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Faster and More Sensitive Determination of *N*-Methylcarbamates in Drinking Water by HPLC

INTRODUCTION

The *N*-methylcarbamates and the *N*-methylcarbamoyloximes are some of the most widely used pesticides in agriculture. The United States Environmental Protection Agency (U.S. EPA) provides guidelines for monitoring the presence of carbamate pesticides and related compounds in raw surface water using EPA Method 531.2. This method uses HPLC with fluorescence detection following postcolumn derivatization to enhance method sensitivity and selectivity compared to UV absorbance detection.¹ Dionex has published a detailed method² that is consistent with the EPA method.

The regulations of the European Union (EU) for drinking water provide a general rule for pesticides (98/83/EC).³ This rule states that the maximum admissible concentration of each individual pesticide component is 0.1 µg/L, and the total concentration is not to exceed 0.5 µg/L. Detection of these regulated compounds at such low concentrations is a challenge faced by many water testing laboratories using the method described in EPA Method 531.2.

The work shown here describes a faster and more sensitive method for the determination of the carbamates specified in EPA Method 531.2. The structures of the compounds measured in EPA Method 531.2 are shown in Figure 1. The separation is performed on an Acclaim[®] Carbamate LC column and the detection is performed on the FLD-3400RS fluorescence detector, which provides higher detection sensitivity compared to the fluorescence detector used in a previous Dionex publication (AN 96).

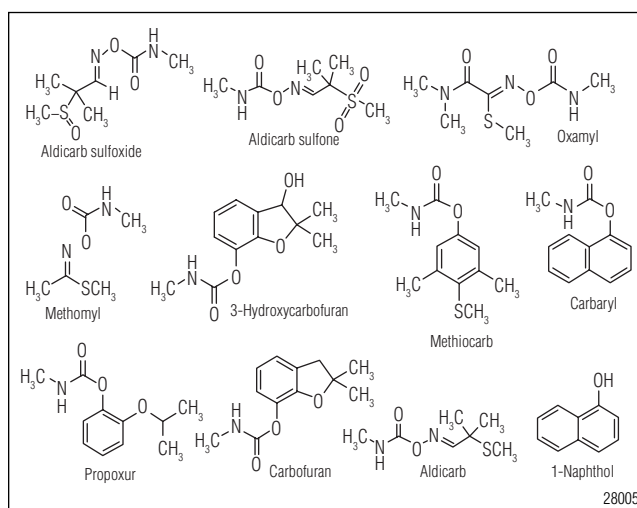


Figure 1. Structures of the carbamates specified in U.S. EPA method 531.2.

The procedures for separation and detection are based on a reversed-phase separation of the carbamates with derivatization by *o*-phthalaldehyde (OPA) followed by fluorescence detection. Baseline separation of these carbamate compounds is observed with resolutions (R_s) ≥ 1.5 and completed within 20 min; method detection limits (MDL) are all ≤ 0.01 µg/L; and the quantification limit is 0.05 µg/L, which exceeds the requirement of EPA Method 531.2 (0.2 µg/L) and meets the restriction in 98/83/EC (0.1 µg/L).

EQUIPMENT

Dionex UltiMate® 3000 HPLC system including:

HPG-3400 Pump with SRD-3600 Solvent Rack with Degasser

WPS-3000TSL Autosampler

TCC-3000 Thermostatted Column Compartment

FLD-3400RS Fluorescence Detector

Chromeleon® Chromatography Data System (CDS) software, Version 6.80, SR9

Pickering PCX 5200 Derivatization Instrument (Pickering Laboratories, Inc.)

REAGENTS AND STANDARDS

Deionized water, Milli-Q® Gradient A10, Millipore Corporation

Methanol (CH₃OH), Fisher

Potassium dihydrogen citrate (KC₆H₇O₇), 98%, Fluka

Sodium thiosulfate (Na₂S₂O₃), 98% Fluka

Sodium hydroxide solution, (NaOH), 50%, Fluka

o-Phthalaldehyde (OPA, C₈H₆O₂, CAS#: 643-79-8), 99%, Pickering

Boric acid, (H₃BO₃, CAS#: 10043-35-3) 99.5%, Fluka

β-Mercaptoethanol, 99%, SCRC, China

EPA Method 531.2 Carbamate Pesticide Calibration Mixture, Restek, 100 µg/mL (P/N 257974)

4-Bromo-3, 5-dimethylphenyl-*N*-methylcarbamate standard, Restek, 100 µg/mL (P/N 32274)

PREPARATION OF REAGENTS AND STANDARDS

Reagent Water

Deionized water, Milli-Q Gradient A10, 18 MΩ-cm resistivity or better

Preserved Reagent Water

Dissolve 4.6 g of potassium dihydrogen citrate and 40 mg of Na₂S₂O₃ in a 50 mL beaker with reagent water, then transfer this solution to a 500 mL volumetric flask and bring to volume with reagent water. Prior to use, filter the solution through a 0.45 µm filter.

Stock Standard Carbamates Calibration Mixture

Pipet 10 µL of EPA 531.2 carbamate calibration mixture (100 µg/mL) into a 1 mL vial and add 990 µL methanol. The concentration for each carbamate in the stock standard mixture is 1.0 µg/mL.

Note: To assist in troubleshooting postcolumn chemistry issues, 1-naphthol is included in the standard carbamate mixture. This compound is naturally fluorescent; therefore, it will be the only peak present in a chromatogram when the postcolumn system is not functioning properly.

Stock Standard of 4-Bromo-3,5-Dimethylphenyl-*N*-Methylcarbamate

Pipet 100 µL of 4-bromo-3,5-dimethylphenyl-*N*-methylcarbamate (surrogate analyte [SUR]) standard (100 µg/mL) into a 1 mL vial and add 900 µL methanol. The concentration of the stock standard solution is 10 µg/mL.

Working Standard Solutions for Calibration

Prepare five working standard solutions by adding the quantities of carbamate mixture stock standard solutions listed in Table 1 to 100 mL or 25 mL volumetric flasks. Add 5 µL of the stock standard solution of 4-bromo-3,5-dimethylphenyl-*N*-methylcarbamate into each flask. Bring to volume with preserved reagent water.

Table 1. Preparation of Calibration Standards

Concentration of Carbamate Mixture Stock Standard (µg/mL)	Volume of Carbamate Mixture Stock Standard (µL)	Concentration of Stock Standard of Surrogate Analyte (SUR) (µg/mL)	Volume of Stock Standard of SUR (µL)	Final Volume of Calibration Standard (mL)	Final Concentration of Carbamate Standard (µg/L)	Final Concentration of SUR (µg/L)
1	5	10	20	100	0.05	2
	10		20	100	0.10	
	5		5	25	0.20	
	15		5	25	0.40	
	20		5	25	0.80	

Sodium Hydroxide Hydrolysis Reagent (Postcolumn Reagent 1)

Sodium hydroxide, 0.2%: dilute 4 mL of 50% w/w sodium hydroxide (NaOH) solution to 1 L with reagent water. The concentration of the hydrolysis solution can dramatically affect the analyte response. Filter and degas with nitrogen just before use.¹

OPA Reagent (Postcolumn Reagent 2) for Postcolumn Derivatization

Prepare boric acid buffer: dissolve 3.0 g of boric acid in a 1 L volumetric flask containing approximately 800 mL of reagent water. Add 1.2 mL of a 50% (w/w) NaOH solution. Bring the volume up to 1.0 L with reagent water. Filter and degas prior to preparation of postcolumn reagent 2.

Add the OPA solution (dissolve 100 mg of OPA in 5 to 10 mL of methanol) to 1 L boric acid buffer, then add 1 mL 2-mercaptoethanol. This solution is postcolumn reagent 2.¹ To review the postcolumn chemistry and see a diagram of the postcolumn system configuration, see AN 96.

TAP WATER SAMPLE PREPARATION

The tap water sample was taken at the Dionex (Shanghai) Applications Lab located in the Pudong District, Shanghai, China.

Add 2.3 g potassium dihydrogen citrate and 20 mg Na₂S₂O₃ to a beaker, then add 250 mL of tap water to the beaker and mix. Prior to use, filter through a 0.45 µm filter.

Time (min)	Flow Rate (mL/min)	Methanol (%)	H ₂ O (%)
-4	0.9	14	86
0		14	86
2		20	80
8		40	60
13.6		70	30
16		70	30

CONDITIONS

Column:	Acclaim® Carbamate, 3.0 × 150 mm, 3 µm, P/N 072926
Column Temperature:	50 °C
Mobile Phase:	Methanol–Water, in gradient (Table 2)
Flow Rate:	0.9 mL/min
Injection Volume:	250 µL
Postcolumn Reagent 1:	0.2% sodium hydroxide, first reaction coil at 100 °C
Postcolumn Reagent 2:	OPA reagent, second reaction coil at room temperature
Flow Rate of Reagents 1 and 2:	0.3 mL/min
Fluorescence:	Excitation/330 nm and Emission/465 nm
	Data Collection Rate: 5
	Response Time: 4
	Sensitivity: 7
	Lamp Mode: High Power
	PMT (Photomultiplier Tube): Pmt1
	Filter Wheel: 280 nm

Conversion of WPS-3000TSL Autosampler for Larger-Volume Injection

A 250 µL injection is needed in this application; therefore, a 250 µL sample loop (P/N 6820.2422) must be installed in the current analytical WPS3000 autosampler. After installing the sample loop, install a buffer loop (P/N 6820.2421) and a 250 µL syringe (P/N 6822.0003) in place of the units that are standard in the common analytical version. For the WPS3000 to successfully make 250 µL injections, all three components (sample loop, buffer loop, and syringe) must be installed.

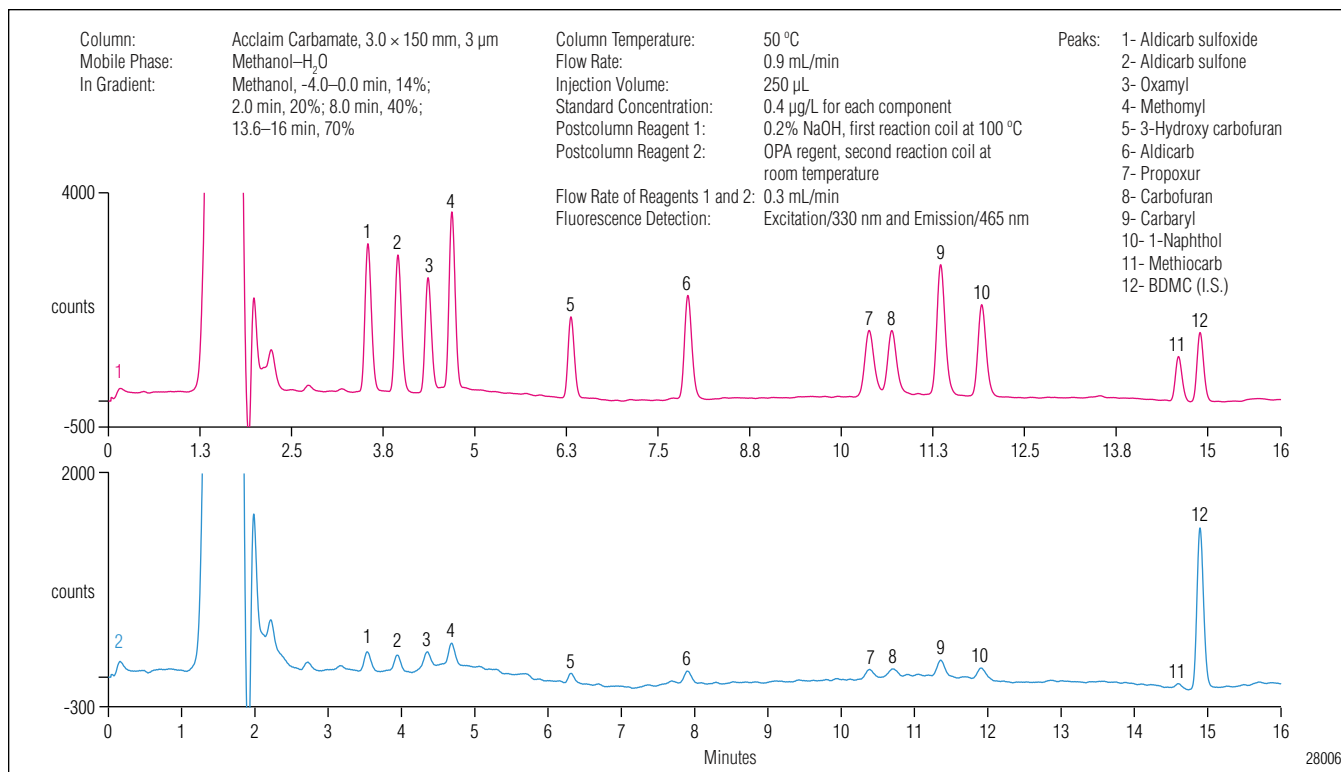


Figure 2. Chromatograms of carbamate standards described in EPA Method 531.2 with concentrations of (A) 0.8 µg/L and (B) 0.05 µg/L.

RESULTS AND DISCUSSION

Separation and Reproducibility

Figure 2 illustrates good separation of the carbamates listed in EPA Method 531.2 using the Acclaim Carbamate LC column, which is designed for the baseline separation of these carbamates. Resolution (R_s) for all peaks is ≥ 1.5 , much better than the values required by the EPA Method (≥ 1.0).

Reproducibility was estimated by making seven replicate injections of a calibration standard with concentration of 0.4 µg/L. The values of relative standard deviation (RSD) of each carbamate for retention time and for peak area are listed in Table 3.

Linearity and Detection Limits

Calibration linearity for the determination of carbamates by this method was investigated by making seven replicate injections of serial standard solutions of carbamates at five different concentrations from 0.05 to 0.8 µg/L. The quantification limit is low—to 0.05 µg/L—which significantly exceeds the requirement of EPA Method 531.2 (0.2 µg/L) and meets the requirements of 98/83/EC (0.1 µg/L).

Table 3. Reproducibility of Retention Times and Peak Areas for Carbamates (n = 7)

Carbamate	Retention Time RSD	Peak Area RSD
Aldicarb sulfoxide	0.071	1.8
Aldicarb sulfone	0.049	1.2
Oxamyl	0.053	2.5
Methomyl	0.027	3.2
3-Hydroxycarbofuran	0.020	1.9
Aldicarb	0.024	2.6
Propoxur	0.022	1.1
Carbofuran	0.025	4.0
Carbaryl	0.026	2.6
1-Naphthol	0.027	2.3
Methiocarb	0.019	3.6

Detection limits of carbamates were calculated by using the equation:

$$\text{Detection limit} = St_{(n-1, 1-\alpha=0.99)}$$

The symbol S represents Standard Deviation (SD) of replicate analyses, n represents number of replicates, $t_{(n-1, 1-\alpha=0.99)}$ represents Student's value for the 99% confidence level with $n - 1$ degrees of freedom.

Table 4. Method Linearity Data and Method Detection Limits (MDL) ^{1,2}

Carbamate	Regression Equation	r^2	Range of Standards µg/L	MDL, µg/L		
				Current Data ³	Data from Dionex AN 96 ⁴	Data Reported in EPA Method 531.2 ⁵
Aldicarb sulfoxide	$A = 2.6763 c - 0.0402$	0.9992	0.05–0.8	0.010	0.018	0.056
Aldicarb sulfone	$A = 2.4889 c - 0.0481$	0.9987		0.008	0.046	0.026
Oxamyl	$A = 1.9727 c - 0.0229$	0.9960		0.007	0.035	0.045
Methomyl	$A = 3.0717 c - 0.0169$	0.9978		0.005	0.028	0.045
3-Hydroxycarbofuran	$A = 1.4316 c - 0.0329$	0.9990		0.009	0.036	0.041
Aldicarb	$A = 2.2111 c - 0.0091$	0.9962		0.004	0.032	0.042
Propoxur	$A = 1.7090 c - 0.0332$	0.9957		0.009	0.031	0.040
Carbofuran	$A = 1.6752 c - 0.0362$	0.9907		0.006	0.059	0.058
Carbaryl	$A = 3.0831 c - 0.0532$	0.9987		0.007	0.026	0.068
1-Naphthol	$A = 2.1117 c - 0.0299$	0.9990		0.008	/	0.034
Methiocarb	$A = 0.8365 c - 0.0205$	0.9974		0.010	0.041	0.036

Note:

1. Detection limits in reagent water with 0.2 µg/L fortified level
2. Using Pickering Model PCX 5200 Postcolumn system
3. Using FLD-3400RS Fluorescence detector
4. Using RF-2000 Fluorescence detector
5. Using Waters Model 474 Fluorescence detector

According to the requirement in Method 531.2, nine replicate injections of reagent water fortified with 0.2 µg/L of carbamate standard mixture were used to determine the minimum detection limits. Table 4 summarizes the MDL data, which show excellent method linearity and sensitivity with detection limits well below those defined in the EPA method. The largely improved detection limits may be attributed to the improvements in fluorescence detector sensitivity and reversed-phase column technology since the original EPA work was completed. The EPA method used a 4 µm, 3.9 × 150 mm column, while this method used a 3 µm, 3.0 × 150 mm column to yield more efficient peaks. These improved detection limits easily allow the analyst to reach the minimum reporting limits (lower than 5× the MDL) of the original method.

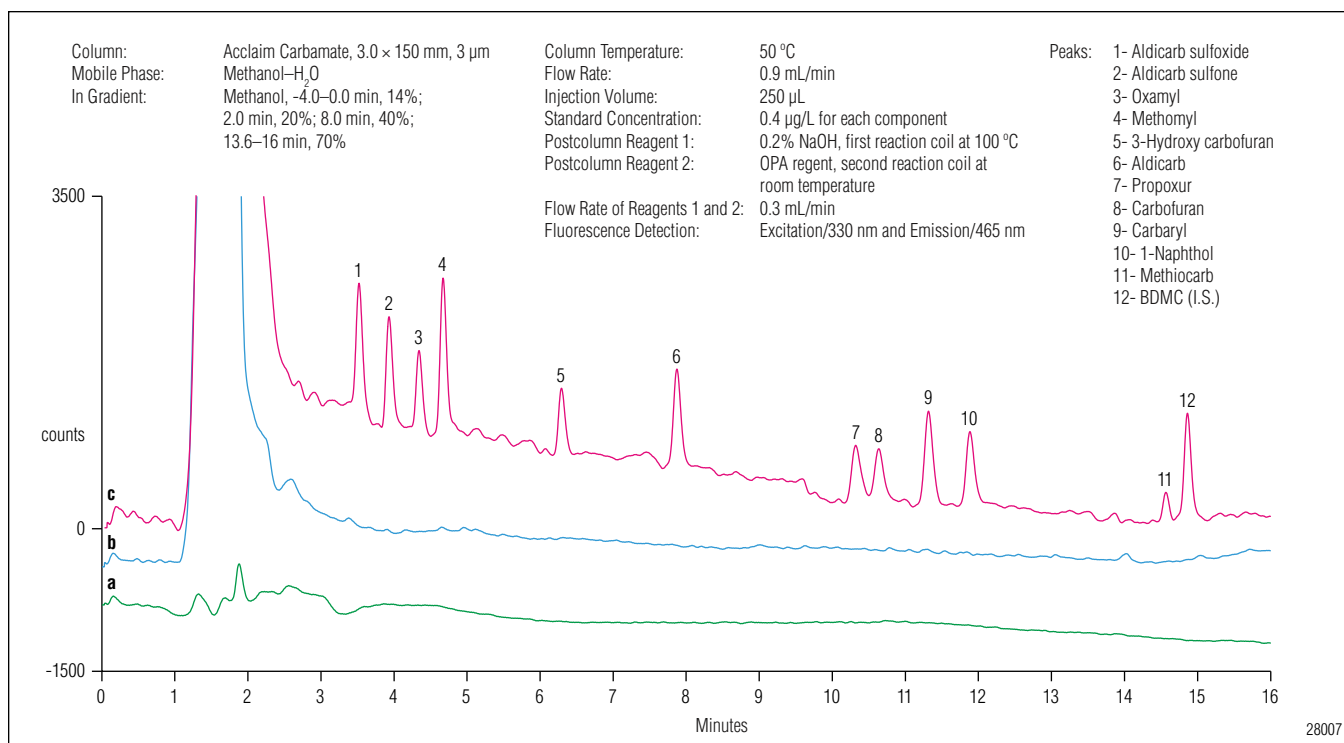


Figure 3. Chromatograms of (a) deionized water, (b) tap water, and (c) sample b fortified with a carbamate standard mixture with 0.4 µg/L for each carbamate. Others conditions are the same as in Figure 2.

Tap Water Sample Analysis

Figure 3 compares the chromatograms of an unadulterated tap water sample with the same sample fortified with 0.4 µg/L carbamate standards. No detectable levels of carbamates were found in the tap water. The related data are summarized in Table 5. These data show excellent spike recovery for each carbamate compound, thereby suggesting method accuracy.

Carbamate	Detected (µg/L)	Added (µg/L)	Found (µg/L)	Recovery (%)
Aldicarb sulfoxide	0.0	0.40	0.43	110
Aldicarb sulfone	0.0		0.41	103
Oxamyl	0.0		0.39	98
Methomyl	0.0		0.42	105
3-Hydroxycarbofuran	0.0		0.41	103
Aldicarb	0.0		0.45	113
Propoxur	0.0		0.41	103
Carbofuran	0.0		0.40	100
Carbaryl	0.0		0.39	98
1-Naphthol	0.0		0.43	110
Methiocarb	0.0		0.40	100

CONCLUSION

This application update describes an optimized method for determining carbamates on a Dionex HPLC system with an Acclaim Carbamate column (3 μm). The significant improvements of method detection limit (<0.01 $\mu\text{g/L}$ or pg-grade per injection) and quantification limit (0.05 $\mu\text{g/L}$ or 12.5 pg per injection) for the carbamates demonstrate that this method is ideally suited for determining these compounds in drinking water.

REFERENCES

1. *Measurement of N-Methylcarbamoyloximes and N-Methylcarbamates in Water by Direct Aqueous Injection HPLC with Postcolumn Derivatization*; U.S. Environmental Protection Agency, U.S. EPA Method 531.2, Revision 1.0, Cincinnati, OH, 2001.
2. Dionex Corporation, *Determination of N-Methylcarbamates by Reversed-Phase HPLC*. Application Note 96, LPN 1935, 2007, Sunnyvale, CA.
3. *Quality of Water Intended for Human Consumption*; European Communities, L 330/42, Official Journal of the European Council Directive 98/83/EC (En), November 1998.

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