

Defining robustness of pesticide analysis in cannabis matrices

Using the SCIEX Triple Quad 6500+ system

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Keep it clean to analyze more green

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 regulations¹⁻⁷ and Canadian regulations⁸. In this study, 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.

Methods

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-MS/MS: A 5-minute gradient was used to inject cannabis flower matrix repeatedly for analysis. An analytical 20-minute gradient representing a typical analysis strategy was injected every 10th sample for comparative analysis. The analytical column used was a Phenomenex 3 µm Luna Omega Polar C18 (3x150 mm) and chromatographic separation was achieved using 5mM ammonium formate with 0.1% formic acid in water and methanol.



Key features of the SCIEX Triple Quad 6500+ system for cannabis analysis

- Stable instrument sensitivity and robustness, demonstrated by 830 injections of a cannabis flower extract
- Peak area variance <5% RSD for carbaryl, carbofuran, myclobutanil and thiacloprid, corrected for with internal standard
- Uncorrected peak area responses at injection 830 were 64-97% of the peak area response of injection 1

Robustness data

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).

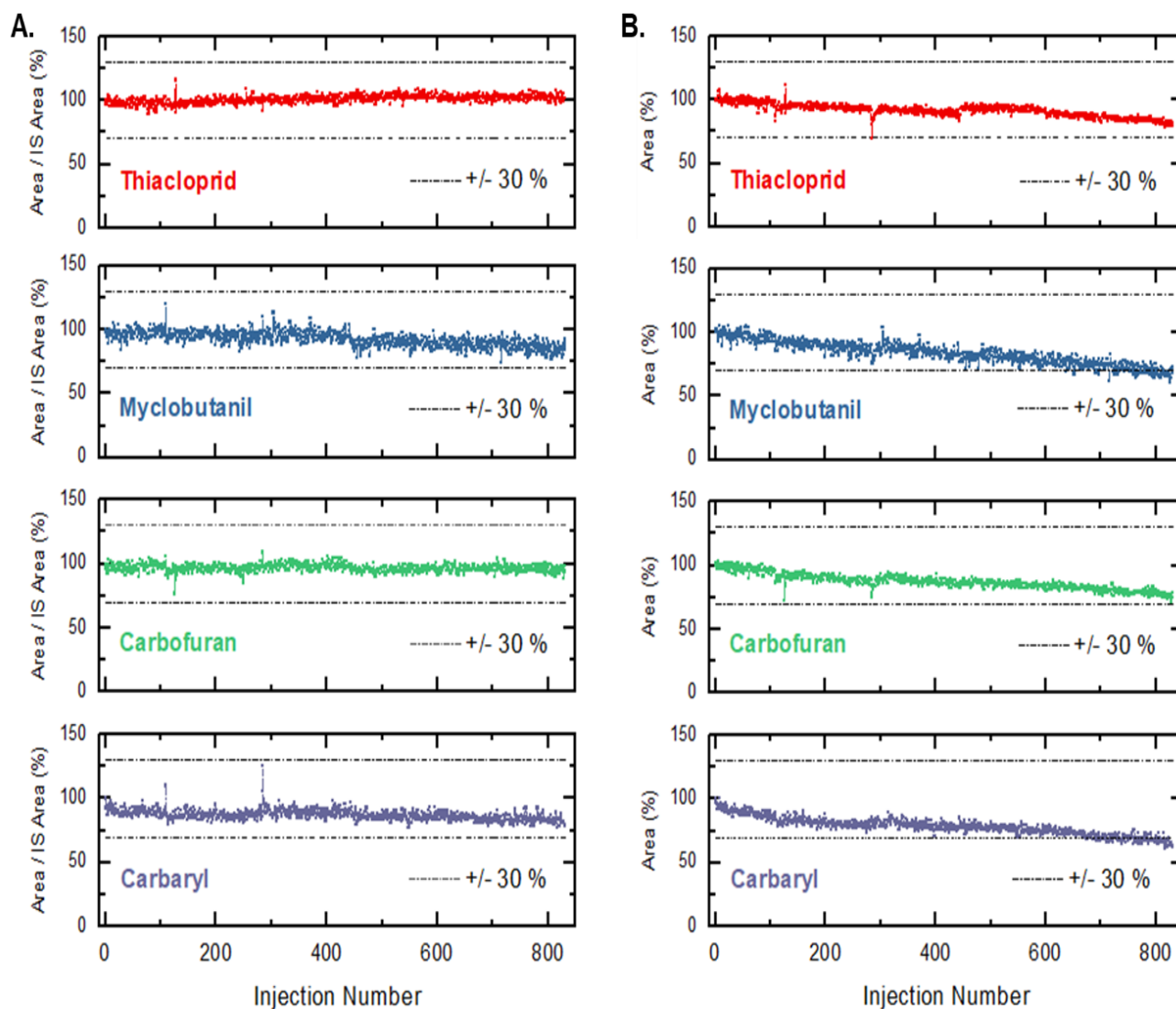


Figure 1. 830 replicate injections of cannabis flower matrix. IS-corrected (A) and raw peak area (B) responses for several pesticides in cannabis flower extracts over 830 injections without instrument maintenance. Raw areas illustrate the true measure of system robustness. Pesticides were fortified to 0.05 ppm in cannabis flower. The $\pm 30\%$ lines are relative to the analyte response for injection 1.

However, these data show that the SCIEX Triple Quad 6500+ system 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, 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 limits¹ (Figure 2, top). Additionally, avermectin B1a, which is known for its thermal lability, was detected at a concentration 10x lower than Oregon

regulatory limits⁵ after the 830 matrix injections (Figure 2, bottom).

Conclusions

The reality of analyzing a highly contaminating matrix is an inevitable decrease in sensitivity. In this application 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.

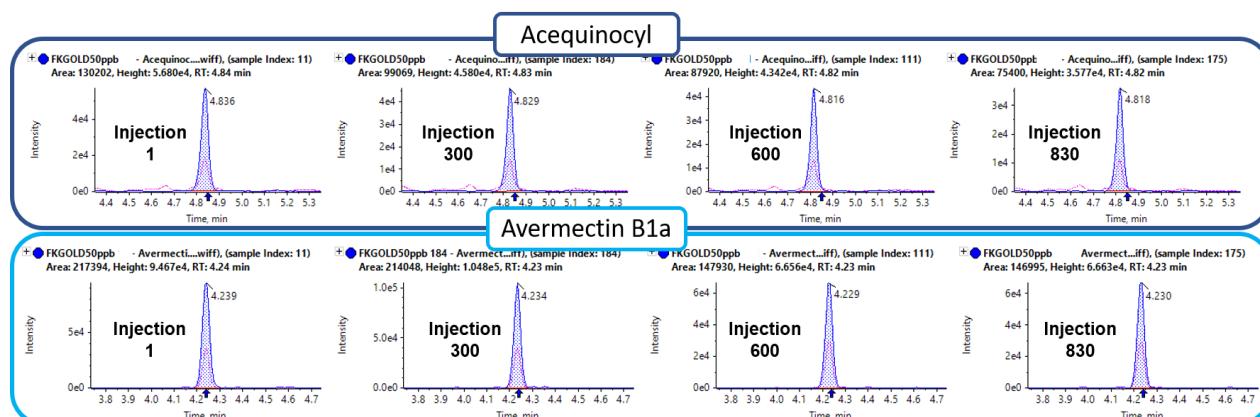


Figure 2: Stable pesticide peak areas across 830 injections. Acequinocyl is the last-eluting compound and normally is susceptible to cannabinoid suppression in matrix (top). Avermectin B1a is the primary component of Abamectin (bottom). Example chromatograms of each are shown throughout the instrument robustness test. Both quantifier and qualifier are clearly visible with no impact on ion ratio (blue and pink overlaid chromatograms) and excellent sensitivity is maintained, even under the extreme conditions employed for this robustness test.

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