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An Automated LC-MS/MS Workflow for High-throughput Pesticide Residue Screening in Cannabis Samples

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Introduction

Recreational and medicinal cannabis has been legalized for use in various U.S. states in recent years. As a result, state lawmakers have introduced legislation detailing minimum acceptable levels of pesticides in retail material. While working toward compliance with regulations, testing laboratories have been challenged by changing target pesticide lists, increasing varieties of complex matrices, growing throughput requirements, and time-consuming data analysis.

To address these difficulties, a workflow has been developed consisting of a sample preparation procedure, an optimized analysis method, and a novel process-control software to automate data acquisition, processing, and reporting. Together, these components allow cannabis testing labs to reliably screen for pesticides with high-throughput. Here, we show results from LC-MS/MS screening of Category 1 and 2 pesticides in cannabis flower extract.

Experimental

Sample Preparation

All reagents and solvents used in this study were HPLC grade. Pesticide mixes representative of California State regulations were obtained from LGC USA at a concentration of 100 µg/mL. The stock solution of pesticides including 66 compounds was prepared at 0.5 µg/mL (ppm) level and used for spiking the cannabis extracts. Concentration of pesticides in matrix matched calibration standards ranged from 0.1 to 50 ng/mL(ppb).

Agilent cannabis sample preparation protocol [1] was used in this workflow to prepare cannabis flower extracts (Figure1):

Step1: Add 1.0 g of chopped cannabis flower into a 45-mL tube and two ceramic homogenizer pellets, then shake for five minutes at high speed.

Step2: Add 15 mL of acetonitrile (ACN) and shake for five minutes at high speed.

Step3: Centrifuge at 5,000 rpm for five minutes.

Step4: Decant supernatant solvent into an unconditioned SampliQ C18 EC SPE Cartridge. Add 5 mL of ACN to the original tube, stirring up the pellet of plant material, and shake for three minutes at high speed. Transfer all solids and ACN to the SampliQ SPE cartridge. Wash the tube with another 5 mL of ACN, and transfer to the SPE cartridge.

Step5: Bring the collected extract up to 25 mL using ACN.

Step6: Take 50 µL of extract and mix with 450 µL of 25% Water: 75% methanol (v/v) in a sample vial.

Experimental

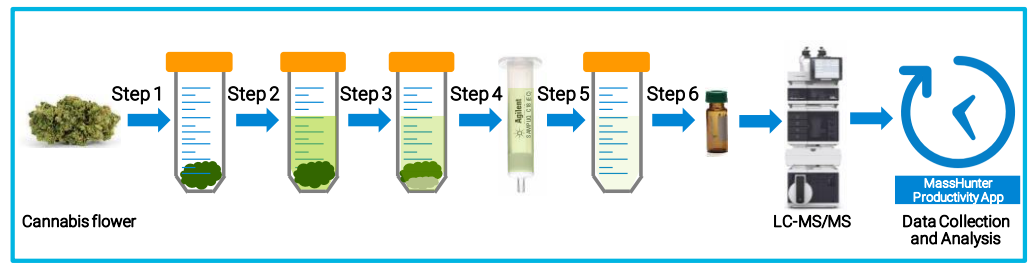

Figure 1. Cannabis sample preparation for LC-MS/MS analysis.

Sample Analysis

Cannabis extract samples were analyzed using an Agilent 1290 Infinity HPLC coupled to an Ultivo B triple quadruple LC/MS system. The Ultivo B was equipped with an Agilent Jet Stream (AJS) ESI source and operated in dynamic MRM (dMRM) with polarity switching.

Figure 2. Agilent Technologies' Ultivo LC/TQ.

Instrument Parameters

Agilent 1290 Infinity II UHPLC Parameters		
Guard Column Column	Poroshell 120 Phenyl-Hexyl, 2.1 × 5 mm, 2.7 µm Poroshell 120 Phenyl-Hexyl, 3.0 × 100 mm, 2.7 µm	
Column temp	55°C	
Injection volume	10 µL (with injector program/pretreatment)	
Mobile phase	A: 0.1% formic acid + 5 mM ammonium formate in water B: 0.1% formic acid in MeOH	
Flow rate	0.500 mL/min	
Gradient	Time (min) 0.00 1.00 2.00 8.00 9.00 9.50 9.51	B% 30 30 75 96 100 100 30
Stop-time	9.51 min	
Post-time	11.0 min	

Injector program/pretreatment	
Draw	10 µL from location 1 with default speed using default offset (100 % deionized water)
Draw	default volume from the sample with default speed using default offset
Wash	Wash needle in flush port for 30 s (100 % methanol)
Draw	10 µL from location 1 with default speed using default offset (100 % deionized water)
Mix	30 µL volume from air with maximum speed five times
Inject	

Agilent Ultivo Triple Quad MS Parameters	
Ionization source	AJS ESI (positive and negative polarities)
Gas temperature	350 (°C)
Drying gas flow	13(L/min)
Nebulizer gas	55 (psi)
Capillary	5500 (V)
Sheath gas temperature	200 (°C)
Sheath gas flow	10 (L/min)
Nozzle voltage	500 (V)

Streamlining Pesticide Screening Using MassHunter Productivity App

MassHunter Productivity App streamlines the sample analysis workflow (Figure 3) and provides a unified user experience from run setup to data review and reporting.

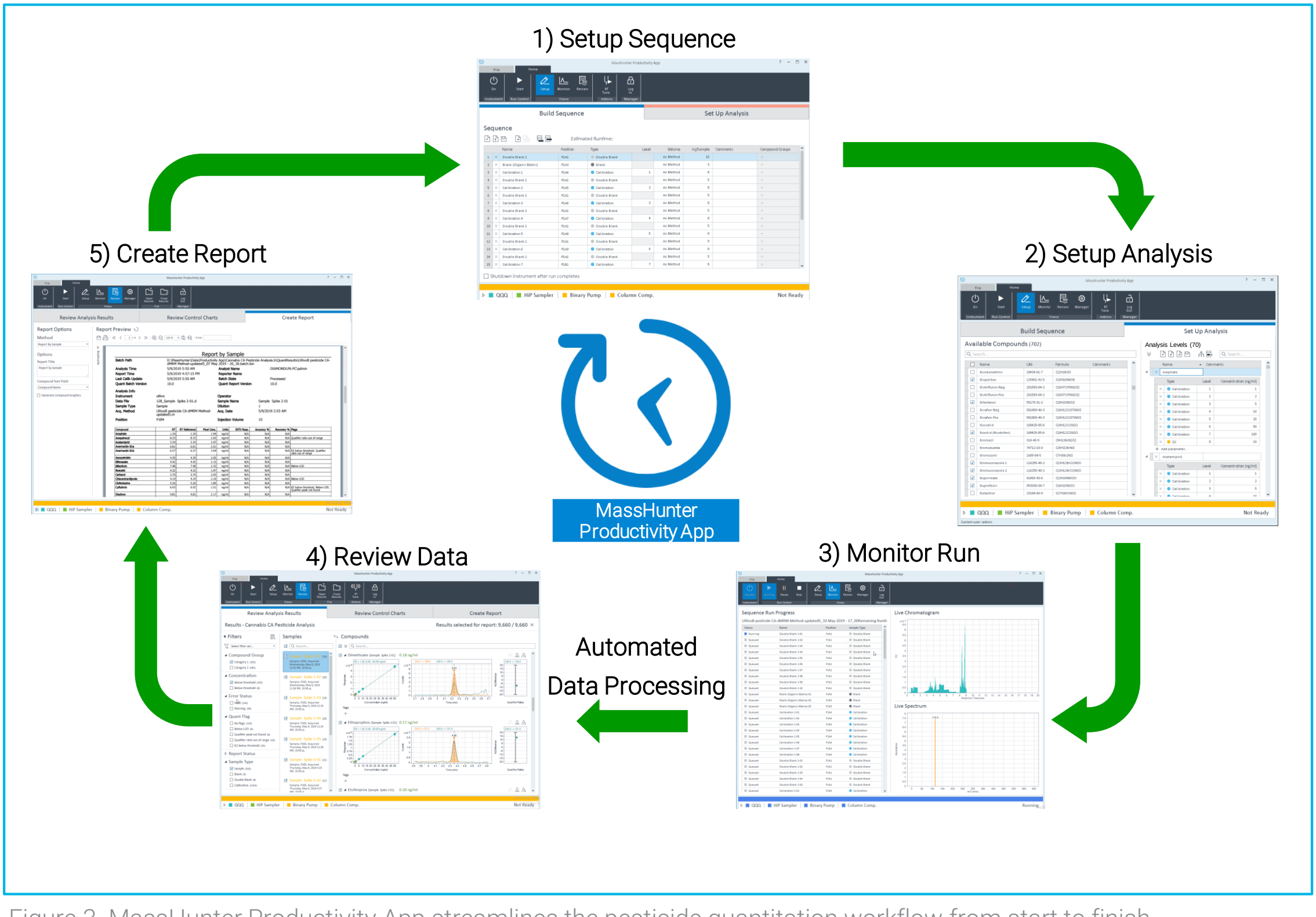

Figure 3. MassHunter Productivity App streamlines the pesticide quantitation workflow from start to finish.

Method Linearity and Precision

California list of pesticides for cannabis testing includes two categories: category 1 and 2. The action limit for pesticides in category 1 is > LOD and for category 2 is 100 ppb or higher [2].

Figure 4 shows a representative MRM chromatogram of pesticides (category 1 and 2) spiked at 5 ng/g in organic cannabis matrix.

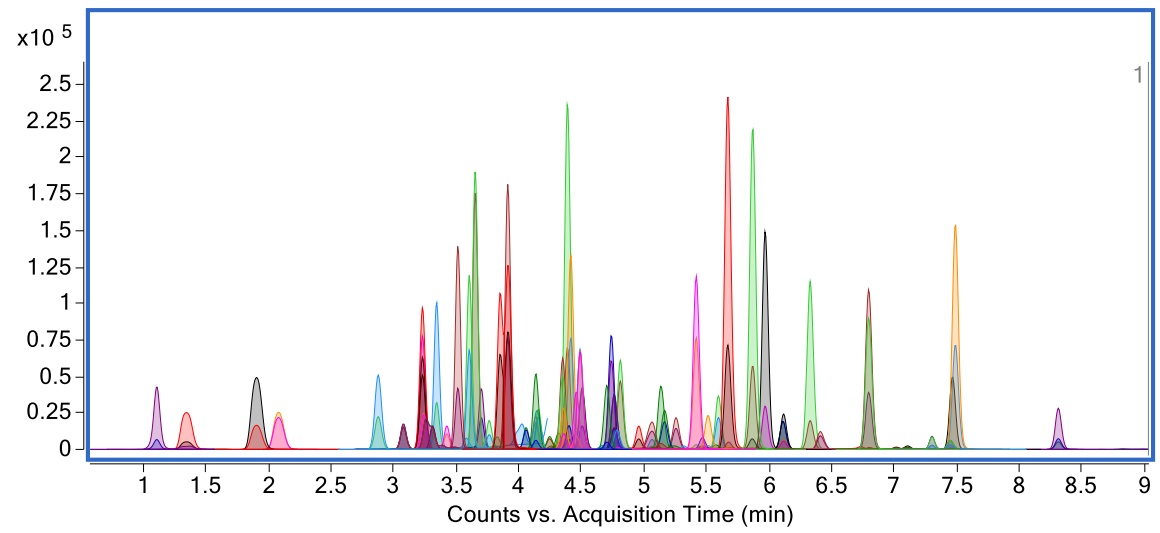

Figure 4. LC-MS/MS MRM chromatogram of spiked (5 ppb) cannabis extract.

Results and Discussion

Matrix extract calibration standards were prepared at concentration levels of 0.1, 0.25, 0.5, 1, 5, 10, and 50 ng/g because sample preparation dilutes the original plant materials by 250 times. All calibration curve yielded correlation coefficient values (R2) >0.99 for the pesticides in the cannabis matrix. In total, 98% of pesticides were detected at concentration 0.1 ppb and in these cases accuracy was in the range of 80–120 % for at least five out of six replicates with relative standard deviations of less than 10 %.

Data Review

With the MassHunter Productivity App's targeted data review capabilities, category 1 and 2 pesticides above their respective action limits could be instantly filtered and reviewed. Figure 5 illustrates an example filter configuration to review all category 1 pesticides above their concentration threshold in the analyzed cannabis samples.

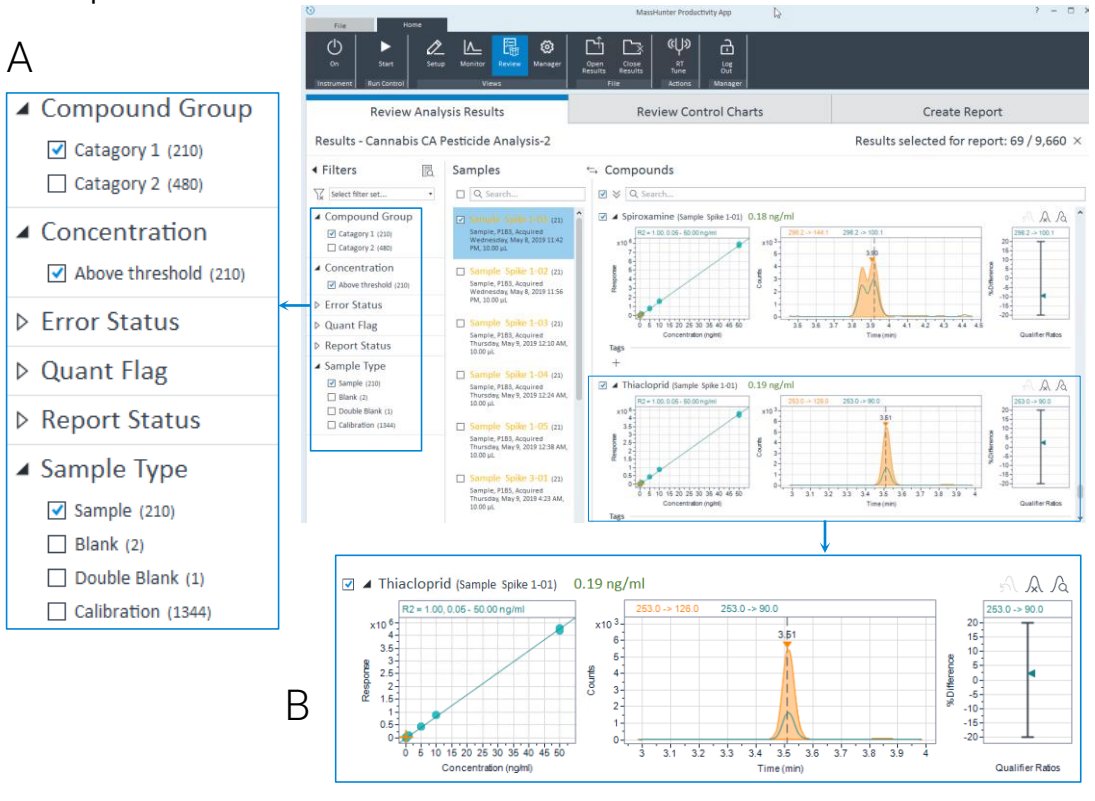

Figure 5. Detected spiked pesticides in a cannabis sample. (A) Type of filter used for reviewing results. (B) Calibration curve, extracted ion chromatogram, and qualifier ratio for detected carbaryl.

Further time savings were made during data processing by using a built-in analysis optimization function in the MassHunter Productivity App. This process uses the calibration samples to calculate the expected retention times and qualifier ratios to improve peak selection and identification in the samples of interest. Figure 6 shows the results of this optimization when there is matrix interference. In Figure 6, both Chlorpyrifos and Coumaphos were detected correctly based on automated peak selection technique in app.

The correct peak selection was further confirmed in the Productivity App by overlaying the quantifier ion chromatograms from calibration samples (Figure 6, A and B).

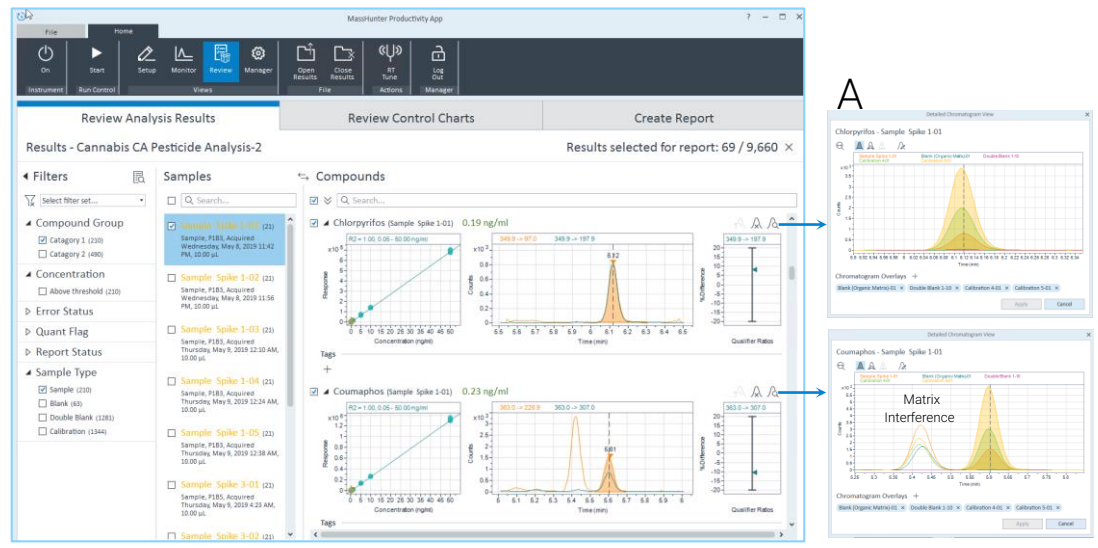

Figure 6. Identified Chlorpyrifos and Coumaphos in cannabis sample. (A, B) Overlaid MRM chromatograms of quantifier ion of identified pesticides in cannabis sample, double blank, organic matrix, and calibration samples.

Conclusions

- The Ultivo triple quadrupole LC/MS provides robust, accurate, and sensitive pesticide testing in challenging matrices such as cannabis.
- Agilent simplified cannabis sample preparation protocol enables accurate pesticides quantification at levels as low as 0.1 ppb.
- The MassHunter Productivity App enables faster targeted screening analysis by automating manual steps, improving automatic peak selection, and offering a simplified data review experience.

References

[1]Stone, P. et al., "Determination of Pesticides and Mycotoxins as Defined by California State Recreational Cannabis Regulations", Agilent Application Note, 5994-0648EN, 2019

[2]Bureau of Marijuana Control Proposed Text of Regulations California Code of Regulations Title 16 Division 42. Bureau of Marijuana Control Chapter 5. Testing Laboratories.

Agilent products and solutions are intended to be used for cannabis quality control and safety testing in laboratories where such use is permitted under state/country law.

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