Analysis of low-level pesticide residues in cannabis concentrates

Quantifying pesticides in wax, isolate and gummy matrices using the SCIEX 7500 system

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In this application note, we leveraged the sensitivity of the SCIEX 7500 system to achieve the low concentrations of pesticides in complex matrices like wax, isolate and gummies needed to meet regulatory guidelines. This added sensitivity allowed for us to combine significant sample dilutions with small injection volumes resulting in improved in-matrix method performance.

The legalization of cannabis and hemp in the United States has driven the need for analytical techniques to support consumer protection. These include chemical tests for potency, heavy metals, residual solvents, terpenes, mycotoxins and pesticides.[1] Accuracy and precision are particularly difficult in cannabis matrices, as the high concentration of cannabinoids (up to 80% in many concentrates), terpenes and other plant metabolites may cause chemical interferences. Additionally, those interferences change with the variety of cannabis and hemp matrices that require testing. This is especially true for pesticides and mycotoxins where the low maximum residue limits (MRLs) and a generally high number of residues required for testing (as seen in Canada[2] and states like Oregon[3], California[4], Pennsylvania[5] and Colorado[1,6]) may lead to disproportion issues with chemical interferences.

One of the simplest and most commonly applied extraction techniques for cannabis matrices involves a simple liquid-liquid extraction with acidified acetonitrile. However, this extraction is relatively non-selective, and many other contaminants are also co-extracted, making these matrices difficult to analyze, especially at the low concentrations required by constantly evolving state regulations.

Key features of the SCIEX 7500 system for cannabis analysis

- Quantification of 102 pesticide residues in three cannabis concentrate matrices using a simple extraction procedure.
- Matrix effects were minimized due to the high sample dilution that is enabled by method sensitivity.
- Reproducibly quantify pesticides at regulatory limits in notoriously difficult cannabis concentrate matrices.

SCIEX 7500 system

Methods

Sample preparation: A 1:100 dilution was performed by extracting 0.2 g of homogenized cannabis matrix in 20 mL of 0.1% formic acid in acetonitrile. Extracts were frozen at -20ºC for 2 hrs before filtration with 0.2 µm PTFE syringe filters. The extracts were then fortified with an analytical pesticide mixture prior to analysis by LC-MS/MS.

LC-MS/MS: A 20 min gradient separation was employed using a Phenomenex 3 µm Luna Omega Polar C18 (3x150 mm) column for both ESI and APCI analyses. Chromatographic separation of a 1 µL injection was achieved using 5mM ammonium formate with 0.1% formic acid in water and methanol for ESI. For APCI, unmodified water and methanol were used and the injection volume was 4 µL. All samples were analyzed in triplicate.

Data processing: Data was acquired and processed using SCIEX OS 3.0 software. The custom calculations feature of SCIEX OS facilitated automatic MRL flagging (as specified by the user) for quick and easy visual analysis of results outside regulatory compliance.

Data and Discussion

Pesticide analysis in cannabis products is challenging due to the variety of sample matrix types, all with unique LC-MS/MS interferences and ion suppression potential. Sensitivity in flower matrices may not translate to sensitivity in a concentrate matrix, such as wax, which typically has a much higher total cannabinoid content. There may also be differences between hemp and cannabis processed products due to the different potency levels of CBD and THC, which can impact ion suppression of pesticides in those matrices. Leveraging the sensitivity of the SCIEX 7500 system, a larger dilution of cannabis matrices is achieved, thereby decreasing the amount of potential contamination introduced to the mass spectrometer. In addition to injecting a smaller amount of material on column, the SCIEX 7500 system enables detection limits of part per billion (ppb) concentration of pesticide residues in cannabis wax, isolate and gummy. To demonstrate the sensitivity of the pesticides in wax, isolate and gummy, the extracts were spiked at 0.01 ppm to 1000 ppm in product. The wide calibration range was used to test for linearity and reproducibility. Replicates at the low end of the calibration curve were then used as a measure of sensitivity in matrix. In Figures 1-4, a matrix blank was shown to differentiate unfortified matrix and spiked matrix. The representative figures show pesticides spiked at or below the MRLs from the Colorado Hemp regulations.[1] Even with a large dilution of 1:100 during sample extraction, sensitivity of pesticides varied from matrix to matrix (Figures 1-4). Benzovindiflupyr has very little matrix suppression differences between the three matrices tested (Figure 2), as opposed to Avermectin B1a which is shown to be more suppressed in wax compared to gummy and isolate (Figure 1). While the large dilution minimized matrix induced ion suppression, it did not completely eliminate it. Therefore, deuterated internal standards are recommended to correct for these matrix effects and to enable accurate quantification of pesticide residues. Kinoprene is a pesticide residue typically derivatized for gas chromatography mass spectrometry (GC-MS/MS) analysis, however, it also can be analyzed by LC-MS/MS using APCI. An interference was observed in the wax matrix that was unable to be separated out using chromatography alone (Figure 4) and may benefit from additional sample clean up to achieve the same sensitivity in the isolate and gummy matrices. The next calibration point was shown for linearity of response for increasing concentrations.

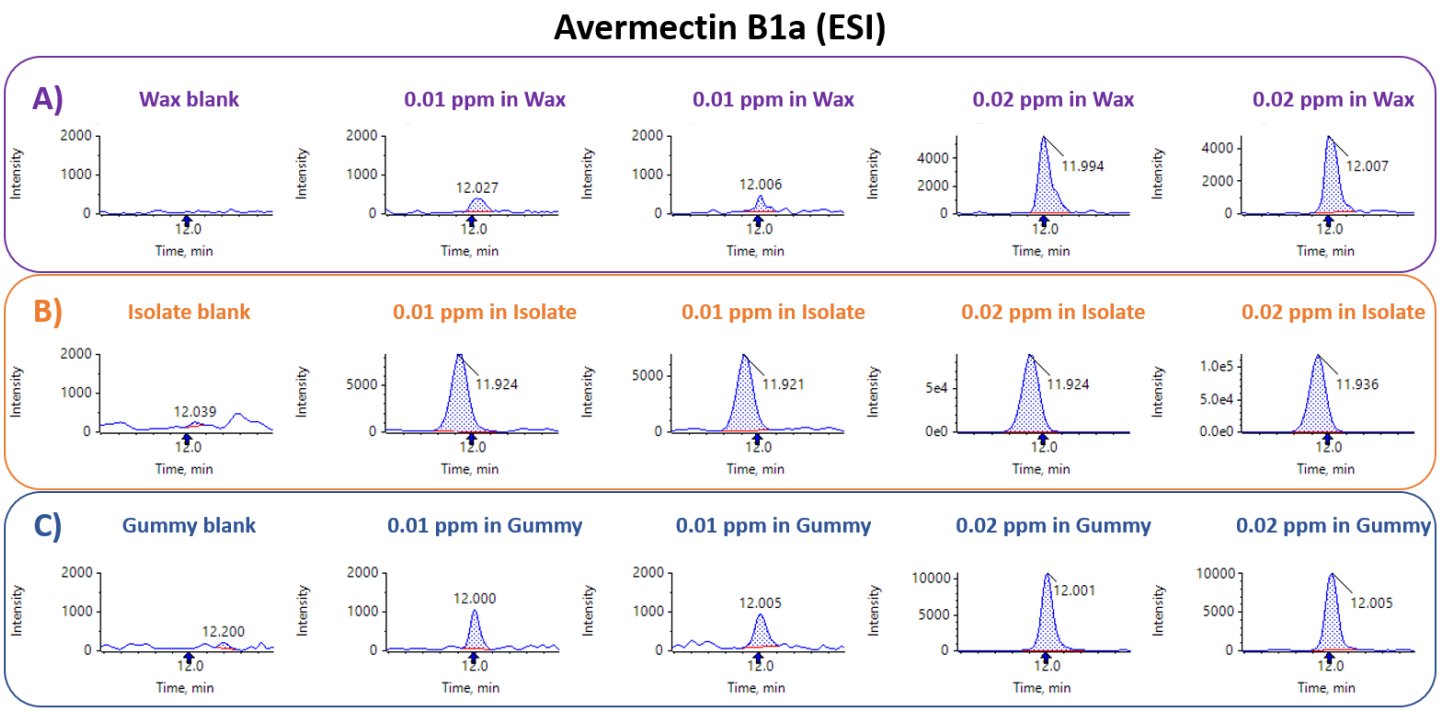

Figure 1. Example chromatograms of avermectin B1a in wax (A), isolate (B) and gummy (C) unfortified as a matrix blank and fortified at two different concentrations analyzed in duplicate to demonstrate reproducibility and sensitivity.

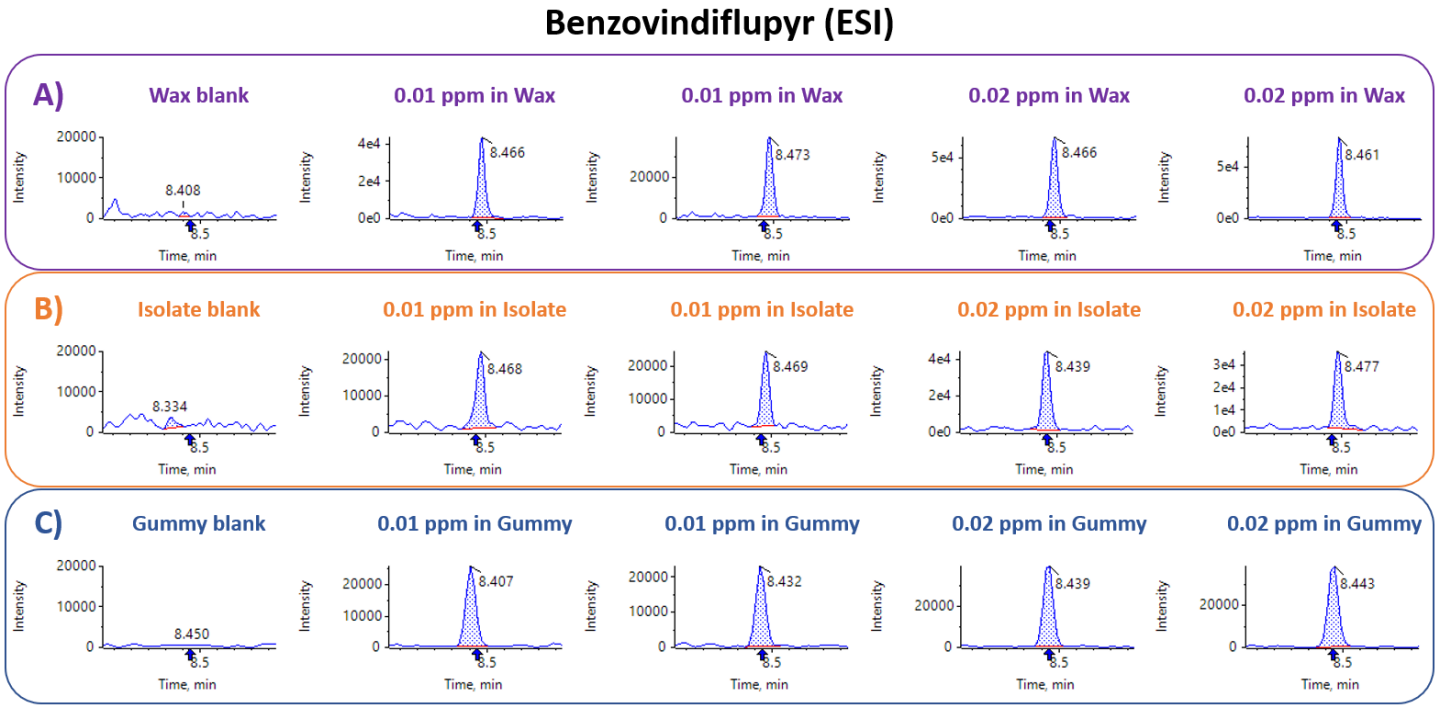

Figure 2. Example chromatograms of benzovindiflupyr in wax (A), isolate (B) and gummy (C) unfortified as a matrix blank and fortified at two different concentrations analyzed in duplicate to demonstrate reproducibility and sensitivity.

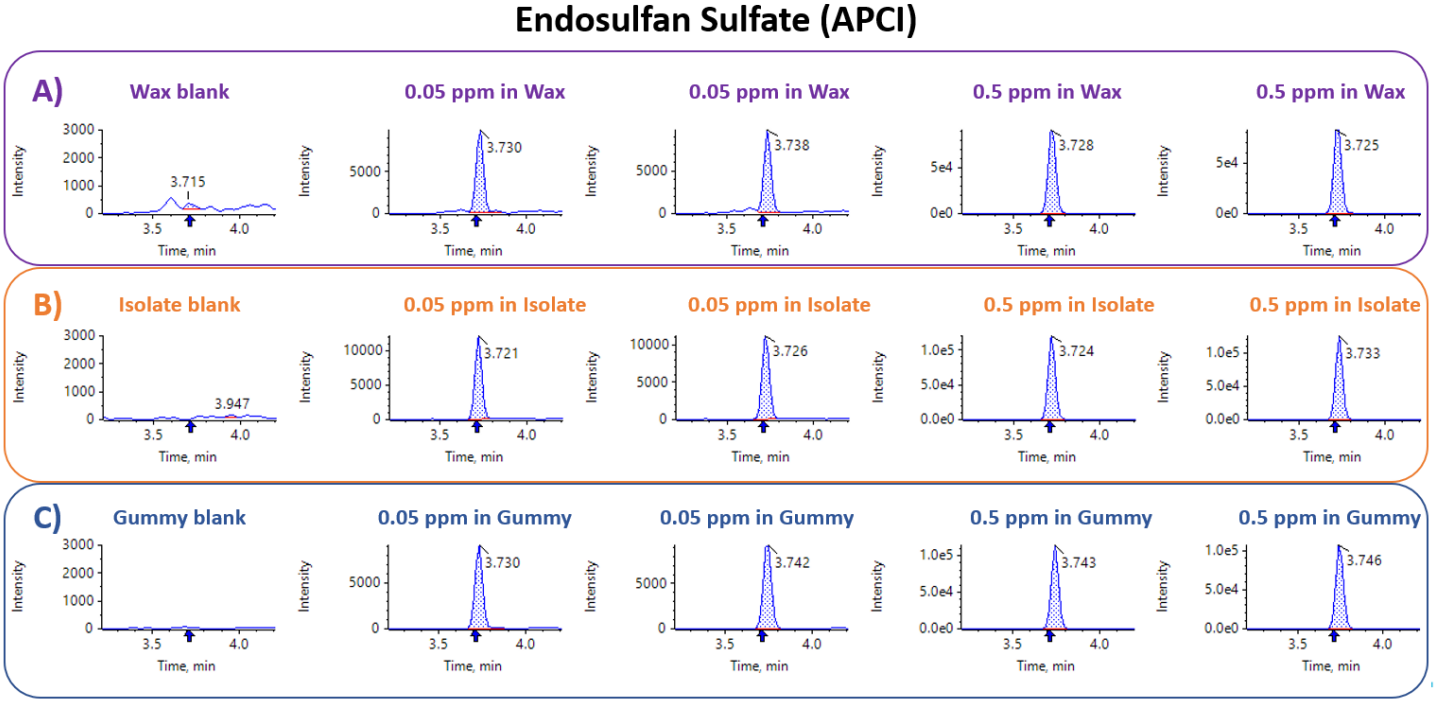

Figure 3. Example chromatograms of endosulfan sulfate (APCI) in wax (A), isolate (B) and gummy (C) unfortified as a matrix blank and fortified at two different concentrations analyzed in duplicate to demonstrate reproducibility and sensitivity.

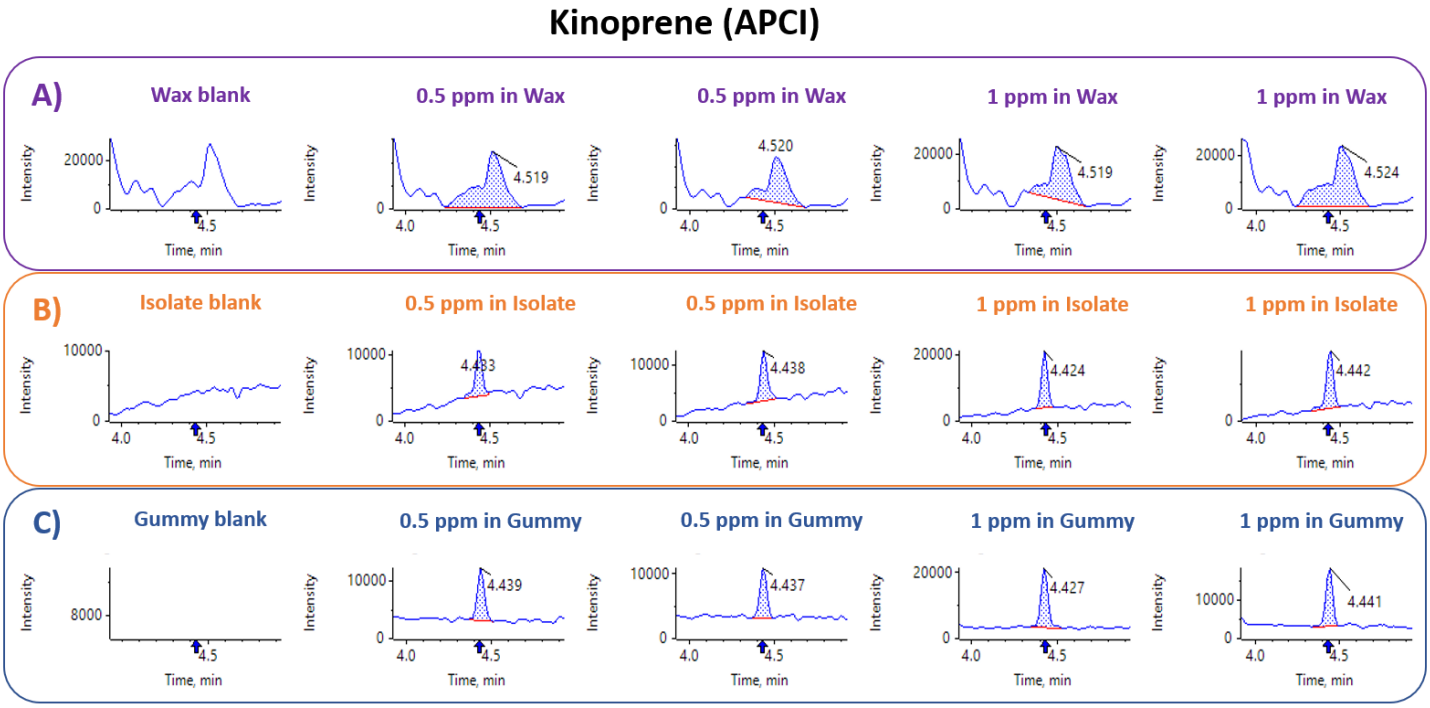

Figure 4: Example chromatograms of kinoprene in wax (A), isolate (B) and gummy (C) unfortified as a matrix blank and fortified at two different concentrations analyzed in duplicate to demonstrate reproducibility and sensitivity.

Conclusion

This study has shown that SCIEX 7500 system can detect all 102 targeted residues in the three different matrices tested using a large sample dilution. The large dilution allowed for the reduction of matrix impacts like high background signal and ion suppression. This reduction in matrix impact was observed in all of the tested cannabis concentrate matrices at varying concentrations. Although additional improvements may be needed to decrease detection limits for certain pesticides, this LC-MS/MS method can be used as a starting point for large pesticide panels in cannabis matrices other than flower.

References

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