

PRODUCT MANUAL

for

Acclaim® Carbonyl RSLC Columns

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PRODUCT MANUAL

FOR

ACCLAIM® CARBONYL RSLC COLUMNS

Acclaim Carbonyl RSLC, 2.2µm, Analytical Column, 2.1 x 100mm (P/N 077972)
Acclaim Carbonyl RSLC, 2.2µm, Analytical Column, 2.1 x 150mm (P/N 077973)
Acclaim Carbonyl RSLC, 2.2 µm, Analytical Column, 3.0 x 100mm (P/N 077974)

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SECTION 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 has a particular target compound list.

The 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.

The Acclaim Carbonyl RSLC columns are available in three formats. The 2.1 mm i.d. columns are recommended for UHPLC instruments and LC/MS applications. The 100 mm length emphasizes speed and the 150 mm length emphasizes high resolution. The 3.0 mm i.d. column is recommended for systems than need higher flow rates or larger injection volumes.

Acclaim Carbonyl Analytical Columns		
Particle Size	Dimensions	Part Number
2.2 µm	2.1 x 100 mm	077972
	2.1 x 150 mm	077973
	3.0 x 100 mm	077974

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SECTION 2 – INSTALLATION

2.1. Preparation of the Mobile Phase

The recommended mobile phases consist of de-ionized water and acetonitrile. For certain applications, methanol can also be used instead of acetonitrile. While "pure" de-ionized water may be used, a low concentration of volatile buffer (e.g. 1 to 5 mM ammonium acetate) is recommended for consistent results and good MS-compatibility. "HPLC grade" solvents and reagents have low UV absorbance and filtered for low particulate count. 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.



NOTE

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

2.1.1. Ammonium acetate buffer

1. Measure 57 ± 3 µL of glacial acetic acid and 144 ± 8 mg of ammonium acetate into 1000 ± 50 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 'idle time', 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 at recommended flow rate for that column dimension, 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 on pages 10 – 14.



NOTE

Mobile phase composition and oven temperature are the two main factors influencing the 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 need to adjust the times for the start and end of the gradient. You may also need 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	% Buffer
2.2 µm, 2.1 x 150 mm Low gradient delay UHPLC; optimized for resolution (Fig. 4)	0.40	28	25	1	-4.5 0.0 8.3 15.0 18.0	52 52 52 100 100	48 48 48 0 0
2.2 µm, 2.1 x 100 mm Low gradient delay UHPLC; optimized for speed (Fig. 1 & 3)	0.75	28	25	1	-1.7 0.0 2.9 5.3 6.2	52 52 52 100 100	48 48 48 0 0
2.2 µm, 3.0 x 100 mm Quaternary HPLC (Fig. 5)	1.00	28	25	2	-3.0 0.0 4.4 8.0 9.8	52 52 52 100 100	48 48 48 0 0

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2.5.2. Standard Methanol conditions

These gradients are satisfactory for EPA 554. Depending on the gradient delay volume of your system, you may need to adjust the times for the start and end of the gradient. You may also need 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	% Buffer
2.2 µm, 2.1 x 150 mm Low gradient delay UHPLC	0.50	42	25	1	-2.5 0.0 5.1 83 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.

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SECTION 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 column. 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)
2.2µm	2.1 x 150mm	800	1.0	0.25 – 0.75	2.5 – 7.5	25 – 35	50
	2.1 x 100mm	700	1.0	0.25 – 0.75	2.5 – 7.5	25 – 35	50
	3.0 x 100mm	600	1.6	0.40 – 1.40	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 2-8). 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 and on most systems. For some special applications, a lower temperature (20 ºC) or a higher temperature (40 ºC) might be needed. 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 2.1-mm i.d. column, gradually increase the flow rate from 0 mL/min up to the desired flow rate in 0.1 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 pre- column filter 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 are for the 2.1 mm i.d. columns; double the flow rate for the 3.0 mm i.d. column.

1. Equilibrate the column with acetonitrile/water v/v 50/50 for 10 column volumes at 0.1 to 0.2 mL/min.
2. Then, wash the column with pure acetonitrile, or acetone for 20 column volumes at 0.1 to 0.2 mL/min.
3. Finally, wash the column with acetonitrile/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.

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SECTION 4 – FREQUENTLY ASKED QUESTIONS

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 2.2µm particle size is used in newer UHPLC systems that operate at higher pressure, and provide high-speed analysis. The 2.1mm 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. The 3.0 mm size is recommended for systems that need to operate with higher flow rates or larger sample injection volumes.

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 the 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.

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6. Can I use D.I. water instead of buffer for the analysis?

Yes. The selectivity changes very little, but the retention time stability is somewhat better with the buffered mobile phase.

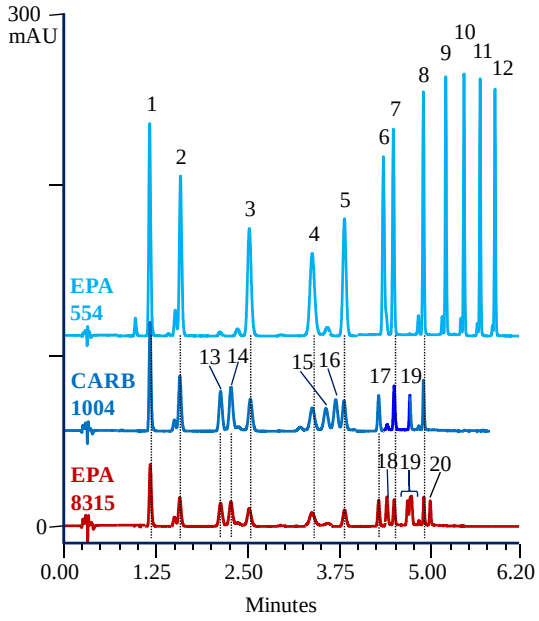
7. When should I use methanol instead of acetonitrile?

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.

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SECTION 5 – EXAMPLE APPLICATIONS

Figure 1. Rapid DNPH Aldehyde and Ketone Standards using Acclaim Carbonyl RSLC 2.2µm, 2.1 x 100mm, Column with Acetonitrile Mobile Phase



Column:	Thermo Scientific Dionex Acclaim® Carbonyl RSLC, 2.2 µm
Dimension:	2.1 x 100 mm
HPLC System:	Thermo Scientific Dionex UltiMate® 3000-RS HPG
Mobile Phases:	A: 1 mM acetic acid + 2mM ammonium acetate 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

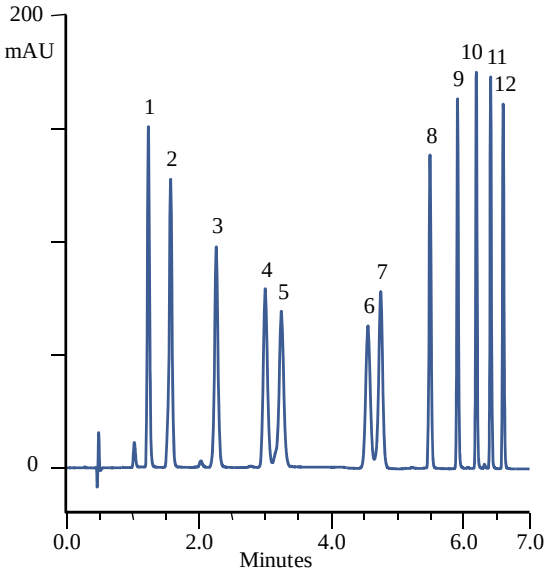
Flow rate:	0.750 mL/min
Injection:	1 µL; bypass at 0.25 min.
Temperature:	28 °C
Detection:	UV at 360 nm, 25 Hz, 0.2 s resp. time
Samples:	Calibration mixes diluted in methanol

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

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Figure 2. EPA 554 Carbonyl-DNPH Standards using Acclaim Carbonyl RSLC 2.2µm, 2.1 x 100mm, Column with Methanol Mobile Phase

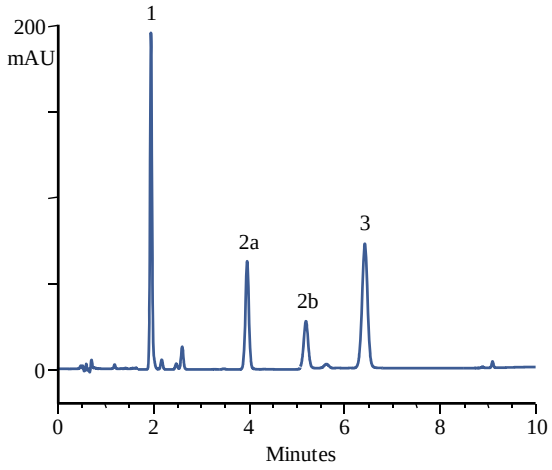


Column:	Thermo Scientific Dionex Acclaim® Carbonyl RSLC				
Dimension:	2.2 µm, 2.1 x 100 mm				
HPLC System:	Thermo Scientific Dionex UltiMate® 3000-RS HPG				
Mobile Phases:	A: 1 mM acetic acid + 2mM ammonium acetate B: Methanol				
Gradient times (min):	-1.7	0.0	3.4	5.5	7.0
%A	30	30	30	0	0
%B	70	70	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

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Figure 3. EPA 1667 DNPH Aldehyde Standards using Acclaim Carbonyl RSLC 2.2µm, 2.1 x 100mm, Column with Acetonitrile Mobile Phase



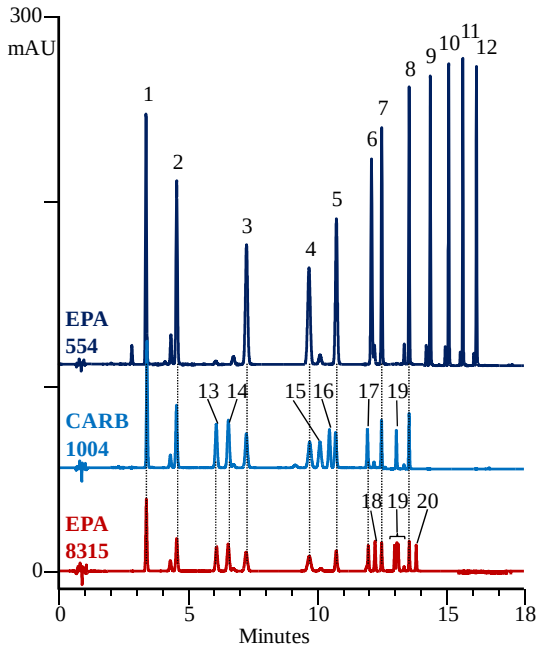
Column:	Thermo Scientific Dionex Acclaim® Carbonyl RSLC, 2.2 µm
Dimension:	2.1 x 100 mm
HPLC System:	Thermo Scientific Dionex UltiMate® 3000-RS HPG
Mobile Phases:	A: 1 mM acetic acid + 2mM ammonium acetate B: Acetonitrile

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

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Figure 4. High-Resolution DNPH Aldehyde and Ketone Standards using Acclaim Carbonyl RSLC 2.2µm, 2.1 x 150mm, Column with Acetonitrile Mobile Phase



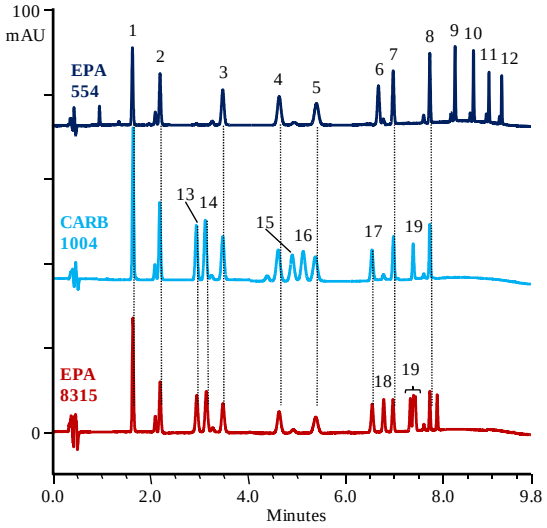
Column:	Thermo Scientific Dionex Acclaim® Carbonyl RSLC, 2.2 µm				
Dimension:	2.1 x 150 mm				
HPLC System:	Thermo Scientific Dionex UltiMate® 3000-RS HPG				
Mobile Phases:	A: 1 mM acetic acid + 2mM ammonium acetate B: Acetonitrile				
Gradient times (min):	-4.5	0.0	8.3	15.0	18.0
%A	48	48	48	0	0
%B	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	11. Nonanal DNPH
2. Acetaldehyde DNPH	12. Decanal DNPH
3. Propionaldehyde DNPH	13. Acetone DNPH
4. Crotonaldehyde DNPH	14. Acrolein DNPH
5. Butyraldehyde DNPH	15. Butanone DNPH
6. Cyclohexanone DNPH	16. Methacrolein DNPH
7. Valeraldehyde DNPH	17. Benzaldehyde DNPH
8. Hexanal DNPH	18. Isovaleraldehyde DNPH
9. Heptanal DNPH	19. Tolualdehyde DNPH
10. Octanal DNPH	20. Xylylaldehyde DNPH

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Figure 5. DNPH Aldehyde and Ketone Standards using Acclaim Carbonyl RSLC 2.2µm, 3.0 x 100mm, Column with Acetonitrile Mobile Phase



Column:	Thermo Scientific Dionex Acclaim® Carbonyl RSLC, 2.2 µm				
Dimension:	3.0 x 100 mm				
HPLC System:	Thermo Scientific Dionex UltiMate® 3000-RS Quaternary				
Mobile Phases:	A: 1 mM acetic acid + 2mM ammonium acetate 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

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