Thermo Scientific

Acclaim Carbonyl C18

Column Product Manual

P/N: 065451-02 April 2013

Thermo SCIENTIFIC

Product Manual

for

Acclaim Carbonyl C18 RSLC, Analytical Columns

2.2 µm, 2.1 x 100mm (P/N 077972)
2.2 µm, 2.1 x 150mm (P/N 077973)
2.2 µm, 3.0 x 100mm (P/N 077974)

Acclaim Carbonyl C18, Analytical Columns

5 µm, 4.6 x 250 mm (P/N083214)
5 µm, 4.6 x 150 mm (P/N 079008)
3 µm, 3.0 x 250mm (P/N 079009)
3 µm, 3.0 x 150mm (P/N 079010)
3 µm, 2.1 x 150mm (P/N 079011)

Acclaim Carbonyl C18, Guard Columns

5 µm, 4.6 x 10mm (P/N 079014)
5 µm, 3.0 x 10mm (P/N 079013)
5 µm, 2.1 x 10mm (P/N 079012)

This document is for Research Use Only. This document is Not for use in Diagnostic Procedures.

Revision History:

Revision 02, April, 2013, Reformatted for Thermo Scientific. Added 3µm and 5µm columns to the product line.

Safety and Special Notices

Make sure you follow the precautionary statements presented in this guide. The safety and other special notices appear in boxes.

Safety and special notices include the following:

 **SAFETY**	***Indicates a potentially hazardous situation which, if not avoided, could result in death or serious injury.***
 **WARNING**	***Indicates a potentially hazardous situation which, if not avoided, could result in damage to equipment.***
 **CAUTION**	***Indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate injury. Also used to identify a situation or practice that may seriously damage the instrument, but will not cause injury.***
 **NOTE**	***Indicates information of general interest.***
IMPORTANT	***Highlights information necessary to prevent damage to software, loss of data, or invalid test results; or might contain information that is critical for optimal performance of the system.***
Tip	***Highlights helpful information that can make a task easier.***

Contents

1. Introduction

Aldehydes and ketones are common pollutants in air and water. The analytical difficulties that need to be overcome include their volatility, their reactivity, and their modest UV absorption. The reaction with dinitrophenylhydrazine (DNPH) is a convenient means of trapping, stabilizing, and tagging these substances. Several standard methods have been developed to apply this chemistry to various environmental situations. Some of the better known ones include CARB 1004 for vehicle exhaust, EPA 554 for drinking water, EPA 1667 for pharmaceutical wastewater, and EPA 8315 for general wastewater. Each standard method has a particular target compound list.

The Thermo Scientific™ Acclaim™ Carbonyl columns are silica-based reversed phase columns designed specifically for separating DNPH derivatives of aldehydes and ketones. They exhibit superior resolution compared with other commercially available columns. All the popular standard methods can be performed with the Acclaim Carbonyl columns.

The Acclaim Carbonyl columns are available in three particle sizes, 5 µm, 3 µm, and 2.2 µm. The 5 µm, 4.6 x 250 mm column is intended for traditional HPLC equipment, and provides excellent resolution. The 3 µm, 3.0x150 mm column gives faster run times and lower solvent consumption, and is compatible with most traditional HPLC systems. The 2.2 µm columns are designed for newer UHPLC systems and feature excellent resolution, speed and solvent consumption.

Acclaim Carbonyl C18 Analytical Columns

Particle Size	Dimensions	Part Number
5 µm	4.6 x 250 mm	083214
	4.6 x 150 mm	079008
3 µm	3.0 x 250 mm	079009
	3.0 x 150 mm	079010
	2.1 x 150 mm	079011
2.2 µm	2.1 x 100 mm	077972
	2.1 x 150 mm	077973
	3.0 x 100 mm	077974

Acclaim Carbonyl C18 Guard Columns

Dimensions	Part Number
4.6 x 10 mm	079014
3.0 x 10 mm	079013
2.1 x 10 mm	079012
Holder, V2	069580

2. Installation

2.1 Preparation of the Mobile Phase

The recommended mobile phase system contains an aqueous portion and a solvent portion. The aqueous portion can be D.I, water or a buffer (e.g., 2 mM ammonium acetate buffer), While D.I. water works well in most cases, a buffered aqueous solution sometimes may yield slightly better consistency of results. The solvent can be acetonitrile or methanol, depending on the application. . All ingredients for the mobile phases are available in "HPLC grade" or better and should be used to make mobile phases. If you use an in-house water purifier, be sure it is correctly maintained.

2.1.1 Preparation of 2 mM ammonium acetate buffer

1. Measure 57 ± 3 µL of glacial acetic acid and 154 ± 5 mg of ammonium acetate into 1000 ± 5 mL of deionized water.

2.2 HPLC System Set-up

The Acclaim Carbonyl columns may be used on any standard HPLC system equipped with an HPLC pump, a column oven, a UV detector, and an injector. To minimize 'downtime', ensure that the whole system is primed before starting your column conditioning.

2.3 Conditioning the Column

When installing a new Carbonyl column for the first time, wash the column with pure methanol for ~ 20 column volumes (or 50 minutes at 1 mL/min), and send the effluent directly to waste. Reconnect the column to the detector, and equilibrate it with the desired mobile phase for at least 20 column volumes before making your first injection.

NOTE

Thermo Scientific recommends that you always read the manual for a new column before installing it for the first time. The manual contains information regarding the operational limits of the column, as well as advice on how to optimize your separation.

2.4 Ensuring Column Performance

Before running any samples, Thermo Scientific recommends that you first confirm the performance of the column by reproducing the lot validation report chromatogram shipped with the column. Compare your results with the one reported in the quality assurance report. At least three injections should be made.

NOTE

Due to various reasons, such as differences in HPLC systems, mobile phases, oven temperature control, etc., you may observe somewhat different peak resolution from those shown in the report. In this case, please contact us with your test chromatogram for technical support and/or optimize chromatographic conditions using the methods stated in "Optimizing Chromatographic Conditions".

2.5 Optimizing Chromatographic Conditions

Some typical chromatograms using the following conditions are shown in sections 2.5.1 and 2.5.2.

NOTE

Mobile phase composition and oven temperature are the two main factors influencing the separation.

NOTE

The mobile phase gradients listed below are intended to provide a starting point, and should be modified as necessary for optimal separation.

2.5.1 Standard Acetonitrile conditions

These gradients are satisfactory for CARB 1004, EPA 554, EPA 1667 and EPA 8315. Depending on the gradient delay volume of your system, you may want to adjust the times for the start and end of the gradient. You may also want to adjust the equilibration time between injections. For EPA 1667, the gradient is optional; you may use simply the initial isocratic conditions if there are no late-eluting interferences.

Column (Notes)	Flow Rate (mL/min)	Temp. (°C)	Data Rate (Hz)	Inj. Volume (µL)	Gradient		
					Time (min)	% Acetonitrile	% Aqueous
5 µm, 4.6 x 250 mm	1.50	28	5	10	-9	52	48
					0	52	48
Standard HPLC					20	52	48
(Fig. 7)					28	90	10
					32	90	10
3 µm, 3.0 x 150 mm	0.90	28	10	5	-3.5	53	47
					0.0	53	47
Standard HPLC; optimized					7.5	53	47
for speed					11.5	90	10
(Fig. 5)					16.0	90	10
3 µm, 3.0 x 250 mm	0.60	28	5	5	-12.0	53	47
					0.0	53	47
Standard HPLC; optimized					20.5	53	47
for resolution					29.0	90	10
					34.0	90	10
3 µm, 2.1 x 150 mm	0.30	28	10	2	-6.0	53	47
					0.0	53	47
Micro HPLC; optimized for					11.0	53	47
low solvent consumption					19.0	90	10
					24.0	90	10
2.2 µm, 2.1 x 150 mm	0.40	28	25	1	-4.5	52	48
Low gradient delay UHPLC;					0.0	52	48
optimized for resolution					8.3	52	48
(Fig. 4)					15.0	100	0
					18.0	100	0
2.2 µm, 2.1 x 100 mm	0.75	28	25	1	-1.7	52	48
Low gradient delay UHPLC;					0.0	52	48
optimized for speed					2.9	52	48
(Fig. 1 & 3)					5.3	100	0
					6.2	100	0
2.2 µm, 3.0 x 100 mm	1.00	28	25	2	-3.0	52	48
					0.0	52	48
Quaternary HPLC					4.4	52	48
					8.0	100	0
					9.8	100	0

2.5.2 Standard Methanol conditions

These gradients are satisfactory for EPA 554. Depending on the gradient delay volume of your system, you may want to adjust the times for the start and end of the gradient. You may also want to adjust the equilibration time between injections.

Column (Notes)	Flow Rate (mL/min)	Temp. (°C)	Data Rate (Hz)	Inj. Volume (µL)	Gradient		
					Time (min)	% Methanol	% Aqueous
5 µm, 4.6 x 250 mm Standard HPLC	1.25	40	5	20	-9 0 20 33 40	72 72 72 100 100	28 28 28 0 0
3 µm, 3.0 x 150 mm Standard HPLC; optimized for speed (Fig. 6)	0.80	40	10	5	-6.0 0.0 7.0 12.5 16.0	72 72 72 100 100	28 28 28 0 0
3 µm, 3.0 x 250 mm Standard HPLC; high resolution	0.53	40	2	5	-12.0 0.0 19.0 32.0 40.0	72 72 72 100 100	28 28 28 0 0
3 µm, 2.1 x 150 mm Micro HPLC; low solvent consumption	0.26	40	5	1	-12.0 0.0 11.5 14.5 24.0	72 72 72 100 100	28 28 28 0 0
2.2 µm, 2.1 x 150 mm Low gradient delay UHPLC	0.50	42	25	1	-1.9 0.0 5.8 8.9 10.5	70 70 70 100 100	30 30 30 0 0
2.2 µm, 2.1 x 100 mm Low gradient delay UHPLC (Fig. 2)	0.50	42	25	1	-1.7 0.0 3.4 5.5 7.0	70 70 70 100 100	30 30 30 0 0
2.2 µm, 3.0 x 100 mm Quaternary HPLC	0.75	42	10	2	-3.5 0.0 3.5 7.0 9.3	70 70 70 100 100	30 30 30 0 0

2.6 Real Sample Analysis

Once satisfactory results have been obtained using your test mix, you are ready to run samples. The same conditions that separate the test mix should be used to analyze your samples.

2.7 Column Storage

After use, the column can be stored in the mobile phase for short periods of time (e.g. overnight). For longer term storage (longer than one week), it is recommended that you store the column in pure methanol or acetonitrile.

3. Column Care

3.1 General Guidelines

These columns should be used with the same precautions you would take for any other silica-based reversed phase columns. Please refer to the table below for recommended operational guidelines.

3.1.1 Recommended Ranges of Operation

Particle Size	Column Dimensions	Maximum Pressure (bar)	Maximum Flow Rate (mL/min)	Typical Flow Rate (mL/min)	pH Range	Typical Temperature (° C)	Maximum Temperature (° C)
5 µm	4.6 x 250 mm	400	2.0	0.8 – 1.5	2.5 – 7.5	25 – 35	50
	4.6 x 150 mm	400	2.0	0.8 – 1.5	2.5 – 7.5	25 – 35	50
3 µm	3.0 x 250 mm	800	1.0	0.4 – 0.8	2.5 – 7.5	25 – 35	50
	3.0 x 150 mm	600	1.0	0.4 – 0.8	2.5 – 7.5	25 – 35	50
	2.1 x 150 mm	600	0.5	0.2 – 0.4	2.5 – 7.5	25 – 35	50
2.2 µm	2.1 x 150 mm	800	01.0	0.25 – 0.75	2.5 – 7.5	25 – 35	50
	2.1 x 100 mm	600	0.1.0	0.25 – 0.75	2.5 – 7.5	25 – 35	50
	3.0 x 100 mm	550	1.5	0.4 – 1.2	2.5 – 7.5	25 – 35	50

3.2 Recommended Operating pH Range

The pH of the mobile phase has little effect on retention times or selectivity on the Acclaim Carbonyl columns, and therefore need not be varied for most samples. To ensure the longest possible lifetime for these columns, a mobile phase that is 'silica friendly' should be used (pH 2.5 – 7.5). In most cases, a simple methanol (or acetonitrile)/water (or ammonium acetate) mobile phase system will work very well.

3.3 Recommended Operating Temperature

The separation of carbonyl DNPH is moderately sensitive to changes in temperature. We have found that optimal separation occurs between 25 ºC and 35 ºC for most applications using acetonitrile gradients, and on most systems. When using methanol gradients, moderately elevated temperatures (35 – 45 °C) are recommended to reduce the back pressure; this permits conventional HPLC equipment to use the 2.2 µm RSLC columns without exceeding the pressure limits of the system. Although the Acclaim Carbonyl columns can be used within a broader temperature range, we have found no practical reason to use them outside the recommended range in order to improve the separation. On the other hand, a mild operating temperature helps to prolong column lifetime.

3.4 Recommended Flow Rate

It is extremely important not to expose the columns to surges in column pressure. When starting up a system from idle, for a 4.6-mm i.d. column, gradually increase the flow rate from 0.5 mL/min up to the desired flow rate in 0.1-0.2 mL/min increments.

3.5 Column Washing Procedure

All samples should be pre-treated and filtered before being injected onto the column. In addition, a guard column is recommended for real sample analysis to prolong the lifetime of the analytical column. If the column does need to be cleaned, such as after long-term storage, the following procedure can be used as a guideline. The flow rates given here are for a 4.6 mm i.d. column; for a 3 mm i.d. column reduce the flow to 40% and for a 2.1 mm i.d. column reduce the flow to 20%.

1. Equilibrate the column with methanol/water v/v 50/50 for 10 column volumes at 0.5 to 1 mL/min.
2. Then, wash the column with pure methanol, acetonitrile, or acetone for 20 column volumes at 0.5 to 1 mL/min.
3. Finally, wash the column with methanol/water v/v 80/20 for 10 column volumes.
4. Before any injection is made, the column should be equilibrated with your initial mobile phase composition for at least 20 column volumes.

4. Frequently Asked Questions (FAQ)

1. How do the Acclaim Carbonyl columns compare with other columns for Aldehydes and Ketones?

   The Acclaim Carbonyl column offers unique selectivity for DNPH derivatives. There is no need for excessively long columns or run times, or for complex gradients to compensate for inadequate selectivity. The Acclaim Carbonyl columns offer a combination of selectivity, resolution and speed that is unmatched by any other column.

2. Which particle size / format should I use?

   The 5-µm particle size is used for standard applications when methods are being developed or if the sample is complex. The 3-µm particle size is used for faster, higher efficiency separations. The 2.2-µm particle size is used in UHPLC systems that operate at higher pressure, and provide high-speed analysis. The 250-mm long columns are optimum for highest resolution and the 150-mm long columns are for high speed separations. The 2.1-mm internal diameter columns are the preferred column for LC/MS applications, or in cases where sample size is limited and greater sensitivity is required, or when solvent savings are desired.

3. My chromatogram shows bad peak shapes and low efficiencies. What is the problem, and how do I resolve it?

   A. Check the system, connections, and tubing for excessive extra column volume. Fix and replace if needed.
   B. Be sure the column is fully equilibrated with mobile phase.
   C. Make sure you are not injecting too large a sample or a sample in the wrong solvent.
   D. Run the column performance test described in the Quality Assurance Report (QAR). Replace the column if it is necessary.

4. Why am I observing high column backpressure?

   A. Check the injection valve and tubing for possible clogging.
   B. Wash the column using the protocol described in “Section 3.5.”
   C. Use a guard column and/or pre-column filter and replace it on a regular basis.

5. Why is the selectivity on my column different from the Quality Assurance Report (QAR), when using the condition described in QAR?

   A. Check your mobile phase composition.
   B. If you are proportioning, try using different lines, or pre-mix the solution and check the selectivity.
   C. Check your column temperature (oven temperature) and make sure it has been calibrated recently.
   D. Use the column within its pH limits. Failing to do so will result in undesirable selectivity change.

6. Should I use D.I. water or buffer for the analysis?

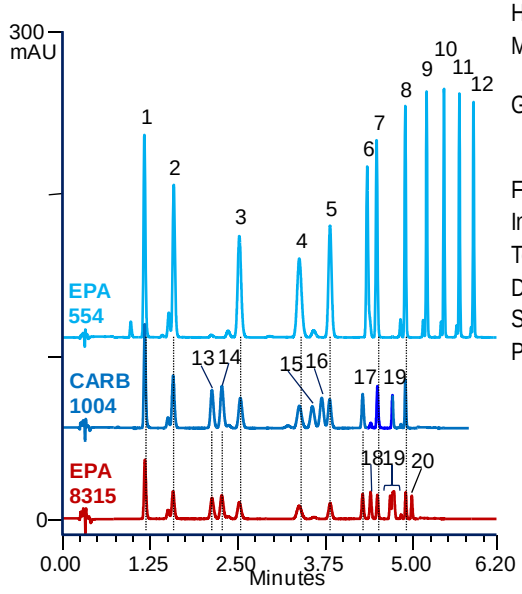
   Both will give good result.. There is virtually no difference in results between D.I. water and buffer. We have observed that in some cases, a slightly buffered aqueous solution may yield marginally better consistency

7. When should I use methanol instead of acetonitrile?

   The chemistry of DNPH derivatives produces isomers for some aldehydes and ketones. These isomers are better resolved with acetonitrile; with methanol, the isomers tend to coelute. (This is a unique feature of the Acclaim Carbonyl columns.) Some pairs of analytes are better resolved using acetonitrile, especially in CARB 1004 and EPA 8315. Acetonitrile usually permits moderately faster analysis times. Methanol is less expensive and less subject to supply problems than acetonitrile. Methanol is more viscous than acetonitrile, and for some column formats, may result in excess pressure. The reference method in EPA 554 uses methanol, and this may be the more comfortable option for regulated laboratories.

5. Applications

Figure 1 Rapid DNPH Aldehyde and Ketone Standards using Acclaim Carbonyl RSLC with Acetonitrile Mobile Phase



Column:	Thermo Scientific Acclaim Carbonyl C18 RSLC, 2.2 µm
Dimension:	2.1 x 100 mm
HPLC System:	Thermo Scientific Dionex UltiMate® 3000-RS HPG
Mobile Phases:	A: D.I. Water B: Acetonitrile

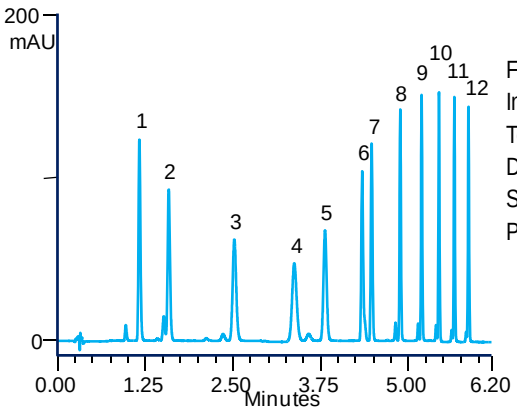
Gradient times (min):	-1.7	0.0	2.9	5.3	6.2
%A	48	48	48	0	0
%B	52	52	52	100	100

Flow rate:	0.750 mL/min
Injection:	1 µL; bypass at 0.25 min.
Temperature:	28 °C
Detection:	UV at 360 nm, 25 Hz, 0.2 s resp. time
Samples:	Calibration mixes diluted in methanol

Peaks:

1. Formaldehyde DNPH
2. Acetaldehyde DNPH
3. Propionaldehyde DNPH
4. Crotonaldehyde DNPH
5. Butyraldehyde DNPH
6. Cyclohexanone DNPH
7. Valeraldehyde DNPH
8. Hexanal DNPH
9. Heptanal DNPH
10. Octanal DNPH
11. Nonanal DNPH
12. Decanal DNPH
13. Acetone DNPH
14. Acrolein DNPH
15. Butanone DNPH
16. Methacrolein DNPH
17. Benzaldehyde DNPH
18. Isovaleraldehyde DNPH
19. Tolualdehyde DNPH
20. Xylylaldehyde DNPH

Figure 2 Rapid EPA 554 DNPH Aldehyde and Ketone Standards using Acclaim Carbonyl RSLC with Acetonitrile Mobile Phase



Column:	Thermo Scientific Acclaim Carbonyl C18 RSLC, 2.2 µm
Dimension:	2.1 x 100 mm
HPLC System:	Thermo Scientific Dionex UltiMate® 3000-RS HPG
Mobile Phases:	A: D.I. Water B: Acetonitrile

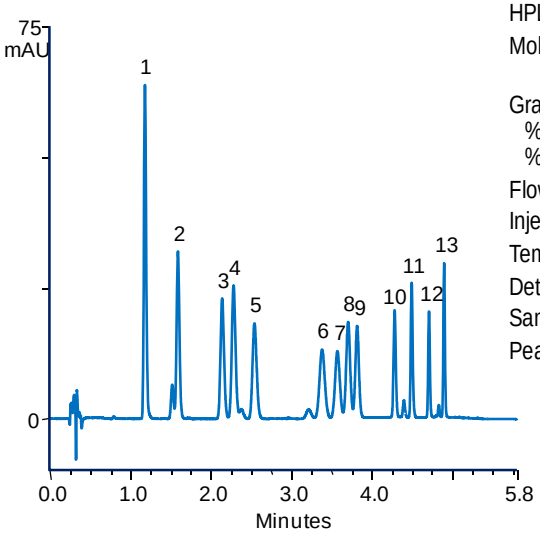
Gradient times (min):	-1.7	0.0	2.9	5.3	6.2
%A	48	48	48	0	0
%B	52	52	52	100	100

Flow rate:	0.750 mL/min
Injection:	1 µL; bypass at 0.25 min.
Temperature:	28 °C
Detection:	UV at 360 nm, 25 Hz, 0.2 s resp. time
Samples:	Calibration mix diluted in methanol

Peaks:

1. Formaldehyde DNPH
2. Acetaldehyde DNPH
3. Propionaldehyde DNPH
4. Crotonaldehyde DNPH
5. Butyraldehyde DNPH
6. Cyclohexanone DNPH
7. Valeraldehyde DNPH
8. Hexanal DNPH
9. Heptanal DNPH
10. Octanal DNPH
11. Nonanal DNPH
12. Decanal DNPH

Figure 3 Rapid CARB-1004 DNPH Aldehyde and Ketone Standards using Acclaim Carbonyl RSLC with Acetonitrile Mobile Phase



Column:	Thermo Scientific Acclaim Carbonyl C18 RSLC, 2.2 µm
Dimension:	2.1 x 100 mm
HPLC System:	Thermo Scientific Dionex UltiMate® 3000-RS HPG
Mobile Phases:	A: D.I. Water B: Acetonitrile

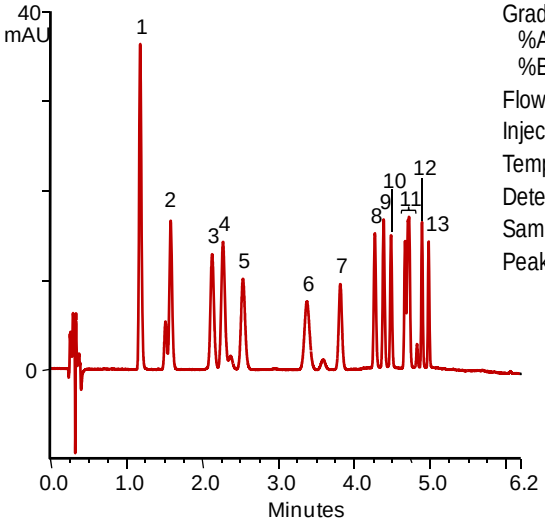
Gradient times (min):	-1.7	0.0	2.9	5.3	5.8
%A	48	48	48	0	0
%B	52	52	52	100	100

Flow rate:	0.750 mL/min
Injection:	1 µL; bypass at 0.25 min.
Temperature:	28 °C
Detection:	UV at 360 nm, 25 Hz, 0.2 s resp. time
Samples:	Calibration mix diluted in methanol

Peaks:

1. Formaldehyde DNPH
2. Acetaldehyde DNPH
3. Acetone DNPH
4. Acrolein DNPH
5. Propionaldehyde DNPH
6. Crotonaldehyde DNPH
7. Butanone DNPH
8. Methacrolein DNPH
9. Butyraldehyde DNPH
10. Benzaldehyde DNPH
11. Valeraldehyde DNPH
12. m-Tolualdehyde DNPH
13. Hexanal DNPH

Figure 4 Rapid EPA 8315 DNPH Aldehyde and Ketone Standards using Acclaim Carbonyl RSLC with Acetonitrile Mobile Phase



Column:	Thermo Scientific Acclaim Carbonyl C18 RSLC, 2.2 µm
Dimension:	2.1 x 100 mm
HPLC System:	Thermo Scientific Dionex UltiMate® 3000-RS HPG
Mobile Phases:	A: D.I. Water B: Acetonitrile

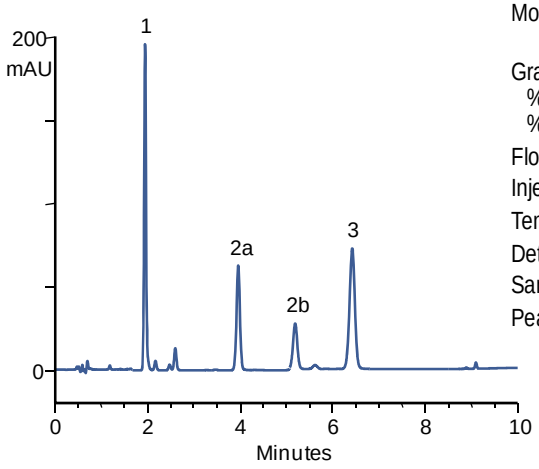
Gradient times (min):	-1.7	0.0	2.9	5.3	6.2
%A	48	48	48	0	0
%B	52	52	52	100	100

Flow rate:	0.750 mL/min
Injection:	1 µL; bypass at 0.25 min.
Temperature:	28 °C
Detection:	UV at 360 nm, 25 Hz, 0.2 s resp. time
Samples:	Calibration mix diluted in methanol

Peaks:

1. Formaldehyde DNPH
2. Acetaldehyde DNPH
3. Acetone DNPH
4. Acrolein DNPH
5. Propionaldehdye DNPH
6. Crotonaldehyde DNPH
7. Butyraldehyde DNPH
8. Benzaldehyde DNPH
9. Isovaleraldehdye DNPH
10. Valeraldehyde DNPH
11. o-, m-, p- Tolualdehydes DNPH
12. Hexanal DNPH
13. Xylylaldehyde DNPH

Figure 5 EPA 1667 DNPH Aldehyde and Ketone Standards using Acclaim Carbonyl RSLC with Acetonitrile Mobile Phase

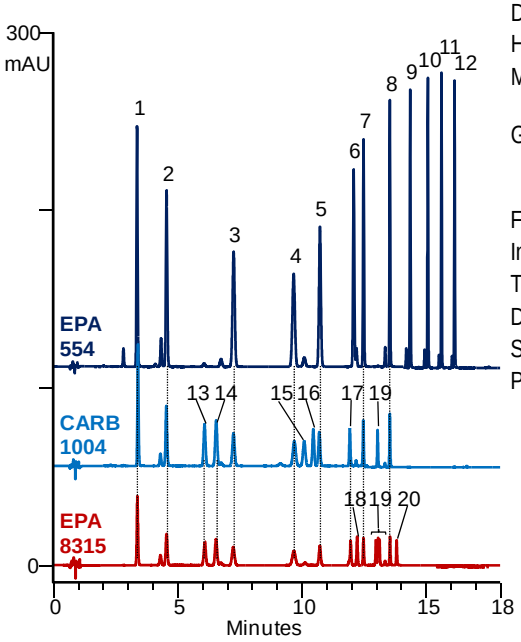


Column:	Thermo Scientific Acclaim Carbonyl C18 RSLC, 2.2 µm
Dimension:	2.1 x 100 mm
HPLC System:	Thermo Scientific Dionex UltiMate® 3000-RS HPG
Mobile Phases:	A: D.I. Water B: Acetonitrile

Gradient times (min):	-4.5	0.0	4.5	7.5	10.0
%A	48	48	48	0	0
%B	52	52	52	100	100

Flow rate:	0.750 mL/min
Injection:	1 µL; bypass at 0.25 min.
Temperature:	28 °C
Detection:	UV at 360 nm, 25 Hz, 0.2 s resp. time
Samples:	Calibration mix diluted in methanol
Peaks:	1. Formaldehyde DNPH 2a. Furfural DNPH 2b. Furfural DNPH 3. Isobutyraldehyde DNPH

Figure 6 High-Resolution DNPH Aldehyde and Ketone Standards using Acclaim Carbonyl RSLC with Acetonitrile Mobile Phase



Column:	Thermo Scientific Acclaim Carbonyl C18 RSLC, 2.2 µm
Dimension:	2.1 x 150 mm
HPLC System:	Thermo Scientific Dionex UltiMate® 3000-RS HPG
Mobile Phases:	A: D.I.Water B: Acetonitrile

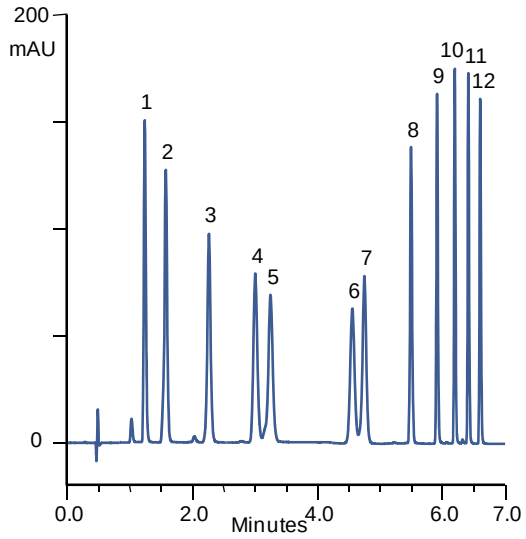
Gradient times (min):	-4.5	0.0	8.3	15.0	18.0
%A	48	48	48	0	0
%B	52	52	52	100	100

Flow rate:	0.400 mL/min
Injection:	1 µL; bypass at 0.25 min.
Temperature:	28 °C
Detection:	UV at 360 nm, 25 Hz, 0.2 s resp. time
Samples:	Calibration mixes diluted in methanol

Peaks:

1. Formaldehyde DNPH
2. Acetaldehyde DNPH
3. Propionaldehyde DNPH
4. Crotonaldehyde DNPH
5. Butyraldehyde DNPH
6. Cyclohexanone DNPH
7. Valeraldehyde DNPH
8. Hexanal DNPH
9. Heptanal DNPH
10. Octanal DNPH
11. Nonanal DNPH
12. Decanal DNPH
13. Acetone DNPH
14. Acrolein DNPH
15. Butanone DNPH
16. Methacrolein DNPH
17. Benzaldehyde DNPH
18. Isovaleraldehyde DNPH
19. Tolualdehyde DNPH
20. Xylylaldehyde DNPH

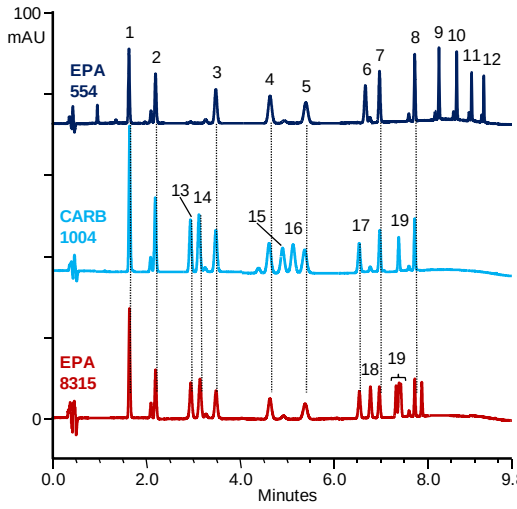
Figure 7 EPA 554 Carbonyl-DNPH Standards using Acclaim Carbonyl RSLC with Methanol Mobile Phase



Column:	Thermo Scientific Acclaim Carbonyl C18 RSLC				
Dimension:	2.2 µm, 2.1 x 100 mm				
HPLC System:	Thermo Scientific Dionex UltiMate® 3000-RS HPG				
Mobile Phases:	A: D.I. Water B: Methanol				
Gradient times (min):	-1.7	0.0	3.4	5.5	7.0
%A	30	30	30	0	0
%B	70	70	70	100	100
Flow rate:	0.5 mL/min				
Injection:	1 µL; bypass at 0.25 min.				
Temperature:	42 °C				
Detection:	UV at 360nm, 10 Hz, 0.4 s resp. time				
Samples:	Calibration mix, 50 µg/mL in methanol				

Peaks:	
1. Formaldehyde DNPH	7. Valeraldehyde DNPH
2. Acetaldehyde DNPH	8. Hexanal DNPH
3. Propionaldehyde DNPH	9. Heptanal DNPH
4. Crotonaldehyde DNPH	10. Octanal DNPH
5. Butyraldehyde DNPH	11. Nonanal DNPH
6. Cyclohexanone DNPH	12. Decanal DNPH

Figure 8 DNPH Aldehyde and Ketone Standards using Acclaim Carbonyl RSLC with Acetonitrile Mobile Phase



Column:	Thermo Scientific Acclaim Carbonyl C18 RSLC, 2.2 µm
Dimension:	3.0 x 100 mm
HPLC System:	Thermo Scientific Dionex UltiMate® 3000-RS Quaternary
Mobile Phases:	A: D.I. Water B: Acetonitrile

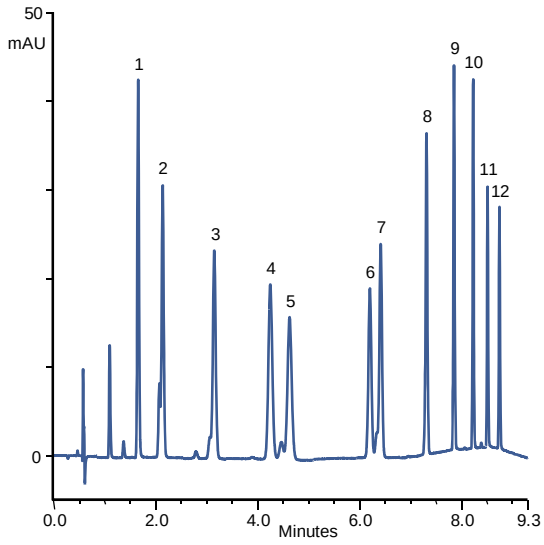
Gradient times (min):	-3.0	0.0	3.4	5.5	9.3
%A	48	48	48	0	0
%B	52	52	52	100	100

Flow rate:	1.00 mL/min
Injection:	2 µL
Temperature:	28 °C
Detection:	UV at 360 nm, 10 Hz, 0.5 s resp. time
Samples:	Calibration mixes diluted in methanol

Peaks:

1. Formaldehyde DNPH
2. Acetaldehyde DNPH
3. Propionaldehyde DNPH
4. Crotonaldehyde DNPH
5. Butyraldehyde DNPH
6. Cyclohexanone DNPH
7. Valeraldehyde DNPH
8. Hexanal DNPH
9. Heptanal DNPH
10. Octanal DNPH
11. Nonanal DNPH
12. Decanal DNPH
13. Acetone DNPH
14. Acrolein DNPH
15. Butanone DNPH
16. Methacrolein DNPH
17. Benzaldehyde DNPH
18. Isovaleraldehyde DNPH
19. Tolualdehyde DNPH
20. Xylylaldehyde DNPH

Figure 9 EPA 554 Carbonyl-DNPH Standards using Acclaim Carbonyl RSLC with Methanol Mobile Phase



Column:	Thermo Scientific Acclaim Carbonyl C18 RSLC
Dimension:	2.2 µm, 3.0 x 100 mm
HPLC System:	Thermo Scientific Dionex UltiMate® 3000-RS Quaternary
Mobile Phases:	A: D.I. Water B: Methanol

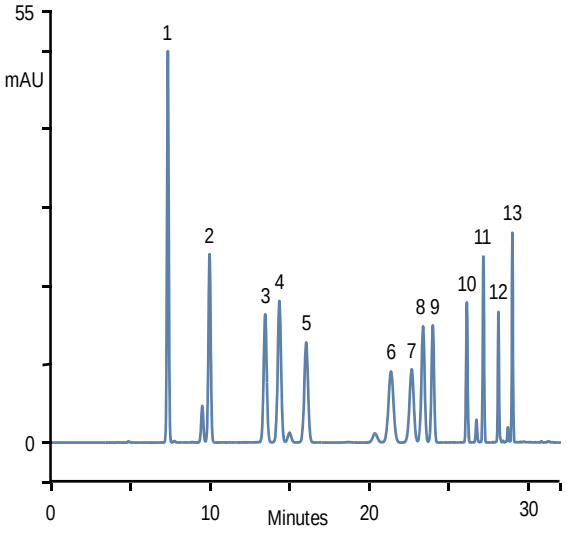
Gradient times (min):	-3.5	0.0	3.5	7.0	9.3
%A	30	30	30	0	0
%B	70	70	70	100	100

Flow rate:	0.75 mL/min
Injection:	2 µL
Temperature:	42 °C
Detection:	UV at 360nm, 10 Hz, 0.4 s resp. time
Samples:	Calibration mix, 50 µg/mL in methanol

Peaks:

1. Formaldehyde DNPH
2. Acetaldehyde DNPH
3. Propionaldehyde DNPH
4. Crotonaldehyde DNPH
5. Butyraldehyde DNPH
6. Cyclohexanone DNPH
7. Valeraldehyde DNPH
8. Hexanal DNPH
9. Heptanal DNPH
10. Octanal DNPH
11. Nonanal DNPH
12. Decanal DNPH

Figure 10 CARB1004 Carbonyl-DNPH Standards using Acclaim Carbonyl 5 µm, 4.6 x 250 mm



HPLC Conditions:

Column:	Thermo Scientific Acclaim Carbonyl C18 RSLC 5µm 4.6x250mm
Pump:	Thermo Scientific Dionex UltiMate LPG-3400A
Mobile Phases:	A: Acetonitrile B: D.I. Water

Gradient:

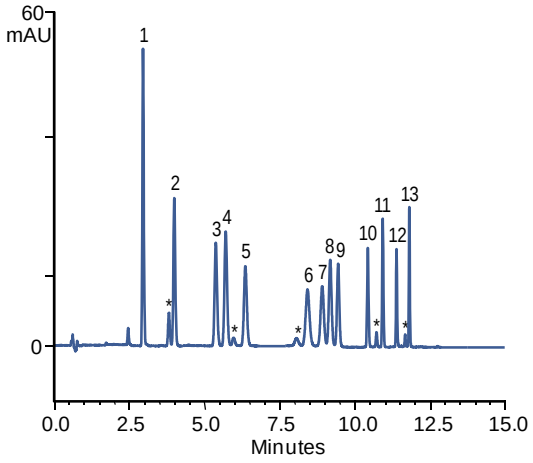
	-9	0	20	28	32
%A	52	52	52	90	90
%B	48	48	48	10	10

Flow rate:	1.5 mL/min
Temperature:	TCC-3100 thermostat at 28 °C
Injection:	WPS-3000 SL sampler, 10 µL
Detection:	VWD-3400, UV 360 nm
Sample:	Standards 1.5 µg/mL in acetonitrile

Peaks:

1. Formaldehyde DNPH
2. Acetaldehyde DNPH
3. Acetone DNPH
4. Acrolein DNPH
5. Propionaldehyde DNPH
6. Crotonaldehyde DNPH
7. Butanone DNPH
8. Methacrolein DNPH
9. Butyraldehyde DNPH
10. Benzaldehyde DNPH
11. Valeraldehyde DNPH
12. m-Tolualdehyde DNPH
13. Hexaldehyde DNPH

Figure 11 CARB1004 Carbonyl-DNPH Standards using Acclaim Carbonyl 3 µm, 3 x 150 mm



HPLC Conditions

Column:	Thermo Scientific Acclaim Carbonyl C18 RSLC 3 µm, 3 x 150mm
Mobile Phases:	A: Acetonitrile B: D.I. Water
Pump:	Thermo Scientific Dionex UltiMate HPG-3400RS

Gradient:

	-3.5	0.0	7.5	11.5	16.0
%A	53	53	53	90	90
%B	47	47	47	10	10

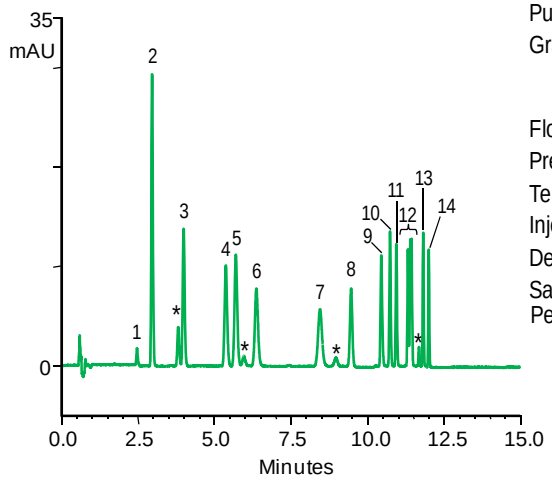
Flow rate:	0.90 mL/min
Pressure:	310 bar max.
Temperature:	28 °C
Injection:	5 µL
Detection:	UV 360 nm, 10 Hz, 0.5 sec.
Sample:	Standards in acetonitrile

Peaks:

1. Formaldehyde DNPH
2. Acetaldehyde DNPH
3. Acetone DNPH
4. Acrolein DNPH
5. Propionaldehyde DNPH
6. Crotonaldehyde DNPH
7. Butanone DNPH
8. Methacrolein DNPH
9. Butyraldehyde DNPH
10. Benzaldehyde DNPH
11. Valeraldehyde DNPH
12. m-Tolualdehyde DNPH
13. Hexaldehyde DNPH

*. Isomers

Figure 12 EPA 8315 Carbonyl-DNPH Standards using Acclaim Carbonyl 3 µm, 3 x 150 mm



HPLC Conditions

Column:	Thermo Scientific Acclaim Carbonyl C18 RSLC 3 µm, 3 x 150mm
Mobile Phases:	A: Acetonitrile B: D.I. Water
Pump:	Thermo Scientific Dionex UltiMate HPG-3400RS

Gradient:

	-3.5	0.0	7.5	11.5	16.0
%A	53	53	53	90	90
%B	47	47	47	10	10

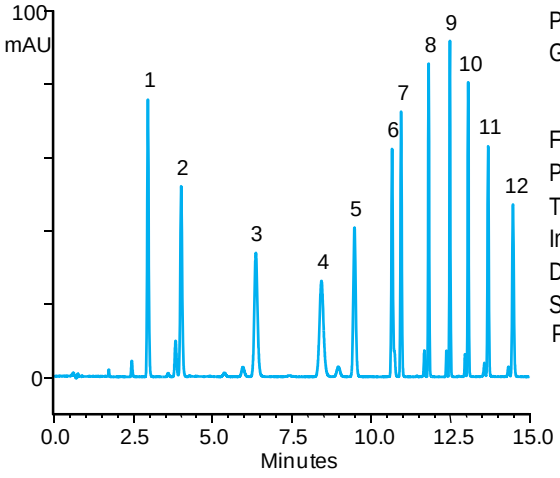
Flow rate:	0.90 mL/min
Pressure:	310 bar max.
Temperature:	28 °C
Injection:	8 µL
Detection:	UV 360 nm, 10 Hz, 0.5 sec.
Sample:	Standards in acetonitrile

Peaks:

1. DNPH
2. Formaldehyde DNPH
3. Acetaldehyde DNPH
4. Acetone DNPH
5. Acrolein DNPH
6. Propionaldehyde DNPH
7. Crotonaldehyde DNPH
8. Butyraldehyde DNPH
9. Benzaldehyde DNPH
10. Isovaleraldehyde DNPH
11. Valeraldehyde DNPH
12. o-, m-, p-Tolualdehydes DNPH
13. Hexaldehyde DNPH
14. Xylylaldehyde DNPH

*. Isomers

Figure 13 EPA 554 Carbonyl-DNPH Standards using Acclaim Carbonyl 3 µm, 3 x 150 mm with Acetonitrile Mobile Phase



HPLC Conditions

Column:	Thermo Scientific Acclaim Carbonyl C18 RSLC 3 µm, 3 x 150 mm
Mobile Phases:	A: Acetonitrile B: D.I. Water
Pump:	Thermo Scientific Dionex UltiMate HPG-3400RS

Gradient:

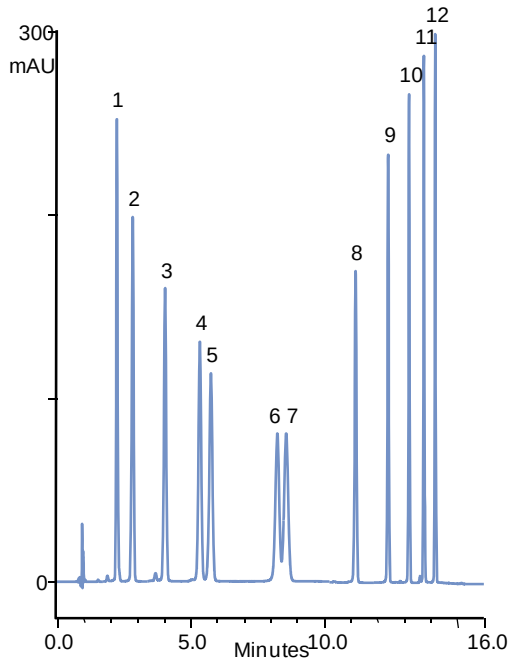
	-3.5	0.0	7.5	11.5	16.0
%A	53	53	53	90	90
%B	47	47	47	10	10

Flow rate:	0.90 mL/min
Pressure:	310 bar max.
Temperature:	28 °C
Injection:	2 µL
Detection:	UV 360 nm, 10 Hz, 0.5 sec.
Sample:	Standards in acetonitrile

Peaks:

1. Formaldehyde DNPH	7. Valeraldehyde DNPH
2. Acetaldehyde DNPH	8. Hexanal DNPH
3. Propionaldehyde DNPH	9. Heptanal DNPH
4. Crotonaldehyde DNPH	10. Octanal DNPH
5. Butyraldehyde DNPH	11. Nonanal DNPH
6. Cyclohexanone DNPH	12. Decanal DNPH

Figure 14 EPA 554 Carbonyl-DNPH Standards using Acclaim Carbonyl 3 µm, 3 x 150 mm with Methanol Mobile Phase

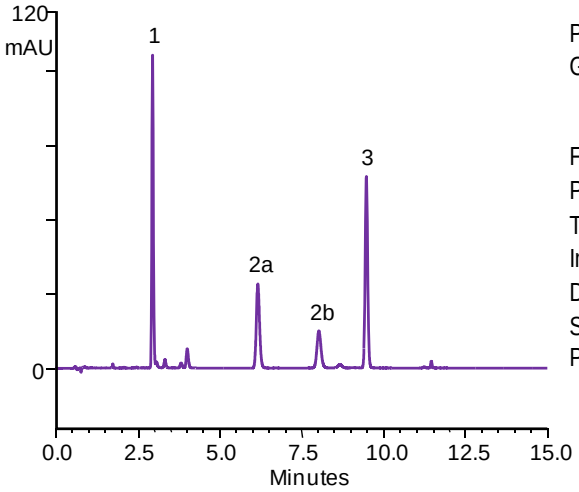


Column:	Thermo Scientific Acclaim Carbonyl C18 RSLC				
Dimension:	3.0 µm, 3.0 x 150 mm				
HPLC System:	UltiMate® 3000-RS				
Mobile Phases:	A: D.I Water B: Methanol				
Gradient times (min):	-6.0	0.0	7.0	12.5	16.0
%A	28	28	28	0	0
%B	72	72	72	100	100
Flow rate:	0.8 mL/min				
Injection:	5 µL				
Temperature:	40 °C				
Detection:	UV at 230nm, 5 Hz, 0.4 s resp. time				
Samples:	Calibration mix, 50 µg/mL in ethanol				

Peaks:

1. Formaldehyde DNPH
2. Acetaldehyde DNPH
3. Propionaldehyde DNPH
4. Crotonaldehyde DNPH
5. Butyraldehyde DNPH
6. Cyclohexanone DNPH
7. Valeraldehyde DNPH
8. Hexanal DNPH
9. Heptanal DNPH
10. Octanal DNPH
11. Nonanal DNPH
12. Decanal DNPH

Figure 15 EPA 1667 Aldehyde-DNPH Standards using Acclaim Carbonyl 3 µm, 3 x 150 mm



HPLC Conditions

Column:	Thermo Scientific Acclaim Carbonyl C18 RSLC 3 µm, 3 x 150mm
Mobile Phases:	A: Acetonitrile B: D.I. Water
Pump:	Thermo Scientific Dionex UltiMate HPG-3400RS

Gradient:

	-3.5	0.0	7.5	11.5	16.0
%A	53	53	53	90	90
%B	47	47	47	10	10

Flow rate:	0.90 mL/min
Pressure:	310 bar max.
Temperature:	28 °C
Injection:	2 µL
Detection:	UV 360 nm, 10 Hz, 0.5 sec.
Sample:	Standards in acetonitrile
Peaks:	1. Formaldehyde DNPH 2a. Furfural DNPH 2b. Furfural DNPH 3. Isobutyraldehyde DNPH