

# A Total Solution to Baseline Separation of 14 Explosives in U.S. EPA Method 8330

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Environmental concerns regarding explosives stem from the mutagenic, carcinogenic, and toxic effects of these nitroaromatic, aminoaromatic, and nitramine compounds, including associated impurities/metabolites, as well as their persistence in the environment.<sup>1</sup> For decades, most expired munitions have been disposed of via direct combustion, which does not quantitatively destroy toxic constituents. As a result, a significant amount of disposed-of explosives contaminate soil and groundwater, requiring thorough characterization of contaminated areas for residual explosives. In addition, explosives analysis is important for the forensic analysis of postexplosion residues and monitoring regulated compounds in munitions wastewater.

A variety of chromatographic techniques have been applied to separate and detect explosives compounds, including GC, thin layer chromatography (TLC), supercritical fluid chromatography (SFC), capillary electrochromatography

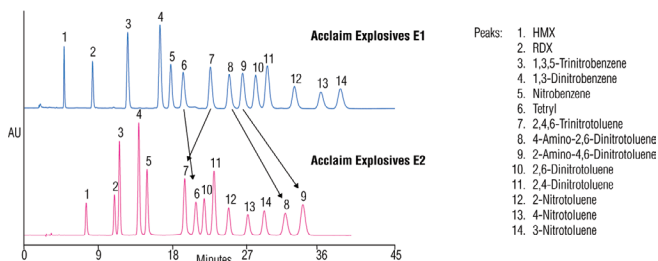


Figure 2 Separation of explosives in U.S. EPA Method 8330 on Acclaim Explosives E1 and E2 columns.

(CEC), and HPLC.<sup>2-7</sup> Among these, HPLC is the preferred method for routine analysis because of its ease of use, high reliability, and ability to detect nitroaromatic and nitramine compounds with good sensitivity (limit of detection [LOD] ~ppb). To facilitate high throughput of large numbers of samples for routine analysis, a simple isocratic HPLC method is often desired. Several HPLC methods for explosives analysis have been standardized as U.S. EPA Method 8330, AOAC International (formerly the Association of Official Analytical Chemists) Official Method 986.221, and American Society for Testing & Materials (ASTM) Method D5143-90. U.S. EPA Method 8330 is the most comprehensive and widely used HPLC method for environmental monitoring.<sup>8</sup> The method is intended for the trace analysis of a collection of 14 explosives residues in water, soil, and sediment matrix. It recommends methanol-water as a mobile phase, a C18 phase as the primary column, and a CN phase as the confirmatory column. The 14 compounds regulated by U.S. EPA Method 8330 are listed in Figure 1.

The main difficulty with U.S. EPA Method 8330 is the coelution of dinitrotoluene (DNT) and amino-dinitrotoluene (AmDNT) isomers on the primary C18 column. Consequently, an additional HPLC run must be performed on a cyano (CN) column, reducing throughput. While several gradient methods were developed to separate DNT and AmDNT isomers that coelute using an isocratic method, the complexity of gradients, long run time, or aggressive chromatographic conditions make these methods undesirable for large-volume analyses. Lang and Burns reported a two-phase approach using a CN guard column in series with a C18 column, in which all 14 explosives listed in U.S. EPA Method 8330 were separated in a single isocratic run

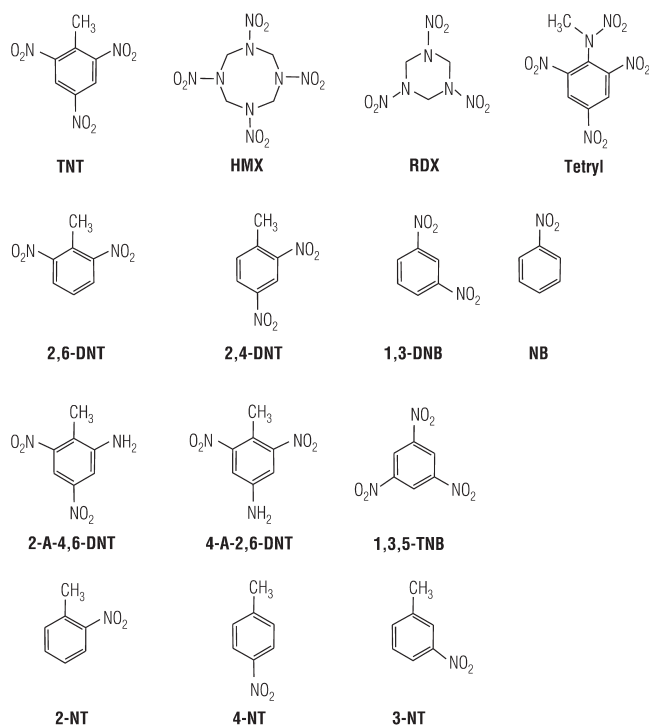


Figure 1 The structures of 14 explosives listed in U.S. EPA Method 8330.

Column: Acclaim Explosives E1  
 5  $\mu$ m, 4.6 x 250 mm  
 Mobile Phase: 43/57 v/v MeOH/H<sub>2</sub>O  
 Temperature: 30 °C  
 Flow Rate: 1 mL/min  
 Inj. Volume: 5  $\mu$ L  
 Detection: UV, 254 nm

Peaks: 1. HMX  
 2. RDX  
 3. 1,3,5-Trinitrobenzene  
 4. 1,2-Dinitrobenzene (I.S.)  
 5. 1,3-Dinitrobenzene  
 6. Nitrobenzene  
 7. Tetryl  
 8. 2,4,6-Trinitrotoluene  
 9. 4-Amino-2,6-Dinitrotoluene  
 10. 2-Amino-4,6-Dinitrotoluene  
 11. 2,6-Dinitrotoluene  
 12. 2,4-Dinitrotoluene  
 13. 2-Nitrotoluene  
 14. 4-Nitrotoluene  
 15. 3-Nitrotoluene

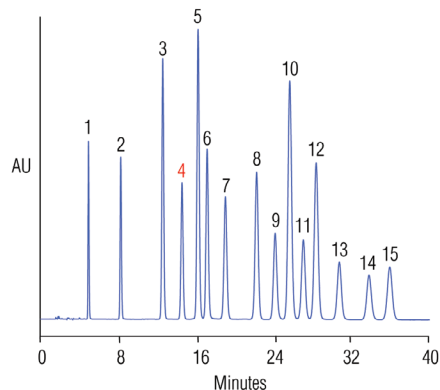


Figure 3 Separation of explosives in U.S. EPA Method 8330 on the Acclaim Explosives E1 column.

using recommended HPLC conditions.<sup>2</sup> Since two different stationary phases are involved, it is not unreasonable to be concerned with issues such as long-term chromatographic performance and column-to-column reproducibility.

To meet the above challenges, a pair of HPLC columns was developed to separate all 14 priority explosives-related compounds in U.S. EPA Method 8330 using a methanol–water mobile phase system, an isocratic method, and a standard HPLC instrument. Both columns can baseline

resolve all 14 explosives, but with complementary selectivities (Figure 2). Thus, the combination of the two columns provides a complete solution for explosives analysis.

## Column features

Acclaim Explosives E1 and E2 columns are a family of high-efficiency silica-based specialty columns designed for baseline separation of all 14 explosives listed in U.S. EPA Method 8330, using methanol–water mobile phases and isocratic methods, in a single chromatographic run. The main features include:

- Baseline resolution of all 14 explosives listed in U.S. EPA Method 8330 on both columns, with complementary selectivities
- The Acclaim Explosives E1 column as the direct replacement of the existing primary (C18) columns
- The Acclaim Explosives E2 column as an alternative or confirmatory column
- High column efficiency, ease of use, and suitability for routine analysis.

1. *Acclaim Explosives E1 column.* The analysis of explosives residues under U.S. EPA Method 8330 calls for C18 reversed-phase columns as the primary column. Despite the fact that many C18 columns are available and being used for explosives analysis, few are capable of baseline resolving all 14 explosives under the conditions recommended in U.S. EPA Method 8330. The Acclaim Explosives E1 column is the solution to this ongoing problem, and provides chromatographers with an effective, simple, and direct replacement for conventional C18 columns. Figure 3 shows the baseline separation

**Table 1 Chromatographic results of explosives analysis on the Acclaim Explosives E1 column**

Explosive	K'	Resolution	R <sup>2</sup> (peak area)	LOD ( $\mu$ g/L)
HMX (cyclotetramethylenetetranitramine)	1.02	12.18	0.9999	0.6
RDX (cyclotrimethylenetrinitramine)	2.37	14.61	1	0.2
1,3,5-TNB (trinitrobenzene)	4.13	5.43	1	0.9
1,3-DNB (dinitrobenzene)	5.61	2.15	1	0.8
NB (nitrobenzene)	5.99	3.96	1	1.3
Tetryl	6.79	3.44	1	2.0
2,4,6-TNT (trinitrotoluene)	8.08	3.13	1	1.5
4-A-2,6-DNT	8.87	2.23	1	2.6
2-A-4,6-DNT	9.48	2.02	1	1.7
2,6-DNT	10.05	1.82	0.9998	2.7
2,4-DNT	10.59	3.15	1	1.4
2-NT (nitrotoluene)	11.57	3.67	1	3.6
4-NT	12.81	2.38	0.9999	4.6
3-NT	13.60	—	0.9999	3.8

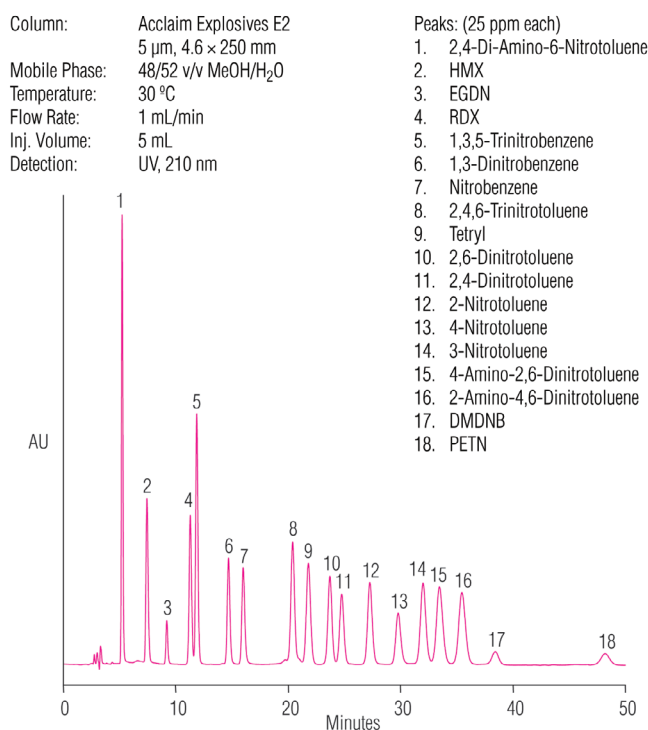


Figure 4 Separation of explosives in U.S. EPA Method 8330 on the Acclaim Explosives E2 column.

tion of an internal standard (1,2-DNB) and 14 target compounds listed in U.S. EPA Method 8330 on an Acclaim Explosives E1 column. In addition, the column provides high efficiency, symmetrical peak shapes, and good linearity for all compounds in U.S. EPA Method 8330. Table 1 lists typical retention, resolutions, correlation coefficients ( $R^2$ ), and estimated LOD. The elution order of these 14 analytes on the column was observed to be similar to that on a conventional C18 column. Thus, the column can be used as an effective replacement for current primary columns. Since both DNT and AmDNT isomers are baseline resolved, the confirmatory column can potentially be eliminated.

2. *Acclaim Explosives E2 column.* Since C18 reversed-phase columns are incapable of providing baseline resolution of the 14 target compounds listed in U.S. EPA Method 8330, the current method requires confirmation of peak assignment using a secondary column with complementary selectivity, such as CN columns. However, because severe peak overlapping is always observed on confirmatory columns, peak identification can be ambiguous and the process is tedious. As illustrated in Figure 4, not only can the Acclaim Explosives E2 column baseline resolve all 14 target compounds listed in U.S. EPA Method 8330 in a single chromatographic run, but also provides selectivity complementary to the Acclaim Explosives E1 column. AmDNT isomers elute last on the Acclaim Explosives E2 column, compared to the Acclaim Explosives E1 column, on which they elute as the eighth and ninth peaks. The reversal elution order of tetryl and TNT was also observed on the Acclaim Explosives E2 column. Table 2 lists typical retention, resolutions, correlation coefficients ( $R^2$ ) (linearity over

a range of 1–1000  $\mu$ g/L), and estimated LOD. While the Acclaim Explosives E2 column can be used alone to completely separate all 14 explosives listed in U.S. EPA Method 8330, in a case in which a confirmatory column is required, its high resolution power combined with its selectivity make it very well suited for this application.

## Practical considerations

Acclaim Explosives E1 and E2 columns (4.6  $\times$  250 mm,  $d_p$  = 5  $\mu$ m) are easy to use and compatible with standard HPLC instrumentation. All chromatographic data in this article were generated on a modular UltiMate<sup>®</sup> 3000 HPLC system equipped with an LPG 3600 gradient pump, WPS-3000 autosampler, TCC-3200 column oven, and VWD 3400 (a UV-VIS multiple-wavelength detector) (all from **Dionex**). With diode array-based detection, a detection wavelength range of 200–400 nm was selected, and peak spectra can be acquired automatically. This provides an individual library to be compiled for each compound. Chromeleon<sup>®</sup> 6.70 chromatography data management software (**Dionex**) was used for system control and data processing. All data acquisition and data evaluation were performed automatically.

Buffers can be used instead of deionized water in the mobile phase without compromising separation. For example, when a mobile phase containing 50 mM ammonium acetate buffer and methanol was used, all 14 explosives were baseline resolved on both Acclaim Explosives columns in a slightly modified mobile phase. This is especially useful for photo-assisted electrochemical detection (PAED), a newly developed sensitive detection method for explosives, in which an acetate buffer is required.

Mobile phase composition affects the separation of explosives compounds. The methanol–water system results in dramatically different selectivity compared to the acetonitrile–water system on all reversed-phase columns evaluated. To comply with the conditions recommended in U.S. EPA Method 8330, the separation of explosives on both columns was optimized in the methanol–water mobile phase system. The Acclaim Explosives E1 column was less sensitive toward the changes in methanol content in the mobile phase. No elution order change was observed when the methanol content varied in the range of 40–46%. To achieve good separation in a reasonable run time, 43% methanol (v/v) should be selected. On the other hand, the separation on the Acclaim Explosives E2 column is more dependent on the methanol content in the mobile phase. With decreasing methanol content, resolution between RDX and 1,3,5-TNB worsens, but resolution between 2,4,6-TNT and tetryl improves. When increasing methanol content in the mobile phase, resolution of RDX and 1,3,5-TNB improves, while resolution between 2,4,6-TNT and tetryl deteriorates. Therefore, for optimal separation on the Acclaim Explosives E2 column, methanol content should be around 48% methanol (v/v).

Separation temperature is another important factor influencing the separation quality. For the Acclaim Explosives E1 column, the optimal temperature for separating all

**Table 2 Chromatographic results of explosives analysis on the Acclaim Explosives E2 column**

Explosive	K'	Resolution	R <sup>2</sup> (peak area)	LOD (µg/L)
HMX	2.03	11.35	1	2.5
RDX	3.57	2.08	1	2.1
1,3,5-TNB	3.86	7.34	1	0.9
1,3-DNB	4.95	4.95	1	0.8
NB	5.43	9.57	1	1.2
2,4,6-TNT	7.36	2.05	1	1.5
Tetryl	7.87	2.73	1	2.6
2,6-DNT	8.57	1.81	1	2.4
2,4-DNT	9.04	3.38	1	1.3
2-NT	9.96	3.46	1	3.1
4-NT	10.99	2.74	0.9999	4.1
3-NT	11.88	2.49	0.9999	3.5
4-A-2,6-DNT	12.81	1.94	0.9997	3.9
2-A-4,6,-DNT	13.64	—	1	2.6

14 explosives compounds in U.S. EPA Method 8330 is around 32 °C with a mobile phase containing 43% methanol (v/v). Separation between 2-Am-4,6-DNT and 2,6-DNT deteriorates gradually when decreasing the temperature below 32 °C. At 25 °C, these two molecules completely coelute. The separation on the Acclaim Explosives E2 column is more sensitive to temperature. The resolution between RDX and 1,3,5-TNB and 2,4,6-TNT and tetryl are affected most by temperature. Higher temperature (40 °C) results in better resolution between RDX and 1,3,5-TNB but worsened resolution between 2,4,6-TNT and tetryl, and a lower temperature (25 °C) gives rise to lower resolution between RDX and 1,3,5-TNB and improved resolution between 2,4,6-TNT and tetryl. The authors found that setting the oven temperature at 28 °C generally provides satisfactory results in a mobile phase containing 48% methanol (v/v).

## Conclusion

Acclaim Explosives (E1 and E2) columns are high-efficiency silica-based columns for explosives analysis that provide baseline resolution of 14 target explosives listed in U.S. EPA Method 8330, but with different selectivities. The E1 col-

umn is an effective direct replacement of the current primary column (C18), while the E2 column is a good alternative that can be used alone and also serve as a confirmatory column for explosives analysis.

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