Improved Separation of Explosives (Nitro Aromatics) for HPLC Analysis Using Synergi™ Hydro-RP and Synergi™ Polar-RP® (EPA Method 8330)

Scott Waite, Phenomenex Inc.

The need for disposal of expired munitions by armed forces has attained considerable importance during the last two decades partially due to the dismantling of military installations and disarmament treaties around the world. Improper storage and disposal techniques have resulted in contamination of soil and groundwater with explosive residues including 2,4,6-trinitrotoluene (TNT) and nitrobenzene. Recent studies have shown that these compounds can cause liver damage and are suspected mutagens (1). Because of these health risks, analytical methods for the thorough identification and characterization of the contaminated areas are in high demand.

EPA Method 8330, introduced in November 1990, is the most common way to analyze samples for the presence of explosives. The method recommends using a C18 phase as a primary column and a CN phase as a confirmation column. Confirmation columns are sometimes needed because of difficulty in separating similar compounds on a single stationary phase. By using two columns with different selectivities, analysts can more accurately identify the compounds of interest. Closely eluting and co-eluting peaks on a typical C18 include 2,4,6-trinitrotoluene, 4-amino-dinitrotoluene, and 2-amino-dinitrotoluene. In addition, separation of 2,6-dinitrotoluene and 2,4-dinitrotoluene can sometimes be difficult.

In this application note, we describe the analysis of 15 explosives using a polar endcapped 80-Å 4-µm C18 reversed phase (Synergi Hydro-RP) as the primary column and a polar endcapped 80-Å 4-µm ether-linked phenyl phase (Synergi Polar-RP) as the confirmation column.

Experimental

The HPLC analysis was carried out using an HP 1100 LC system (Agilent Technologies, Palo Alto, California) equipped with a quaternary pump, in-line degasser, multi-wavelength detector, and autosampler. HP Chemstation software (Version A.08.03) was used for data analysis. The HPLC columns used for this analysis were Synergi Hydro-RP 250 × 4.6 mm and Synergi Polar-RP 250 × 4.6 mm (Phenomenex, Torrance, California). The mobile phase composition is listed in Table I. The detection wavelength used was 254 nm, and the column oven temperature was 35 °C. Standards were obtained from Restek (Bellefonte, Pennsylvania).

Table I: HPLC method conditions and peak identification

Columns	(1) Synergi Hydro-RP 4µ C18 80 Å 250 × 4.6 mm (2) Synergi Polar-RP 4µ Phenyl 80 Å 250 × 4.6 mm
Samples	1 = HMX 2 = RDX 3 = 1,3,5-trinitrobenzene 4 = 1,2-dinitrobenzene (internal standard) 5 = tetryl 6 = 1,3-dinitrobenzene 7 = nitrobenzene 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
Mobile phase	(1) A: 51% water, B: 45% methanol, C: 4% acetonitrile (2) A: 60% water, B: 5% methanol, C: 35% acetonitrile
Flow	(1) 0.8 mL/min (2) 1.0 mL/min
Detection	UV at 254 nm
Column temperature	35 °C

Results and Discussion

Experiments performed resulted in a major improvement in the separation of common explosives in EPA 8330 compared with typical C18 phases. Figure 1 shows a chromatogram of the explosives standard injected at about 25 ng on-column. The Synergi Hydro-RP column is able to achieve separation of all of the EPA Method 8330 compounds (Figure 1) versus the standard method, which describes the use of two columns (a C18 phase combined with a cyano phase). Dense bonding coupled with unique polar endcapping in the Synergi Hydro-RP phase gives added retention and improved selectivity for neutral to polar compounds. This results in improved separation of EPA Method 8330 compounds. Quantitation of individual components using Synergi Hydro-RP is more accurate due to the resolution achieved. A second confirmation run can be avoided due to the improved separation of the explosives mix. This will improve sample throughput and decrease analysis time. We also decreased the flow rate from 1.5 mL/min to 0.8 mL/min, which will decrease solvent consumption.

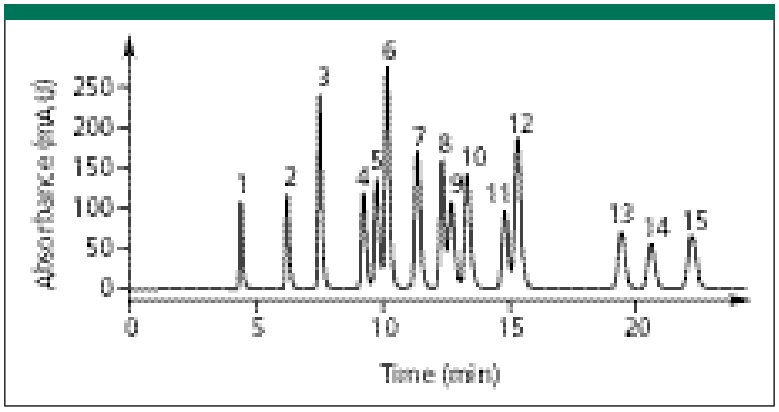

Figure 1: EPA explosive mix using Synergi Hydro-RP. Standard is a mixture of the following components: 1 = HMX, 2 = RDX, 3 = 1,3,5 trinitrobenzene, 4 = 1,2-dinitrobenzene (internal standard), 5 = tetryl, 6 = 1,3-dinitrobenzene, 7 = nitrobenzene, 8 = 2,4,6-trinitrotoluene, 9 = 4-amino-2,6 dinitrotoluene, 10 = 2-amino-4,6 dinitrotoluene, 11 = 2,6-dinitrotoluene, 12 = 2,4-dinitrotoluene, 13 = 2-nitrotoluene, 14 = 4-nitrotoluene, 15 = 3-nitrotoluene.

If a confirmation column is needed, then Synergi Polar-RP can be used to confirm peak identity. As shown in Figure 2, Polar-RP was able to improve separation of 2,4,6-trinitrotoluene (peak 7) from 4-amino-dinitrotoluene (peak 8), tetryl (peak 4) from 1,3-dinitrobenzene (peak 5), and 2,6-dinitrotoluene (peak 10) from 2,4-dinitrotoluene (peak 11) versus what is typically observed using a cyano (CN) column.

Using Synergi Hydro-RP combined with Synergi Polar-RP as a confirmation column will ensure proper identification of EPA Method 8330 compounds with improved quantitation, lower solvent consumption, and higher throughput.

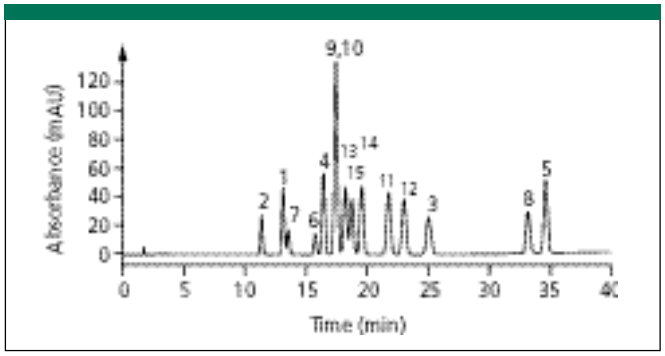

Figure 2: EPA explosive mix using Synergi Polar-RP. Standard mixture is the same as in Figure 1. Note the change in elution order for many of the components: 2 = RDX, 1 = HMX, 7 = nitrobenzene, 6 = 1,3-dinitrobenzene, 4 = 1,2-dinitrobenzene (internal standard), 9 = 4-amino-2,6 dinitrotoluene, 10 = 2-amino-4,6 dinitrotoluene, 13 = 2-nitrotoluene, 15 = 3-nitrotoluene, 14 = 4-nitrotoluene, 11 = 2,6-dinitrotoluene, 12 = 2,4-dinitrotoluene, 3 =1,3,5-trinitrobenzene, 8 = 2,4,6-trinitrotoluene, 5 = tetryl.

Reference

(1) L. Berthe-Corti, H. Jacobi, S. Kleihauer, and I. Witte, "Cytotoxicity and Mutagenicity of (TNT) and Hexogen Contaminated Soil in *S. typhimurium* and Mammalian Cells," *Chemosphere* **2,** 209–218 (1998).

Phenomenex Inc.
USA tel. (310) 212-0555, USA fax (310) 328-7768
UK tel. +44 1625 501367, UK fax +44 1625 501796
Germany tel. +49 6021 58830-0, Germany fax +49 6021 58830-11
e-mail international@phenomenex.com
www.phenomenex.com