Introduction
Nanospray LC-MS is subject to many of the same issues as High Performance Liquid Chromatography (HPLC); however, due to the low flow rates and small volumes involved, these issues are often more difficult to isolate. This document provides information for diagnosing common Thermo Scientific™ EASY-Spray™ column issues allowing the identification and resolution of many issues common to nanoLC and also describes preventative steps to prevent recurrence.

Preventative action references
AB21900 Reverse phase SPE conditions optimized for peptide purification
AB21955 EASY-Spray Best Practices
## EASY-Spray Preventative Maintenance

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Solution</th>
<th>Preventative action</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Spray</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sputtering (no spray)</td>
<td>Replace column</td>
<td>Use positioning tool AB21955</td>
</tr>
<tr>
<td>No spray with visible organic material buildup on the tip</td>
<td>Cleaning gradients</td>
<td>Address sample quality AB21900</td>
</tr>
<tr>
<td>Spray tip visibly damaged (i.e. peeling, cracking, or missing tip)</td>
<td>Replace column</td>
<td>Use positioning tool AB21955</td>
</tr>
<tr>
<td>No flow at the tip</td>
<td>Ensure flow to column</td>
<td>Verify LC performance</td>
</tr>
<tr>
<td><strong>Pressure</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immediate high pressure error: usually observed due to the presence of particulates</td>
<td>Flush system, autosampler maintenance</td>
<td>Filter samples Use inline filter AB21961</td>
</tr>
<tr>
<td>Elevated operating pressure due to bed damage because column was not conditioned appropriately before use</td>
<td>Condition column</td>
<td>Condition column AB21955</td>
</tr>
<tr>
<td>Slow pressure increase due to insoluble or hydrophobic contaminants on stationary phase</td>
<td>Flush system, autosampler maintenance</td>
<td>AB21961 Address sample quality AB21900</td>
</tr>
<tr>
<td>Low/no pressure due to no flow or physical damage to column</td>
<td>Ensure flow to column</td>
<td>Verify LC performance</td>
</tr>
<tr>
<td></td>
<td>Check for physical damage to column</td>
<td>Use positioning tool AB21955</td>
</tr>
<tr>
<td><strong>Chromatography</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Background interference and noisy baseline</td>
<td>Cleaning gradients</td>
<td>AB21961</td>
</tr>
<tr>
<td>Slow creeping up of baseline - indicative of a hydrophobic contaminant such as a lipid or undigested protein</td>
<td>Flush system, autosampler maintenance</td>
<td>AB21961 Address sample quality AB21900</td>
</tr>
<tr>
<td>Ion suppression (low signal intensity)</td>
<td>Verify MS performance</td>
<td>Address sample quality AB21900</td>
</tr>
<tr>
<td></td>
<td>Cleaning gradients</td>
<td>Cleaning gradients and blanks between runs AB21961</td>
</tr>
<tr>
<td>Resolution</td>
<td>Cleaning gradients</td>
<td>AB21961</td>
</tr>
<tr>
<td>Peak shape</td>
<td>Cleaning gradients</td>
<td>AB21961</td>
</tr>
<tr>
<td>Retention time reproducibility – check column equilibration volume</td>
<td>Update method</td>
<td>Equilibrate appropriately AB21955</td>
</tr>
<tr>
<td>Retention time reproducibility – column contamination</td>
<td>Cleaning gradients</td>
<td>Address sample quality AB21900</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cleaning gradients and blanks between runs AB21961</td>
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Current versions of product instructions are available at separatedbyexperience.com/chromexpert.

Learn more about EASY-Spray columns at thermofisher.com/EASYspray

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