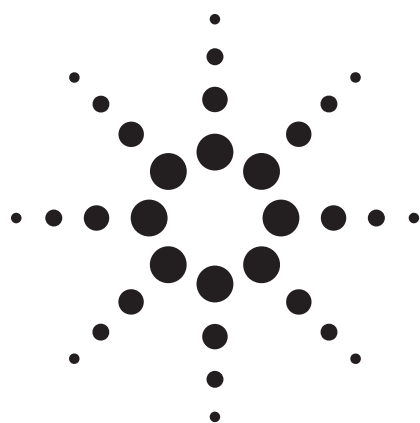




Separation of Explosives in EPA 8330: Column Choices Optimize Speed, Resolu- tion, and Solvent Use

Application

Environmental

Authors

John W. Henderson Jr. and William J. Long
Agilent Technologies, Inc.
2850 Centerville Road
Wilmington, DE 19808-1610
USA

Abstract

ZORBAX Extend-C18 columns separate the explosive compounds in EPA method 8330, and the variety of column configurations available allows customized HPLC methods based on resolution, speed, and even solvent usage. For example, a fast method for the explosive-materials standard (EPA 8330) uses 1.8- μ m, short length columns. The method was then customized using two other Extend-C18 column configurations. Each column highlights a combination of resolution, speed, and/or solvent savings. The advantage is being able to choose which combination of resolution, speed, and solvent usage is needed by simple column substitution.

Introduction

The ZORBAX Rapid Resolution High Throughput (RRHT, 1.8 μ m) LC column line has over 120 column choices, including 11 bonded phases and silica, three column diameters, and six lengths. In addition, there are another 150+ Rapid Resolu-

tion (3.5 μ m) column choices, allowing customization of HPLC methods to meet the analyst's tailored objectives. Many ZORBAX column choices are available because the stationary phase chemistry (both silica support and bonded phase) between 5-, 3.5- and 1.8- μ m particles is uniform.

EPA 8330 explosives residues are typically analyzed by a 4.6 mm $\times$ 250 mm, 5 μ m C18 column [1] but can be improved by newer technology: smaller 1.8- μ m or 3.5- μ m ZORBAX particles and Extend-C18 bonded phase. Many different Extend-C18 columns can be chosen (the combination of column length, diameter, and particle size) to provide a satisfactory separation, and each separation exemplifies a newer column technology's benefit and supports the end user's choice of speed, resolution, and solvent usage.

High-efficiency 1.8- μ m particles in 100-mm length columns reduce analysis time and have about the same efficiency compared to 5- μ m particles in 250-mm columns. Therefore, they are helpful by saving time in method development or generating more data in a limited amount of time. But these columns will generate a higher back pressure that some people may not desire. It is still possible to obtain the same resolution but using a longer 3.5- μ m column. The end result is an analysis time still shorter than that achieved with a 250-mm, 5- μ m column.



Agilent Technologies

Experimental

The Agilent 1200 Rapid Resolution LC (RRLC) system:

- G1312B binary pump SL with mobile phase
A: 5 mM ammonium formate in water,
B: methanol
- G1376C automatic liquid sampler (ALS) SL
- G1316B Thermally Controlled Column (TCC) Compartment SL using the low-volume heat exchanger kit (PN G1316-80003)
- G1365C multiwavelength detector (MWD) at 254 nm, with a G1315-60024 micro flow cell (3-mm path, 2- μ L volume), response time setting of 0.5 s

ZORBAX columns:

- Rapid Resolution High Throughput (RRHT) Extend-C18, 4.6 mm $\times$ 100 mm, 1.8 μ m, PN 728975-902
- Rapid Resolution (RR) Extend-C18, 4.6 mm $\times$ 100 mm, 3.5 μ m, PN 764953-902
- Solvent Saver Plus Extend-C18, 3.0 mm $\times$ 100 mm, 3.5 μ m, PN 764953-302

The sample is a 1:1 mix of EPA 8330 Mix A (cat. no. 47283) and EPA 8830 Mix B (cat. no. 47284) from Sigma-Aldrich (Bellefonte, PA), diluted in methanol:water.

Results and Discussion

Selectivity, or the relative band spacing between two peaks, is different among C18 columns. In many cases the difference is small, so adjusting mobile phase organic strength can fine tune the retention to achieve comparable resolution between one C18 column and an alternative C18 column. Temperature may also influence selectivity, and small adjustments in temperature can fine tune the resolution.

For complex mixtures, fine tuning organic strength and temperature could be used to improve resolution and ultimately make a method more robust. Determining the combination of temperature, % organic, and what column (stationary phase) is best is frequently discovered by experimentation. This is time consuming at the very least and often daunting. Fortunately, research narrows the testing.

Consider an explosive residue standard of 14 nitroaromatic and nitramine compounds. Trace residues of these explosives were analyzed by time-of-flight LCMS by Kinghorn et al. using an Extend-C18, 4.6 mm $\times$ 250 mm, 5- μ m column and a methanol/water gradient at a temperature of 40 $^{\circ}$ C [2]. Additionally, EPA method 8330 describes an HPLC method for the 14 compounds using an isocratic methanol/water mobile phase and a C18 column. Temperature is not specified, but the method states, *"If column temperature control is not employed, special care must be taken to ensure that temperature shifts do not cause peak misidentification."* [1]

In both methods a lack of selectivity required a TOF detector or additional analysis by an orthogonal stationary phase to confirm peak identity.

We separated the 14 compounds with enough resolution to make the MS detector or secondary analysis by a different stationary phase redundant.

The above methods narrowed our method-development starting conditions to:

- Extend-C18 (from successful Kinghorn method)
- Isocratic mobile phase A: 5 mM ammonium formate, B: Methanol (so new method is similar to EPA 8330). The ammonium formate was selected based on recommendations from a pre-existing method. The difference between water and 5 mM ammonium formate was not investigated.
- 40 $^{\circ}$ C controlled temperature (to ensure constant selectivity)
- RRHT column configuration 4.6 mm $\times$ 100 mm, 1.8 μ m (for rapid analyses with efficiency comparable to the 4.6 mm $\times$ 250 mm, 5- μ m columns used in the Kinghorn and EPA methods)

The methanol composition of the mobile phase was lowered incrementally from 50 to 25% until all 14 were reasonably resolved. A critical pair (peaks 6 and 7) persisted as partially resolved. Further decreasing organic strength would result in excessive retention of peaks 12, 13, and 14. Temperature was then optimized. A one-degree temperature increase (41 $^{\circ}$ C) provided enough selectivity to resolve the critical pair. Figure 1 demonstrates temperature's selectivity effect on these compounds.

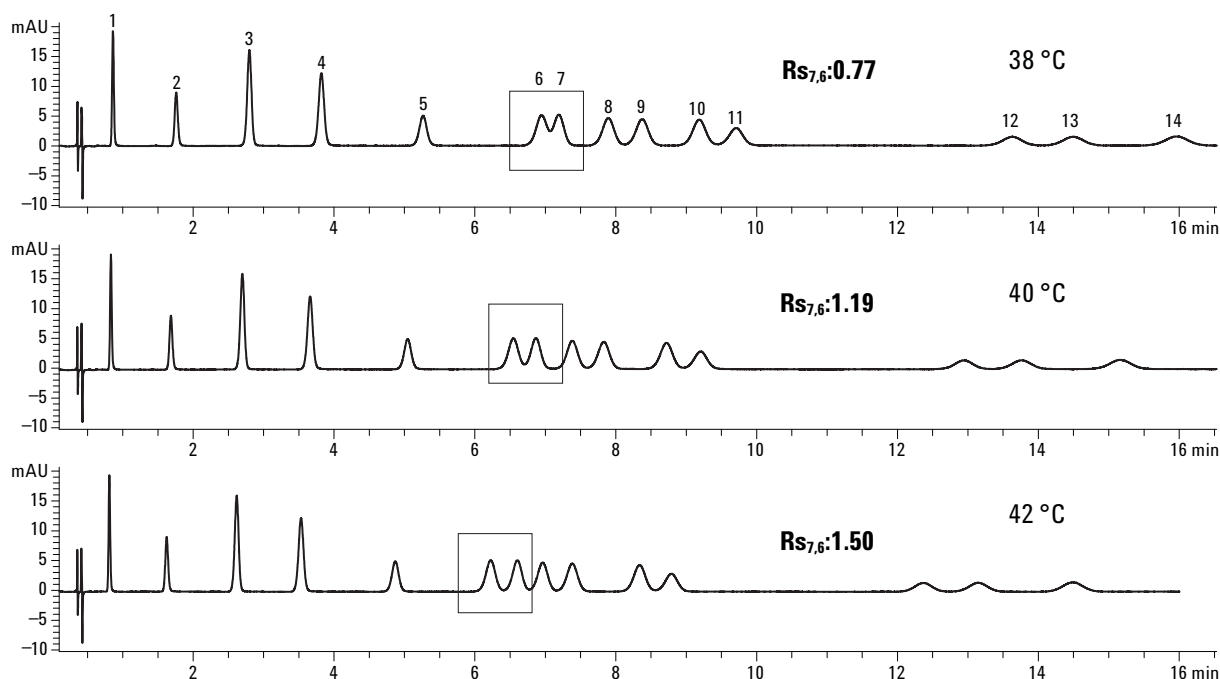


Figure 1. Temperature optimizes critical pair resolution.

Extend-C18 provides ample selectivity for the 14 nitroaromatics and nitramines identified in the EPA 8330 method; excellent resolution is obtained in a reasonable time. Figure 2 shows the separation using a RRHT 4.6 mm × 100 mm, 1.8 μm, Extend-C18. Resolution of all peaks is baseline or better ($R_s > 1.5$). High resolution makes it easier to quantify the analytes. For example, the EPA 8330 method warns, “2,4-DNT and 2,6-DNT elute at similar retention times ($R_s < 1.5$) and a large

concentration of one isomer may mask the other; therefore, if it is not apparent that both isomers are present, an isomeric mixture should be reported” [1]. When baseline resolution is obtained, retention times differ significantly, avoiding peak masking. If higher resolution is the most important objective, then the Extend-C18 4.6 mm × 100 mm, 1.8-μm column using the conditions in Figure 2 is an excellent choice.

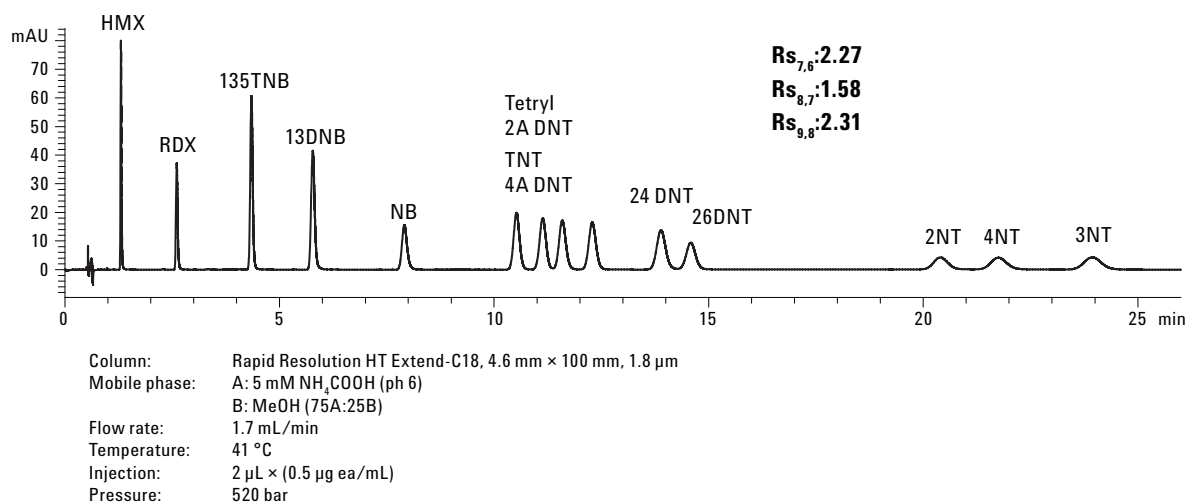


Figure 2. EPA 8330 explosive standard high-resolution separation on Extend-C18.

Table 1 names the 14 explosives and their abbreviations used in the figures.

Table 1. EPA 8330 Explosives and Their Abbreviations

Name	Abbreviation
Cyclotetramethylene-tetranitramine	HMX
Cyclotrimethylene-trinitramine	RDX
1,3,5-trinitrobenzene	135TNB
1,3-dinitrobenzene	13DNB
Nitrobenzene	NB
2,4,6-trinitrophenyl-N-methylnitramine	tetryl
2,4,6-trinitrotoluene	TNT
2-amino-4,6-dinitrotoluene	2A DNT
4-amino-2,6-dinitrotoluene	4A DNT
2,4-dinitrotoluene	24 DNT
2,6-dinitrotoluene	26 DNT
2-nitrotoluene	2NT
4-nitrotoluene	4NT
3-nitrotoluene	3NT

If higher throughput is important, isocratic methods can be sped up by increasing flow rate. The 25% methanol mobile phase flowing 1.7 mL/min through the 4.6 mm × 100 mm, 1.8-μm column generates a system pressure of about 500 bar, leaving

a small range to increase flow rate. An alternative is to substitute the 1.8-μm column with a 3.5-μm column. Pressure decreases substantially, allowing faster flow rates.

Figure 3 overlays two Extend-C18 chromatograms. The top chromatogram is a 4.6 mm × 100 mm column with 3.5-μm particles at a 2.5 mL/min flow rate. Compared to Figure 2, the 32% increase in flow rate reduces analysis time by roughly 40%. The price for the considerable time savings is less resolution of closely neighboring peaks. Resolution is still sufficient, as a resolution factor (R_s) of 1.25 for equally sized peaks means 99.4% of peak area is not overlapped. If one peak is 1/32 as tall as the other, an R_s of 1.0 still means 99.2% of the peak areas do not overlap [3].

Figure 3's bottom chromatogram is a different column substitution, replacing the 4.6-mm-id column with the Solvent Saver 3.0-mm-id column. Flow rate was reduced from 2.5 to 1.1 mL/min. for equivalent mobile phase linear velocity. The outcome is similar retention and resolution, but only half of the solvent is consumed.

Table 2 summarizes the customization benefits.

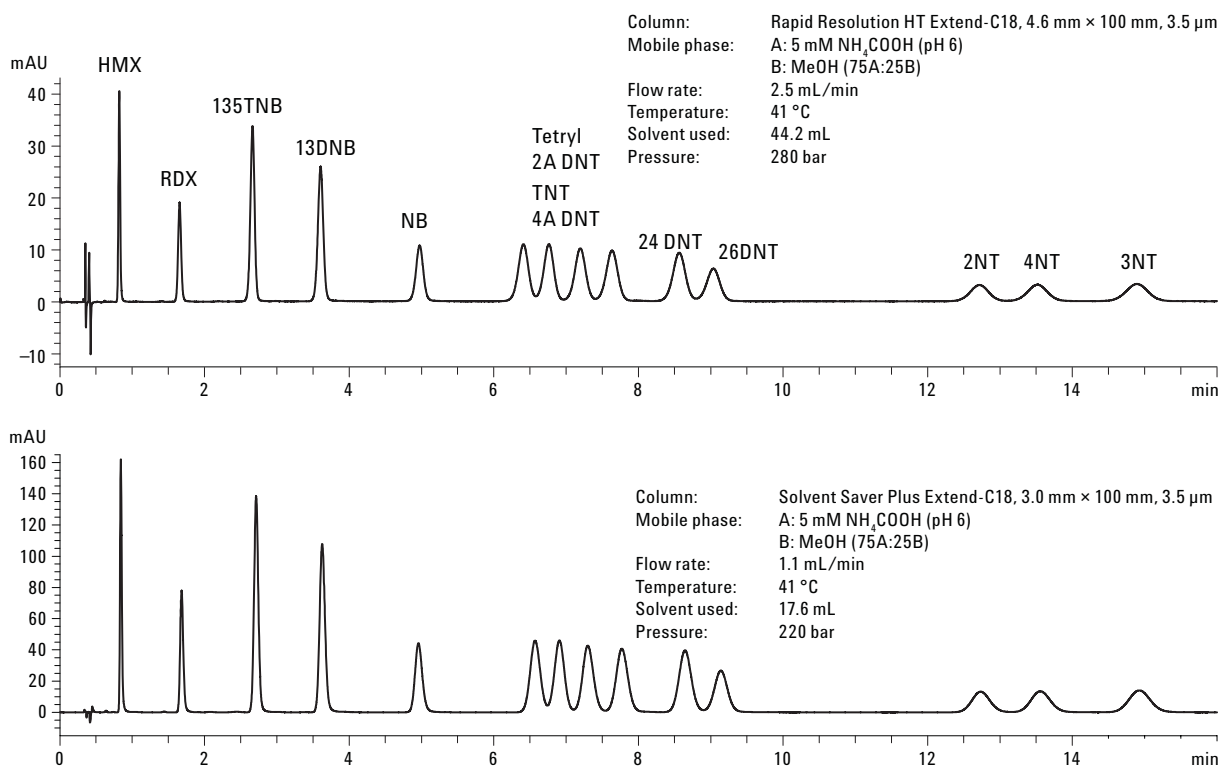


Figure 3. Rapid resolution options for EPA 8330 explosive standard on Extend-C18.

Table 2. Column Dimensions Highlight Resolution, Speed, and Solvent Savings

	RRHT (4.6 mm id, 1.8 µm)	RR (4.6 mm id, 3.5 µm)	Solvent Saver Plus (3.0 mm id, 3.5 µm)
Resolution: Rs 7,6	2.3	1.3	1.3
Resolution: Rs 8,7	1.6	1.6	1.4
Resolution: Rs 9,8	2.3	1.5	1.6
Analysis time	26 min	16 min	16 min
Solvent consumption	44.2 mL/analysis	40 mL/analysis	17.6 mL/analysis

Table 2 suggests that another column configuration could be valuable for this analysis: Solvent Saver HT Extend-C18, 3.0 mm × 100 mm, 1.8-µm column (PN 728975-302). This would produce high resolution like the RRHT column and produce time and solvent savings from the smaller column diameter. The Solvent Saver HT Extend-C18 column was not evaluated in this work.

Conclusions

Highly efficient (1.8 µm) short columns (100 mm) are ideal for method development compared to 5-µm, 150-mm or 250-mm columns because shorter analysis time increases productivity and allows more analyses to be performed in a fixed time frame.

Selectivity is manipulated by changing stationary phase, mobile phase, and temperature.

An isocratic HPLC method for complex mixtures of explosive materials was quickly created from highly efficient 100-mm columns, Extend-C18's unique selectivity, and temperature optimization. The selectivity and column configurations make Extend-C18 a compelling choice for the analysis of explosive substances named in EPA method 8330. Extend-C18's selectivity provides ample resolution with negligible peak coelution; this may eliminate an additional analysis to confirm peak identity.

The ZORBAX column family, including Extend-C18, has consistent stationary-phase chemistry between 3.5- and 1.8-µm particles, enabling simple column substitution for method customization. The high-resolution 4.6 x 100, 1.8-µm configuration, however, requires flexibility to work at operating pressures above 400 bar. The chromatographer can choose benefits such as higher resolution, faster analysis time, or less solvent usage based on column dimensions.

References

1. EPA Method 8330, *Nitroaromatics and Nitramines by High Performance Liquid Chromatography (HPLC)*, revision 0, September 1994
2. R. Kinghorn, C. Milner, J. Zweigenbaum, "Analysis of Trace Residues of Explosive Materials by Time-of-Flight LC/MS," Agilent publication 5989-2449EN (2005)
3. L. R. Snyder, J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, 2nd ed., pp. 38–42, 1979

For More Information

For more information on our products and services, visit our Web site at www.agilent.com/chem.

Agilent shall not be liable for errors contained herein or for incidental or consequential damages in connection with the furnishing, performance, or use of this material.

Information, descriptions, and specifications in this publication are subject to change without notice.

© Agilent Technologies, Inc. 2007

Printed in the USA
November 21, 2007
5989-7632EN



Agilent Technologies