

## CARBAMATES



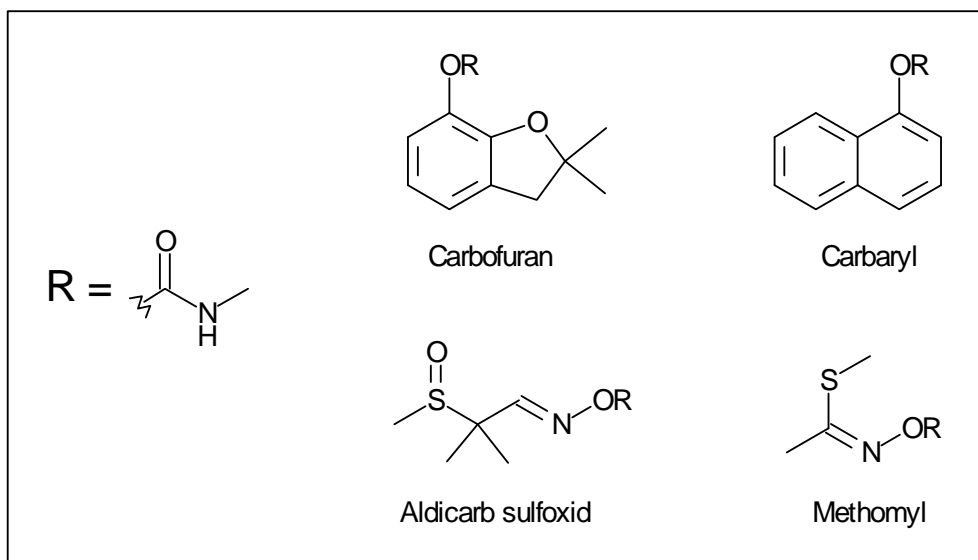
The *N*-methyl carbamates are frequently used pesticides. Their extensive application provoked the American environmental agency (EPA) to include *N*-methyl carbamates in their water control programme at the beginning of the 80s (EPA 531.1, Update EPA 531.2). For food, the AOAC- method 985.23 applies. Both regulations are based on "Reversed Phase HPLC" in combination with post-column derivatization.

The American regulations demand the separation of ten *N*-methyl carbamates plus 1-naphthol and an internal standard (4-bromo-3,5-dimethylphenyl-*N*-methyl carbamate = BDMC).

For these regulations, PICKERING offers complete methods (PINNACLE PCX and application kits). The kit includes C18 columns. For the analysis of additional carbamates, however, a C8 column with greater selectivity is available, which enables the separation of up to 23 carbamates and their metabolites, respectively. The carbamate kits contain, in addition to the columns, reagents, diluents and a standard.

### Description of the Method

All *N*-methyl carbamates possess an *N*-methyl-substituted urethane structure (=R) and are only distinguished by the residue of the ester group.

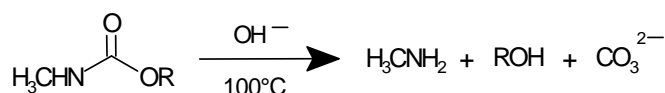


Basic structure of *N*-methyl carbamates

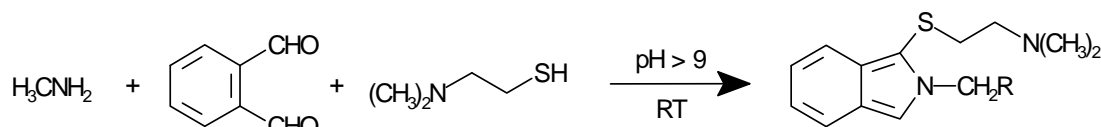
# APPLICATION NOTE

The derivatization is performed in a two-step reaction. In the first step, the carbamate function is hydrolysed with the hydrolysis reagent (NaOH), whereby methyl amine (primary amine) is formed, which reacts in the second step with *o*-phthalaldehyde (OPA) and Thiofluor® (2-mercaptoethanol derivative) resulting in a fluorescent isoindole derivative.

## 1. Hydrolysis:



## 2. Derivatisation:



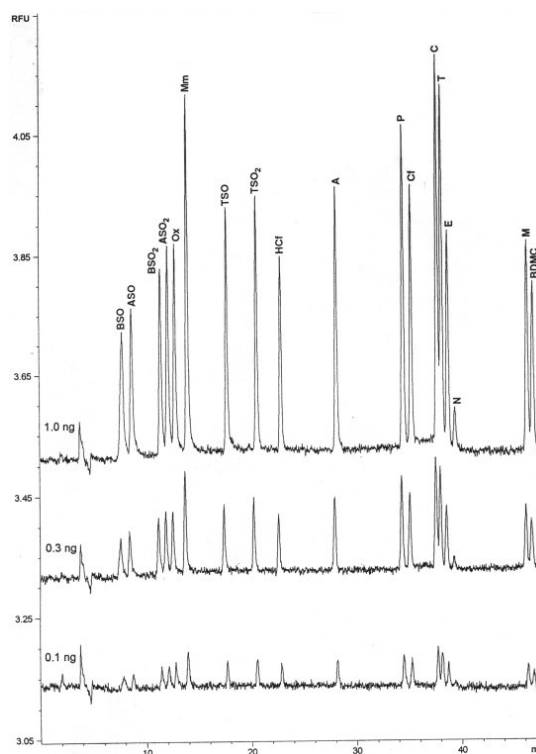
Derivatization of *N*-methyl carbamates to Fluorescent Isoindole Derivatives

## Chromatograms

Chromatogram of a Carbamate Standard at Different Concentrations

Concentrations (abs.) per carbamate:  
1.0 ng (top), 0.3 ng (middle) and 0.1 ng (bottom). Thiofanox (T) 5.0 ng, 1.5 ng and 0.5 ng.

Column: Pickering C8-Reversed Phase Column (P/N 0840250)  
Eluents: Water/Acetonitrile gradient (see method 03.6 below)



# APPLICATION NOTE

Abbreviations for all used *N*-methyl carbamates:

A	= Aldicarb	HCf	= 3-Hydroxycarbofuran
ASO	= Aldicarb sulphoxide	M	= Methiocarb
ASO <sub>2</sub>	= Aldicarb sulphone	Mm	= Methomyl
BDMC*	= Internal Standard	N	= 1-Naphtol
BSO	= Butocarboxim sulphoxide	Ox	= Oxamyl
BSO <sub>2</sub>	= Butocarboxim sulphone	P	= Propoxur
C	= Carbaryl	T	= Thiofanox
Cf	= Carbofuran	TSO	= Thiofanox sulphoxide
E	= Ethiofencarb	TSO <sub>2</sub>	= Thiofanox sulphone

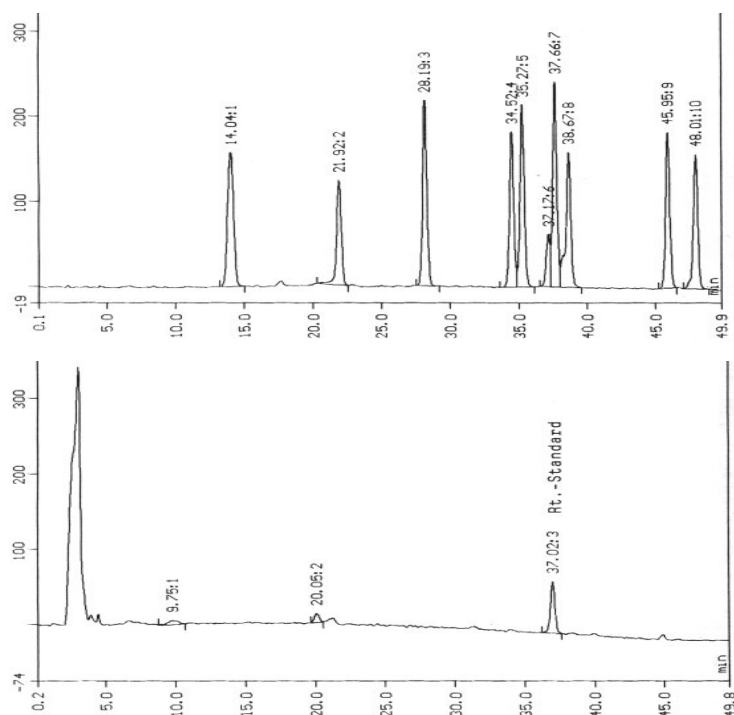
\*4-Brom-3,5-dimethylphenyl-*N*-methyl carbamate

Chromatogram of a Fruit Sample (Mango) Spiked with a Carbamate Standard (0.01 µg/kg) after Clean-up by Gel Permeation Chromatography and a Fruit Sample Blank (Mango) after the same Clean-up

Column: Pickering C8-Reversed Phase Column (P/N 0840250)  
Eluents: Water/Acetonitrile gradient (developed by the user)

The standard used for spiking the mango sample contains:

Methomyl, Dioxacarb, Aldicarb, Propoxur, Carbofuran, Carbaryl, Thiofanox, Mercaptodimethur, Promecarb  
The signals at 37.17 min resp. 37.02 min are a retention time standard.



## HPLC Conditions and Derivatization Parameters

<b>HPLC</b>	
Operating Mode	binary gradient
Eluent	Water/Methanol or Water/Acetonitril
Degassing	Helium- or vacuum degassed
HPLC Column	RP-C18- or C8 -Column with Guard column
Column Oven	55 °C
Flow Rate	0.8 mL/min bzw. 1 mL/min
Inject Volume	Up to 100 µL
<b>Post-column Derivatization</b>	
Pinnacle PCX	Dual-pump
Reactor volume	0.5 mL
Reactor temperature	42 resp. 37 °C
Reagent 1	CB130 hydrolysis reagent 0.05M (aqueous NaOH solution) respectively CB130.2 hydrolysis reagent 0.075M (for EPA 531.2)
Reagent 2	Solution of <i>o</i> -phthalaldehyde (OPA) and Thiofluor <sup>®</sup> (2-mercaptoethanol derivative) in CB910-diluent
Reagent Flow	0.3 mL/min
<b>Detection</b>	
Detection Mode	Fluorescence detection
Excitation Wavelength	330 nm
Emission Wavelength	465 nm
Flowcell	Analytic; pressure stable up to 7 bar

## Gradient programs

The C18 columns (15 and 25 cm) are driven with a water/methanol gradient. There are two different gradient programs for both columns. One for large-volume injections (up to 4000 µL) of aqueous samples with "on-column" concentration of the carbamates and another for methanolic samples following "off-line" clean up and concentration.

As a consequence of the low limits in Europe (0.1 ppb per carbamate) compared to the USA, it is not possible to achieve the required sensitivity by direct injection of large sample volumes without previous concentration of the sample. Consequently, the gradient program for this method is not specified here.

### **Kit for 12 Carbamates (AOAC-Method 985.23): 0352-0004:**

**C18 Column; 4,6 x 150mm;** sample solved in methanol

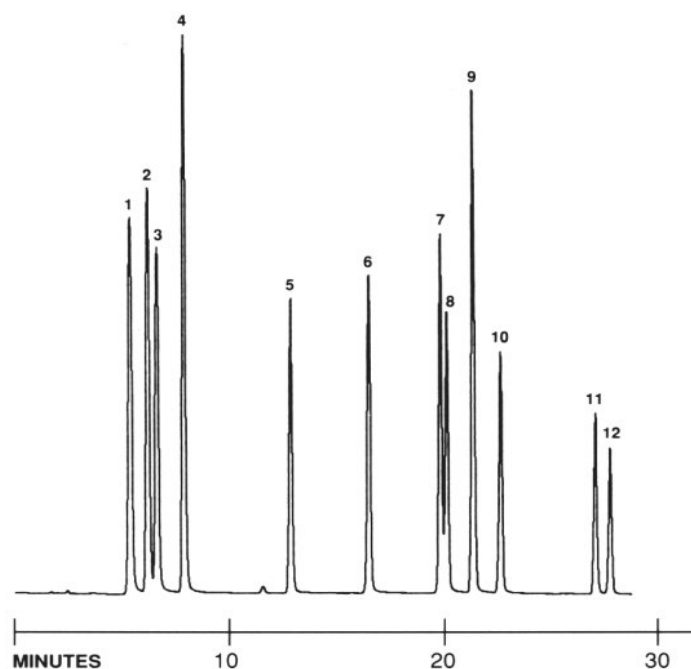
Flow rate: 1.0 mL/min; column temperature: 42°C

Step	Time [min]	Interval [min]	Water [%]	MeOH [%]	
Equil.			82	18	Equilibration
0	0	0	82	18	Injection up to 10 µl
1	0 – 0.5	0.5	82	18	Isocratic
2	0.5 - 29	28.5	30	70	Linear gradient
3	29.1 - 31	1.9	0	100	Step / Rinsing of column
4	31.1 -	5 - 8	82	18	Reequilibration

## APPLICATION NOTE

### Chromatogram of a Carbamate Standard on the C18 Column (150 x 4.6 mm)

Carbamate concentration (abs.): 25 ng per carbamate



Abbreviations for all used *N*-methyl carbamates:

6	=	Aldicarb	5	=	3-Hydroxycarbofuran
1	=	Aldicarb sulphoxide	11	=	Methiocarb
2	=	Aldicarb sulphone	4	=	Methomyl
12	=	BDMC (Internal standard)	10	=	1-Naphtol
9	=	Carbaryl	3	=	Oxamyl
8	=	Carbofuran	7	=	Propoxur

\*4-Brom-3,5-dimethylphenyl-*N*-methylcarbamate

# APPLICATION NOTE

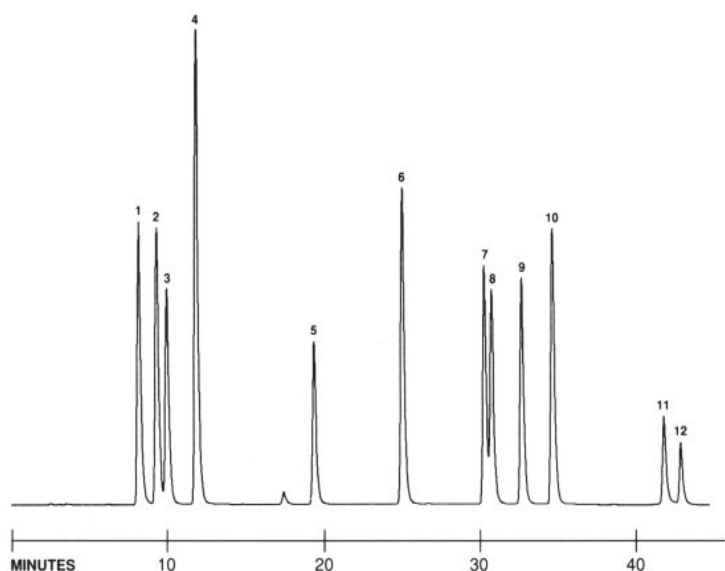
## Kit for 12 Carbamates: EPA Method 531.1 (Kit P/N 0352-0003) and EPA Method 531.2 (Kit P/N 0352-0009):

**C18 Column; 4,6 x 250mm;** sample solved in methanol  
Flow rate: 1.0 mL/min; Column Temperature: 42°C

Step	Time [min]	Interval [min]	Water [%]	MeOH [%]	
Equil.			80	20	Equilibration
0	0	0	80	20	Injection up to 10 µl
1	0 - 1	1	80	20	Isocratic
2	1 - 44	43	25	75	Linear gradient
3	44,1 - 49	4,9	0	100	Step change/Rinsing of column
4	49,1 -	5 - 8	82	18	Re-equilibration

### Chromatogram of a Carbamate Standard on the C18 Column (250 x 4.6 mm)

Carbamate concentration (abs.): 25 ng per carbamate. List of Carbamates used: s. above



## APPLICATION NOTE

### **Kit for 23+ Components: 0352-0002:**

**C8 Column; 4 x 250mm;** sample solved in methanol  
Flow rate: 0.8 mL/min; column temperature: 37°C

Step	Time [min]	Interval [min]	Water [%]	MeOH [%]	
Equil.			88	12	Equilibration
0	0	0	88	12	Injection up to 10 µl
1	0 - 2	2	88	12	Isocratic
2	2 - 42	40	66	34	Linear gradient
3	42 - 46	4	66	34	Isocratic
4	46.1 - 49	2.9	0	100	Step /Rinsing of column
4	49.1 -	5 - 8	88	12	Reequilibration

**C8 Column; 4 x 250mm;** sample solved in methanol  
Flow rate: 0.8 ml/min; column temperature: 37°C

Step	Time [min]	Interval [min]	Water [%]	ACN [%]	
Equil.			90	10	Equilibration
0	0	0	90	10	Injection up to 10 µl
1	0 - 2	1	90	10	Isocratic
2	2 - 46	44	51	49	Linear gradient
3	46.1 - 49	2.9	0	100	Step / Rinsing of column
4	49.1 -	5 - 8	90	10	Re-equilibration

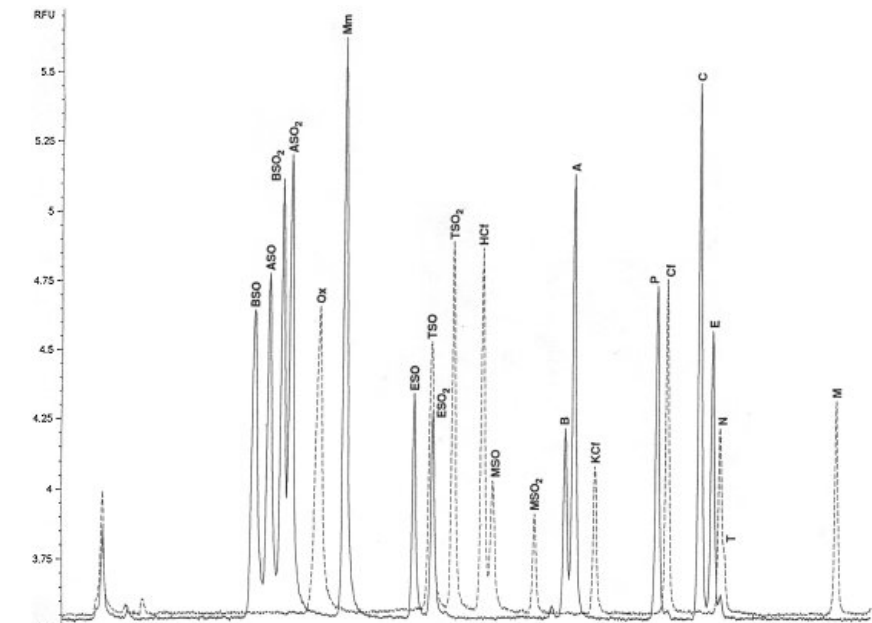
There is a choice of two gradients for the C8 column: *water/methanol* or *water/acetonitrile*. Acetonitrile generates a higher sensitivity, but its purchase and disposal are expensive.

# APPLICATION NOTE

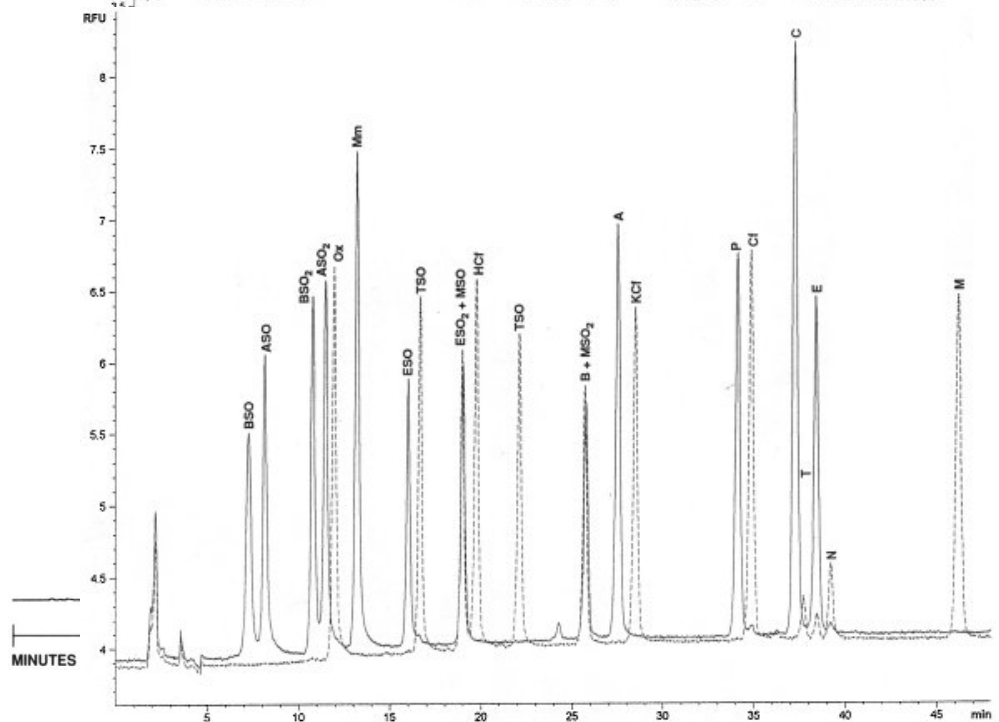
Two carbamate pairs co-elute with each gradient (see chromatograms), however, these differ in the methods. Hence, a change of method may help clarify as to which carbamate is present in the sample

## Chromatogram of a Carbamate Standard on the C8 Column (250 x 4.0 mm) Carbamate Concentration (abs.): 25 ng per carbamate

Water/Methanol  
Gradient



Wasser/Acetonitril  
Gradient



Abbreviations for all used *N*-methyl carbamates:

A	=	Aldicarb	KCf	=	3-Ketocarbofuran
ASO	=	Aldicarb sulphoxide	M	=	Methiocarb
ASO <sub>2</sub>	=	Aldicarb sulphone	MSO	=	Methiocarb sulphoxide
B	=	Butocarboxim	MSO <sub>2</sub>	=	Methiocarb sulphone
BSO	=	Butocarboxim sulphoxide	Mm	=	Methomyl
BSO <sub>2</sub>	=	Butocarboxim sulphone	N	=	1-Naphtol
C	=	Carbaryl	Ox	=	Oxamyl
Cf	=	Carbofuran	P	=	Propoxur
E	=	Ethiofencarb	T	=	Thiofanox
ESO	=	Ethiofencarb sulphoxide	TSO	=	Thiofanox sulphoxide
ESO <sub>2</sub>	=	Ethiofencarb sulphone	TSO <sub>2</sub>	=	Thiofanox sulphone
HCf	=	3-Hydroxycarbofuran			

## Literature

B. D. McGarvey, *J. Chromatogr.* **1993**, 642, 89 – 105 (**Review**).

### Food Samples

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W. Blaß, C. Philipowski, *Pflanzenschutzachr. Bayer* **1992**, 45, 277 – 318.

A. de Kok, M. Hiemstra, *J. Assoc. Off. Anal. Chem.* **1992**, 75, 1063 – 1072.

M. J. Page, M. French, *J. Assoc. Off. Anal. Chem.* **1992**, 75, 1073 – 1083.

### Water Samples

M. Hiemstra, A. de Kok, *J. Chromatogr. A* **1993**, 667, 155 – 166.

V. A. Simon, K. S. Pearson, A. Taylor, *J. Chromatogr.* **1993**, 643, 317 – 320.

A. de Kok, M. Hiemstra, U. A. Th. Brinkman, *J. Chromatogr.* **1992**, 623, 265 – 276.

# APPLICATION NOTE

## Chemicals and Columns

### Post-Column Derivatization Unit

Catalog No.	Description
1153-1052	PINNACLE PCX; dual-pump; 500 µL reactor

### Carbamate Kits

Catalog No.	Description
<b>0352-0003</b>	<b>Application Kit for 12 Carbamates (EPA Method 531.1)</b>
1846250	Analytical column, RP C18, 4.0 x 250 mm
18ECG001	Guard column holder with 3 guard cartridges for carbamate analysis
O120	<i>o</i> -Phthalaldehyde (OPA), "Chromatographic Grade™", 5 g
CB910	OPA diluent, "Chromatographic Grade™", 4 x 950 mL
3700-2000	Thiofluor®, "Chromatographic Grade™", 10 g
CB130	Hydrolysis reagent, 4 x 950 mL
1700-0132	ChlorAC® buffer for preservation of aqueous samples, 250 mL
1700-0063	Carbamate Standard, qualitative, 1.5 mL, 2.5 µg/mL each compound
<b>0352-0004</b>	<b>Application Kit for 12 Carbamates (AOAC Method 985.23):</b>
1846150	Analytical Column, RP C8, 4.0 x 150 mm
18ECG001	Guard column holder with 3 guard cartridges for carbamate analysis
O120	<i>o</i> -Phthalaldehyde (OPA), "Chromatographic Grade™", 5 g
CB910	OPA diluent, "Chromatographic Grade™", 4 x 950 mL
3700-2000	Thiofluor®, "Chromatographic Grade™", 10 g
CB130	Hydrolysis Reagent for carbamate analysis, 4 x 950 ml
1700-0132	ChlorAC®-Buffer for preservation of aqueous samples, 250 ml
1700-0063	Carbamate Standard, qualitative, 1.5 mL, 2.5 µg/mL each compound
<b>0352-0002</b>	<b>Application Kit for 23+ Carbamates (Expanded Resolution)</b>
0840250	Analytical column, RP C8, 4.0 x 250 mm
18ECG001	Guard column holder with 3 guard cartridges for carbamate analysis
O120	<i>o</i> -Phthalaldehyde (OPA), "Chromatographic Grade™", 5 g
CB910	OPA diluent, "Chromatographic Grade™", 4 x 950 mL
3700-2001	Thiofluor®, "Chromatographic Grade™", 10 g
CB130	Hydrolysis Reagent for carbamate analysis, 4 x 950 mL
1700-0132	ChlorAC®-Buffer for preservation of aqueous samples, 250 mL
1700-0063	Carbamate Standard, qualitative, 1.5 mL, 2.5 µg/mL each compound

## Analytical Columns for Carbamate Analysis

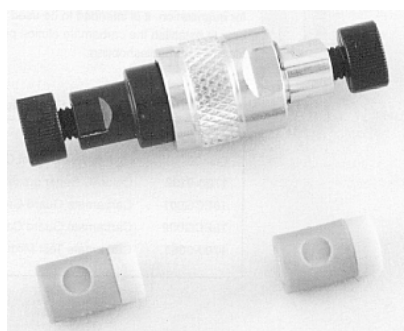
- 1) Chromatographically-certified for carbamate analysis
- 2) Two-solvent linear gradient methods
- 3) High resolution and precision
- 4) Absence of "bleeding" (stable phase)

The reversed-phase LC separation of the ten *N*-Methyl carbamates, plus 1-Naphthol and BDMC, mandated by the USEPA or AOAC methods can be accomplished with either of two Pickering C18 carbamate columns.

A number of other carbamate pesticide compounds occur worldwide which are not included in the ten compounds mandated. Pickering's expanded-resolution C8 column separates as many as 23 carbamates with either Water/Acetonitrile or Water/Methanol gradients. The differences in peak selectivity between the Methanol and Acetonitrile protocols enable the user to employ both solvent systems as a means of confirming peak identification.

Catalog No.	Description
1846150	Analytical column, RP C18, 4.6 x 150 mm
1846250	Analytical column, RP C18, 4.6 x 250 mm
0840250	Analytical column, RP C8, 4.0 x 250 mm

## Guard column holder with three guard cartridges



- 1) Proven protection for the carbamate column
- 2) No effect upon peak shape and resolution
- 3) Easy replacement of cartridge
- 4) Corrosion- and leak-proof construction

The potential for unintentional destruction of the analytical column is extremely high; guard cartridges are a fraction of the cost of a new column. The Cartridge Holder can be used over and over again. Normally, only the cartridges need replacement, as back-pressures increase or chromatographic performance appears to deteriorate.

Catalog No.	Description
18ECG001	Guard column holder with 3 guard cartridges
18ECG002	2 Guard cartridges

## o-Phthalaldehyde Reagent

Primary amines such as amino acids form highly fluorescent compounds when reacted with o-Phthalaldehyde (OPA) and a mercaptan under basic conditions. At a pH >9 and ambient temperature, reaction is generally complete within 1 - 30 seconds. The products of this reaction, 1-alkyl-2-alkylthio-substituted isoindoles, exhibit optimal excitation at 330 nm and maximal emission at 465 nm.



For an oxygen-sensitive reagent like OPA to remain stable for days instead of hours, it is important to start with the purest and most stable ingredients available, and to store and use the reagent under anaerobic conditions.

Using the chemicals described below, a long-lasting (up to ten days) OPA reagent for post-column derivatization of primary amines can be prepared. Each chemical is accompanied by clear instructions for formulating the reagent in your laboratory within minutes.

**For the preparation of o-Phthalaldehyde reagent PICKERING offers:**

- 1) Ultra-pure, crystalline OPA
- 2) Borate diluent; free of heavy metals, particulates and amines
- 3) Thiofluor; crystalline substitute for 2-Mercaptoethanol

## o-Phthalaldehyde

Catalog No.	Description
O120	o-Phthalaldehyde (OPA), "Chromatographic Grade™", 5 g

## o-Phthalaldehyde Diluent

For the carbamate pesticide analysis Pickering offers a sodium borate buffer with pH 9,1 capable to buffer neutral eluents like water or methanol/acetonitrile.

Catalog No.	Description
CB910	OPA diluent, "Chromatographic Grade™", 4 x 950 mL

## APPLICATION NOTE

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### Thiofluor<sup>®</sup>

Pickering's Thiofluor<sup>®</sup>, a solid, nearly odorless nucleophile, is a superior substitute for 2-Mercaptoethanol in the preparation of OPA reagents. It forms a more stable and longer-lasting fluorophore with OPA than does 2-Mercaptoethanol, while possessing the same fluorescence properties.

Unlike the volatile 2-Mercaptoethanol, Thiofluor<sup>®</sup> will not migrate through the gas manifold and regulator of the OPA reagent pressurization system.

Catalog No.	Description
3700-2000	Thiofluor <sup>®</sup> , "Chromatographic Grade™", 10 g

### Hydrolysis Reagent

- 1) Prevents mineral precipitation from water samples
- 2) Guaranteed free of fluorescing impurities
- 3) Consistent quality, bottle to bottle, lot to lot

**This 0.05 M NaOH/C47 reagent is applied in the first stage of post-column carbamate derivatization. Hydrolysis Reagent C47 is a unique formulation which prevents the accumulation of insoluble mineral deposits - usually Calcium or Magnesium hydroxides - in the post-column reactor. Analysis of "hard" drinking water and groundwater samples invariably results in the blocking of the post-column reactor due to precipitation of these insolubles. The C47 formulation maintains these minerals in solution, saving time and effort in frequent remedial cleanup procedures.**

Catalog No.	Description
CB130	Hydrolysis Reagent for carbamate analysis, 4 x 950 ml

### ChlorAC<sup>®</sup> Buffer

- 1) Preserves aqueous carbamate samples
- 2) Free of contaminants which fluoresce at low detection levels
- 3) Convenient and ready to use

## APPLICATION NOTE

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Since several of the common carbamates - carbaryl, oxamyl, hydroxycarbofuran - are labile in water due to oxidation or hydrolysis, the samples and standards must be preserved in order to obtain valid results.

USEPA Method 531.1 specifies pH adjustment, dechlorination and cold-storage to preserve the samples. The optimum pH is  $3.0 \pm 0.2$ . The recommended preservative buffer is made from monochloroacetic acid and Potassium acetate.

Unfortunately, the commercial grades of monochloroacetic acid are not specified to be free of contaminants that interfere with carbamate analysis. Laboratories have reported varying levels of fluorescent interferences from "99%" crystalline material.

ChlorAC buffer from Pickering Laboratories is a highly purified Chromatographic Grade™ preservative. It is ready to use and prepared to EPA specifications. ChlorAC is guaranteed to be free of co-eluting interferences for the analytes in EPA 531.1.

### Suggested Sampling Protocol

Add 1.8 mL of ChlorAC Buffer to each pre-cleaned 60 mL sample vial.

If the water sample is chlorinated, dechlorinate with 5 mg of sodium thiosulfate per 60 mL sample.

Fill the sample vials with the dechlorinated water. Seal and mix well.

Maintain the samples at 4 °C for transportation and at -10 °C during storage for up to 28 days.

When preparing standards and blanks for chromatographic analysis, use ChlorAC buffer diluted 10 mL in 1000 mL of HPLC-grade water.

The shelf life of a factory-sealed 250 mL bottle of ChlorAC is estimated at two years.

### Reference

M.W. Dong, M.V. Pickering, M.J.I. Mattina, H.M. Pylypiw, Jr., LC•GC, 1992, 10(6), 442-446.

Catalog No.	Description
1700-1320	ChlorAC® buffer for preservation of aqueous samples, 250 mL

# APPLICATION NOTE

## Carbamate Test Mixture

The carbamate test mixture is a 1.5 mL methanol solution of ten carbamates, plus 1-Naphthol and BDMC at a concentration of approximately 2.5 µg/mL.

**Please Note:** This qualitative standard is not for quantitation. It is intended to be used only to establish the carbamate elution profile and for troubleshooting.

Standard contains:

Aldicarb	3-Hydroxycarbofuran
Aldicarb Sulfone	Methiocarb
Aldicarb Sulfoxide	Methomyl
BDMC* (internal standard)	1-Naphthol
Carbaryl	Oxamyl
Carbofuran	Propoxur

\* 4-Bromo-3,5-dimethylphenyl-*N*-methylcarbamate)

Catalog No.	Description
1700-0063	Carbamate Standard, qualitative, 1.5 mL, 2.5 µg/mL

## Referencenes

H.A. Moye, S.J. Scherer, P.A. St. John, *Analy. Letters*, 1977, 10, 1049-1073.  
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