Figure 1: Injection valve in inlet position, left is standard configuration and right is the Online-Diluting and Refocusing (ODR) configuration.

**ABSTRACT**

In high-throughput laboratories, it is highly desirable to minimize sample preparation procedures to reduce sample analysis time and labor. However, the result is often to use highly organic samples that can be analyzed by UHPLC. In other situations where sensitivity is a prime factor, such as LC-MS/MS, a larger injection volume is often desired. Both challenges are often encountered in MicroLC applications since most MicroLC users investigate sensitive analysis.

This study investigates the feasibility of utilizing Online Diluting and Refocusing (ODR) for the analysis of samples in microflow LC and MS systems. A hybrid system was used with Eksigent’s microLC to evaluate the ODR configuration with a large volume sample loop in the flow path, enabling diluting the highly organic sample and focusing of analytes on the top of the column. This configuration uses a large volume sample loop in the flow path, enabling diluting the highly organic sample and focusing of analytes on the top of the column. The ODR configuration uses a large volume sample loop in the flow path, enabling diluting the highly organic sample and focusing of analytes on the top of the column.

**INTRODUCTION**

In high-throughput laboratories it is highly desirable to minimize sample preparation procedures to reduce sample analysis time and labor, which often leads to samples with a high organic solvent content to be analyzed by LC-MS systems. Typical sample loops used in microflow LC systems are 2-2.5 µL. When the sample is high in organic, the injected analytes can start eluting from the column when the equilibrium is disrupted by the high organic injection which serves as strong eluent. Experimental chromatograms of large volume injections with MicroLC in one configuration (Figure 1A) shows a split, with the majority of the peak eluting in the void volume. Other early eluting peaks had poor peak shape or the peak was smears out as a broadly distorted front or tail on the peak, or splits. This is illustrated in figure 2A, a chromatogram of large volume injection with MicroLC where triazenes mix was prepared in 20% methanol at 1 ng/mL.

**MATERIALS AND METHODS**

**LC and MS systems**

Eksigent™ microLC – ESIAB, SCIEX, Framingham, MA

**EXPERIMENTAL CONDITIONS**

<table>
<thead>
<tr>
<th><strong>Sample</strong></th>
<th><strong>Flow Rate</strong></th>
<th><strong>Mobile Phase</strong></th>
<th><strong>Column</strong></th>
<th><strong>Gradient Conditions</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample A</td>
<td>25 µL/min</td>
<td>CH3CN: 5% H2O</td>
<td>Hypersil C18, 150 mm x 2.1 mm</td>
<td>0% B to 100% B in 2.5 min</td>
</tr>
<tr>
<td>Sample B</td>
<td>50 µL/min</td>
<td>CH3CN: 2% H2O</td>
<td>Luna 100A, 150 mm x 2.1 mm</td>
<td>0% B to 100% B in 5 min</td>
</tr>
</tbody>
</table>

**RESULTS**

In the standard microLC configuration (Figure 1A), sample is loaded on the small (<10 µL) sample loop then injected on the column. Due to the low delay volume, sample prepared in high organic content can cause peak splitting when injected on the column. Figure 2A shows Online Diluting and Refocusing (ODR), configured with a small volume loop (<1.5 µL), the highly organic sample partially fills the loop. It flows through the entire sample loop before reaching the top of the column. A gradient through the loop in figure 3A shows the chromatogram of a sample prepared in 100% methanol.

**CONCLUSIONS**

This study demonstrates the use of Online-Diluting and Refocusing (ODR) for the analysis of samples prepared in high organic content. The ODR configuration uses a large volume sample loop in the flow path, enabling diluting the highly organic sample and focusing of analytes on the top of the column. Highly organic samples can be injected directly on a microLC column with ODR configuration while maintaining excellent chromatographic performance.

**REFERENCES**


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