. H. Van Middelen, *J. Agr. Food Chem.*, 18 (1970) 154.
15 R. L. Argauer, *J. Agr. Food Chem.*, 17 (1969) 888.
16 S. C. Lau and R. L. Marxmiller, *J. Agr. Food Chem.*, 18 (1970) 413.
17 L. I. Butler and L. M. Mcdonough, *J. Ass. Offic. Anal. Chem.*, 53 (1970) 495.
18 J. N. Seiber, *J. Agr. Food Chem.*, 20 (1972) 443.
19 J. H. Ruzicka, *Proc. Soc. Anal. Chem.*, 10 (1973) 32.
20 H. Miyata and T. Kashimoto, *Shokuhin Eiseigaku Zasshi*, 15 (1974) 485.
21 L. Wong and F. M. Fisher, *J. Agr. Food Chem.*, 23 (1975) 317.
22 H. W. Dorough and J. H. Thorstenson, *J. Chromatogr. Sci.*, 13 (1975) 212.
23 M. Oda, N. Shida and T. Kashiwa, *Noyaku Kensasho Hokoku*, 16 (1976) 60.

24 J. F. Lawrence, *J. Chromatogr.*, 123 (1976) 287.
25 J. F. Lawrence, D. A. Lewis and H. A. McLeod, *J. Chromatogr.*, 138 (1977) 143.
26 K. Nagasawa, H. Uchiyama, A. Ogamo and T. Shinozuka, *J. Chromatogr.*, 144 (1977) 77.
27 H. G. Loebering, L. Weil and K. E. Quentin, *Vom Wasser*, 51 (1978) 265.
28 R. C. Hall and D. E. Harris, *J. Chromatogr.*, 169 (1979) 245.
29 G. H. Tjan and J. T. A. Jansen, *J. Ass. Offic. Anal. Chem.*, 62 (1979) 769.
30 A. D. Thruston Jr., *EPA Rep.*, EPA-R2-72-079, U.S. Environmental Protection Agency, Corvallis, OR, 1972.
31 R. W. Frei and J. F. Lawrence, *J. Chromatogr.*, 83 (1973) 321.
32 R. W. Frei, J. F. Lawrence, J. Hope and R. M. Cassidy, *J. Chromatogr. Sci.*, 12 (1974) 40.
33 B. M. Colvin, B. S. Engdahl and A. R. Hanks, *J. Ass. Offic. Anal. Chem.* 57 (1974) 648.
34 J. N. Seiber, *J. Chromatogr.*, 94 (1974) 151.
35 H. A. Moye, *J. Chromatogr. Sci.*, 13 (1975) 268.
36 C. M. Sparacino and J. W. Hines, *J. Chromatogr. Sci.*, 14 (1976) 549.
37 C. F. Aten and J. B. Bourke, *J. Agr. Food Chem.*, 25 (1977) 1428.
38 J. F. Lawrence, *J. Agr. Food Chem.*, 25 (1977) 211.
39 H. A. Moye, S. J. Scherer and P. A. St. John, *Anal. Lett.*, 10 (1977) 1049.
40 J. F. Lawrence and D. Turton, *J. Chromatogr.*, 159 (1978) 207.
41 I. Stoeber and R. Reupert, *Vom Wasser*, 51 (1978) 273.
42 R. T. Krause, *J. Chromatogr. Sci.*, 16 (1978) 281.
43 J. F. Lawrence and R. Leduc, *J. Ass. Offic. Anal. Chem.*, 61 (1978) 872.
44 W. P. Cochrane, *J. Chromatogr. Sci.*, 17 (1979) 124.
45 G. R. Pieper, *Bull. Environ. Contam. Toxicol.*, 22 (1979) 167.
46 R. T. Krause, *J. Chromatogr.*, 185 (1979) 615.
47 J. Muth and J. Giles, *Altex Chromatogram*, 3 (1980) 5.
48 R. T. Krause, *J. Ass. Offic. Anal. Chem.*, 63 (1980) 1114.
49 W. H. McDermott, *J. Ass. Offic. Anal. Chem.*, 63 (1980) 650.
50 J. L. Anderson and D. J. Chesney, *Anal. Chem.*, 52 (1980) 2156.