PRODUCT MANUAL

for

Acclaim® Explosives Columns

DIONEX

IC | HPLC | MS | EXTRACTION | PROCESS | AUTOMATION

PRODUCT MANUAL

FOR

ACCLAIM® EXPLOSIVES COLUMNS

Acclaim Explosives E1, 5 µm, Analytical Column, 4.6 x 250 mm (P/N 064305)
Acclaim Explosives E2, 5 µm, Analytical Column, 4.6 x 250 mm (P/N 064309)
Acclaim Explosives E2, 3µm, Analytical Column, 3.0 x 250mm (P/N 070081)
Acclaim Explosives E2, 3µm, Analytical Column, 3.0 x 150mm (P/N 070082)
Acclaim Explosives E2, 3µm, Analytical Column, 2.1 x 150mm (P/N 070083)
Acclaim Explosives E2 RSLC, 2.2µm, Analytical Column, 2.1 x 100mm (P/N 076225)
Acclaim Explosives E2 RSLC, 2.2µm, Analytical Column, 2.1 x 150mm (P/N 076226)
Acclaim Explosives E2 RSLC, 2.2µm, Analytical Column, 3.0 x 100mm (P/N 076227)

Acclaim Explosives E1 Guard Column, 4.3 x 10 mm (P/N 064303)
Acclaim Explosives E2 Guard Column, 4.3 x 10 mm (P/N 064307)
Acclaim Explosives E1, 5µm, Guard Column, 4.6 x 10mm (P/N 069702)
Acclaim Explosives E2, 5µm, Guard Column, 4.6 x 10mm (P/N 069703)
Acclaim Explosives E2, 5µm, Guard Column, 3.0 x 10mm (P/N 071989)

Acclaim Explosives Kit (E1 and E2 5 µm, 4.6 x 250 mm Analytical Columns and Guards) (P/N 064312)

Thermo Fisher Scientific, 2011
Document No. 025205
Revision 03
July 2011

TABLE OF CONTENTS

SECTION 1 – INTRODUCTION

The Acclaim® Explosives (E1 and E2) columns are silica-based reversed phase columns designed specifically for separating the 14 explosives compounds listed in EPA SW-846 Method 8330. Both the Acclaim E1 and E2 columns exhibit superior resolution compared with other commercially available columns, with selectivity that is complementary to each other. The selectivity of the Acclaim E1 column resembles that offered by reversed phase C18 columns, with the advantage of baseline separation of all 14 compounds. Thus it is an excellent choice for the EPA Method 8330 primary analysis. The Acclaim E2 column also provides baseline separation of the 14 explosives listed in EPA 8330, but with selectivity that is complementary to the Acclaim E1 column. This makes the Acclaim E2 an excellent alternative column for the EPA Method 8330 confirmatory analysis.

The unique selectivity of the Acclaim E2 column not only provides baseline resolution of all 14 compounds targeted by EPA Method 8330, but also allows for simultaneous separation of some nitrate esters explosives of great importance (e.g. PETN and EGDN).

The Acclaim E2 column is available in three particle sizes, 5µm, 3µm, and 2.2 µm. The 3 µm, 3 x 150 mm E2 column allows for a threefold acceleration of EPA Method 8330A. The 3 µm 3 x 250 mm format provides a significant increase in resolution for EPA Method 8330A. The 2.1 x 150mm column is designed for high-throughput LC/MS applications. The new 2.2 µm RSLC formats provide the highest throughput, LC/MS compatibility and reduced solvent consumption.

Acclaim Explosives Analytical Columns			
Stationary Phase	Particle Size	Dimensions	Part Number
E1	5 µm	4.6 x 250 mm	064305
E2	5 µm	4.6 x 250 mm	064309
	3 µm	3.0 x 250 mm	070081
		3.0 x 150 mm	070082
		2.1 x 150 mm	070083
	2.2 µm	2.1 x 100 mm	076225
		2.1 x 150 mm	076226
		3.0 x 100 mm	076227

NOTE

The Acclaim guard columns come in two series, the original and the new V2. The holders and cartridges are not interchangeable between series. The V2 series have more dimensions available, higher performance and better reliability.

Acclaim Explosives Guard Columns				
	Old Guard Holder		New V2 Guard Holder	
Stationary Phase	Dimensions	Part Number	Dimensions	Part Number
E1, 5 µm	4.3 x 10 mm	064303	4.6 x 10 mm	069702
E2, 5 µm	4.3 x 10 mm	064307	4.6 x 10 mm	069703
			3.0 x 10 mm	071989
	Holder, old	059456	Holder, V2	069580

SECTION 2 – INSTALLATION

2.1. Preparation of the Mobile Phase

The mobile phase for both the Acclaim E1 and the Acclaim E2 columns consists of methanol and D.I. water, as recommended in EPA 8330. Both pre-mixed and proportioning valve generated mobile phases will give satisfactory results. The use of a proportioning valve provides better flexibility in method optimization, but the pre-mixed mobile phase provides more reproducible results.

NOTE

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

2.1.1. Pre-mixed Mobile Phase

1. Acclaim Explosives E1: Mix methanol (340 g or 430 mL) and D.I. water (570 g or 570 mL)
2. Acclaim Explosives E2: Mix methanol (379 g or 480 mL) and D.I. water (520 g or 520 mL)

2.1.2. Mobile Phase Generated In-situ by a Proportioning Valve

1. Acclaim Explosives E1: Proportion methanol (43%) with D.I. water (57%)
2. Acclaim Explosives E2: Proportion methanol (48%) with D.I. water (52%)

2.2. HPLC System Set-up

The Acclaim Explosives 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 explosives 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

Dionex 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, Dionex recommends that you first confirm the performance of the column by reproducing the separation of the EPA Method 8330 explosives mix (14 explosives) shown in the lot validation report chromatogram shipped with the column. Run the EPA 8330 mix (14 components) under the same conditions specified in the report. 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

NOTE

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

2.5.1. Acclaim Explosives E1

1. Prepare your mobile phase as recommended in Section 2.1. With the oven temperature set to 31 ºC, you should obtain satisfactory separation for 14 explosives compounds in EPA 8330.
2. If further improvement is required, gradually adjust the mobile phase composition. You should find an optimal separation in the range of 40/60 to 45/55 (methanol/water v/v).
3. If further improvement is required, start to adjust the oven temperature, while keeping your mobile phase composition as optimized in the previous step. If your oven is calibrated, you will find an optimal temperature in the 28 to 35 ºC range. See figures 1 and 2 for reference.

2.5.2. Acclaim Explosives E2

1. Prepare your mobile phase composition as recommended in Section 2.1. With the oven temperature set to 30 ºC, you should obtain satisfactory separation for 14 explosives compounds in EPA 8330.
2. If further improvement is required, adjust the oven temperature in the range 25 to 32 ºC, until your optimal resolution is achieved.
3. If further improvement is required, start to adjust mobile phase composition, while keeping the temperature constant and at the temperature set in the previous step. You should find optimum resolution in the range of 45/55 to 52/48 (methanol/water v/v).
4. If further improvement is still needed, repeat “2” and “3.” See figures 3 and 4 for reference.

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.

SECTION 3 – COLUMN CARE

3.1. General Guidelines

The Acclaim Explosives Columns are silica-based reversed phase columns designed for separating the 14 explosives listed in U.S. EPA method 8330. In some cases, depending upon the compounds in the sample, the column can be used for other explosives applications, including some additional nitramines and nitrate esters. 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

Column	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)
Acclaim E1	5µm	4.6 x 250mm	400	2.0	0.8 – 1.5	3.5 – 7.0	25 – 35	50
Acclaim E2	5µm	4.6 x 250mm	400	2.0	0.8 – 1.5	2.5 – 7.5	25 – 35	50
	3µm	3.0 x 250mm	800	1.0	0.4 – 0.6	2.5 – 7.5	25 – 35	50
		3.0 x 150mm	600	1.3	0.4 – 0.8	2.5 – 7.5	25 – 35	50
		2.1 x 150mm	600	0.8	0.2 – 0.5	2.5 – 7.5	25 – 35	50
	2.2µm	2.1 x 150mm	800	1.0	0.25 – 0.7	2.5 – 7.5	25 – 35	50
		2.1 x 100mm	700	1.0	0.25 – 0.7	2.5 – 7.5	25 – 35	50
		3.0 x 100mm	600	1.6	0.4 – 1.4	2.5 – 7.5	25 – 35	50

3.2. Recommended Operating pH Range

The pH of the mobile phase has little or no effect on retention times or selectivity on the Acclaim Explosives 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. 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 explosives using the Acclaim Explosives columns is highly 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 Explosives 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 (for a 4.6-mm i.d. column)

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:

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.

For assistance, contact Technical Support for Dionex products. In the U.S., call 1-800-346-6390. Outside the U.S., call the nearest Thermo Fisher Scientific office.

SECTION 4 – FREQUENTLY ASKED QUESTIONS

1. How do the Acclaim Explosives columns compare with other columns for EPA 8330?

The Acclaim Explosives columns are the only commercially available columns to provide "true" baseline separation for all 14 explosives listed in EPA 8330 under the conditions recommended by the EPA, and on a standard HPLC system. Baseline separation allows for fast calibration and more accurate results. All other currently used columns provide less than satisfactory separation for EPA 8330.

2. Which Acclaim Explosives column should I use?

Should I use E1 or E2?
You cannot make a mistake when it comes to Acclaim Explosives columns. Both the Acclaim E1 and E2 columns provide baseline separation for all 14 explosives listed in EPA 8330. They have complementary selectivity which is required by the current EPA method. If no confirmation column is required, either column is good to use. When resolution of RDX and 1,3,5-TNB is crucial, the Acclaim E1 column is ideal. When resolution between 4-A-2,6-DNT, 2-A-4,6-DNT, 2,6-DNT and 2,4-DNT is important, the Acclaim E2 column is the best choice.

3. Which particle size / format should I use?

The 5µm particle size is used for standard applications when methods are being developed or if the sample is complex. The 3µm particle size is used for faster, higher efficiency separations. The 2.2µm particle size is used in newer UHPLC systems that operate at higher pressure, and provide high-speed analysis. The 250mm long columns are optimum for highest resolution and the 150mm long columns are for high speed separations. 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.

4. 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) Run the column performance test described in the Quality Assurance Report (QAR). Replace the column if needed.

5. 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.

6. 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.

7. How do I improve the resolution between 2-A-4,6-DNT and 2,6-DNT on the E1 column?

Increase the column temperature by 1 to 3 ºC. See Figure 1 for reference.

8. How do I improve the resolution between 2,4-DNT and 2,6-DNT on the E1 column?

Decrease the column temperature by 1 to 3 ºC. See Figure 1 for reference.

9. How do I improve the resolution between RDX and 1,3,5-TNB on the E2 column?

a) Increase the column temperature by 1 to 3 ºC. See Figure 3 for reference.
b) Or/and increase mobile phase organic content. See Figure 4 for reference.

10. How do I improve the resolution between TNT and Tetryl on the E2 column?

a) Decrease the column temperature by 1 to 3 ºC. See Figure 3 for reference.
b) Or/and decrease mobile phase organic content. See Figure 4 for reference.

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

Yes. We found that pH 4.0 – 5.5 acetate buffer/methanol systems provide equally good result with minor modifications.

12. Can I use a higher flow rate than 1 mL/min?

Yes, provided that the column backpressure is maintained below the maximum recommended pressure as shown in Section 3.1.1.

SECTION 5 – EXAMPLE APPLICATIONS

Figure 1

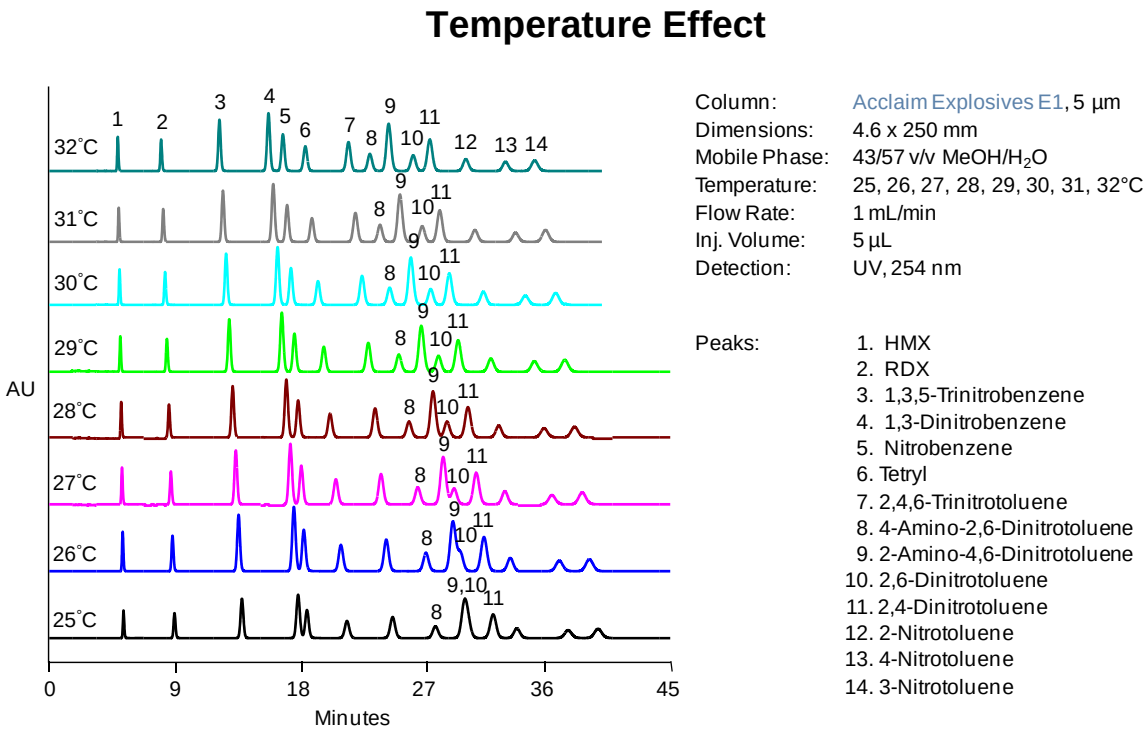
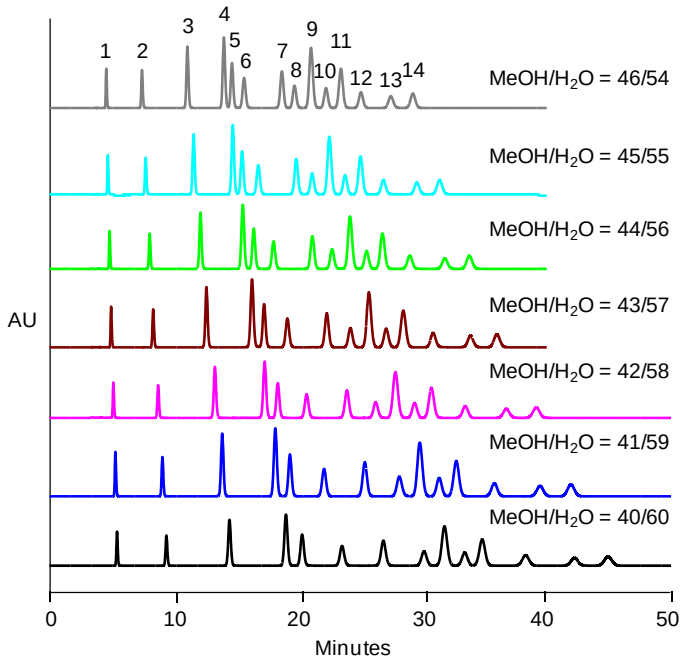


Figure 2

Mobile Phase Organic Content



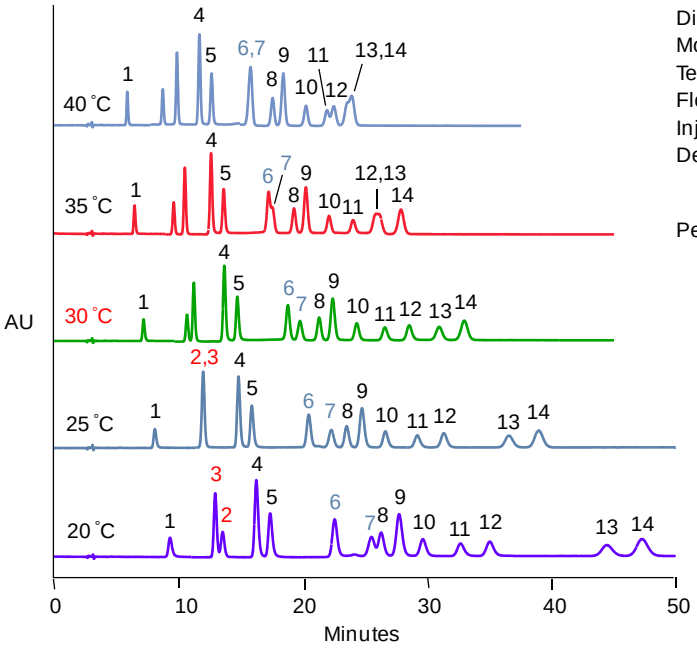
Column:	Acclaim Explosives E1, 5 µm
Dimensions:	4.6 x 250 mm
Mobile Phase:	$MeOH/H_2O$
Temperature:	30 °C
Flow Rate:	1 mL/min
Inj. Volume:	5 µL
Detection:	UV, 254 nm

Peaks:

1. HMX
2. RDX
3. 1,3,5-Trinitrobenzene
4. 1,3-Dinitrobenzene
5. Nitrobenzene
6. Tetryl
7. 2,4,6-Trinitrotoluene
8. 4-Amino-2,6-Dinitrotoluene
9. 2-Amino-4,6-Dinitrotoluene
10. 2,6-Dinitrotoluene
11. 2,4-Dinitrotoluene
12. 2-Nitrotoluene
13. 4-Nitrotoluene
14. 3-Nitrotoluene

Figure 3

Temperature Effect



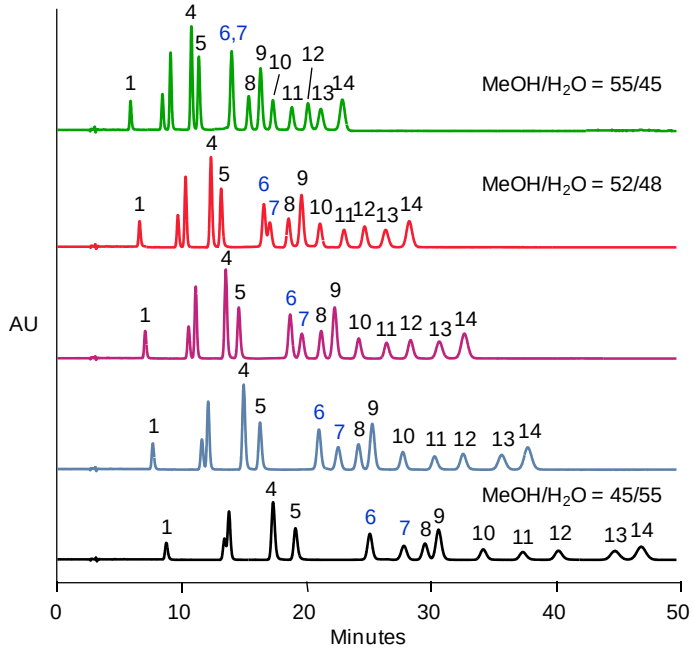
Column:	Acclaim® Explosives E2, 5 µm
Dimensions:	4.6 x 250 mm
Mobile Phase:	48/52 v/v MeOH/H_2O
Temperature:	20, 25, 30, 35, and 40 °C
Flow Rate:	1 mL/min
Inj. Volume:	5 µL
Detection:	UV, 254 nm

Peaks: (EPA 8330 mix, 50 ppm each)

1. HMX
2. RDX
3. 1,3,5-Trinitrobenzene
4. 1,3-Dinitrobenzene
5. Nitrobenzene
6. 2,4,6-Trinitrotoluene
7. Tetryl
8. 2,6-Dinitrotoluene
9. 2,4-Dinitrotoluene
10. 2-Nitrotoluene
11. 4-Nitrotoluene
12. 3-Nitrotoluene
13. 4-Amino-2,6-Dinitrotoluene
14. 2-Amino-4,6-Dinitrotoluene

Figure 4

Mobile Phase Organic Content

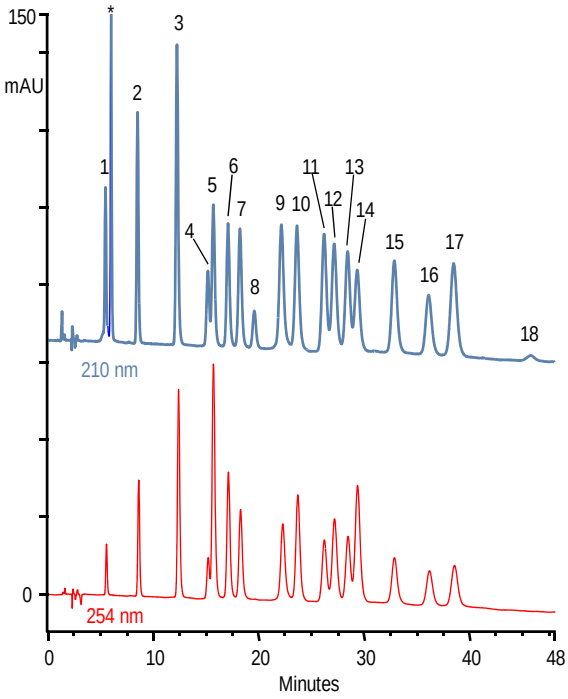


Column:	Acclaim® Explosives E2, 5 µm
Dimensions:	4.6 x 250 mm
Mobile Phase:	MeOH/H_2O
Temperature:	30 °C
Flow Rate:	1 mL/min
Inj. Volume:	5 µL
Detection:	UV, 254 nm
Peaks:	(EPA 8330 mix, 50 ppm each)

1. HMX
2. RDX
3. 1,3,5-Trinitrobenzene
4. 1,3-Dinitrobenzene
5. Nitrobenzene
6. 2,4,6-Trinitrotoluene
7. Tetryl
8. 2,6-Dinitrotoluene
9. 2,4-Dinitrotoluene
10. 2-Nitrotoluene
11. 4-Nitrotoluene
12. 3-Nitrotoluene
13. 4-Amino-2,6-Dinitrotoluene
14. 2-Amino-4,6-Dinitrotoluene

Figure 5

EPA8330B Standards on Acclaim Explosives E1



HPLC Conditions

Column:	Acclaim Explosives E1, 5 µm 4.6x250 mm
Pump:	Summit P680A DGP
Isocratic:	Acetonitrile:methanol:water 3:33:64 v:v:v
Flowrate:	1.25 mL/min
Injection:	ASI-100 sampler, 15 µL
Temperature:	36 °C; resolution may be fine-tuned by temperature adjustments.
Detection:	UVD-340U, UV 254 & 210 nm, spectrum scan 200-600 nm

Peaks

1. HMX
2. RDX
3. 1,3,5-TNB
4. 1,2-DNB (i.s.)
5. 1,3-DNB
6. NB
7. 3,5-DNA
8. Nitroglycerine
9. Tetryl
10. 2,4-TNT
11. 2,6-DNT
12. 2,4-DNT
13. 4-A-2,6-DNT
14. 2-A-4,6-DNT
15. 2-NT
16. 4-NT
17. 3-NT
18. PETN

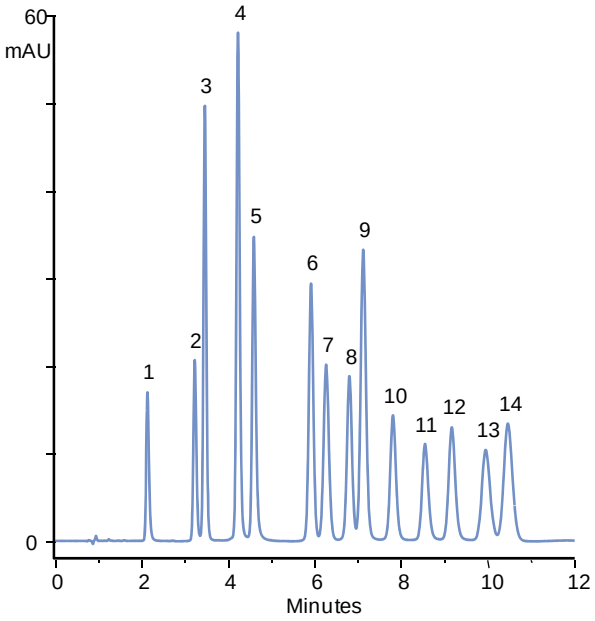
*. solvent artifact

Samples 300 µg/mL in acetonitrile except Nitroglycerine and PETN, 100 µg/mL

HPLC conditions graciously provided by Keith Tanguay of Katahdin Analytical Services, Scarborough ME.

Figure 6

Acceleration of EPA Method 8330A using Acclaim Explosives E2 Column



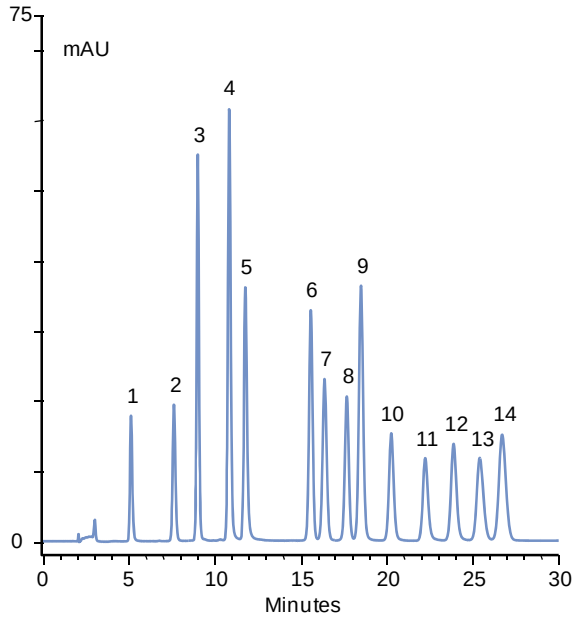
Standards in
50% MeCN: 50% NH4OAc buffer
50 µg/mL

Column:	**Acclaim Explosives E2**
Dimensions:	3 µm, 3.0 x 150 mm
Mobile Phase:	49/51 v/v MeOH/H_2O
Temperature:	26 ºC
Flow Rate:	0.90 mL/min (520 bar)
Inj. Volume:	2 µL
Detection:	UV, 254 nm

Peak	Compound	Resolution	Asymm.	Plates
1	HMX	9.01	1.32	5343
2	RDX	1.88	1.15	10278
3	1,3,5-TNB	5.93	1.09	13117
4	1,3-DNB	2.51	1.11	14765
5	NB	7.63	1.21	14419
6	2,4,6-TNT	1.65	1.06	14834
7	Tetryl	2.41	1.05	11943
8	2,6-DNT	1.44	n.a.	16130
9	2,4-DNT	2.91	1.05	16017
10	2-NT	2.91	1.09	16536
11	4-NT	2.26	1.15	16571
12	3-NT	2.43	1.10	16575
13	2-A-4,6-DNT	1.40	n.a.	12062
14	4-A-2,6-DNT	n.a.	n.a.	12325

Figure 7

High Resolution for EPA Method 8330A with Acclaim Explosives E2 Column



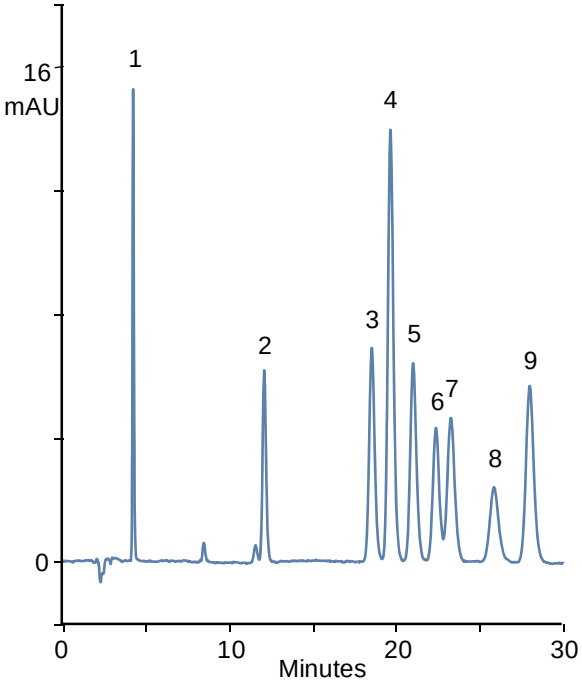
Standards in
50% MeCN: 50% NH4OAc buffer
50 µg/mL

Column:	**Acclaim Explosives E2**
Dimensions:	3 µm, 3.0 x 250 mm
Mobile Phase:	49/51 v/v MeOH/H_2O
Temperature:	27 ºC
Flow Rate:	0.54 mL/min (470 bar)
Inj. Volume:	2.6 µL
Detection:	UV, 254 nm

Peak	Compound	Resolution	Asymm.	Plates
1	HMX	9.10	1.19	6784
2	RDX	5.16	1.18	10368
3	1,3,5-TNB	6.77	1.07	22034
4	1,3-DNB	3.02	1.17	21816
5	NB	10.72	1.28	21071
6	2,4,6-TNT	1.94	1.04	26203
7	Tetryl	2.97	1.05	21901
8	2,6-DNT	1.87	1.02	26587
9	2,4-DNT	3.69	1.04	26039
10	2-NT	3.77	1.08	26204
11	4-NT	2.92	1.20	25990
12	3-NT	2.36	1.12	26290
13	2-A-4,6-DNT	1.76	1.12	20427
14	4-A-2,6-DNT	n.a.	1.09	20455

Figure 8

Gun Surveillance Standards on Acclaim Explosives E2



HPLC Conditions

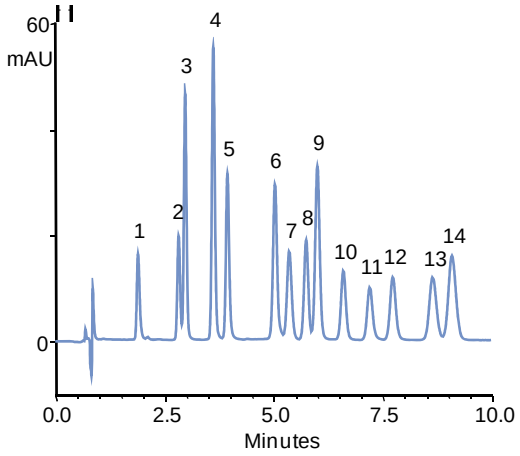
Column:	Acclaim Explosives E2, 5 µm 4.6x250 mm
Pump:	Summit P680A DGP
Mobile Phase:	37% 2mM NH4OAc, pH 5 58% methanol 5% acetonitrile
Flowrate:	1.25 mL/min
Injection:	ASI-100 sampler, 30 µL sample in 50% acetonitrile
Detection:	UVD 340U, UV 254 nm and spectra 200-595 nm

Peaks		
	1. Dimethylphthalate	20 µg/mL
	2. N-Nitrosodiphenylamine	5
	3. 2,2'-Dinitrodiphenylamine	5
	4. Diphenylamine	20
	5. 2,4'-Dinitrodiphenylamine	5
	6. 2,4-Dinitrodiphenylamine	5
	7. 4-Nitrodiphenylamine	5
	8. 4,4'-Dinitrodiphenylamine	5
	9. 2-Nitrodiphenylamine	5

Note: Resolution may be fine-tuned by temperature adjustment

Figure 9

Rapid EPA 8330A Explosives on Acclaim E2 RSLC



Column:	Acclaim® Explosives E2 RSLC, 2.2 µm
Dimension:	2.1 x 100 mm
HPLC System:	UltiMate® 3000-RS HPG
Mobile Phases:	Methanol:Water 48:52 (v/v)
Flow rate:	0.34 mL/min (293 bar)
Injection:	1 µL
Temperature:	31 °C
Detection:	UV at 254nm, 10 Hz, 0.4 s resp. time
Sample:	Calibration mix, 25 µg/mL in 50% acetonitrile
Peaks:	1. HMX 2. RDX 3. 1,3,5-TNB 4. 3,5-DNB 5. NB 6. 2,4,6-TNT 7. Tetryl 8. 2,6-DNT 9. 2,4-DNT 10. 2-NT 11. 4-NT 12. 3-NT 13. 4-Am-2,6-DNT 14. 2-Am-4,6-DNT

