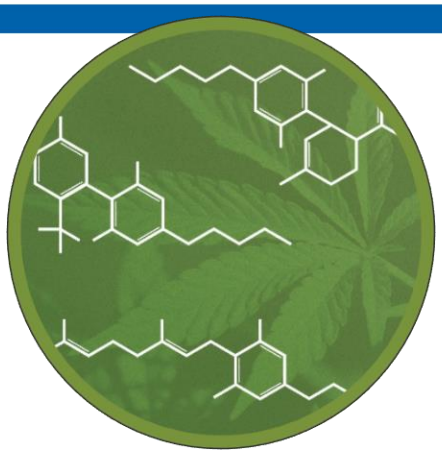

TN-1326

Defining Robustness of Pesticide and Mycotoxin Analysis in Cannabis Matrices

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Introduction

Sample clean-up is an important step for high-throughput LC-MS/MS analyses. The more contaminants that are removed during the clean-up step, the longer the LC-MS/MS will be able to maintain the required sensitivity. Unfortunately, cannabis matrices contain high concentrations of cannabinoids, waxes, terpenes, and other secondary metabolites which present a significant analytical challenge. These compounds have the potential to interfere with the analysis of pesticides, making it difficult to meet the ng/g sensitivity levels required by most recreational United States regulations and Canadian regulations.

In this technical note, the unique selectivity and increased retention for both polar and non-polar analytes of the Luna Omega Polar C18 column and the robustness of the SCIEX® Triple Quad™ 6500+ system was evaluated by injecting a cannabis flower extract 830 times with no system maintenance. The cannabis flower was spiked with a mixture of commonly monitored cannabis pesticides and the peak area of these pesticides was monitored over time, with and without internal standard correction.

Sample Preparation

A 1:10 dilution was performed using 5 g of homogenized cannabis flower extracted in 50 mL of 0.1 % Formic Acid in Acetonitrile. Extracts were winterized at -20 °C for 2 hours before filtration with 0.2 µm PTFE syringe filters. The extract was fortified with an analytical pesticide mixture and vortexed before being dispensed into equal 1 mL aliquots to be stored at 4 °C prior to LC-MS/MS analysis.

LC Conditions

Column: Luna™ Omega 3 µm Polar C18
Dimensions: 150 x 3.0 mm
Part No.: 00F-4760-Y0
Mobile Phase: A: 5 mM Ammonium Formate + 0.1 % Formic Acid in Water
B: 5 mM Ammonium Formate + 0.1 % Formic Acid in Methanol

Gradient:

Time (min)	%B
0	55
0.5	55
2.5	80
8.5	90
12.5	100
16.5	100
17	0
20	0

Flow Rate: 0.8 mL/min
Injection Volume: 2 µL
Temperature: 40 °C
LC System: SCIEX ExionLC™
Detection: MS/MS
Detector: SCIEX Triple Quad 6500+

Results and Discussion

Cannabis flower extracts are a particularly challenging matrix. Very few LC-MS/MS robustness studies have been conducted with this matrix, without MS system maintenance over a prolonged duration. When determining instrument stability using this type of robustness test, normalizing the analyte peak area to an internal standard (IS) area can be misleading, as the response from the internal standard and the native pesticide(s) are likely to change proportionately. Therefore, the IS ratio will stay consistent across many injections, as shown for Carbofuran (**Figure 1**, left), inaccurately suggesting ideal system performance despite the harsh conditions employed in this study.

The true measure of instrument robustness must be an evaluation of the uncorrected peak area as a function of time. Without MS system maintenance and given the conditions of this study, a decrease in peak area may be expected, as observed when the raw Carbofuran area is plotted (**Figure 1**, right).

However, these data show that the SCIEX Triple Quad 6500+ system, coupled with the Luna Omega Polar C18 column, achieves sensitivity that meets regulatory limits and reliably detects pesticides of interest in a complex matrix. These features persist over the analysis of 830 cannabis samples without cleaning the MS system.

An example of this robust sensitivity can be seen with Acequinocyl, which is hydrolytically unstable. It has poor ionization efficiency and coelutes with numerous cannabinoids late in the gradient. For all 830 injections, Acequinocyl was detected at a concentration 40x lower than Oregon Regulatory Limits (**Figure 2**, top). Additionally, Avermectin B1a, which is known for its thermal lability, was detected at a concentration 10x lower than Oregon Regulatory Limits after the 830 matrix injections (**Figure 2**, bottom).

It should be noted that although this study using this instrument shows that system maintenance isn't required during the study, regular maintenance of the column, LC system, and MS system should be done.

Figure 1. 830 Replicate Injections of Cannabis Flower Matrix Using a Luna™ Omega Polar C18 Column.

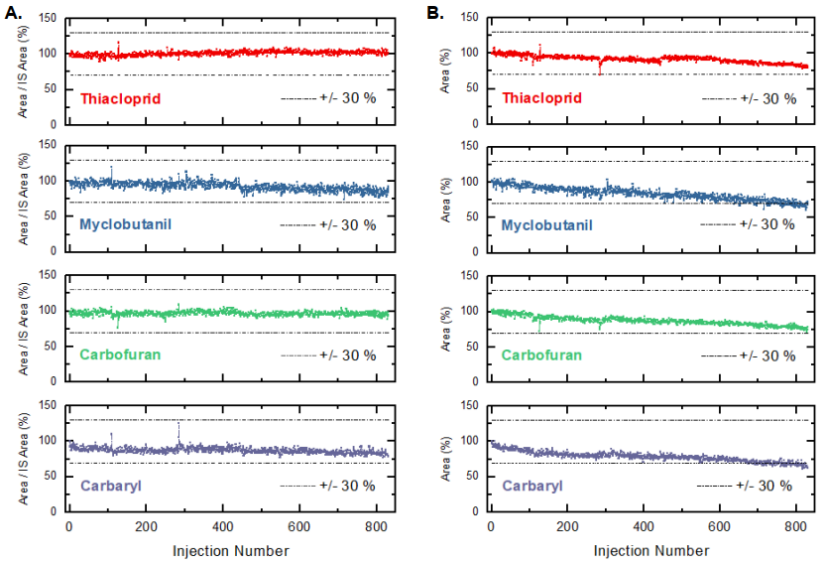
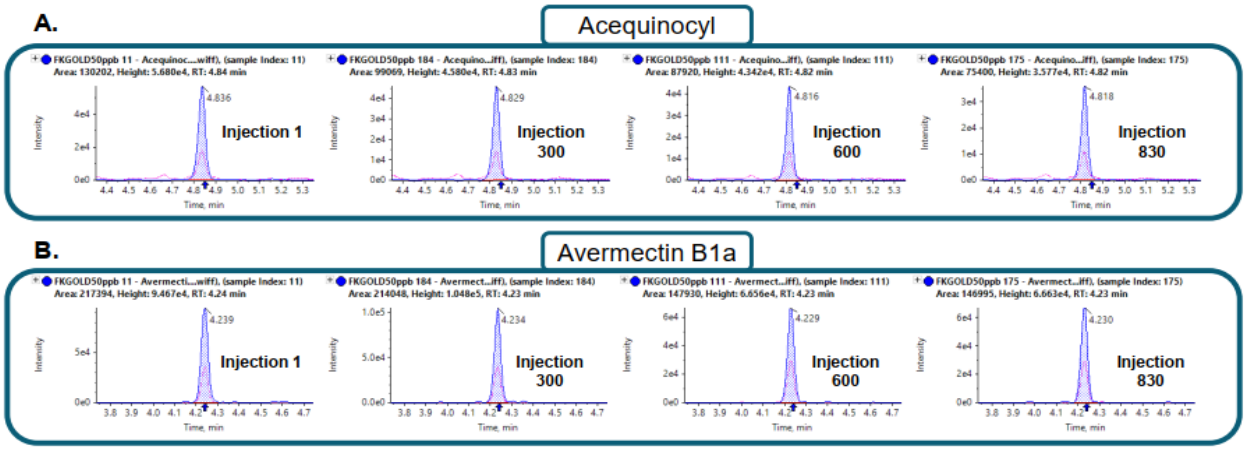


Figure 2. Stable Pesticide Peak Areas Across 830 Injections Using a Luna Omega Polar C18 Column.



Conclusion

The reality of analyzing a highly contaminating matrix is an inevitable decrease in sensitivity. In this technical note, it is shown that the way instrument robustness data is organized and presented can fail to capture changes in sensitivity over time. It is therefore important to assess both the ion ratio reproducibility (**Figure 2**) and the raw peak area reproducibility (**Figure 1**), as this will inform practical considerations in a testing lab such as how often an MS system must be cleaned to maintain sensitivity requirements.

Luna™ Omega Ordering Information

3 µm MidBore™ Columns (mm)			SecurityGuard™ Cartridges (mm)	
Phases	**50 x 3.0**	**100 x 3.0**	**150 x 3.0**	**4 x 2.0* /10pk**
Polar C18	00B-4760-Y0	00D-4760-Y0	00F-4760-Y0	AJ0-7600
PS C18	00B-4758-Y0	00D-4758-Y0	00F-4758-Y0	AJ0-7605
C18	00B-4784-Y0	00D-4784-Y0	00F-4784-Y0	AJ0-7611
SUGAR	—	—	00F-4775-Y0	AJ0-4496

for ID: 2.0 – 3.0 mm

*SecurityGuard Analytical Cartridges require holder, Part No.: KJ0-4282

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