**Torus DEA Column Startup Guide for Polar Pesticide Separations**

**NOTE:** Torus DEA Columns are manufactured and QC-tested for supercritical chromatography, not for the hydrophilic chromatographic separation of polar pesticides.

**CONTENTS**

I. INTRODUCTION
II. GETTING STARTED
III. ANALYTICAL METHOD
   a. Reagent Preparation
   b. LC Conditions
   c. Mass Spectrometer Parameters
IV. TROUBLESHOOTING
V. APPLICATIONS FOR OTHER POLAR ANALYTES

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### I. INTRODUCTION

Thank you for choosing the Torus™ DEA Column. To ensure successful use of this column for polar pesticide analysis, detailed procedures of system washing, column startup and washing are demonstrated in this guide. The analysis of glyphosate and its related compounds (as shown in Figure 1) has been demonstrated using the parameters listed below for this specific column, Waters ACQUITY® UPLC® I-Class System and Xevo® TQ-XS Mass Spectrometer. Chromatographic performance and reproducibility will not be preserved for separation conditions and instrument configurations that are not specified in this guide.

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![Chemical structures of glyphosate, aminomethyl phosphonic acid (AMPA), and glufosinate.](image)

*Figure 1. Chemical structures of glyphosate, aminomethyl phosphonic acid (AMPA), and glufosinate.*
For the analysis of polar pesticides, such as glyphosate, the column sorbent requires initial mobile phase purging and equilibration to achieve reproducible results. This guide provides details for the column mobile phase, column equilibration and separation conditions required to analyze polar pesticides using the Torus DEA Column. The conditions apply to the 2.1 mm x 100 mm column configuration (p/n 186007616).

II. GETTING STARTED

Before installing the column, the LC system may require passivation using an acid wash (detailed in Section IV) to remove metal ions and other interferences accumulated in the system, which may interact with polar pesticides. Depending on the sample type or the quality of the reagents used, peak shapes of analytes may deteriorate over time, which may require repassivation of the system. Please refer to Section IV Troubleshooting for detailed instructions.

Column cleaning protocol

To obtain the retention time and peak shape performance, it is necessary to flush, equilibrate and condition the column using the procedure outlined below. Failure to follow this protocol may result in broad peak shape for very polar compounds such as glyphosate and AMPA (See Section IV Troubleshooting).

1. Put lines A1 into 50 mM ammonium formate pH 2.9 and B1 into 0.9% formic acid in acetonitrile, and prime each line for 2 minutes.
2. Flush 40 column volumes (~13.8 mL for 2.1 x 100 mm column) of 50:50 A1:B1 at flow rate of 0.5 mL/minute for 30 minutes.
3. Put lines A1 and B1 into water and prime each line for 4 minutes.
4. Prepare the column wash solution (5 mM Na₂EDTA prepared in 80:20 water:acetonitrile) as shown in Section IIIa.
5. Divert the LC flow to waste, as Na₂EDTA can suppress the signal of the mass spectrometer.
6. Put lines A1 and B1 in the wash solution and prime each line for 6 minutes.
7. Flush the column with 50:50 A1:B1 at a flow rate of 0.5 mL/min for 20 minutes.
8. Once complete, prime the solvent lines A1 and B1 each with water for 3 minutes.
   Note: Since the separation is operated under Hydrophilic Interaction Chromatography (HILIC) mode, do not allow water to flow through the column after priming the solvent lines.
9. Put lines A1 in 50 mM ammonium formate pH 2.9 and B1 in 0.9% formic acid in acetonitrile and prime each line for 5 minutes.
10. Run 50:50 A1:B1 for 10 minutes at 0.5 mL/min.
11. Connect the LC flow to the MS. Equilibrate the column with the initial gradient conditions and allow the pressure and temperature to stabilize (delta pressure <30 psi) for a minimum of 15 minutes.

III. ANALYTICAL METHOD

a. Reagent preparation

1. Preparation of column wash solution 5 mM Na₂EDTA
   a. Weigh 1.86 g of ethylenediaminetetraacetic acid disodium salt dihydrate (CAS 6381-92-6) and make up to 1 L with 80:20 water:acetonitrile.
   b. Sonicate the solution for 5 minutes.

2. Preparation of sample
   a. Solvent standards:
      Glyphosate, AMPA and glufosinate were purchased from vendor. The individual stock solution was prepared at 1 mg/mL in water. A mixture of three compounds was prepared at 100 ppb (ng/mL) in 50:50 acetonitrile:water with 0.25 mM Na₂EDTA.
      Note: Since the separation is operated under Hydrophilic Interaction Chromatography (HILIC) mode, using 100% aqueous solution as sample diluents is not recommended. The injected sample should contain a minimum of 50% organic solvents such as acetonitrile.
   b. Sonicate the solution for 5 minutes.

3. Preparation of mobile phases
   a. Mobile phase A, 50 mM Ammonium formate pH 2.9
      1) Weigh 3.15 g of ammonium formate (reagent grade or better) and add 500 mL of water to the volumetric flask.
      2) Sonicate the solution until all ammonium formate dissolved.
      3) Add 400 mL of water.

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<table>
<thead>
<tr>
<th>Column</th>
<th>Stationary phase</th>
<th>Particle shape</th>
<th>Particle size (µm)</th>
<th>Pore size (Å)</th>
<th>Pore volume (cc/g)</th>
<th>Surface area (m²/g)</th>
<th>Endcapped</th>
</tr>
</thead>
<tbody>
<tr>
<td>Torus DEA</td>
<td>Diethylamine</td>
<td>Spherical</td>
<td>1.7</td>
<td>130</td>
<td>0.7</td>
<td>185</td>
<td>Proprietary</td>
</tr>
</tbody>
</table>

Torus DEA Column Startup Guide for Polar Pesticide Separations
4) Using a magnetic stirrer and calibrated pH meter, adjust the pH to 2.9 by gently adding formic acid. Add water to make up the total volume of 1 L.

5) Note: Prepare fresh buffer regularly to prevent contamination and to maintain the pH of 2.9 which is crucial to obtain the desired chromatography.

b. Mobile phase B, 0.9% Formic acid in acetonitrile

1) Pipette 9 mL of formic acid into ~800 mL acetonitrile. Mix well.

2) Add acetonitrile to make up the total volume of 1 L.

b. LC conditions

LC system: ACQUITY UPLC I-Class with Sample manager with Flow-Through Needle (FTN)

Column: Torus DEA, 1.7 µm, 2.1 x 100 mm

Mobile phase A: 50 mM ammonium formate pH 2.9

Mobile phase B: 0.9% Formic acid in acetonitrile

Strong wash: 10:90 Acetonitrile:water

Weak wash: 90:10 Acetonitrile:water

Seal wash: 10% Methanol in water

Column temp.: 50 °C

Sample temp.: 10 °C

Injection vol.: 10 µL

Flow rate: 0.5 mL/min

Gradient:* 

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Flow rate (mL/min)</th>
<th>%A</th>
<th>%B</th>
<th>Curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>0.50</td>
<td>10.0</td>
<td>90.0</td>
<td>-</td>
</tr>
<tr>
<td>4.50</td>
<td>0.50</td>
<td>60.0</td>
<td>40.0</td>
<td>2</td>
</tr>
<tr>
<td>8.50</td>
<td>0.50</td>
<td>60.0</td>
<td>40.0</td>
<td>6</td>
</tr>
<tr>
<td>20.0</td>
<td>0.50</td>
<td>10.0</td>
<td>90.0</td>
<td>1</td>
</tr>
</tbody>
</table>

Note: In the gradient table, the long column equilibration time from 8.50 minutes to 20.0 minutes is necessary to ensure consistent chromatographic performance such as retention times and peak shape.

* There is a gradient start delay by 320 µL after injection as shown in the picture below.

6) Using a magnetic stirrer and calibrated pH meter, adjust the pH to 2.9 by gently adding formic acid. Add water to make up the total volume of 1 L.

5) Note: Prepare fresh buffer regularly to prevent contamination and to maintain the pH of 2.9 which is crucial to obtain the desired chromatography.

b. Mobile phase B, 0.9% Formic acid in acetonitrile

1) Pipette 9 mL of formic acid into ~800 mL acetonitrile. Mix well.

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Mobile phase A: 50 mM ammonium formate pH 2.9

Mobile phase B: 0.9% Formic acid in acetonitrile

Strong wash: 10:90 Acetonitrile:water

Weak wash: 90:10 Acetonitrile:water

Seal wash: 10% Methanol in water

Column temp.: 50 °C

Sample temp.: 10 °C

Injection vol.: 10 µL

Flow rate: 0.5 mL/min

Gradient:* 

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Flow rate (mL/min)</th>
<th>%A</th>
<th>%B</th>
<th>Curve</th>
</tr>
</thead>
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<tr>
<td>Initial</td>
<td>0.50</td>
<td>10.0</td>
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<td>4.50</td>
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<td>8.50</td>
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</tr>
<tr>
<td>20.0</td>
<td>0.50</td>
<td>10.0</td>
<td>90.0</td>
<td>1</td>
</tr>
</tbody>
</table>

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* There is a gradient start delay by 320 µL after injection as shown in the picture below.

c. Mass spectrometer parameters

The mass spectrometry settings and MRM transitions of the polar pesticides are listed below. System-to-system variations will required optimization of these parameters to achieve the best performance and sensitivity.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Transitions</th>
<th>Cone voltage (V)</th>
<th>Collision energy (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glyphosate</td>
<td>168.0 &gt; 62.9</td>
<td>30</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>168.0 &gt; 80.9</td>
<td>30</td>
<td>18</td>
</tr>
<tr>
<td>AMPA</td>
<td>110.0 &gt; 62.9</td>
<td>30</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>110.0 &gt; 80.0</td>
<td>30</td>
<td>12</td>
</tr>
<tr>
<td>Glufosinate</td>
<td>179.5 &gt; 95</td>
<td>30</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>179.8 &gt; 85</td>
<td>30</td>
<td>16</td>
</tr>
</tbody>
</table>

Figure 2. Example of chromatographic separation of AMPA, glufosinate, and glyphosate with retention time (minutes) labeled on peak apex.
IV. TROUBLESHOOTING

Loss in sensitivity or deterioration of peak shape

The peak shape of the analytes may be affected after several sample injections due to metal ion contamination in the LC system and column. Metal contamination increases peak tailing and wider peaks that results in decreased signal intensity. Cleaning the column using the column startup protocol in Section II is recommended.

Following the column cleaning procedure, peak shape should improve as shown in Figure 3B. If peak shape remains unacceptable, contamination of the LC system should be suspected. For cleaning of the Waters LC system, refer to the Section “Cleaning to Eliminate Contamination” (Cleaning Conditions 3 using phosphoric acid wash) in Waters Document 715001307, Controlling Contamination in LC-MS Systems. Please contact your local Waters representative if you need more guidance and advice on troubleshooting or cleaning the liquid chromatography system. For non-Waters LC systems, please contact your supplier for specific information on liquid chromatography system cleaning.

Figure 3. The effect of column wash by using disodium EDTA solution. The peak tailing has improved by flushing column with disodium EDTA as indicated by the circles. (A) Column without disodium EDTA wash; (B) Column with disodium EDTA wash.
V. APPLICATION FOR OTHER POLAR PESTICIDES

The same method has been applied for the analysis of other polar pesticides. Example retention times for selected analytes are shown in Figure 4.

![Figure 4](image_url)

Figure 4. Example of chromatography achieved on the Torus DEA Column for an expanded suite of 13 anionic polar pesticides.