Isolation, Purification and Chromatography of Nitro Compounds and Explosives

Technical Overview

Introduction

Explosives and structurally related nitro compounds are a class of compounds that are generally toxic. Therefore, they are monitored by the EPA and other environmental agencies. Since these compounds can migrate from soil into the waterways, Agilent’s SampliQ DVB solid phase extraction (SPE) tube was evaluated for isolation and cleanup of these compounds from water. Simultaneously, Agilent’s PoroShell 120 EC-C18 column was evaluated for the chromatographic separation of these components.

A dilute aqueous solution of EPA Method 8330 Calibration Mix A and B standards (14 explosive and nitro compounds) was applied to Agilent’s SampliQ DVB SPE tubes for isolation and sample cleanup. Samples were eluted with acetonitrile (ACN). The eluent was diluted to 30% acetonitrile/water and analyzed on Agilent’s Poroshell 120 EC-C18 column.

An Agilent SampliQ DVB SPE tube (3 mL tube, 60 mg, p/n 5982-3136) was used for isolation and concentration of the sample (1 µg/mL) from the 2% acetonitrile/water solution. Twelve milliliters of the sample were applied to the SampliQ DVB SPE tube as illustrated in Figure 2. The combined acetonitrile effluent was diluted to 30% acetonitrile/water, and analyzed at 210 nm and 254 nm using external standards on Agilent’s Poroshell 120 EC-C18 column (3.0 mm × 100 mm, 2.7 µm, p/n 695975-302).

Recoveries for the 14 compounds ranged from 90% to 105%. The RSDs were equal to or less than 1.2% for three replicates. The results presented here show that Agilent’s SampliQ DVB SPE tubes, coupled with Agilent’s PoroShell 120 EC-C18 column is an excellent combination for the isolation and analysis of these compounds.
Study Purpose and Methodology

The purpose of the study was to evaluate the effectiveness of Agilent’s SampliQ DVB SPE tubes for the isolation and purification of nitro-containing compounds and explosives (Figure 1). Given the similarity of many of the structures, this sample provides an excellent opportunity to evaluate the separation on Agilent’s Poroshell 120 EC-C18 line of columns.

An aliquot of 14 standards in acetonitrile (50 µg/mL each) was diluted to provide a 2% acetonitrile/water solution of standards (1 µg/mL each std). This solution was used as the sample for application to the SampliQ DVB SPE tube.

Figure 1. Structures.
**Results and Discussion**

The average recoveries of triplicate samples ranged from 90% to 105% (Table 1). The highest RSDs for the three replicate recoveries was 1.2%.

**Table 1. Recovery Data from Agilent’s Polymer SampliQ DVB SPE Tubes**

<table>
<thead>
<tr>
<th>Number</th>
<th>Compound ID</th>
<th>Sample % Recovery</th>
<th>Replicate % RSD (n = 3)</th>
<th>Full name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HMX</td>
<td>104</td>
<td>0.8</td>
<td>1,3,5,7-tetranitro-1,3,5,7-tetrazocane</td>
</tr>
<tr>
<td>2</td>
<td>RDX</td>
<td>103</td>
<td>0.6</td>
<td>1,3,5-trinitro-1,3,5-triazinane</td>
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<tr>
<td>3</td>
<td>1,3,5-tnb</td>
<td>105</td>
<td>0.6</td>
<td>1,3,5-trinitrobenzene</td>
</tr>
<tr>
<td>4</td>
<td>1,3-dnb</td>
<td>103</td>
<td>0.7</td>
<td>1,3-dinitrobenzene</td>
</tr>
<tr>
<td>5</td>
<td>nb</td>
<td>101</td>
<td>1.2</td>
<td>Nitrobenzene</td>
</tr>
<tr>
<td>6</td>
<td>Tetryl</td>
<td>90</td>
<td>1.2</td>
<td>N-methyl-N-(2,4,6-trinitrophenyl) nitramide</td>
</tr>
<tr>
<td>7</td>
<td>2,4,6-trt</td>
<td>103</td>
<td>0.6</td>
<td>2,4,6-trinitrotoluene</td>
</tr>
<tr>
<td>8</td>
<td>2A-4,6-dnt</td>
<td>103</td>
<td>0.6</td>
<td>2-amino-4,6-dinitrotoluene</td>
</tr>
<tr>
<td>9</td>
<td>4A-2,6-dnt</td>
<td>101</td>
<td>0.6</td>
<td>4-amino-2,6-dinitrotoluene</td>
</tr>
<tr>
<td>10</td>
<td>2,4-dnt</td>
<td>102</td>
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<td>2,4-dinitrotoluene</td>
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<tr>
<td>11</td>
<td>2,6-dnt</td>
<td>102</td>
<td>0.7</td>
<td>2,6-dinitrotoluene</td>
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<tr>
<td>12</td>
<td>2-nt</td>
<td>98</td>
<td>1.2</td>
<td>2-nitrotoluene</td>
</tr>
<tr>
<td>13</td>
<td>4-nt</td>
<td>98</td>
<td>1.0</td>
<td>4-nitrotoluene</td>
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<tr>
<td>14</td>
<td>3-nt</td>
<td>97</td>
<td>1.2</td>
<td>3-nitrotoluene</td>
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</table>

These compounds are a mixture of the EPA Method 8330 Calibration Mix A and B.
Excellent separation of the compounds was achieved with the Agilent Poroshell 120 column as illustrated in Figure 3. 

**Figure 3.** Representative standard chromatogram, 5 µg/mL, 254 nm.

**HPLC Analysis**
- **Column:** Agilent Poroshell 120 EC-C18, 3.0 mm × 100 mm, 2.7 µm (Agilent p/n 695975-302)
- **Mobile phase:** 25% MeOH/water (isocratic)
- **Flow rate:** 1.0 mL/min
- **Detection DAD:** 254, 210 nm
- **Column temperature:** 44 °C
- **Injection volume:** 30 µL
- **Flow cell:** 10 mm, 13 µL
Conclusion

The use of Agilent’s SampliQ DVB SPE tubes provided high recoveries with excellent reproducibility. This, coupled with the separation achieved with Agilent’s Poroshell 120 column, provides a complete package for the analysis of nitro compounds and explosives. One would expect this procedure to be applicable to additional nitro compounds and explosives.

Agilent SPE part numbers

<table>
<thead>
<tr>
<th>Description</th>
<th>Part number</th>
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</thead>
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<tr>
<td>SampliQ DVB SPE tube, 3 mL tube, 60 mg</td>
<td>5982-3136</td>
</tr>
<tr>
<td>Poroshell 120 EC-C18, 3.0 mm × 100 mm, 2.7 µm</td>
<td>695975-302</td>
</tr>
</tbody>
</table>

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