Analyzing Highly Organic Samples, Polar Analytes, and Large Volume Injections using microflow chromatography coupled with mass spectrometry

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ABSTRACT

In high-throughput laboratories, it is highly desirable to minimize sample preparation procedures to reduce sample analysis time and labor, which often leads to highly organic samples to be analyzed by LC/MS. In other situations where sensitivity is the primary goal of a LC/MS method, a larger injection volume is often desired. Both challenges are often encountered in MicroLC applications since most MicroLC users investigate specifically for improvements in sensitivity and throughput. This study investigates the feasibility of utilizing online-diluting-and-refocusing configuration to analyze highly organic samples, polar analytes and large volume injection with MicroLC in one-dimensional format to achieve high-throughput and ultra-sensitive analysis.

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. Typical sample loops used in microflow LC systems are 2-5 µL. When the sample is in high organic, the injected analyte(s) could start eluting from the column when the equilibration is disrupted by the high organic injection which serves as strong eluent. Experimental results showed that injecting 2 µL of sample prepared in 100% methanol caused the earliest eluting peak to split, with the majority of the peak eluting in the void volume. Other early eluting peaks had poor peak shape with excessive tailing. In applications where sensitivity is important, a larger injection volume is often desired. In microflow chromatography columns have limited capacity, which limits the amount of injected volume to prevent volume overloading of the column. Both challenges are often encountered in microflow LC applications, since microflow LC is often looked at for improvements in sensitivity. This study investigates the feasibility of utilizing an online-diluting-and-refocusing configuration to analyze large volume samples with a high organic content using microLC/MS to achieve high-throughput and ultra-sensitive analysis. To overcome these challenges, the LC system was configured with a much larger sample loop. This configuration is called “Online Diluting and Refocusing”, i.e. ODR. In this configuration the highly organic sample will be continuously diluted while traveling through the sample loop before reaching the analytical column. Injecting analytes dissolved in up to 100% organic solution showed excellent peak shape with no peak dispersion and excellent retention time and peak area reproducibility, while maintaining sensitivity.

MATERIALS AND METHODS

LC and MS systems

Eksigent ekspert™ microLC 200 system

AB SCIEX Triple Quad™ 5500 LC/MS/MS System

Experimental Conditions

LC

Column:	HALO C18, 0.5 × 50 mm, 2.7 µm
Flow Rate:	40 µL/min
Mobile Phase:	A) water, 0.1% formic acid
	B) acetonitrile, 0.1% formic acid
Column temperature:	40 ˚C
Sample loop:	20 µL
Gradient conditions:	Detailed in table 1

MS

Interface:	AB Sciex TurboIonSpray® ion source with 50 µm hybrid electrode
Temperature (TEM):	350 ˚C
Curtain Gas (CUR):	20
Collision Gas (CAD):	Medium
Ion Spray Voltage (IS):	5500
Ion Source Gas 1 (GS1):	20
Ion Source Gas 2 (GS2):	20

Time (min)	B %
0	5
0.5	5
2.5	90
3.0	90
3.1	5
5.0	5

Table 1. Gradient conditions for triazenes mix experiments

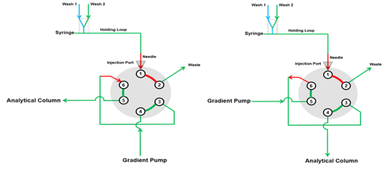

Figure 1. Injection valve in inject position, left is standard configuration and right is the Online-Diluting-and-Refocusing (ODR) configuration

RESULTS

Online focus

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 solvent smears out as a broadly distorted front or tail on the peak, or splits. This is illustrated in figure 2A, a chromatogram of triazenes sample prepared in 100% methanol. Figure 1B shows Online-Diluting-and-Refocusing (ODR) configuration with a sample loop volume > 10 µL. In this configuration, the highly organic sample partially fills the loop. It travels through the entire sample loop before reaching the top of the column. A gradient through the loop helps in focusing the analytes on the column. Figure 2B is a chromatogram of triazenes sample prepared in 100% methanol injected using the ODR configuration.

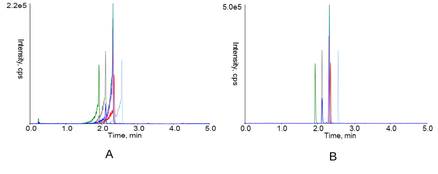

Figure 2. Comparison of the chromatography obtained in standard microLC injection configuration (A) and ODR configurations (B). 1 µL of triazenes mix in 100% methanol was injected for both configurations.

Large volume injection

In certain situations, sensitivity of standard UHPLC can be increased by injecting higher volume of sample. The same gains can be achieved using microLC 200. Figure 3 shows a chromatogram with 20 µL large volume injection with microLC where triazenes mix was prepared in 20% methanol at 1 ng/mL.

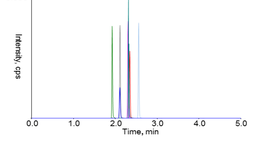

Figure 3. Chromatogram of large volume injection with microLC. 20 µL of 1 ng/mL triazenes mix in 20% methanol.

Figure 3 illustrates the ability to inject sample volumes as large as 20 µL to improve sensitivity. No peak distortion was observed with up to 20% MeOH

Polar analytes injection

Injecting poorly retained polar compounds prepared in high organic solvent may cause peak splitting as in figure 4A where a 2 µL of salbutamol in 25% acetonitrile is injected using a standard microLC injection configuration (figure 1A). Figure 4B is a chromatogram of 1 µL of the same sample injected using the ORD configuration (figure 1B), no peak splitting was observed.

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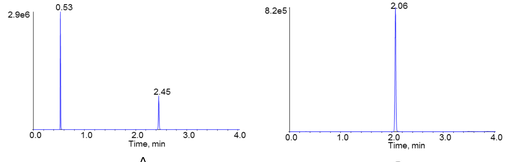

Figure 4. Chromatogram A: 2 µL salbutamol in 25% CH3CN. B: 1 µL salbutamol in 25% CH3CN: the majority of analyte elutes in the void.

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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