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Note

Liquid chromatographic determination of N-methylcarbamate pesticides using a single-stage post-column derivatization reaction and fluorescence detection

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The high-performance liquid chromatographic (HPLC) method for post-column derivatization and fluorescence detection of N-methylcarbamate pesticides introduced by Moye *et al.*[1] has been widely recognized for its sensitivity and selectivity for these compounds. Refined and collaboratively studied by Krause[2–6] and optimized by Engelhardt and Lillig[7], the method has achieved widespread acceptance and has been adopted by a number of laboratories for multiresidue analysis of N-methylcarbamate pesticides in foods. The number of N-methylcarbamates which may be analyzed by the method was expanded by De Kok *et al.*[8], who also introduced a simple cleanup method which can be applied to all types of crop samples.

The post-column derivatization technique consists of a two-stage reaction which converts the carbamate into a fluorescent derivative which is detected by a fluorescence detector. The first stage is hydrolysis of the carbamate molecule to release methylamine. This is accomplished by the introduction of an alkali solution, usually sodium hydroxide or potassium hydroxide, by means of a reagent delivery pump connected to the flow stream after the analytical column. The flow then passes through a reaction coil in a heater at 80–100°C where hydrolysis occurs. The second stage is derivatization of the released methylamine with *o*-phthalaldehyde (OPA) and 2-mercaptoethanol or 3-mercaptopropionic acid[9] to form a highly fluorescent substituted isoindole. This is accomplished using a second reagent delivery pump connected to the flow stream after the reaction coil.

Nondek *et al.*[10,11] explored the potential of replacing the use of alkali solution to hydrolyze the carbamates with a catalytic solid-phase reactor packed with an anion exchange resin. This approach eliminates one post-column reagent delivery pump and the attending mixing problems and flow pulsations. It also eliminates band broadening due to dilution of the analyte in the mobile phase. The cost of instrumentation is reduced, but this is partially offset by the cost of the solid-phase reactor. In spite of the potential advantages, this approach has not gained widespread acceptance.

This paper reports a simplification of the post-column derivatization technique which realizes the advantages of the solid-phase reactor technique by eliminating one post-column reagent delivery pump, but without the expense and potential problems of the solid-phase reactor. The method was evaluated for 11 commonly analyzed N-methylcarbamate pesticides. Results of this evaluation are presented here.

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EXPERIMENTAL

Materials

Analytical-grade standards of aldicarb, aldicarb sulfoxide and aldicarb sulfone were supplied by Union Carbide (Research Triangle Park, NC, U.S.A.). Oxamyl was supplied by E. I. du Pont de Nemours (Wilmington, DE, U.S.A.), carbofuran by FMC (Middleport, NY, U.S.A.) and carbaryl by City Chemical (New York, NY, U.S.A.). Methiocarb, bufencarb, methomyl and propoxur were supplied by Agriculture Canada, Laboratory Services Division (Ottawa, Canada).

Standard solutions were prepared by diluting 100 μg/ml stock solutions in methanol with HPLC-grade water to appropriate concentrations. Since oxamyl and propoxur were not well resolved from aldicarb sulfone and carbofuran, respectively, by the HPLC system used in this study, two series of mixed standards were used. One consisted of oxamyl and propoxur, and the other contained the remaining nine compounds.

Methanol, acetonitrile and water were HPLC-grade and obtained from J. T. Baker through Johns Scientific (Toronto, Canada). OPA and 2-mercaptoethanol were obtained from BDH (Toronto, Canada), and 3-mercaptopropionic acid from Aldrich (Milwaukee, WI, U.S.A.). Potassium hydroxide and sodium carbonate were obtained from Fisher Scientific (Don Mills, Canada).

The derivatization reagent was prepared by adding 10 mg OPA in 1 ml methanol to 400 ml 0.01 *M* potassium hydroxide solution in HPLC-grade water. This solution was degassed under vacuum with magnetic stirring, after which 0.05 ml 2-mercaptoethanol was added.

Instrumentation

A Hewlett-Packard 1090 liquid chromatograph equipped with a 79835A solvent delivery system, 79846A autoinjection module and 79847A autosampler was used. The injection volume was 10 μl and the flow-rate 1 ml/min. A mobile phase gradient of 20–80% acetonitrile in water over 16 min was employed. A Spherisorb 5 ODS-2 column (250 × 4.6 mm I.D.) was supplied by Phenomenex (Torrance, CA, U.S.A.). The single-stage post-column reactor, illustrated schematically in Fig. 1, consisted of the following. From a reservoir (A), the derivatization reagent was delivered at a rate of 0.6 ml/min by an SSI Model 350 pump equipped with a prime/purge valve (B)

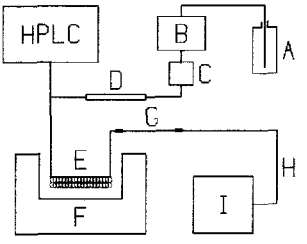

Fig. 1. Schematic diagram of post-column reactor. A = Derivatization reagent reservoir; B = reagent delivery pump equipped with prime/purge valve; C = pulse damper; D = spent PRP-1 column (to give back pressure to pulse damper); E = two delay tubes in series; F = hydrolysis heater; G = single-bead string reactor; H = 1 m × 0.5 mm I.D. PTFE tubing; I = fluorescence detector.

through a LO-Pulse pulse damper (C) connected to the flow stream from the HPLC column through a Valco zero volume tee. A spent 150 × 4.1 mm I.D. Hamilton PRP-1 column (D) inserted between the pulse damper and the tee provided back pressure which increased the efficiency of the pulse damper. The two streams were mixed in two knitted PTFE delay tubes (E) (3 m × 0.5 mm I.D., Supelco, Oakville, Canada) which were immersed in series in a 140°C dry bath consisting of glass beads in a Pierce Reacti-Therm heating module (Chromatographic Specialties, Brockville, Canada), hereafter called the reaction heater (F). The delay tubes were followed by a single-bead string reactor (G) and 1 m × 0.5 mm I.D. PTFE tubing (H) (both from Supelco) in which the flow stream cooled before it entered the flow cell of a Kratos FS 970 fluorescence detector (I). The excitation wavelength was 229 nm and a 418 nm emission filter was used. The chromatogram was recorded and integrated on a Shimadzu C-R3A Chromatopac (not shown in Fig. 1).

The following parameters were evaluated in order to optimize chromatographic peak heights of the carbamates: aqueous solution used to prepare the derivatization reagent; reaction heater temperature; delay tube length; choice of mercaptan in the derivatization reagent; and choice of organic mobile phase modifier.

RESULTS AND DISCUSSION

Selection of the optimum aqueous solution for the derivatization reagent made it possible to hydrolyze the carbamates and derivatize the released methylamine in a single step. Table I summarizes the relative peak heights of nine of the carbamates, using five derivatization reagent solutions of varying pH, normalized on the highest peak for each compound. While the 0.01 *M* sodium tetraborate solution (A) produced

TABLE I

EFFECT OF AQUEOUS SOLUTION USED TO PREPARE DERIVATIZATION REAGENT ON PEAK HEIGHTS OF 9 CARBAMATES (0.2 μg/ml EACH)

Solutions: A = 0.01 *M* sodium tetraborate, pH 9.2; B = 0.001 *M* potassium hydroxide, pH 9.5; C = 0.05 *M* sodium carbonate, pH 10.7; D = 0.01 *M* potassium hydroxide, pH 11.4; E = 0.06 *M* potassium hydroxide, pH 12.4. Reaction heater temperature 140°C.

Carbamate	*Relative peak heights (%)*[a]				
	A	*B*	*C*	*D*	*E*
Aldicarb sulfoxide	100	74	79	99	72
Aldicarb sulfone	100	79	72	91	66
Methomyl	39	17	91	100	85
3-Hydroxycarbofuran	100	77	68	95	59
Aldicarb	44	13	89	100	86
Carbofuran	100	63	61	84	56
Carbaryl	100	84	60	86	58
Methiocarb	100	82	56	85	43
Bufencarb	100	69	49	70	43
Mean	87	62	69	90	63

[a] Normalized on the highest peak (100%) for each compound.

the greatest number of maximum (100%) peaks (7 out of the 9 compounds tested), the peaks for methomyl and aldicarb were judged to be unacceptably low. The solution which produced the best peak heights overall, including the highest peaks for methomyl and aldicarb, was 0.01 *M* potassium hydroxide (D).

The method was evaluated at five reaction heater temperatures using the derivatization reagent prepared in 0.01 *M* potassium hydroxide. The results (Table II) indicate that methomyl and aldicarb exhibited the greatest resistance to hydrolysis, and the highest peaks for these compounds were obtained at the highest temperature studied. At 140°C seven of the eleven compounds exhibited their highest peaks, but the methomyl and aldicarb peaks were only about 60% of the maximum height obtained. However, since the absolute peak heights of methomyl and aldicarb at 140°C were comparable to those of the other compounds at the same temperature (see Fig. 2), the overall improvement in peak height at 150 and 160°C was not felt to be sufficient to justify using these higher temperatures. Based on these considerations it was decided to use a reaction heater temperature of 140°C. The single-bead string reactor positioned immediately downstream from the reaction heater provided adequate back-pressure to prevent boiling of the derivatization reagent/mobile phase mixture in the delay tubes.

Of all the carbamates studied, methomyl and aldicarb were most affected by the length of the delay tube (Table III). At a reaction heater temperature of 140°C the peak heights obtained for these two compounds were almost 90% higher with two delay tubes than with one. The peak heights for the other compounds were somewhat lower with two delay tubes than with one. Perhaps this was caused by band broadening due to diffusion of the analytes in the added volume. However, because of the significant improvement in peak height of methomyl and aldicarb, two delay tubes were used in the optimized single-stage reactor.

TABLE II

EFFECT OF REACTION HEATER TEMPERATURE ON PEAK HEIGHTS OF 11 CARBAMATES (0.2 μg/ml EACH)

Derivatization reagent prepared in 0.01 *M* aq. potassium hydroxide.

Carbamate	*Relative peak height (%)*[a]				
	100°C	*130°C*	*140°C*	*150°C*	*160°C*
Aldicarb sulfoxide	24	86	100	94	88
Aldicarb sulfone	32	94	100	95	91
Oxamyl	31	100	97	100	88
Methomyl	0	35	60	84	100
3-Hydroxycarbofuran	30	98	100	99	94
Aldicarb	0	34	59	82	100
Propoxur	21	97	100	98	98
Carbofuran	22	97	100	90	97
Carbaryl	52	99	100	98	97
Methiocarb	32	100	98	95	94
Bufencarb	26	97	100	98	94
Mean	23	85	92	94	95

[a] Normalized on the highest peak (100%) for each compound.

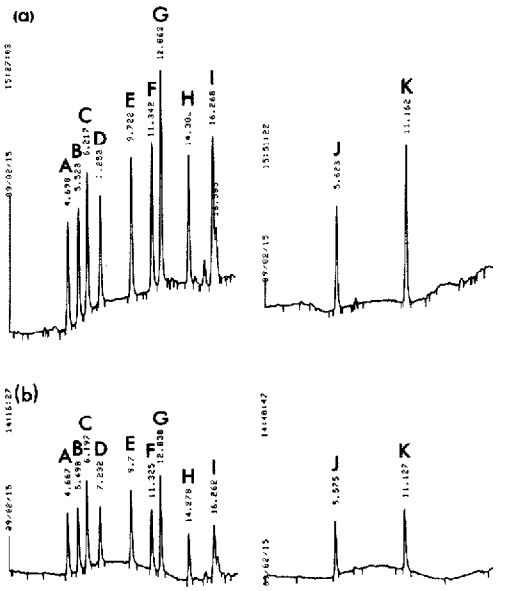

Fig. 2. Chromatograms of carbamate pesticides (0.2 μg/ml each): (a) single-stage method; (b) two-stage method. A = Aldicarb sulfoxide; B = aldicarb sulfone; C = methomyl; D = 3-hydroxycarbofuran; E = aldicarb; F = carbofuran; G = carbaryl; H = methiocarb; I = bufencarb; J = oxamyl; K = propoxur.

TABLE III

EFFECT OF LENGTH OF DELAY TUBE ON PEAK HEIGHTS OF 9 CARBAMATES (0.2 μg/ml EACH)

Derivatization reagent prepared in 0.01 *M* potassium hydroxide. Reaction heater temperature 140°C.

Carbamate	*Relative peak height (%)*[a]	
	One delay tube[b]	*Two delay tubes*[c]
Aldicarb sulfoxide	100	96
Aldicarb sulfone	100	95
Methomyl	53	100
3-Hydroxycarbofuran	100	86
Aldicarb	54	100
Carbofuran	100	85
Carbaryl	100	89
Methiocarb	100	84
Bufencarb	100	83
Mean	90	91

[a] Normalized on the highest peak (100%) for each compound.
[b] Approximate total length 3 m.
[c] Approximate total length 6 m.

TABLE IV

REPRODUCIBILITY OF RETENTION TIMES AND PEAK HEIGHTS OF 9 CARBAMATES (0.2 μg/ml EACH) USING THE SINGLE-STAGE POST-COLUMN DERIVATIZATION METHOD

Carbamate	*Coefficient of variation (%)*[a]	
	Retention time	*Peak height*
Aldicarb sulfoxide	0.18	1.08
Aldicarb sulfone	0.39	1.37
Methomyl	0.28	1.15
3-Hydroxycarbofuran	0.20	1.77
Aldicarb	0.21	2.14
Carbofuran	0.16	1.68
Carbaryl	0.20	3.42
Methiocarb	0.12	4.56
Bufencarb [b]	0.08	3.15

[a] Based on five consecutive 10-μl injections.
[b] Based on four injections only.

Acetonitrile and methanol were compared as modifiers of the aqueous LC mobile phase. A mobile phase gradient of 30–100% methanol in water over 16 min resulted in retention times which approximated those produced by the 20–80% acetonitrile gradient described earlier. The total peak height was about 90% higher when acetonitrile was used, indicating that the choice of organic mobile phase modifier made a significant difference in the performance of the method.

The choice of mercaptan in the derivatization reagent, either 2-mercaptoethanol or 3-mercaptopropionic acid (at the same concentration), did not have a significant effect on peak heights of the carbamates.

The reproducibility of the method in terms of coefficients of variation of both retention times and peak heights (Table IV) was adequate. The limit of detection was on the order of 0.1 ng analyte injected. Calibration curves for the carbamates were linear up to 5 ng analyte injected.

Fig. 2 illustrates chromatograms of the mixed standards obtained with the single-stage method (Fig. 2a) and the conventional two-stage method (Fig. 2b). The conditions used in the two-stage method were as follows. The hydrolyzing reagent (0.05 *M* aq. potassium hydroxide) was introduced at a flow-rate of 0.6 ml/min. The hydrolysis heater temperature was 140°C. The derivatizing reagent (5 mg OPA and 50 μl 2-mercaptoethanol in 200 ml 0.01 *M* aq. sodium tetraborate was introduced at a flow-rate of 0.6 ml/min. All other conditions were the same as those for the single-stage method. The total peak height for all the carbamates was 119% higher with the single-stage method than with the two-stage method at the same recorder attenuation.

The single-stage post-column derivatization method presented here is therefore an improvement over the conventional two-stage method in terms of both sensitivity and simplicity. The hardware requirements and cost are reduced and the daily operation is streamlined by the requirement of preparation of only one derivatization reagent.

REFERENCES

1 H. A. Moye, S. J. Scherer and P. A. St. John, *Anal. Lett.*, 10 (1977) 1049.
2 R. T. Krause, *J. Chromatogr. Sci.*, 16 (1978) 281.
3 R. T. Krause, *J. Chromatogr.*, 185 (1979) 615.
4 R. T. Krause, *J. Assoc. Off. Anal. Chem.*, 63 (1980) 1114.
5 R. T. Krause, *J. Assoc. Off. Anal. Chem.*, 68 (1985) 726.
6 R. T. Krause, *J. Assoc. Off. Anal. Chem.*, 68 (1985) 734.
7 H. Engelhardt and B. Lillig, *Chromatographia*, 21 (1986) 136.
8 A. de Kok, M. Hiemstra and C. P. Vreeker, *Chromatographia*, 24 (1987) 469.
9 P. Kucera and H. Umagat, *J. Chromatogr.*, 225 (1983) 563.
10 L. Nondek, U. A. Th. Brinkman and R. W. Frei, *Anal. Chem.*, 55 (1983) 1466.
11 L. Nondek, R. W. Frei and U. A. Th. Brinkman, *J. Chromatogr.*, 282 (1983) 141.