PRODUCT MANUAL

for

Acclaim® Carbamate Column

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Product Manual

for

Acclaim® Carbamate Analytical Columns

5 µm, 4.6 x 250 mm, P/N 072924
3 µm, 4.6 x 150 mm, P/N 072925
3 µm, 3.0 x 150 mm, P/N 072926
3 µm, 2.1 x 150 mm, P/N 072927
2.2 µm, 2.1x150 mm RSLC, P/N 075596
2.2 µm, 2.1x100 mm RSLC, P/N 075597

Acclaim® Carbamate Guard Columns

5 µm, 4.6 x 10 mm, P/N 072928
5 µm, 3.0 x 10 mm, P/N 072929
5 µm, 2.1 x 10 mm, P/N 072930

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SECTION 1 – INTRODUCTION

Acclaim® Carbamate columns are reversed-phase columns designed for the separation of N-Methylcarbamate and N-Methylcarbamoyloxime pesticides. These columns are designed and tested to meet or exceed all the requirements for US EPA Method 531.2.

- 5 µm, 4.6x250 mm Carbamate columns set the standard for high resolution routine analysis with post-column reaction (PCR) and fluorescence detection (FLD).
- 3 µm, 4.6x150 mm Carbamate columns provide excellent resolution with increased throughput.
- 3 µm, 3.0x150 mm Carbamate columns offer higher analysis throughput with reduced solvent consumption.
- 3 µm, 2.1x150 mm Carbamate columns are suited to LC/MS methods for the best detection limits.
- 2.2 µm, 2.1x150 mm Carbamate columns are designed for LC/MS methods for highest resolution and best detection limits.
- 2.2 µm, 2.1x100 mm Carbamate columns provide ultra-fast LC/MS methods with excellent resolution.

1.1. Features

1. Baseline-resolution of all critical pairs in EPA 531.2 using simple, linear gradients
2. Compatible with methanol/water, acetonitrile/water, and methanol/acetonitrile/water mobile phase systems for baseline separations.
3. Low bleed for compatibility with post-column reaction or MS detection.
4. Lot tested for reproducible retention times and selectivity.

1.2. Physical data

Bonding Chemistry: Proprietary covalently bonded layer
Silica Substrate: Spherical, high-purity
Particle size – 5µm, 3µm, and 2.2µm
Surface area – 300 m^2/g
Pore size – 120 Å

	Particle Size	Column Dimensions	P/N	Required Holder
Analytical	5µm	4.6 x 250mm	072924	N/A
	3µm	4.6 x 150mm	072925	N/A
		3.0 x 150mm	072926	N/A
		2.1 x 150mm	072927	N/A
	2.2 µm	2.1 x 150mm	075596	N/A
		2.1 x 100mm	075597	N/A
Guard	5µm	4.6 x 10mm	072928	P/N 069580
		3.0 x 10mm	072929	P/N 069580
		2.1 x 10mm	072930	P/N 069580

1.3. Specifications and Recommended Operational Parameters

Shipping solution: 90/10 v/v methanol / DI water
Storage solution: 90/10 v/v acetonitrile / DI water
Buffer pH Range: pH 3 – 7 (recommended)
Temperature Range: < 60° C

Stationary Phase	Particle size	Column Dimension	Part number	Maximum Recommended Pressure	Typical Flow Rate
Acclaim Carbamate	5µm	4.6 x 250mm	072924	6000 psi	0.8 – 1.6 mL/min
	3µm	4.6 x 150mm	072925	6000 psi	0.8 – 1.6 mL/min
		3.0 x 150mm	072926	6500 psi	0.4 – 0.8 mL/min
		2.1 x 150mm	072927	6000 psi	0.2 - 0.4 mL/min
	2.2 µm	2.1 x 150 mm	075596	11000 psi	0.2 – 0.6 mL/min
		2.1 x 100 mm	075597	10000 psi	0.2 – 0.65 mL/min

SECTION 2 – GETTING STARTED – A STEP-BY-STEP PROCEDURE

Step 1 – Become familiar with the systems and methods

A full discussion of the analysis of carbamate pesticides is beyond the scope of this manual. Read and understand the relevant method requirements (for example EPA 531.2). Review your laboratory's SOP. Read the operator's manuals for your HPLC equipment. Pay special attention to the instructions for the post-column reaction system, since this is considered an advanced HPLC technique.

In this manual, we will assume that your HPLC system is properly configured, and concentrate on the columns and chromatography.

Step 2 – Visually inspect the column

Upon receiving the new column, perform a visual inspection immediately. Report any damage to your local Dionex representative. Depending upon the nature of the damage, we may request that you ship the damaged column back to us for a replacement.

Step 3 – Mobile phase preparation

The Acclaim Carbamate columns typically use water and methanol, and optionally acetonitrile for mobile phases. The PCR/FLD technique is highly sensitive and requires very high-quality solvents; traces of ammonia or amines will cause problems. For the organic solvents, use a grade that is rated for HPLC and if possible also for trace residue analysis; no further treatment is necessary. If you use a water purification system, keep it well maintained on a regular schedule. Make sure that the solvent reservoirs, filters and tubing are clean. Clean the water reservoir and replace the water weekly.

Step 4 – Install the column and guard

To install the guard column you will need either the guard cartridge holder V2 (P/N 069580) or the kit with holder V2 and coupler (P/N 069707). If you plan on operating the column more than 10° C above ambient temperature, a mobile phase pre-heater is recommended. Orient the analytical column so the flow is in the direction of the arrow on the label.

Step 5 – Condition the column

When a new column is used for the first time, it should be washed thoroughly with 90% methanol at the normal flow rate for the column. For the first 10 minutes, direct the column outlet to waste. Then connect it to the detector, and continue until the baseline is stable.

Step 6 – Reproduce the chromatogram in the Quality Assurance Report

Perform the column performance test using the conditions described in the Quality Assurance Report and compare the results with the one in the report. For the column performance test mix, individual components dissolved in methanol are available from Absolute Standards, Inc, (www.absolutestandards.com).. The components are diluted in 5mM, pH3 phosphate buffer. After the column is fully equilibrated, multiple injections should be made until reproducible chromatograms are obtained. Also record the operating pressure.

Later, if you suspect that the column is damaged, you may repeat this performance test.

Due to various reasons, such as difference of LC systems, mobile phases, oven temperature control, etc, you may observe slightly different results from those in the report. If you have questions, please contact your local Dionex representative.

NOTE ***At the factory, Dionex uses a UV detector instead of PCR/FLD for the column performance test. This separates the column performance from the performance of the PCR system. If you must use the PCR/FLD detection for your column performance test, be aware that the retention times will be longer and the peaks wider than if you use a UV detector. Also, if you use PCR/FLD, you should dilute the test mix 100x with methanol.***

Step 7 – Real sample analysis

Once the satisfactory result is obtained, the column is ready for use. Please contact your local Dionex representative for answers to any technical questions.

SECTION 3 – METHOD DEVELOPMENT

The following sections describe the suggested operating conditions for the different columns. Use these as the starting points for any optimizations you may wish to perform. Usually, a minor adjustment of the gradient times will be sufficient to produce a satisfactory chromatogram.

- The resolution of early peaks (aldicarb sulfoxide, aldicarb sulfone, oxamyl, and methomyl) depends on the percent of methanol at the initial conditions.
- The resolution of propoxur and carbofuran depend on the gradient slope.
- The injection volumes for aqueous samples are very large and do affect retention times. The injection volume necessary to reach the required detection limits will depend on the performance of the detector.
- The gradient delay volume of the HPLC system may be significant, especially for the 3.0 and 2.1 mm diameter columns.
- Different models of fluorescence detectors often differ significantly in their spectral response curves, so optimization of the excitation and emission wavelengths may be beneficial.
- Methanol and acetonitrile give somewhat different selectivity.

3.1. 5 µm, 4.6 x 250 mm

Guard column	5 µm, 4.6 x 10 mm, P/N 072928
Mobile phases	**A.** Water **B.** Methanol
Column temperature	30 °C
Injection volume	500 µL (up to 1000 µL) for samples in water Up to 20 µL for samples in organic solvent
Flow rate	1.20 mL/min
Gradient program	**Time** -8 0 6 7 21 24 32 **(minutes)** **%A** 80 80 77 57 53 30 30 **%B** 20 20 23 43 47 70 70
Post-column reaction	1. Sodium hydroxide, 0.1 molar a. 0.3 mL/min b. 1.0 mL reactor at 100 °C 2. o-Phthalaldehyde/thiol reagent a. 0.30 mL/min b. 0.3 mL reactor at ambient temperature
Fluorescence detection	Excitation: 330 nm Emission: 465 nm Data rate: 2 Hz

3.2. 3 µm, 4.6 x 150 mm

Guard column	5 µm, 4.6 x 10 mm, P/N 072928
Mobile phases	**A.** Water **B.** Methanol
Column temperature	30 °C
Injection volume	500 µL (up to 1000) µL for samples in water Up to 20 µL for samples in organic solvent
Flow rate	1.20 mL/min
Gradient program	**Time** -5.7 0.0 3.2 3.8 12.2 14.0 19.2 **(minutes)** **%A** 83 83 83 30 30 10 10 **%B** 17 17 17 70 70 90 90
Post-column reaction	1. Sodium hydroxide, 0.1 molar a. 0.3 mL/min b. 1.0 mL reactor at 100 °C 2. o-Phthalaldehyde/thiol reagent a. 0.30 mL/min b. 0.3 mL reactor at ambient temperature
Fluorescence detection	Excitation: 330 nm Emission: 465 nm Data rate: 4 Hz

3.3. 3 µm, 3.0 x 150 mm

Guard column	5 µm, 3.0 x 10 mm, P/N 072929
Mobile phases	**A.** Water **B.** Methanol
Column temperature	50 °C
Injection volume	100 µL (up to 400 µL) for samples in water Up to 8 µL for samples in organic solvent
Flow rate	0.90 mL/min
Gradient program	**Time** -4.0 0.0 2.0 2.5 8.0 13.6 16.0 **(minutes)** **%A** 85 85 80 62 60 30 30 **%B** 15 15 20 38 40 70 70
Post-column reaction	1. Sodium hydroxide, 0.1 molar a. 0.3 mL/min b. 1.0 mL reactor at 100 °C 2. o-Phthalaldehyde/thiol reagent a. 0.30 mL/min b. 0.3 mL reactor at ambient temperature
Fluorescence detection	Excitation: 330 nm Emission: 465 nm Data rate: 4 Hz

3.4. 3 µm, 2.1 x 150 mm

These are typical conditions for a single-quadrupole mass spectrometer with an electrospray interface. Many of the MS parameters are dependent on the exact model, and will need to be empirically determined.

Guard column	5 µm, 2.1 x 10 mm, P/N 072930						
Mobile phases	**A.** Water **B.** 5 mM ammonium formate **C.** Methanol						
Column temperature	30 °C						
Injection volume	20 µL (up to 100 µL) for samples in water Up to 4 µL for samples in organic solvent						
Flow rate	0.30 mL/min						
Gradient program	**Time**	-4	0	2	15	15.1	20 **(minutes)**
	%A	85	85	85	30	5	5
	%B	5	5	5	5	5	5
	%C	10	10	10	65	90	90
Mass Spectrometer	**Name**			**SIM**	**Ion**		**Time Range**
	Aldicarb sulfoxide			224	[M+NH4]+		5.0 - 6.8
	Aldicarb sulfone			240	[M+NH4]+		6.8 - 7.8
	Oxamyl			237	[M+NH4]+		6.8 - 7.8
	Methomyl			163	[M+H]+		7.8 - 9.0
	3-Hydroxycarbofuran			255	[M+NH4]+		9.0 - 12.0
	Aldicarb			208	[M+NH4]+		12.0 - 14.0
	Propoxur			210	[M+H]+		14.0 - 16.5
	Carbofuran			222	[M+H]+		14.0 - 16.5
	Carbaryl			219	[M+NH4]+		14.0 - 16.5
	Methiocarb			169	[M-CH3NHCOO+NH4]+		16.5 - 18.0

3.5. 2.2 µm, 2.1 x 150 mm

These are typical conditions for a triple-quadrupole mass spectrometer with an electrospray interface. Many of the MS parameters are dependent on the exact model, and will need to be empirically determined.

Guard column	N/A
Mobile phases	A. Water B. 1 mM ammonium formate C. Methanol
Column temperature	50 °C
Injection volume	20 µL (up to 100 µL) for samples in water Up to 4 µL for samples in organic solvent
Flow rate	0.60 mL/min
Gradient program	**Time** -5 0 0.4 7.2 7.3 10.0 **(minutes)** **%A** 52 52 52 40 0 0 **%B** 5 5 5 5 5 5 **%C** 20 20 20 55 95 95
Mass Spectrometer	Name — Mass Transition — Time Range Aldicarb sulfoxide — 207.2 → 131.8 — 0 – 3 Aldicarb sulfone — 240.1 → 85.9 — 0 – 3 Oxamyl — 237.2 → 72.0 — 0 – 3 Methomyl — 163.1 → 87.9 — 0 – 3 3-Hydroxycarbofuran — 238.1 → 162.9 — 3 – 5 Aldicarb — 208.2 → 115.9 — 5 – 7 Propoxur — 210.2 → 111.0 — 7 – 8 Carbofuran — 222.2 → 165.0 — 7 – 8 Carbaryl — 202.1 → 144.9 — 7 – 8 Carbaryl-d_7 — 209.2 → 151.9 — 7 – 8 (internal standard) 1-Naphthol — 143.0 → 114.5 — 8 – 9 Methiocarb — 226.2 → 168.9 — 9 – 10

SECTION 4 – COLUMN CARE

4.1. Mobile phases

All chemicals and solvents should be at the highest available quality. All mobile phases should be filtered before use. In-liner filters are recommended. Microbial contamination of water is a common source of interferences; clean the reservoir and replace the water weekly.

4.2. Guard cartridges

The guard cartridge must be used with the analytical column, and replaced at a frequency depending on the nature of the sample. Failing to do so will result in rapid deterioration of column performance, and short column lifetime.

4.3. Column storage

You may leave the column in the HPLC in the mobile phase at the end of the gradient. If you plan on storing the column for over a month, flush it with un-buffered methanol until the pressure is stable, then remove it from the HPLC and securely plug it.

4.4. Recommended operating pH range – pH 3 to 7

To obtain better column lifetime, it is highly recommended to use "silica friendly" mobile phases in the range of pH 3 to 7. The maximum pH limit of the column is pH 2 to 8.

4.5. Recommended operating temperature limit – up to 60 ºC

For longer column life, turn off the column oven when the column is not in use.

4.6. Flow rate and pressure limit

Refer to Section 1.3.

4.7. Column washing procedures

Columns may become contaminated in a variety of ways, and sometimes washing will restore them to a useful condition.

- Amines or ammonia: These can cause the baseline to be high or irregular with PCR/FLD. Prepare a solution of 0.05% formic acid in 30:70 methanol:water. Flush the column while monitoring the PCR/FLD signal until the baseline returns to normal.
- Hydrophobic substances: This can cause unusual peak shapes or high pressure. Flush the column to waste first with acetone for 20 minutes, then with 70:30 methanol:water until all the acetone is removed.
- Particulate matter: This can cause high column pressure. Replace the guard cartridge. Reverse the column and flush it to waste with mobile phase at reduced flow rate while monitoring the pressure.
- If the above procedures fail to resolve the issue, replace the column.

4.8. Sample Preparation

The EPA Method 531.2 is designed for drinking water, but the analytical technique has often been applied to other sources of water. Samples may have colloids (silt, clay, humus, etc.) that pass through common 0.45 µm membrane filters. For that reason, filter samples with the smallest porosity filters that are practical for use.

Similar chromatographic methods have been applied to carbamate pesticide residues in food, soil and other complex matrices. These entail some kind of extraction and cleanup steps prior to HPLC analysis. Careful attention the sample preparation will protect your HPLC column from early failure.

SECTION 5 – FREQUENTLY ASKED QUESTIONS

5.1. Which Acclaim Carbamate column should I use?

Dionex offers the Carbamate columns in four formats. The 5 µm 4.6 x 250 mm column is closest to the format used in US EPA Method 531.2. The 3 µm 4.6 x 150 mm and the 3 µm 3.0 x 150 mm columns offer reductions in time and solvent consumption. If you have a scaled-down PCR/FLD system, the 3 µ 3.0 x 150 mm column also offers reduced reagent consumption. Finally, the 3 µm 2.1 x 150 mm column is intended for LC/MS applications where ultimate sensitivity is the goal. The RSLC 2.2 µm 2.1 x 150 mm column is intended for LC/MS applications where the highest resolution is the goal. The RSLC 2.2 µm 2.1 x 100 mm column offers the highest throughput for LC/MS applications.

5.2. What factors should I consider for method development using this column?

Refer to Section 3.

5.3. What mobile phases should I use with this column?

Acclaim Carbamate columns can be used in several mobile phase systems, such as methanol/water, acetonitrile/water, or methanol/ecetonitrile/water, to provide excellent separation for all carbamate pesticides regulated by EPA Method 531.2. If you are using PCR/FLD then use pure water and methanol. Some laboratories use water, methanol, and acetonitrile in a ternary gradient. Other additives have little effect on the chromatography, and may adversely affect the PCR chemistry.

If you are using electrospray LC/MS then use water, methanol, and 1 mM ammonium formate. Several compounds are best detected as the $[M+NH_4]^+$ adduct.

5.4. What should I do before starting using Acclaim Carbamate column?

Read this User's Guide carefully, and contact Dionex Technical Support if you have any questions regarding using this column.

5.5. How to store the column?

You may leave the column in the HPLC in the mobile phase at the end of the gradient. If you plan on storing the column for over a month, flush it with methanol until the pressure is stable, then remove it from the HPLC and plug it securely.

5.6. Do I need a guard column with an Acclaim Carbamate analytical column?

Yes. It is highly recommended to use guard cartridges when analyzing environmental samples. The guard cartridge protects the more expensive analytical column by trapping highly retained components and particulates from the mobile phase or the sample. Since guard columns are not available for the RSLC columns (at the time of this writing), you should use a pre-column filter of 0.5 µm or smaller porosity.

5.7. What should I do if the column shows deteriorated performance?

Refer to "Section 4.7 Column washing procedure" for details.

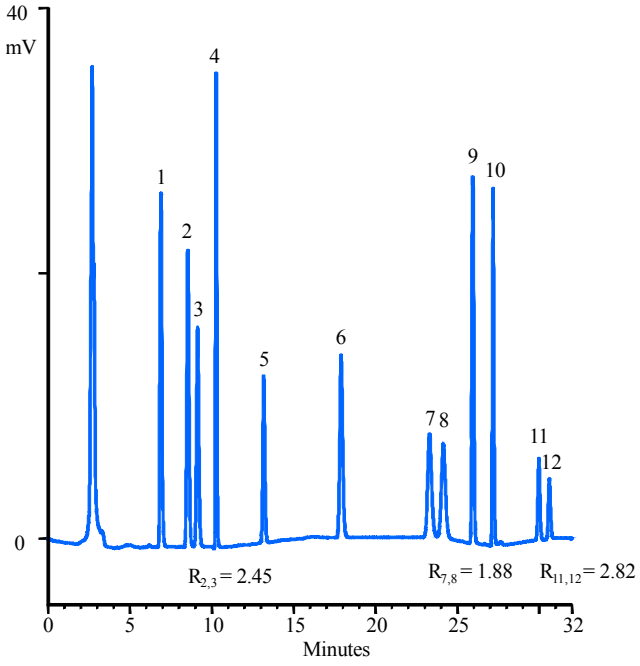
5.8. What should I do if the column exhibits excessively high backpressure?

First, make sure that the mobile phase is freshly prepared and filtered before using it, and that the samples are free of particulate matter. Replace the guard cartridge and/or filter. Then, back flush the column at reduced flow rate while monitoring the change in column pressure. If all above fail, replace it with a new column.

APPENDIX A

Typical results for Acclaim Carbamate 5 µm, 4.6 x 250 mm with post-column reaction and fluorescence detection.

Carbamate Pesticides by EPA Method 531.2



Column:	Acclaim Carbamate, 5 µm
Dimensions:	4.6 x 250 mm
LC System:	Ultimate 3000
Mobile Phases:	A: Water
	B: Methanol

Gradient:

Time (min)	-8.0	0.0	6.0	7.0	21.0	24.0	32.0
%A	80	80	77	57	53	30	30
%B	20	20	23	43	47	70	70

Flow:	1.20 mL/min
Temperature:	30 °C
Injection:	10 µm
Detector:	Post-column reaction
	Fluorescence, ex. 330 nm, em. 465 nm
Sample:	Calibration mix 100 µg/L in citrate buffer

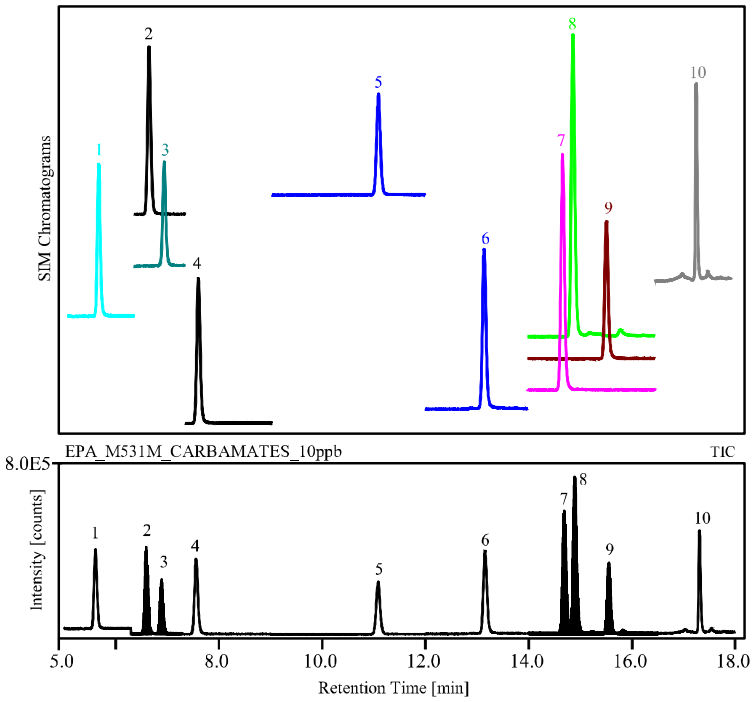
Peaks:

1. Aldicarb sulfoxide
2. Aldicarb sulfone
3. Oxamyl
4. Methomyl
5. 5-Hydroxycarbofuran
6. Aldicarb
7. Propoxur
8. Carbofuran
9. Carbaryl
10. 1-Naphthol
11. Methiocarb
12. BDMC (surrogate)

APPENDIX B

Typical results for Acclaim Carbamate 3 µm, 2.1 x 150 mm with electrospray mass-spectrometric detection.

N-Methyl Carbamates in EPA Method 531.1 by LC-MS



Top: SIM chromatograms of 10 carbamates; bottom: total ion chromatogram (TIC);
Shaded peaks in TIC indicate multiple SIM scans in those time ranges.

Chromatographic Conditions

Column:	Acclaim Carbamate, 2.1x150 mm, 3 µm
Column Temp.:	Ambient
Mobile Phase:	A: CH_3CN, B: 1 mM NH_4OOCH, C: H_2O

Time	%A	$B	%C
-4.00	10	5	85
2.00	10	5	85
15.00	65	5	30
15.01	90	5	5
20.00	90	5	5

Flow Rate:	300 µL/min
Injection:	20 µL, 10 ppb of each analyte

Mass Spectrometric Conditions

Interface:	Electrospray Ionization
Scan Mode:	Selected Ion Monitoring (SIM)
Needle Voltage:	1 kV
Probe Temp:	500 °C
Nebulizer Gas:	
Cone Voltage:	

