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Determination of N-methylcarbamates and N-methylcarbamoyloximes in water by high-performance liquid chromatography with the use of fluorescence detection and a single *o*-phthalaldehyde post-column reaction

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ABSTRACT

A single-stage post-column fluorescence HPLC method for the analysis of N-methylcarbamates and N-methylcarbamoyloximes is presented as an alternative to the traditional two-stage method. The two-stage technique involves post-column hydrolysis with sodium hydroxide followed by derivatization with *o*-phthalaldehyde and β-mercaptoethanol in a borate buffer. In the proposed method only one reagent, consisting of sodium hydroxide, *o*-phthalaldehyde, and N,N-dimethyl-2-mercaptoethylamine hydrochloride (Thiofluor), is used. The stability of the reagent is compared to single-stage alternatives involving β-mercaptoethanol or 3-mercaptopropionic acid, which have been previously reported. It is reported that Thiofluor provides the best stability and is satisfactory for overnight chromatographic runs. The sensitivity of the method is reported to be equal to that of the two-stage method. This innovation simplifies reagent preparation and equipment maintenance, while shortening start-up time without any loss in sensitivity.

INTRODUCTION

The extensive agricultural application of toxic N-methylcarbamates, *e.g.*, carbofuran and carbaryl, and of toxic N-methylcarbamoyloximes, *e.g.*, aldicarb and methomyl, has led the United States Environmental Protection Agency (U.S. EPA) to regulate levels of carbofuran [1], and to require monitoring the levels of oxamyl, methomyl, 3-hydroxycarbo-furan, and carbaryl [2] in community drinking water sources. The prevalent analytical technique for these compounds is the high-performance liquid chromatography (HPLC) fluorescence method first developed by Moye *et al.* [3] and extensively studied by Krause [4–7].

N-Methylcarbamates and N-Methylcarbamoyloximes in environmental samples are normally determined using two post-column reactions. In the first reaction the analytes are hydrolyzed using sodium hydroxide to methylamine and in the second the latter reacts with β-mercaptoethanol and *o*-phthalaldehyde (OPA) in a borate buffer to form a fluorescent isoindole.

Attempts at simplifying carbamate HPLC analysis have involved replacement of the hydrolysis reagent by a basic solid-phase catalyst contained in a heated cartridge in the post-column stream [8,9]. Ion-exchange resins in the basic form [8] and magnesium oxide [9] have been used in these schemes, which eliminate the need for one of the two post-column pumps normally used.

McGarvey [10] has eliminated one pump by placing the base together with the OPA and β-mercaptoethanol in the same bottle. The use of 3-mercaptopropionic acid is presented as an alternative to

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β-mercapto-ethanol. McGarvey's paper does not address the critical issue of reagent stability over a time period needed to process a group of samples. Our work indicates that the stability of the β-mercaptoethanol reagent is unsatisfactory, and that N,N-dimethyl-2-mercaptoethylamine hydrochloride (Thiofluor) gives a somewhat more stable reagent than does 3-mercaptopropionic acid. The odor associated with either β-mercapto-ethanol or 3-mercaptopropionic acid is not present with Thiofluor.

We have found that both reactions can be carried out using a single reagent consisting of o-phthalaldehyde and Thiofluor in 0.05 *M* sodium hydroxide. The single reagent is adequately stable for runs of as many as 30 injections, without the need to fabricate and maintain solid-phase cartridges. Use of a single-stage post-column reaction decreases equipment cost, simplifies reagent preparation, equipment set-up, and system maintenance without any loss in sensitivity.

EXPERIMENTAL

Chemicals

All carbamate analytes used in the preparation of calibration standards were supplied by U.S. EPA (Research Triangle Park, NC, USA). The method was tested with a carbamate mixture of the ten analytes plus 1-naphthol purchased from Crescent Chemical (Hauppauge, NY, USA). The internal standard, 4-bromo-3,5-dimethylphenyl N-methylcarbamate (BDMC), was supplied by Aldrich (Milwaukee, WI, USA). Aldrich also supplied 3-mercaptopropionic acid and β-mercaptoethanol. Pickering Labs. (Mountain View, CA, USA) supplied Thiofluor. Fisher Scientific (Orlando, FL, USA) provided HPLC-grade water, acetonitrile, and all other chemicals, which were of reagent grade.

Instrumentation

Samples were injected using a Perkin Elmer ISS-100 autosampler (Perkin Elmer, Norwalk, CT, USA). The analytical pump was a Perkin Elmer Series 410 with gradient capability. The signal was monitored with a Perkin Elmer LS-1 fluorescence detector. Data handling was carried out using the Perkin Elmer OMEGA IV Workstation equipped with a GP-100 Graphics Printer. The post-column derivatization equipment including pump, flow conditioners, reaction coils, and oven were supplied by Pickering Labs.

Chromatographic conditions

An Uptight guard column (Upchurch Scientific, Oak Harbor, WA, USA), packed with C_{18} bonded-phase preceded a Perkin Elmer 150 × 4.6 mm I.D. HS-3C_{18}, 3 μm column. Well water samples were directly injected after being filtered. The injection volume was always 400 μl. A water–acetonitrile gradient began at 5% acetonitrile and reached 20% in 13 min and 65% during the next 15 min. The original composition of 5% was restored during the next 2 min and held for 8 min before the next injection. All changes were made in linear fashion. Pumping was at 1 ml/min throughout the gradient program. Mobile phase components were continuously sparged with helium during chromatography. Excitation wavelength of the pulsed xenon source was set at 340 nm, and emission was set at 460 nm. The detector gain was set at 2 and the response at 3.

Post-column reaction conditions. In two-stage studies both post-column pumps were run at approximately 0.1 ml/min. The hydrolysis reaction was carried out at 95°C in a 500-μl coil and the OPA reaction at ambient temperature in a 200-μl reaction coil. In single-stage studies the post-column pump was set at approximately 0.1 ml/min and the reaction was carried out at 95°C in a 500-μl reaction coil. A 34.4-bar pressure-release valve was installed ahead of the detector in all studies to enhance performance of post-column pumping.

Reagent for single-stage method. To approximately 100 ml of water in a 250-ml volumetric flask was added 1.25 ml of 10 *M* sodium hydroxide. A solution of 180 mg of Thiofluor in *ca.* 10 ml of water and a solution of 25 mg of OPA in 2.5 ml of methanol were added with water rinsings. The solution was diluted to 250 ml, filtered through a 0.45-μm nylon filter, and degassed with helium for approximately 10 min before starting the sample run. The reagent was prepared fresh daily.

Reagents for the two-stage post-column reaction method. The conditions are essentially those recommended in U.S. EPA method 531.1 [11]. The reagent used in the first (95°C) hydrolysis reaction is 0.05 *M* sodium hydroxide. The second reagent was prepared by dissolving 4.78 *g* sodium borate deca-

hydrate in 250 ml water and adding 25 mg of OPA in 2.5 ml of methanol. Both reagents were filtered and degassed as in the single-stage method. Just before use, 25 μl of 50% (v/v) β-mercaptoethanol in acetonitrile was added to the OPA reagent. The OPA reagent was prepared fresh daily.

Preservation of standards and samples. The preservative used was that recommended in U.S. EPA method 531.1 [11] and was prepared by mixing 156 ml 2.5 *M* monochloroacetic acid and 100 ml 2.5 *M* potassium acetate. Each 10 ml of standard or control solution contained 300 μl of preservative. Samples were stored at −23°C.

RESULTS AND DISCUSSION

Comparison of chromatograms developed using the present single-stage method and using the traditional two-stage post-column method is shown in Fig. 1. Examination of Fig. 1 reveals that the chromatograms are essentially equivalent. Retention times, recoveries, and estimated detection limits (EDLs) for the ten analytes are shown in Table I. Both single-stage and two-stage estimated detection limits for a signal-to-noise ratio of 5 were found to be in the range of 0.5 to 1.2 μg/l. This is in reasonably close agreement to the literature values of 0.2 to 0.6 μg/l [12].

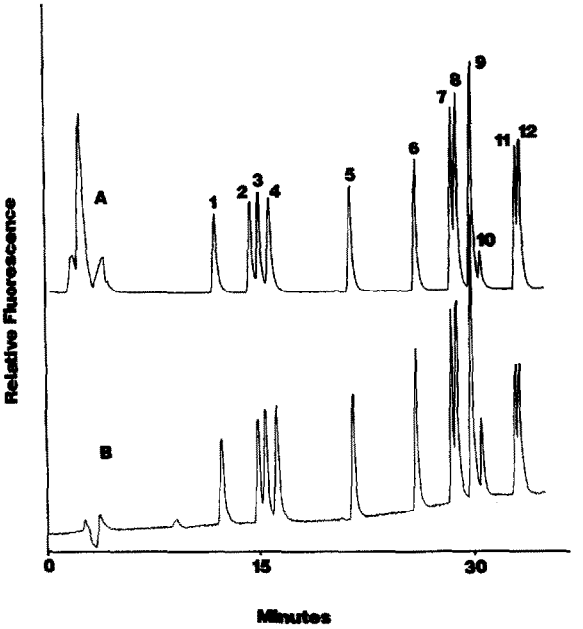

Fig. 1. Chromatogram using two-stage post-column reaction method (A) and using single-stage post-column reaction method (B). For both chromatograms: 1 = aldicarb sulfoxide, 2 = aldicarb sulfone, 3 = oxamyl, 4 = methomyl, 5 = 3-hydroxycarbofuran, 6 = aldicarb, 7 = propoxur, 8 = carbofuran, 9 = carbaryl, 10 = 1-naphthol, 11 = methiocarb, 12 = BDMC (internal standard). Both A and B resulted from the injection of 400 μl of water containing 20 μg/l of each analyte.

TABLE I

CHROMATOGRAPHIC RESULTS FOR TEN ANALYTES USING A SINGLE-STAGE POST-COLUMN REACTION

Retention times and recoveries were based on seven measurements at 10 μg/l. EDL is the estimated detection limit at a signal-to-noise ratio of 5.

Analyte	Retention time (min)	Recovery at 10 μg/l (%)	EDL (μg/l)
Aldicarb sulfoxide	12.08	100.4	1.2
Aldicarb sulfone	14.38	101.0	1.0
Oxamyl	15.07	99.4	0.9
Methomyl	15.69	99.6	0.9
3-Hydroxycarbofuran	21.23	102.1	1.1
Aldicarb	25.79	99.6	0.7
Propoxur	28.35	100.6	0.6
Carbofuran	28.71	100.4	0.5
Carbaryl	29.78	101.0	0.4
Methiocarb	32.92	99.4	0.7

Recoveries at 10 μg/l, averaged over seven determinations, were in the narrow range from 99.4 to 102%. Recoveries were based on the observed concentrations of analytes in a quality control mixture purchased from Crescent Chemical, after a single-point calibration using a standard prepared in our laboratory.

The precision at 10 μg/l was demonstrated by determining the relative standard deviation for seven measurements. The relative standard deviation varied from 1.8% for methomyl to 3.1% for 3-hydroxycarbofuran, with an average value of 2.1%.

Linearity of the single-stage post-column reaction method was tested using four concentrations, 3, 10, 20, and 50 μg/l. The r value was found to be 0.999 for each of the ten analytes.

In order to compare the stability of the single-stage post-column reagent used in the present method with that used by McGarvey [10], peak heights were followed over the course of twenty injections. In McGarvey's work the reagent was prepared using either β-mercaptoethanol or 3-mercaptopropionic acid. Using McGarvey's β-mercaptoethanol formulation peak heights for the ten analytes decreased to 35% of their original magnitude, on average, during 20 injections. Using McGarvey's preparation employing 3-mercaptopropionic acid peak heights were, on average, 91% of their starting magnitude after twenty injections. Use of the present method gave peak heights, on average, of 97% of their original magnitude after twenty injections. Sparging with helium throughout the run resulted in improvement for β-mercaptoethanol bringing peak heights to 71% of their initial values, but did not improve stability for Thiofluor or 3-mercaptopropionic acid. Though use of 3-mercaptopropionic acid is practical, we recommend the use of Thiofluor and suggest recalibration after every ten injections for best accuracy. Thiofluor, besides providing a somewhat more stable reagent, does not have an objectionable odor as does 3-mercaptopropionic acid.

In our laboratory we process as many as thirty vials containing samples and controls in a single overnight run. Provided that reasonably fresh water was used in making the gradient, we have encountered no interferences or unusual behavior with this method.

CONCLUSIONS

Carbamates and carbamoyloximes can be determined in the μg/l range using a single-stage reagent containing Thiofluor and OPA dissolved in 0.05 M sodium hydroxide. The single-stage method has no disadvantages compared to the traditional two-stage technique and leads to faster reagent preparation and considerable operational simplification.

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