Determination of *N*-Methylcarbamates by Reversed-Phase HPLC

INTRODUCTION

N-methylcarbamates and *N*-methylcarbamoyloximes are among the most widely used pesticides in the world. U.S. EPA method 531.2 provides guidelines for monitoring these compounds in ground and surface waters as well as drinking water.[1] Reversed-Phase High Performance Liquid Chromatography (RP-HPLC) is the preferred method for separating carbamates. Most alternate methods for analysis have significant limitations. Gas chromatographic analysis has proven unsuccessful due to degradation of the analyte compounds during vaporization. HPLC with UV detection does not offer the sensitivity or specificity required for the sample matrices of interest. HPLC with positive mode electrospray ionization mass spectrometry has been shown to be a promising alternative, but it increases the expense and expertise required for the analysis.

Postcolumn derivatization with fluorescence detection after a reversed-phase HPLC separation is consistent with U.S. EPA Method 531.2. This method delivers highly sensitive determinations of carbamate compounds.

SUMMARY OF CHROMATOGRAPHIC METHOD

After direct injection of the sample onto the HPLC column, carbamates are separated using a water/methanol/acetonitrile gradient. After separation, they pass through a postcolumn reaction system where they are derivatized with a fluorescent reagent, then quantified using fluorescence detection. The Acclaim® 120 C18 column provides reliable separation of the analyte compounds. The reagents specified are quality controlled by the manufacturer to ensure minimal background interference.

INSTRUMENTATION

Dionex Summit® HPLC system* consisting of:
- DPG-680 Dual Pump
- ASI-100 Automated Sample Injector
- TCC-100 Thermostatted Column Compartment
- RF-2000 Fluorescence Detector
- Chromeleon® 6.80 Chromatography Workstation
- Pickering PCX 5200 Derivatization Instrument

*This application can also be performed on an UltiMate® 3000 HPLC with the following components:
- DPG 3600A Dual Pump SRD 3600
- WPS-3000TSL Automated sample injector
- TCC-3200 Thermostatted Column Compartment
- RF-2000 Fluorescence Detector

REAGENTS AND STANDARDS

Water, Milli-Q water from Milli-Q Gradient A10
Methanol (CH_3OH), Fisher, HPLC grade
Acetonitrile (CH_3CN), Fisher, HPLC grade
Potassium dihydrogen citrate ($KC_6H_7O_7$), Fluka, ≥ 98%
Sodium thiosulfate ($Na_2S_2O_3$), Fluka, ≥ 98%
Hydrolysis Reagent (0.2% NaOH), Pickering, chromatographic grade (P/N CB130)
o-Phthalaldehyde (OPA, $C_8H_6O_2$), Pickering, chromatographic grade, (P/N O120)
OPA Diluent (0.4% sodium borate solution), Pickering, chromatographic grade (P/N CB910)
Mercaptan Reagent, (Thiofluor™$(CH_3)_2NCH_2CH_2SH \cdot HCl$), Pickering, chromatographic grade (P/N 3700-2000)
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 WaterStock Standard Solutions

Deionized water, Type I reagent grade, 18 MΩ-cm resistivity or better

Preserved Reagent Water

Dissolve 4.7 g potassium dihydrogen citrate and 160 mg $Na_2S_2O_3$ in a 50 mL beaker with reagent water, 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 Solutions of Carbamate Pesticide Calibration Mixture

Pipet 10 µL and 100 µL 531.2 carbamate pesticide calibration mixture (100 µg/mL) into two 1 mL vials, add 990 µL to vial one and 900 µL methanol to vial two. The concentrations of stock standard solutions of the calibration mixture are 1.0 µg/mL and 10 µg/mL, respectively.

Stock Standard Solution of 4-Bromo-3,5-dimethylphenyl-*N*-methylcarbamate Standard (Surrogate Analyte, SUR)

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

Table 1: Preparation of calibration curve standards

Stock std. of carbamate cal. mixture (µg/mL)	Vol. of stock std. of carbamate cal. mixture (µL)	Vol. of 10 µg/mL stock std. of SUR (µL)	Final vol. of cal. std. (mL)	Final conc. of cal. std. (µg/L)	Final conc. of SUR (µg/L)
1.0	5.0	5.0	25	0.20	2.00
1.0	12.5	5.0	25	0.50	2.00
1.0	25.0	5.0	25	1.00	2.00
10.0	5.0	5.0	25	2.00	2.00
10.0	12.5	5.0	25	5.00	2.00
10.0	25.0	5.0	25	10.0	2.00

Working Standard Solutions

Prepare six working standard solutions by adding the quantities of carbamate mixture stock standard solutions listed in Table 1 to separate 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.

Sodium Hydroxide Hydrolysis Reagent (Post Column Reagent 1)

Decant the hydrolysis reagent into a clean reagent reservoir that has been rinsed with methanol. Because high-purity sodium hydroxide is difficult to purchase as well as prepare, we strongly recommend the use of this reagent for optimum system performance. This reagent also contains an antifouling additive to prevent mineral buildup inside the reactor.

OPA Reagent (Postcolumn Reagent 2)

Decant the contents of the OPA diluent into a clean reagent bottle that has been rinsed with methanol. Sparge the diluent for approximately 10 min to remove oxygen.

Note: The remaining steps should be accomplished quickly because the prepared reagents are sensitive to oxygen and light. Weigh approximately 100 mg of *o*-phthalaldehyde into a small beaker, dissolve in 10 mL methanol, and add to the OPA diluent. Rinse the beaker with 1 to 2 mL of methanol and add this to the diluent. Add 2 g of Thiofluor to the reagent bottle, replace the cap, and sparge for 1 to 2 min. Swirl the bottle gently to ensure complete mixing.

Tap Water Sample Preparation

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

Place 2.32 g potassium dihydrogen citrate and 80 mg $Na_2S_2O_3$ in a 500 mL beaker, then add approximately 250 mL tap water and mix. The treated tap water sample should be stored in the dark at -10°C until analyzed. Prior to use, filter through a 0.45-µm filter.

CONDITIONS

Column: Acclaim® 120 C18, 3 µm, 4.6 × 150 mm (P/N 059133)

Column temperature: 42 °C

Mobile phase: A: water, B: acetonitrile, C: methanol

Gradient:

Time (min)	A (%)	B (%)	C (%)	Curve
0.0	85	0	15	
3.0	85	0	15	5
14.0	60	20	20	1
15.3	40	30	30	5
21.0	40	30	30	5
22.0	85	0	15	5
30.0	85	0	15	5

Flow rate: 1.0 mL/min

Injection volume: 250 µL

Post column reagent 1: 0.2% sodium hydroxide, first reaction coil at 100 °C

Flow rate of reagent 1: 0.3 mL/min

Post column reagent 2: OPA reagent, second reaction coil at ambient temperature

Flow rate of reagent 2: 0.3 mL/min

Fluorescence: Excitation: 330 nm
Emission: 465 nm
Response: 2 (0.5 s)
Sensitivity: 2 (medium)
Gain: 1 (×1)

RESULTS AND DISCUSSION

Postcolumn Chemistry

The postcolumn system features two reagent pumps, two reactors (one heated), and a column oven. A built-in pressure switch will shut down the reagent pumps when it senses that the analytical pump pressure has dropped below 3.4 Mpa (500 Psi). This feature prevents backflow of sodium hydroxide onto the analytical column when the analytical pump fails to pump or deliver mobile phase. A schematic diagram of the system hardware is shown in Figure 1.

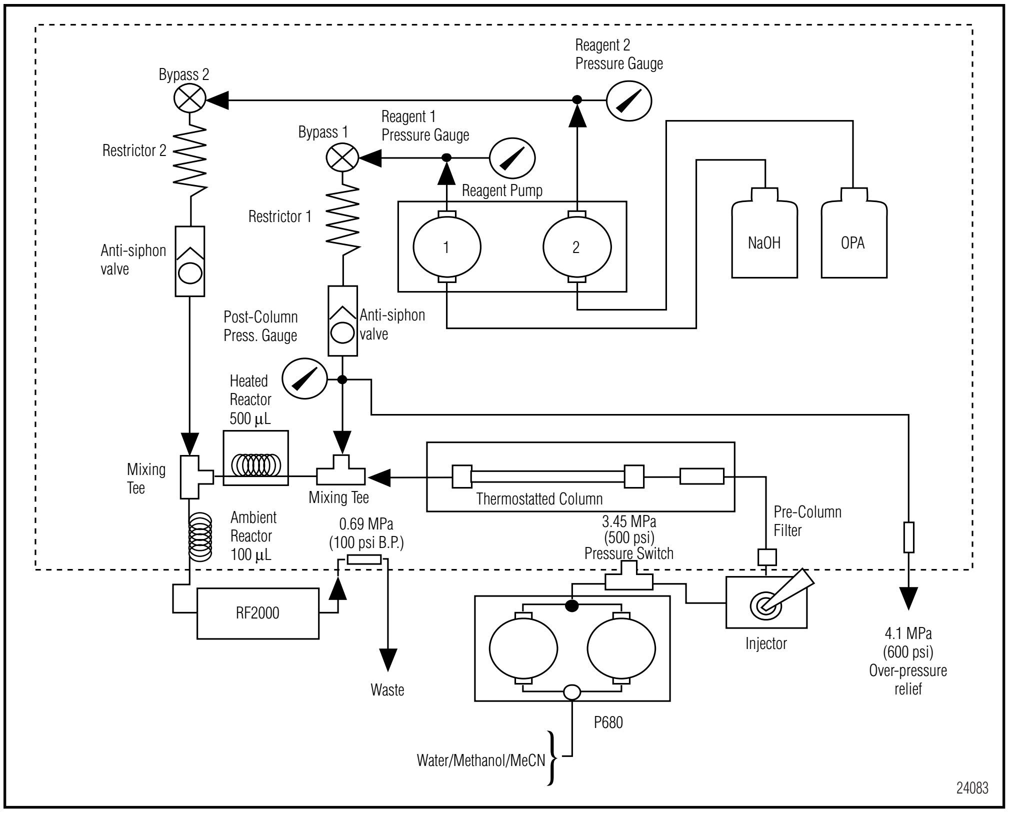


Figure 1. Schematic of carbamate analysis system setup. The chromatography column and postcolumn reactor system are represented by the portion of diagram inside the dotted line.

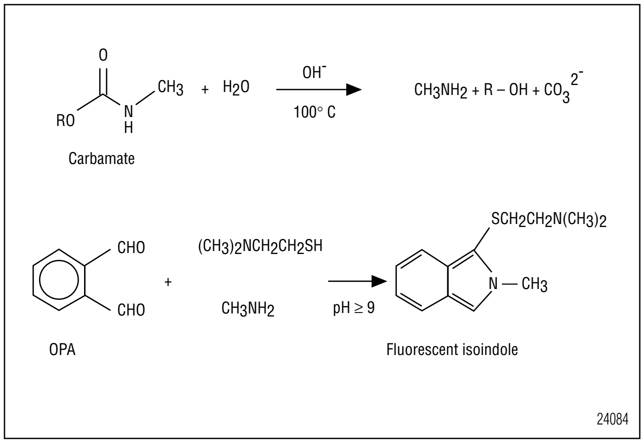


Figure 2. Postcolumn reaction chemistry of carbamate.

After separation on the C18 column, carbamates are hydrolyzed by sodium hydroxide at 100° C. The resulting methylamines are then reacted with o-phthalaldehyde and N, N'-dimethyl-2-mercaptoethylamine hydrochloride (Thiofluor) to form a fluorescent isoindole compound. The details of this chemistry are shown in Figure 2.

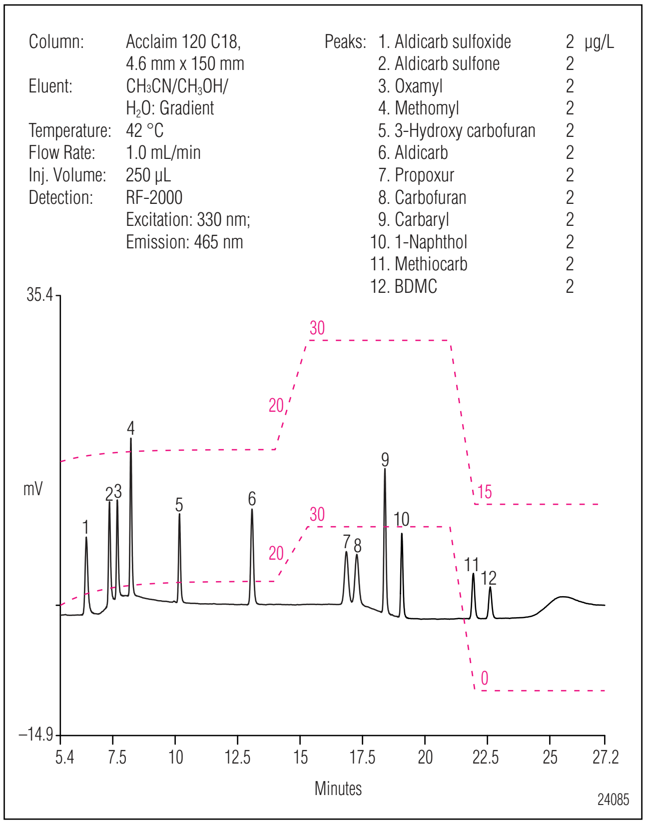
Note: The Thiofluor reagent replaces 2-mercaptoethanol, a reagent sometimes used for this application. The advantage of using Thiofluor is that it is more stable and relatively odorless.

To assist in troubleshooting postcolumn chemistry issues, 1-naphthol is included in some standard carbamate mixtures, as shown in Figure 3 (peak 10). This compound is naturally fluorescent; therefore it will be the only peak present in a chromatogram when the postcolumn system is not functioning properly.

Resolution and Reproducibility

Figure 3 illustrates good separation of the carbamates listed in U. S. EPA Method 531.2 using the Acclaim 120 C18. Resolution for all peaks is much better than the values required in the EPA Method (≥ 1.0).

Reproducibility was estimated by making 7 replicate injections of a calibration standard with concentration of 1.0 µg/L. The values of relative standard deviation (RSD) of each carbamate for retention time and for peak area are listed in Table 2.



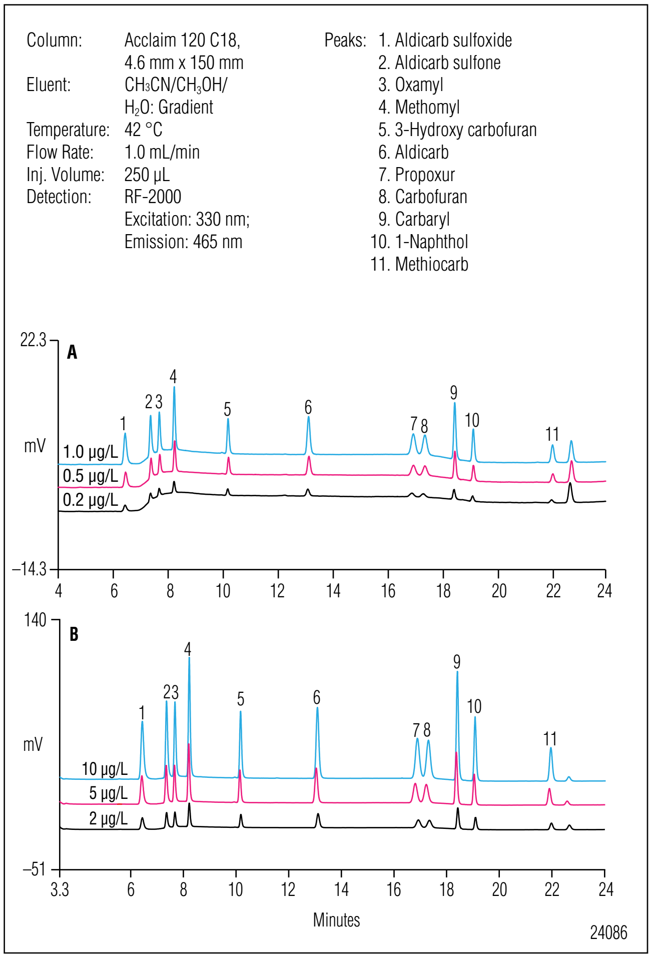
Column: Acclaim 120 C18, 4.6 mm x 150 mm
Eluent: $CH_3CN/CH_3OH/H_2O$: Gradient
Temperature: 42 °C
Flow Rate: 1.0 mL/min
Inj. Volume: 250 µL
Detection: RF-2000 Excitation: 330 nm; Emission: 465 nm

Peaks:		µg/L
1.	Aldicarb sulfoxide	2
2.	Aldicarb sulfone	2
3.	Oxamyl	2
4.	Methomyl	2
5.	3-Hydroxy carbofuran	2
6.	Aldicarb	2
7.	Propoxur	2
8.	Carbofuran	2
9.	Carbaryl	2
10.	1-Naphthol	2
11.	Methiocarb	2
12.	BDMC	2

Figure 3. Standard mixture of 10 carbamates (2 µg/L) plus two reference compounds (peaks 10 and 12) with 250 µL injection.

Table 2: Reproducibility of retention time and peak areas for ten carbamates and two reference components

Carbamates	Retention Time RSD (%)	Peak Area RSD (%)
Aldicarb sulfoxide	0.11	3.09
Aldicarb sulfone	0.08	1.39
Oxamyl	0.08	1.04
Methomyl	0.06	0.99
3-Hydroxycarbofuran	0.06	1.39
Aldicarb	0.05	1.06
Propoxur	0.07	2.04
Carbofuran	0.08	2.98
Carbaryl	0.04	0.83
1-Naphthol	0.03	0.79
Methiocarb	0.03	2.78



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Temperature: 42 °C
Flow Rate: 1.0 mL/min
Inj. Volume: 250 µL
Detection: RF-2000
Excitation: 330 nm;
Emission: 465 nm

Peaks:
1. Aldicarb sulfoxide
2. Aldicarb sulfone
3. Oxamyl
4. Methomyl
5. 3-Hydroxy carbofuran
6. Aldicarb
7. Propoxur
8. Carbofuran
9. Carbaryl
10. 1-Naphthol
11. Methiocarb

Figure 4. An overlay of chromatograpms of carbamates with concentrations of (A) 0.2, 0.5 and 1.0 µg/L, respectively; and (B) 2.0, 5.0 and 10 µg/L.

Linearity and Detection Limits

Figure 4 shows an overlay of chromatograms of the serial standard solutions of carbamates. Calibration linearity for the determination of carbamates by this method was investigated by making replicate injections (n = 7) of serial standard solutions of carbamates at six different concentrations. Detection limits of carbamates were calculated by using the equation found in EPA 531.1 and 531.2:

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

Where:

S = standard deviation of replicate analyses

$t_{(n-1,\ 1-\alpha=0.99)}$ = Student's value for the 99% confidence level with n -1 degrees of freedom,

n = number of replicates

Table 3: Method linearity data and method detection limits (MDL)

Carbamates	Correlation coefficient (R)	Method 531.1 MDL requirements (µg/L)	Method 531.1 MDL (µg/L)	Dionex Method MDL (µg/L)
Aldicarb sulfoxide	0.9992	2.0	0.59	0.018
Aldicarb sulfone	0.9995	2.0	1.00	0.046
Oxamyl	0.9994	2.0	0.86	0.035
Methomyl	0.9995	0.5	0.29	0.028
3-Hydroxycarbofuran	0.9994	2.0	1.90	0.036
Aldicarb	0.9995	1.0	0.22	0.032
Propoxur	0.9994	1.0	1.00	0.031
Carbofuran	0.9993	1.5	0.52	0.059
Carbaryl	0.9995	2.0	1.30	0.026
Methiocarb	0.9993	4.0	1.90	0.041

* *When n = 10,* $t_{(n-1,\ 1-\alpha=0.99)} = 3.17$

Table 4: Tap water sample analysis

Carbamates	Detected (µg/L)	Added 1 (µg/L)	Found (µg/L)	RSD (%)	Recovery (%)	Added 2 (µg/L)	Found (µg/L)	RSD (%)	Recovery (%)
Aldicarb sulfoxide	0.0	0.20	0.20	6.25	102	10.00	10.66	4.14	107
Aldicarb sulfone	0.0	0.20	0.21	8.42	103	10.00	10.65	4.60	107
Oxamyl	0.0	0.20	0.19	13.4	93	10.00	10.60	4.52	106
Methomyl	0.0	0.20	0.18	5.11	92	10.00	10.88	4.61	109
3-Hydroxycarbofuran	0.0	0.20	0.19	4.25	94	10.00	10.46	4.43	105
Aldicarb	0.0	0.20	0.18	6.16	88	10.00	10.88	4.30	109
Propoxur	0.0	0.20	0.19	6.92	92	10.00	10.56	4.67	106
Carbofuran	0.0	0.20	0.21	8.11	103	10.00	10.60	4.78	106
Carbaryl	0.0	0.20	0.17	6.28	87	10.00	10.54	4.85	105
1-Naphthol	0.0	0.20	0.20	4.47	99	10.00	10.22	4.52	102
Methiocarb	0.0	0.20	0.20	4.82	100	10.00	10.46	4.80	105

Ten replicate injections of reagent water fortified with 0.2 µg/L carbamate standard were used in this method. Table 3 summarizes the data, which show excellent method linearity and sensitivity, with detection limits well below those defined in the EPA method. (The improved detection limits are largely due to improvements in fluorescence detector sensitivity and reversed-phase column technology since the original EPA work was completed. The EPA method used a 5 µm 4.6 x 250 mm column while this method used a 3 µm 4.6 x 150 mm column to yield more efficient peaks.) These improved limits easily allow the analyst to reach the minimum reporting limits (3-5 times the MDL) of the original method.

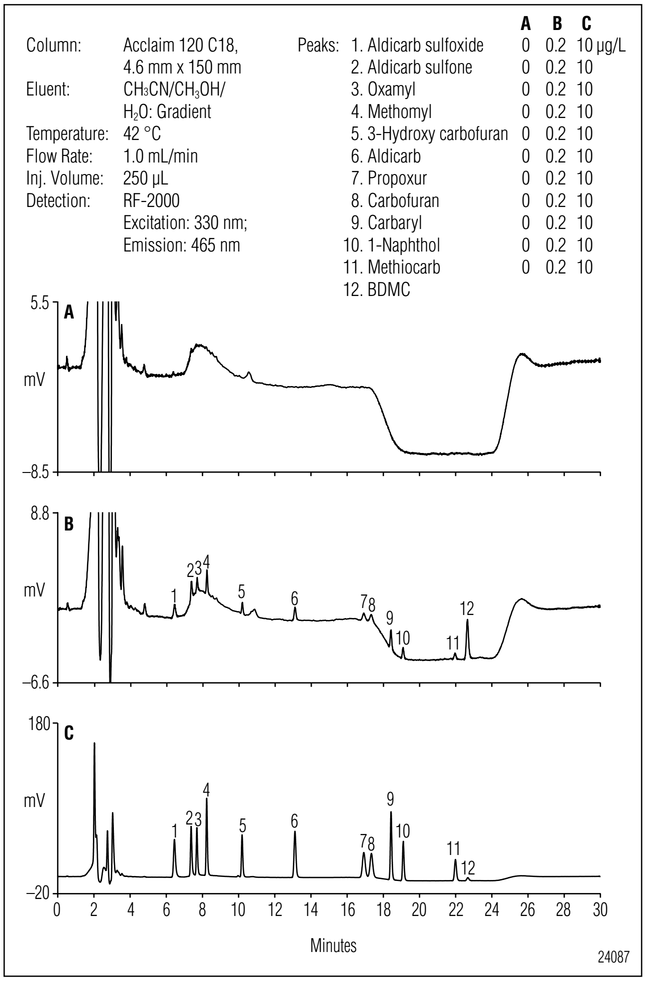

Figure 5. Chromatograms (A) a tap water sample, (B) tap water (A) + 0.2 µg/L carbamate standard mix (C) tap water (A) + 10 µg/L carbamate standard mix.

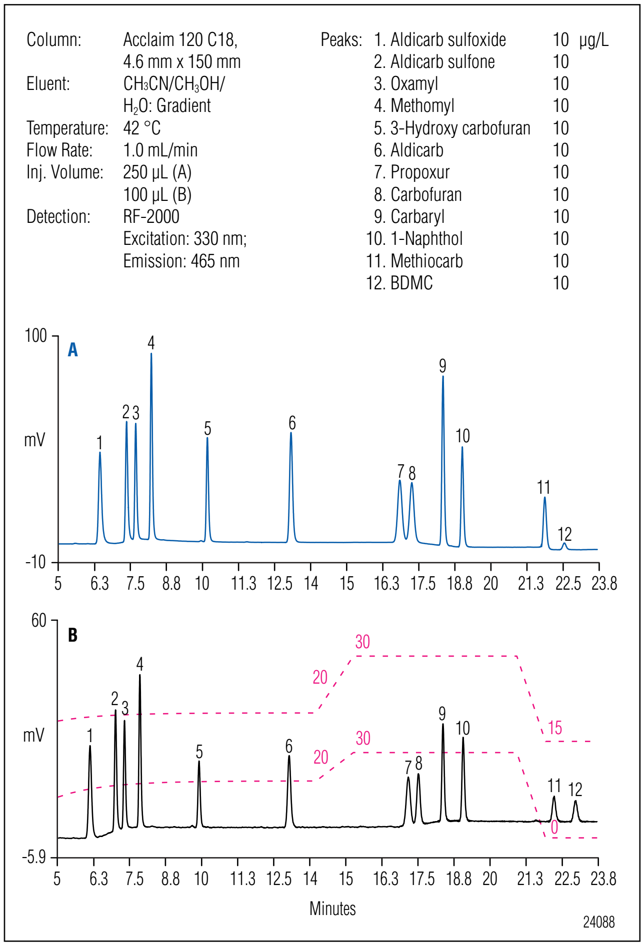

Figure 6. Chromatograms of a standard mixture of 10 carbamates (2.5 µg/mL) plus two reference compounds (peaks 10 and 12) on (A) Summit and (B) UltiMate Systems, respectively, under the same chromatographic condition except for injection volume, (A) 250 µL and (B) 100 µL.

Tap Water Sample Analysis

Figure 5 compares the chromatograms of an unadulterated tap water sample with two samples spiked with 0.2 µg/L and 10 µg/L carbamate standards. No detectable levels of carbamates were found in the tap water. The related data are summarized in Table 4. This data shows excellent spike recovery for each carbamate compound.

Chromatography with the UltiMate 3000 HPLC system

This application can also be performed on the UltiMate 3000 HPLC system. Figure 6 shows the chromatograms of a 10 µg/L carbamate standard on the Summit and UltiMate 3000 systems, respectively, using the same chromatographic conditions except for injection volume. (The UltiMate 3000 used a 100 µL sample loop while the Summit used a 250 µL sample loop.) The UltiMate 3000 can be equipped with a 250 µL sample loop (P/N 6820.2422) and 250 µL syringe (P/N 6822.0003). Good separation of carbamates is achieved on both HPLC systems.

CONCLUSION

This application note describes an optimized method for determining carbamates on a Dionex HPLC system with an Acclaim 120 C18 column (3µm). The method meets or exceeds the chromatographic requirements of the U. S. EPA 531.2 monograph method for carbamates, demonstrating that it is ideally suited for determining these compounds in drinking water.

PRECAUTIONS

Mobile Phase Precautions

- Avoid touching the interior of the mobile phase reservoirs and the dip tubes; the amino acids present in fingerprints will cause contamination. Gloves are suggested.
- Do not leave caps and lines exposed to the atmosphere. To fill the reservoir, transfer caps and lines into a spare bottle or an Erlenmeyer flask filled with deionized water.
- Change the water in the solvent reservoir every 3 to 4 days to prevent possible bacterial growth.
- Do not purge the system with 100% acetonitrile as this reagent can promote precipitation of borate salt in the reactor.

Column Maintenance and Precautions

- If the column backpressure is high, isolate the source—guard, analytical column, or the 0.5-µm in-line filter. Replace items causing the increased pressure.
- At shutdown, flush the column with pure methanol; do not store the column in water.
- Organic contaminants may be removed from the column by first washing with methanol, followed by dichloromethane, followed with a final methanol rinse.
- Never disconnect any fitting between the HPLC pump and the column until the postcolumn system has been shut down and depressurized by loosening the fitting at the “To Detector” port.
- Replace any fittings that leak between the HPLC pump and the column in order to prevent backflow in the event of an unattended shutdown.
- When removing the column, disconnect the outlet fitting first.

Reagent, Sample and Standard Precautions

- Always wear gloves when preparing reagents. Both the hydrolysis reagent and Thiofluor cause skin irritation. Fingerprints also contaminate reagents.
- The hydrolysis reagent is stable and can be replaced as it is used. The OPA reagent is sensitive to oxidation, and degrades over time. It should be prepared fresh for optimum sensitivity.
- Thiofluor is extremely hygroscopic. Store in a tightly closed container.
- Filter all samples through a 0.45 µm membrane filter. Some samples may require even more thorough filtration, e.g. 0.2 µm, especially if colloids are present.
- Aqueous samples must always be properly buffered. Consult EPA Method 531.2 for details.

REFERENCES

1. Measurement of *N*-methylcarbamoyloximes and *N*-methylcarbamates in Water by Direct Aqueous Injection HPLC with Postcolumn Derivatization” U.S. EPA Method 531.2, Revision 1.0, U.S. Environmental Protection Agency: Cincinnati, OH, 2001.
2. PCX 5200 Postcolumn Analysis of Carbamate, Application Manual, Version 3.0, Pickering Laboratories: Mountain View, CA, 2003.

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