Future-proofing Cannabis analysis with the SCIEX Triple Quad™ 7500 LC-MS/MS System – QTRAP® Ready

Low-level pesticide analysis, achievable with SCIEX mass spectrometry and powered by SCIEX OS Software

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Since 2015, certain states in the USA and some nations have implemented or begun discussions on the legalization of Cannabis. With this comes responsibility for ensuring the safety of consumer products. Compliance requirements around pesticide residues—allowance, detection, and tolerance limits—are varied among regions for which regulations exist. The trend, however, seems to be towards increasingly rigid requirements (more pesticides and lower detection limits). The Cannabis regulatory landscape is continuously evolving. Anticipation of more aggressive analytical requirements necessitates development of pesticide quantification methods which are as sensitive and robust as possible. Cannabis flower as a matrix represents an analytical challenge in complexity. Matrix interference and suppression affect the ability of current methods to deliver the required results. Advancements in analytical technologies represent promising avenues for residue detection in Cannabis in a changing regulatory landscape.

In order to assess the latest advancements in triple quadrupole technology and its potential for residue analysis in Cannabis, the Canadian regulated pesticide list (excluding Kinoprene) was used as a panel for quantitative analysis. The Canadian approach to pesticide regulation in Cannabis is uniquely characterized by a large panel of analytes and very low tolerance limits required in testing.[2,3] With the method developed here, very low level detection limits were achieved using very small injection volumes, which illustrates the performance of the SCIEX Triple Quad 7500 LC-MS/MS System – QTRAP Ready.[1]

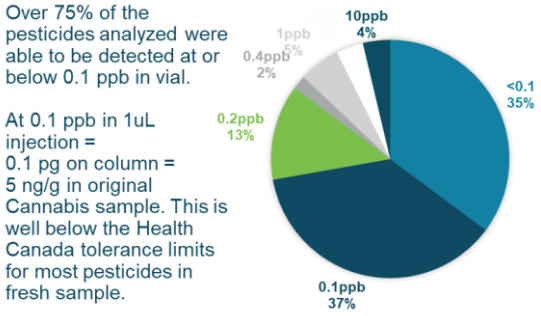


Figure 1. Extremely low concentration detection of pesticides. The pie chart shows the percentage of the analytes in the pesticide panel which were measured at each LOQ value. The SCIEX 7500 LC-MS/MS System pushes the ability to see commonly analyzed pesticide residues to very low levels, even allowing the method to leverage very low injection volumes of 1 µL. The translation to detection limits in fresh sample is significant to regulatory language.

Key features of the SCIEX 7500 System for pesticides analysis

- Limits of detection at or below 0.1 ppb in vial in neat standards for many of the compounds on the Health Canada list of pesticides in Cannabis
- Low level quantification achieved in complex fortified Cannabis matrices
- Additional sensitivity allows use of larger dilutions of sample, protecting the front end from contamination and extending uptime between routine cleanings (robustness)
- Added sensitivity also provides flexibility to adapt to future tightening of regulations and tolerance limits—"future proof"
- Low volume injection keeps matrix introduction minimal over time, robustness testing demonstrated exemplary consistency over >2000 injections[4]

Methods

Sample preparation: Calibration standards of known concentrations of the pesticide mixture were prepared in neat solvent, while samples of Cannabis flower were minimally prepared using solvent extraction in acetonitrile and dilution for LC-MS/MS analysis. Testing Cannabis samples, which have gone through very little matrix cleanup, was done to assess method performance in challenging matrix conditions.

The analyte panel designated by the Canadian regulations regarding pesticide testing was used to define the pesticide targets for this method.

Sample preparation followed previous protocols for Cannabis analysis.[2,3]

Chromatography: An injection volume of 1 µL was separated on a Phenomenex Luna Omega Polar C18 (2.6 µm x 100 mm) using a flow rate of 0.4 mL/min (Table 1).

Mass spectrometry: The SCIEX 7500 System was employed to assess trace level quantitative performance of the analyte panel in both neat standards and the flower extracts.

Two Multiple Reaction Monitoring (MRM) transitions were used for each analyte in the 95-compound panel. For each transition, voltages were optimized per analyte for compound-specific parameters such as collision energy (CE). Ion source parameters are broadly applied and hence more generic settings are selected which will work well for all analytes in the method (Table 2).

Data processing: All data were processed using the SCIEX OS Software. SCIEX OS Software for data acquisition is the latest software development for the SCIEX triple quadrupole platform and has integrated all quantitative data processing functions, enabling a single software platform to be used from the start of sample analysis through to results reporting.

Table 1. LC gradient.

Time (min)	B (%)
0.75	*5*
8	*100*
8.5	*100*
9	*5*
10	*End*

Mobile phase A - 0.1% formic acid in water, 5mM ammonium formate
Mobile phase B - 0.1% formic acid in acetonitrile, 5mM ammonium formate

Table 2. OptiFlow® Pro Ion Source Parameters.

Parameter	Value
CAD	*10*
CUR	*32 psi*
GS1	*40 psi*
GS2	*70 psi*
IHT	*200*
IS	*1500 v*
TEM	*400°C*

SCIEX 7500 System for Cannabis analysis

The primary goal of this work was to both assess the system sensitivity in the solvent-based calibration curves, and also the performance in the highly challenging Cannabis matrix. Another important test of the system includes the robustness of the hardware and its resistance to performance decline with accumulated matrix. In other work, the robustness of the novel entrance optics was assessed by comparing analyte signals in a complex matrix over continuous injections.[4]

Gains in ion transfer efficiency can result in increased baseline, which can impact quantitative performance. Typical signal gains observed on the system ranged from a factor of 3 to more than 10 times.[1] Compounds were examined over a wide linear dynamic range covering up to 5 orders of magnitude.[1]

The robustness and selectivity of any Cannabis method is absolutely critical, given the historical challenges that this very complex matrix poses. Figures 2-6 illustrate the sensitivity and performance of the method for some example pesticides. The chromatograms shown represent increasing concentrations of standard calibrators in neat solvent solution.

The sensitivity of the instrument enables the assessment of method performance using very small injection volumes of 1 µL. Comparison of “mass on column” must be accounted for when comparing sensitivity between analytical methods. The translation of the in-vial concentration to the mass on column and also the corresponding concentration in the original fresh sample (based on the sample preparation procedure) can be seen in Table 3.

Table 3. Translation of in-vial LOQ values to on-column analyte mass and original concentration in Cannabis sample. The mass on column value is critical for comparing LOQs across acquisition methods that differ by platform, injection volume, or separation strategy. The value for LOQ in sample what most regulatory limits are based on, and its relationship to the LOQ in vial is dependent on sample extraction and preparation as well as injection volume.

LOQ in vial (ppb)	Mass on column for 1 µL injection (pg)	LOQ in sample (ng/g)
0.1	*0.1*	*5*
0.2	*.0.2*	*10*
1	*1*	*50*

Sensitivity: Most pesticides in the 95-compound panel were detected at levels below 1 ppb in the vial. Roughly half of them were measured at a lower limit of 0.1 ppb or lower (Figure 1). This is particularly notable as these values were achieved using a dilute-and-shoot method with a small (1 µL) injection volume.

Figure 1 is a breakdown of the lowest concentration limits observed, and how many pesticides were quantifiable at each calibration level. The lowest calibration level tested for this method was 0.1 ppb, and while 37% of the analyzed species had LOQs observed at this level, 35% of the analyzed species had a high enough signal at this level that the actual LOQ could be assumed to be a lower concentration. Figure 3 illustrates the sensitivity of this method on the SCIEX Triple Quad 7500 LC-MS/MS System – QTRAP Ready for three example pesticides. The chromatograms shown represent increasing concentrations of standard calibrators in neat solvent solution.

Linear response: The lower levels of detection observed for much of this panel result in a calibration curve which is linear in its range to a lower concentration than previous methods on earlier mass spectrometry platforms. However, this can impact the linear response of the upper concentration range as it becomes limited by detector saturation. In Figure 2, two examples of calibration curves are shown. For oxamyl, the response from 0.1 to 100 ppb remains linear. For spirotetramat, however, the signal saturation is apparent at the highest concentrations, as seen by a plateau in the calibration curve. The observed linear range for the majority of the panel extends from 0.1 to 100 ppb; at higher concentrations than 100 ppb, detector saturation starts to be observed for most analytes.

Reproducibility and robustness: Hardware robustness is absolutely critical for routine analysis of low-level residues in a matrix as challenging as Cannabis. Many laboratories struggle with maintaining instrument uptime and performance with constant injection of this matrix, as cleaning and establishing decontamination can be time consuming and frequent. The sample volume is a critical aspect of business operations, and many matrix cleanup procedures have been demonstrated to have detrimental impact to method sensitivity and analyte recoveries.

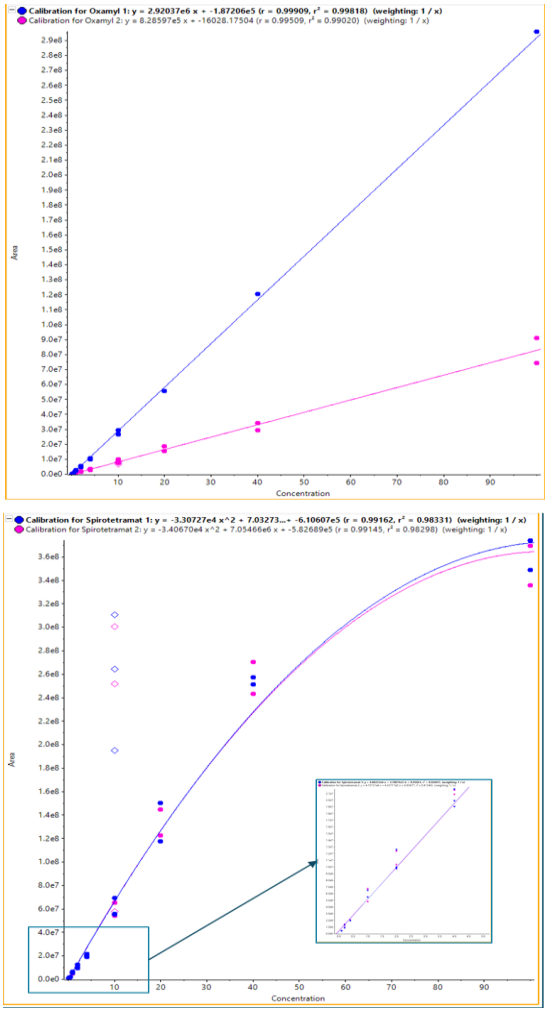
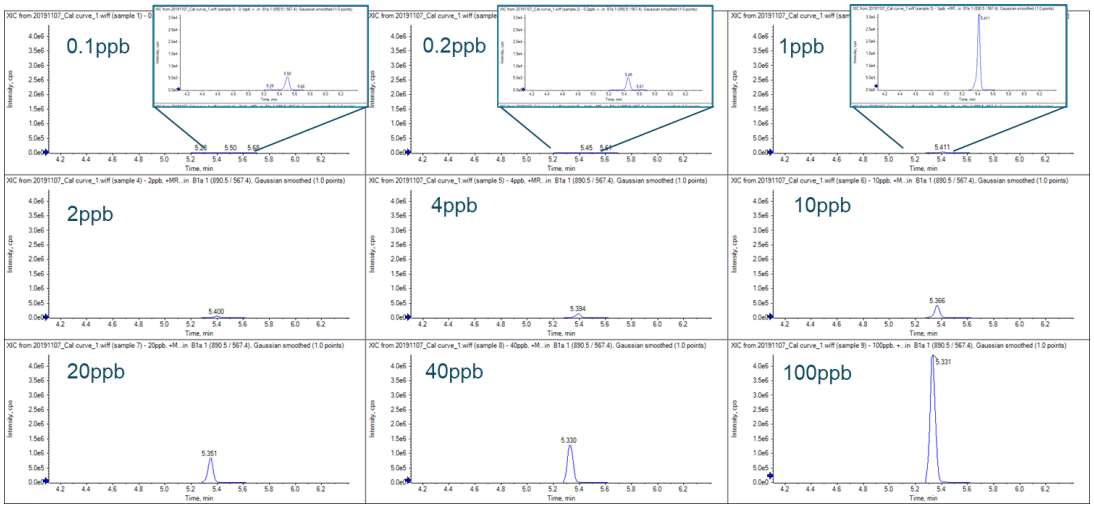


Figure 2. Two calibration curves demonstrate the difference between compounds in the increased sensitivity and its impact on the linear range. (Top) Oxamyl maintains a linear response across the concentration range of 0.1 to 100 ppb. (Bottom) Spirotetramat calibration curve plateaus at the high concentration end due to detector saturation. The low end of the concentration range still behaves linearly.

Avermectin B1a



Chlorfenapyr

MGK-264

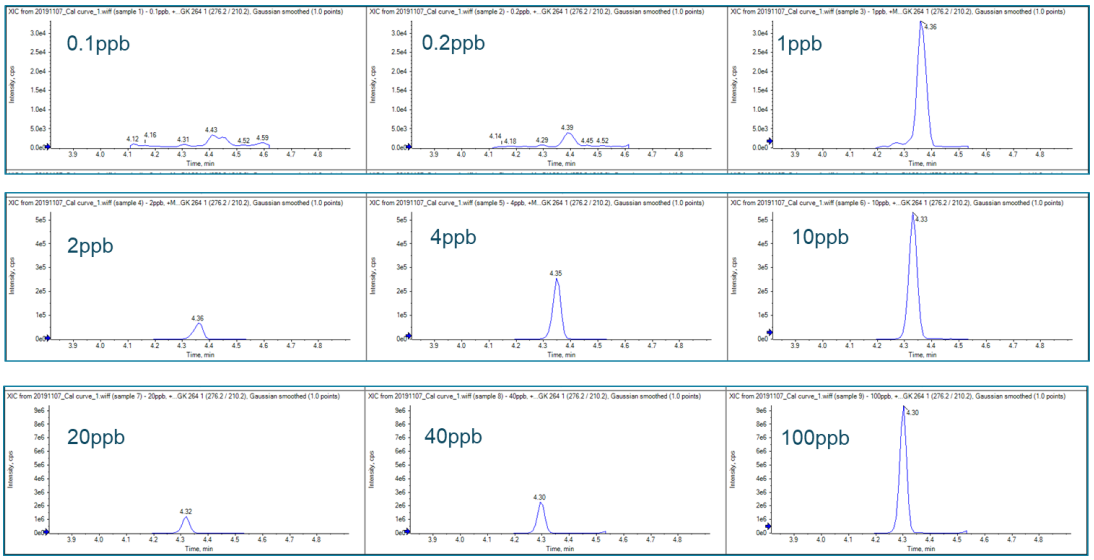


Figure 3. Examples of low-level detection of pesticides. Data is shown for three selected pesticides at increasing concentrations of standard calibrators in neat solvent solution, for avermectin B1a (top), chlorfenapyr (middle), and MGK-264 (bottom).

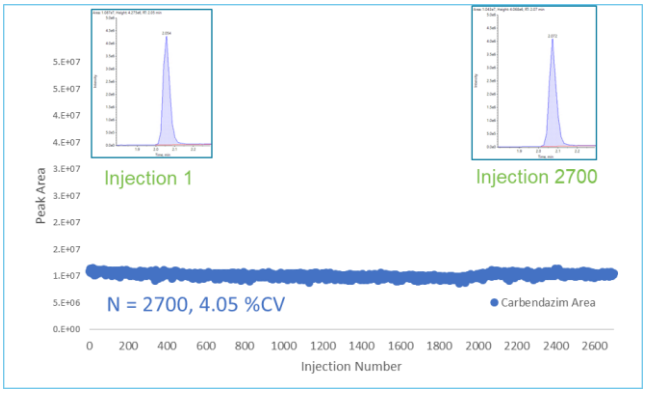


Figure 4. Excellent reproducibility and robustness in matrix. A short LC-MS run was performed (4 min gradient at 400 µL/min flow rate) for this specific test. The raw peak area was plotted across 2700 injections. The S/N also remained constant across injections.

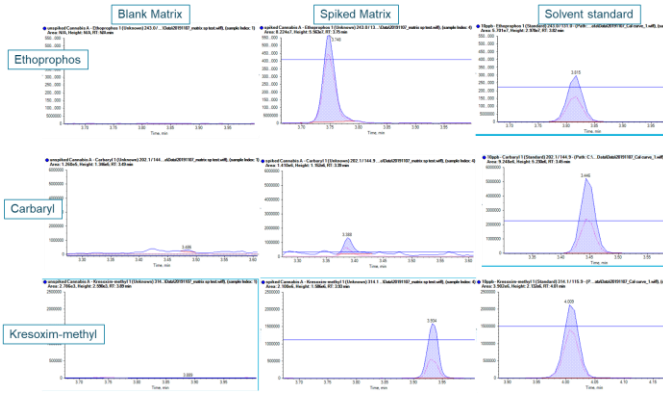


Figure 5. Examples of pesticide peak quality and detection in matrix vs. in solvent.

This Cannabis analysis method utilizes a "dilute-and-shoot" procedure, in order to test how the instrument will fare, but a small injection volume and limited sample size restrict the conclusions that can be made with regards to robustness. However, a test was executed in order to demonstrate extreme robustness and reproducibility across a great number of injections in a complex matrix. This test injected over 2000 samples of pesticide mixture in a black tea matrix used exclusively for this purpose. Figure 4 shows exemplary consistency from the first to the last injections with no cleaning or maintenance having taken place over the course of the test.

Analysis in Cannabis: The calibration standards and curves were built with standards made in neat solvent. This is a typical practice in food and Cannabis testing, which allows a calibration curve to be applicable to a variety of matrix types. In order to additionally assess how the analytes will look in Cannabis samples, three different flower samples were measured with and without fortification. Figure 5 illustrates three example pesticides as they are observed in unspiked matrix, spiked matrix, and neat solvent standard of corresponding in-vial concentration.

As discussed above, Health Canada has set limits for pesticides in Cannabis products which currently represent the most stringent in the world of Cannabis regulation. These limits are typically defined as a mass of analyte per mass of sample. Therefore, comparison of method sensitivities must take into account the sample preparation protocol, as well as the injection volume. These parameters are easily adjusted for optimization or method development, and as such, can vary widely between laboratories monitoring the same or similar panels, but must be understood to make direct comparisons regarding detection limits. Table 3 shows the direct correlation between the LOQs determined with this method and the mass on column and corresponding LOQ in sample. The mass on column value is critical for comparing LOQs across acquisition methods that differ by platform, injection volume, or separation strategy.

Health Canada limits in Cannabis plant samples vary across the compound panel but range from 0.01 to 1.5 µg/g, or 10 to 1500 ng/g.

$$\left(10\frac{ng}{g}\right) x\ (1g\ of\ sample) = 10\ ng\ analyte$$

$$\frac{10ng}{5\ mL\ extraction\ solvent} = \frac{2ng}{mL}(ppb) in\ extract$$

In this method, the extract was then diluted 1:10

$$\frac{\frac{2ng}{mL}}{10} = 0.2\frac{ng}{mL}(ppb) LOQ\ in\ vial\ required\ for\ the\ lowest\ limits\ of\ the\ Canadian\ list$$

$$OR: 0.2\ pg\ mass\ on\ column$$

Conclusions

The SCIEX Triple Quad 7500 LC-MS/MS System – QTRAP Ready was used to analyze a panel of pesticides from the Health Canada regulations for residues in Cannabis. It was foremost important to evaluate and understand the absolute sensitivity of the LC-MS/MS system and to investigate how the method would perform in a challenging matrix extract. It was found that sub-ppb levels were easily achievable even with sample dilution and using a small injection volume. This suggested that the SCIEX 7500 System is highly promising for the industry of residue testing in the routine laboratory, addressing the specific challenges faced by these laboratories. Using larger dilutions and smaller injection volumes will help these laboratories maintain maximum uptime and reduce the need for cleaning and decontamination.

The SCIEX OS Software platform for acquisition and processing makes the data collection and processing seamless from sample to report, and advanced features accelerate and streamline the quantification process. Having access to the greatest possible sensitivity in an analytical platform allows the testing lab to be agile and adaptable to dynamically changing regulatory landscape—"future-proofing" the lab's analytical capability.

References

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