Application Note

Food Testing & Agriculture

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# Optimizing Sample Preparation in Pesticides Analysis for Cannabis


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## Abstract

Many U.S. state-regulated pesticides lists for cannabis can be analyzed exclusively by LC/MS/MS. Notable exceptions include California, Florida, and Nevada, where GC/MS/MS is also required. The states requiring GC/MS are expected to grow as more compounds and lower detection limits are required. In this work, the detection and quantitation of all LC-amenable pesticides and mycotoxins were reliably met by at least 50% of the current California legislative safety action limits in cannabis dried flower samples (limits of detection (LODs) range between 0.5 to 50 ppb; Malathion's LOD = 100 ppb). Forty three GC-amenable pesticides regulated by the Bureau of Cannabis Control in California met the established limits of quantitation (LOQs) with the Agilent 8890 GC combined with an Agilent 7010B triple quadrupole GC/MS system.

The Agilent standardized sample preparation procedure aligned with the Agilent multiplatform approach provides a rapid return on investment (ROI) and a stable foundation to meet current and future testing requirements.

## Introduction

Cannabis is a complex plant containing many endogenous chemicals representing numerous chemical classes. Compared to other plants and vegetables, cannabis has higher amounts of potential interferences, and notably high concentrations of terpenes, cannabinoids, flavonoids, phenols, and fatty acids. The complexity of the cannabis matrix makes detection and accurate quantification of trace levels of pesticides more challenging. Interfering compounds can negatively impact ionization in the mass spectrometer, affect signal-to-noise ratios (S/N), and build up in the instrument source and consumables, thus decreasing productivity while increasing maintenance and operating costs. To overcome this challenge, a combination of optimized sample preparation and state-of-the-art instrumentation is required.

## Experimental

Pesticide residue analysis in cannabis requires state-of-the-art LC and GC triple quadrupole mass spectrometry. For LC/MS/MS analysis, the Agilent 1290 Infinity II LC can be coupled to either the Agilent 6470 triple quadrupole LC/MS or the Agilent Ultivo triple quadrupole LC/MS. These MS systems are equipped with the Agilent Jet Stream (AJS) ESI source. The LC/MS/MS analysis instrumental and method parameters can be found in Agilent application notes.[1,2] For GC/MS/MS analysis, we recommend the Agilent 8890 GC system coupled to an Agilent 7010B triple quadrupole GC/MS. The GC/MS/MS analysis instrumental and method parameters can be found in an Agilent Application Note.[1]

## Detailed sample prep

To analyze a representative sample, the cannabis must be fully homogenized prior to its extraction. This can be done by adding two ceramic homogenizers (part number 5982-9313) or stainless-steel beads to a tube of chopped cannabis, and mechanically shaking for five minutes or more at high speed (ideally on a vertical shaking device, such as a Geno/Grinder-type machine). The homogenizers will help turn the dry cannabis into fine powder.

1. Weigh 1 g of homogenized cannabis sample in a 50 mL centrifuge tube (Figure 1).

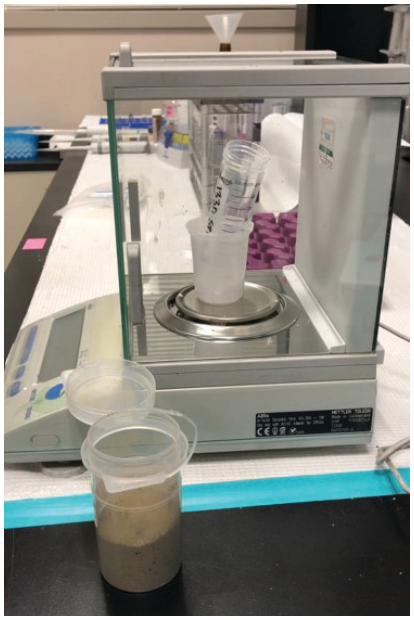

Figure 1.

2. If precleanup spiked matrix samples are to be prepared, pipette the pesticide standard solution(s), isotopically labeled standards (such as captan-$d_6$), and mycotoxin standards into the dry cannabis powder, then vortex for 30 seconds (Figure 2).

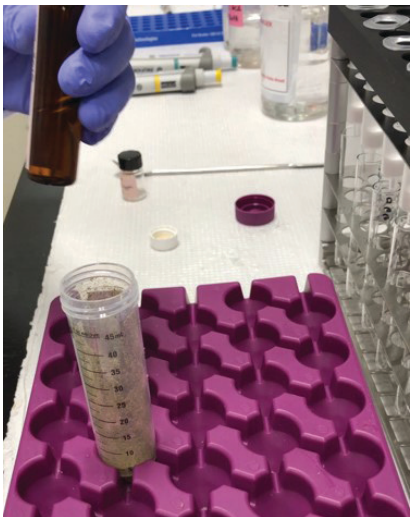

Figure 2.

3. Add two ceramic homogenizers or stainless-steel beads.
4. Add 15 mL of pesticide-grade acetonitrile to the tube and cap.
5. Mechanically shake for 3 to 5 minutes using a Geno/Grinder or similar mechanism at high speed (Figure 3).


Figure 3.

6. Set up the SPE manifold, and place a SampliQ C18 EC 6 mL 500 mg SPE cartridge (part number 5982-1365) onto the manifold; manifold used in application:
   - SPE cartridge rack, 6 mL, for PPM-48 (p/n 5191-4104)
   - Collection rack, 16 × 100 mm tubes, PPM-48 (p/n 5191-4108)
   - Waste rack and three waste bins, for PPM-48 (p/n 5191-4112)
7. Place collection tubes in the holder to collect the eluent.
8. Decant/transfer the supernatant from the tube (color may vary from sample to sample). The sample will flow by gravity (Figure 4).

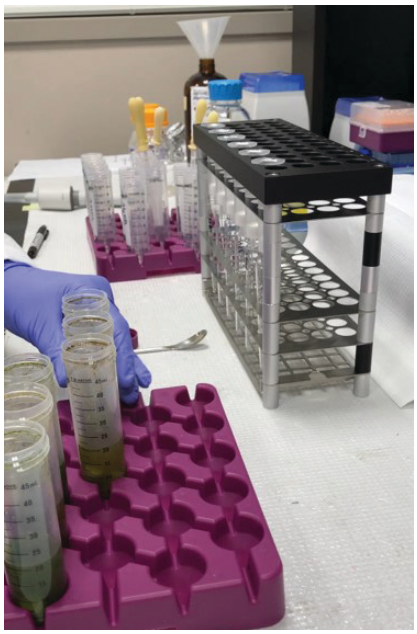

Figure 4.

9. After the solvent has gone through the C18 cartridges, add 5 mL of acetonitrile to each of the empty sample tubes (Figure 5).

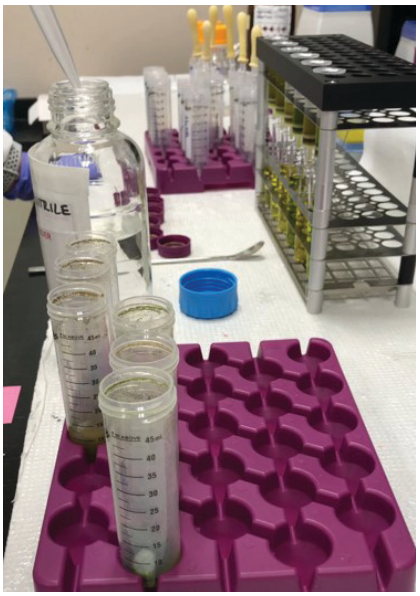

Figure 5.

10. Mechanically shake for 3 to 5 minutes using Geno/Grinder or similar mechanism at high speed.
11. Decant/transfer the supernatant from the tube into the same C18 cartridge (Figure 6).

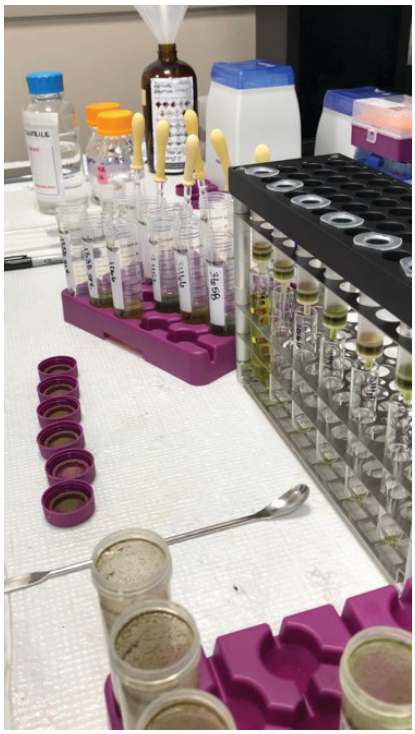

Figure 6.

12. After the solvent has gone through the C18 cartridges, rinse the empty sample tube with an additional 5 mL of acetonitrile and decant into the same C18 cartridge (Figure 7).

Figure 7.

13. Dilute up to a final volume of 25 mL (now the sample has been diluted by a factor of 25; Figure 8).

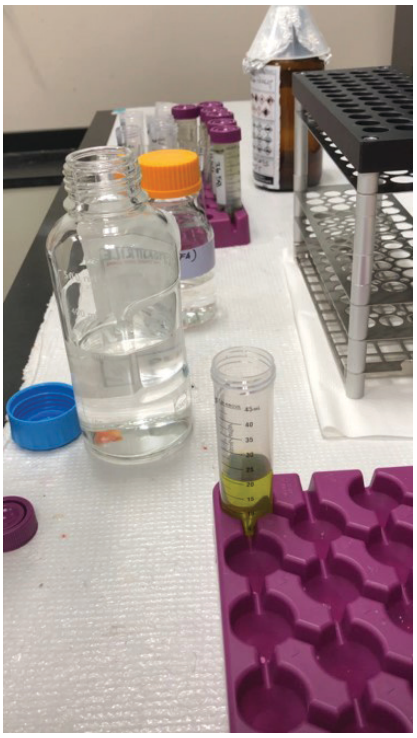

Figure 8.

**Detailed dilution unique to LC/MS/MS (Figure 9)**

Mix 50 µL of diluted extract with 450 µL of 25:75 water/methanol (v:v) containing 0.1% formic acid in a 2 mL autosampler vial (250-fold dilution)

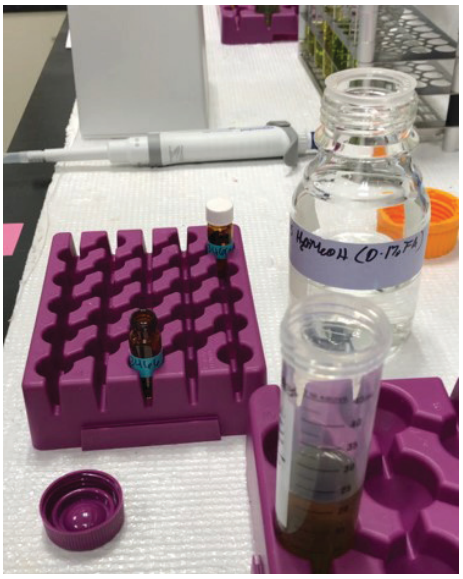

Figure 9.

**Detailed dilution unique to GC/MS/MS (Figure 10)**

Mix 200 µL of diluted extract with 800 µL of 50:50 hexane/acetone (v:v) containing 0.1% formic acid in a 2 mL autosampler vial (125-fold dilution)


Figure 10.

Figure 11 shows the benefits of different dilution factors of the complex cannabis matrix.

## Analysis[1,2,3]

### LC/MS/MS analysis

Detection and quantitation of all LC-amenable pesticides and mycotoxins were reliably met by at least 50% of the current California legislative safety action limits cannabis dried flower samples (LODs range between 0.5 and 50 ppb; Malathion's LOD = 100 ppb).

Figure 12 shows overlaid chromatograms of California pesticides list and mycotoxins in extracted flower matrix, actual concentration 500 ppt (pre-extraction concentration = 125 ppb.)

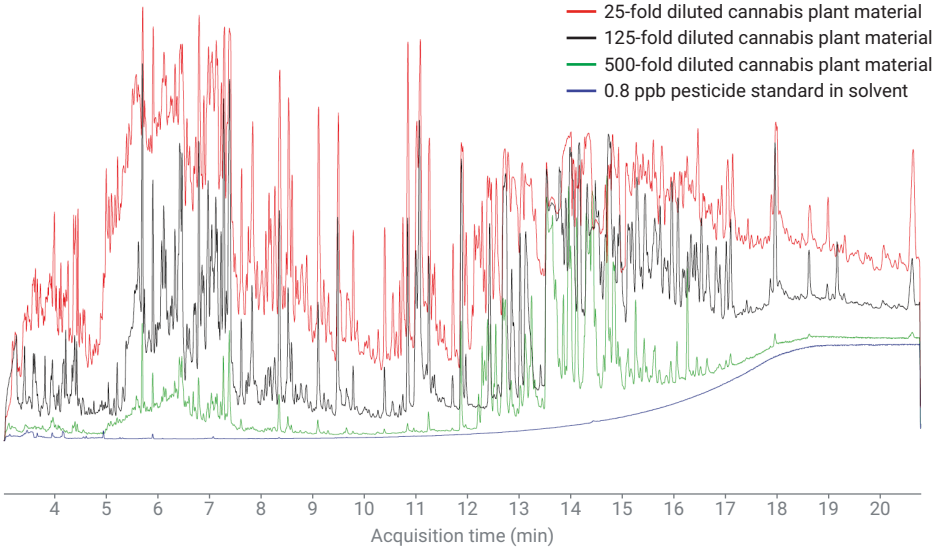


**Figure 11.** The benefits of different dilution factors of the complex cannabis matrix.

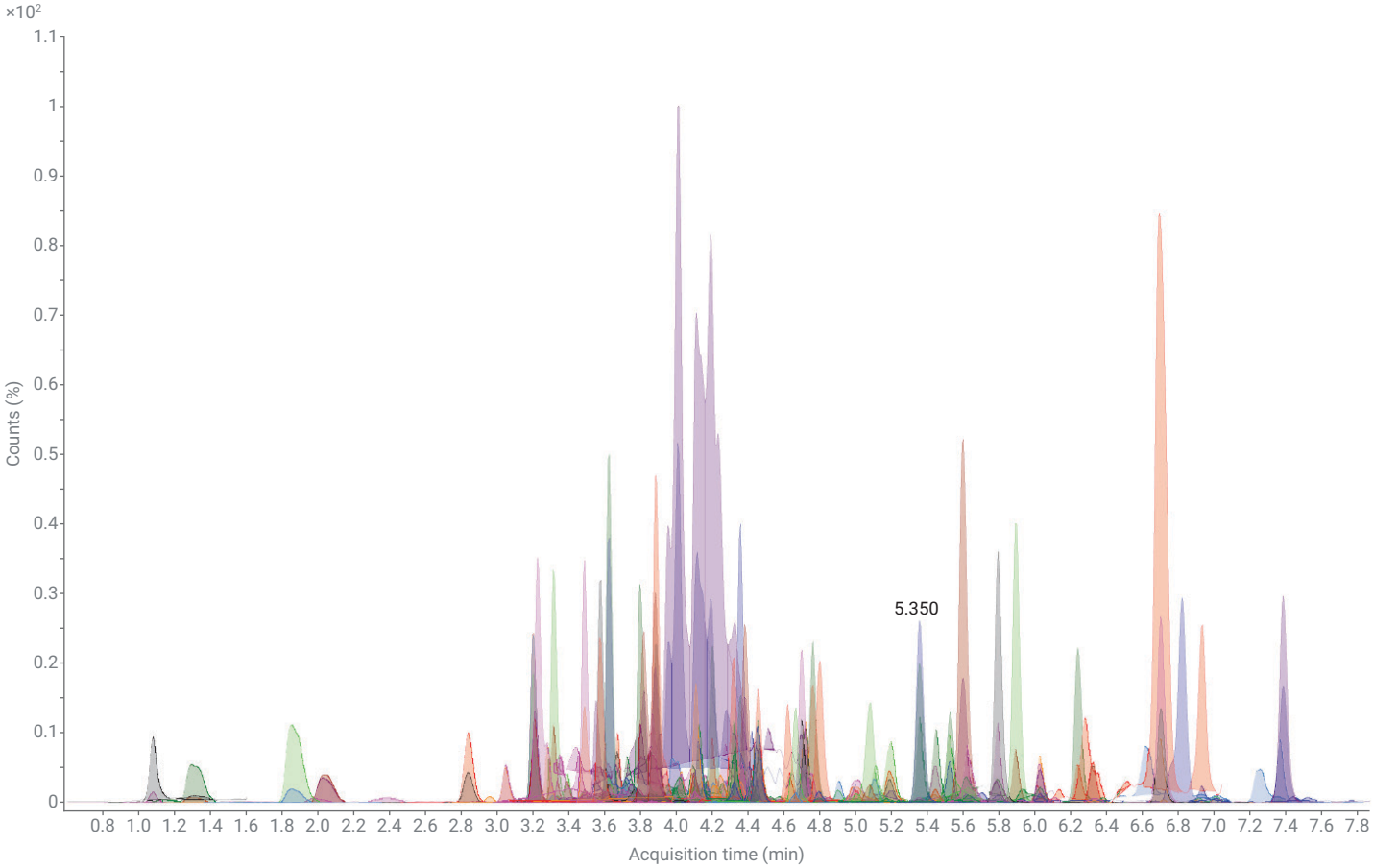


**Figure 12.** Overlaid chromatograms of California pesticides list and mycotoxins in extracted flower matrix.

## GC/MS/MS analysis

LOQs for 43 LC- and GC-amenable pesticides had LOQs ≤0.8 ppb in-vial (≤100 ppb in dried cannabis plant material) when analyzing a 125-fold diluted cannabis extract.

Figure 13 shows MRM chromatograms of 10 sequential injections for pentachloronitrobenzene (A), captan (B), and chlordane-*cis*/*trans* (C) at LOQ level.

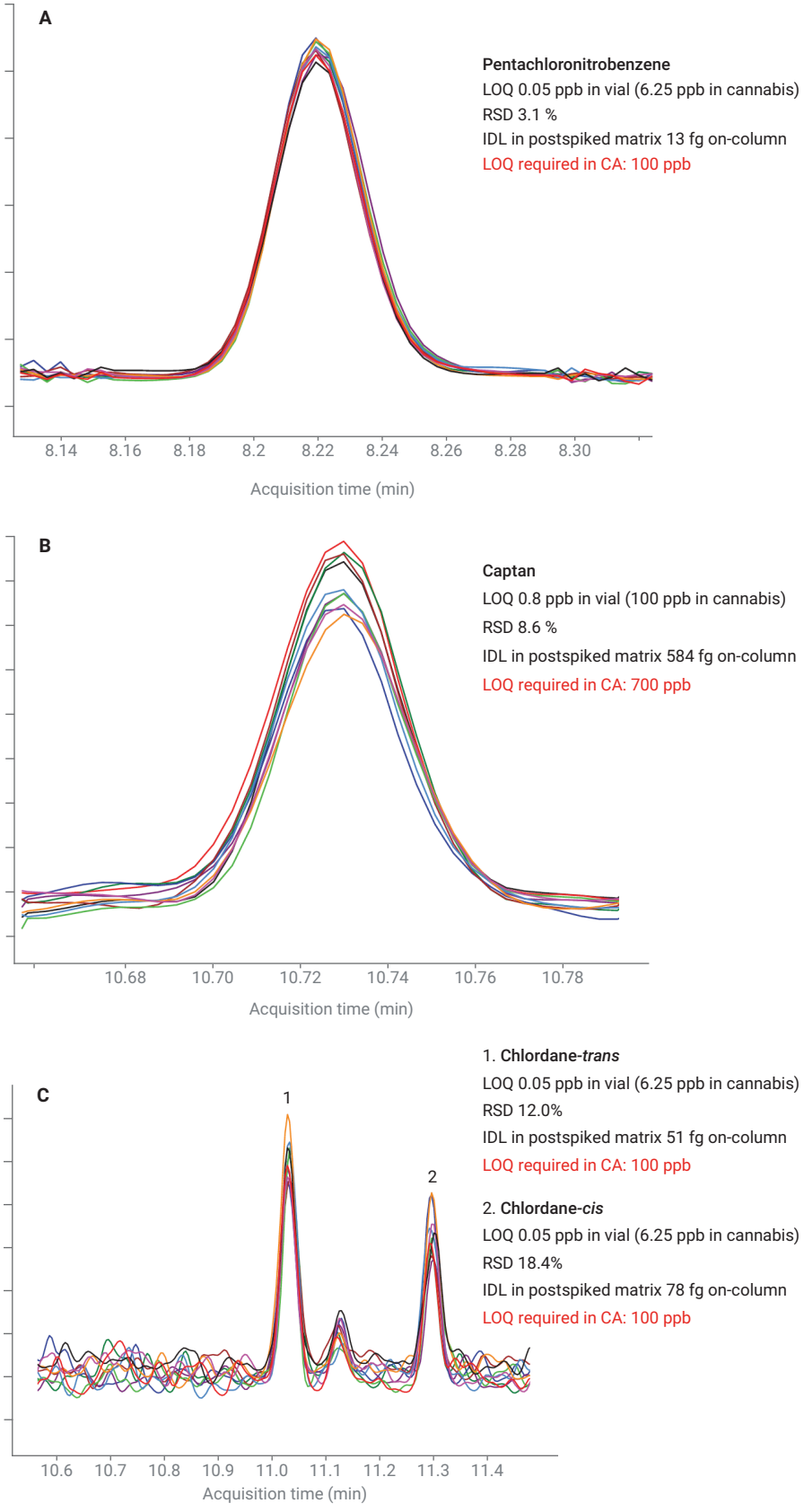


**Figure 13.** MRM chromatograms of 10 sequential injections for pentachloronitrobenzene (A), captan (B), and chlordane-*cis/trans* (C) at LOQ level.

## Conclusion

The complexity of the cannabis matrix makes detection and accurate quantification of trace levels of pesticides more challenging. The Agilent standardized sample preparation procedure aligned with the Agilent multiplatform approach provides a rapid ROI and a stable foundation to meet current and future testing requirements.

## References


1. Roy, J.-F.; *et al.* A Sensitive and Robust Workflow to Measure Residual Pesticides and Mycotoxins from the Canadian Target List in Dry Cannabis Flower, *Agilent Technologies Application Note*, publication number 5994-0429EN, **2018**.
2. Andrianova, A.; Westland, J.; Churley, M.; Sensitive and Robust Detection of Pesticides Regulated in California in Dried Cannabis Plant Material, *Agilent Technologies Application Note*, publication number 5994-0568EN, **2019**.
3. Stone, P.J.W.; *et al.* Determination of Pesticides and Mycotoxins as Defined by California State Recreational Cannabis Regulations - A combined LC/MS/MS analysis method, *Agilent Technologies Application Note*, publication number 5994-0648EN, **2019**.

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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