Robust GS-MS/MS Analysis of Pesticides in Cannabis by Using High Sample Dilution

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Introduction

The challenge of quantifying pesticide residues in cannabis flower is a complex problem that is due in part to the great disparity between concentration levels of naturally-occurring cannabinoids and incurred pesticide residues, as well as the high terpene content of the plant. The typical extraction process gives rise to the potential for low pesticide recoveries and deleterious effects on the analytical instrumentation caused by co-extracted material.

Our approach to sample preparation exploits the benefits of highly sensitive instruments allowing for diluting the sample extract by several hundred-fold. Extraction of dried cannabis is followed by cartridge SPE. The eluate, a portion of which is also utilized for LC-MS/MS analysis, is then diluted with a less polar solvent mixture and dispersive SPE performed. A final dilution step is made prior to analysis to yield an overall sample dilution of 500-fold. The GC is equipped with columns of differing polarities and mid-point column backflush capability. The system utilizes a High Efficiency Source (HES), which results for the creation of up to 20x more ions. Hence, higher sample dilution is possible while still maintaining LOQs, which were determined to be 0.1 mg/kg for 85% of the target list. Performance of the method with incurred samples is presented. In addition to highly diluting the sample extract, the addition of small molecule analyte protectants (AP) prior to injection was evaluated.

Experimental

Extraction

1g of sample and 15 mL ACN, extract 2-5 min
Add to polymeric SPE 500 mg cartridge, gravity flow
5 mL ACN cartridge rinse x 2
q.s. volume to 25 mL

Experimental, cont.

Dispersive SPE with high dilution

100 µL of extract added to 900 µL of hexane:acetone, 1:1
Add to Agilent Bond Elut Universal dSPE [PSA, C18, GCB]
(Hexane:acetone deters from planar pesticide retention by GCB)

Dilute 300 µL of dSPE extract with 300 µL hexane:acetone, 1:1 into autosampler vial.

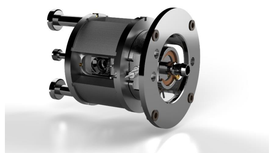

Figure 1. High Efficiency Source of the 7010 GC-MS/MS

GC-MSMS Analysis

An Agilent 7890 GC coupled to a 7010 Triple Quadrupole GC/MS system equipped with High Efficiency Source (HES) was used. The GC system was equipped with a Multi-Mode Inlet (MMI) with air cooling and a back flushing system based on a Purged Ultimate Union controlled by an AUX EPC module. GC columns were 15 m x 250 µm x 0.25 µm. Column 1 was a DB-35MSUI (35% phenyl) and column 2 was a DB-5MSUI (5% phenyl). The He flows were 1.2/1.25 mL per min. Injection volume was 1 – 2 µL using ramped hot splitless injection into a 4mm single taper gooseneck liner containing a wisp of deactivated glass wool.

Method Performance

The instrument calibration range (ESTD) was 0.2 - 20 ng/mL. Pesticide recoveries were acceptable (70-120%) for the list of over 70 targets. LOQs were 0.1 mg/kg for 85% of the target list. The use of analyte protectants (AP) in routine analysis is now being evaluated but has not yet been implemented.

Results and Discussion

Quant MRM transitions for 0.2 ppb calibration standard

Examples of more challenging analytes spiked in cannabis show that acceptable chromatography was maintained at the lowest concentration in vial of 0.2 ppb. (No added AP) Refer to Figure 2.

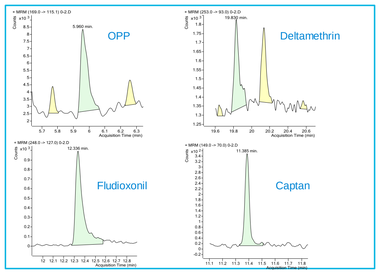

Figure 2. Chromatography examples (0.2 ppb spiked in cannabis)

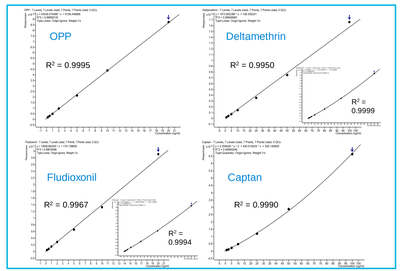

Figure 3. Representative calibration data (cannabis based)

Matrix matched calibration: 0.2 – 20 ppb (ESTD method)

Excellent correlation coefficients assured accurate quantitation. Quadratic fits were used in some instances to improve quantitation at the lowest concentrations. Refer to Figure 3.

Evaluation of Analyte Protectants

Addition of L-gulonolactone and D-sorbitol (~600 and 300 µg/mL, respectively) improved the peak shape and/or response for the majority of target analytes. In terms of S/N, some targets showed a decrease or no change with added AP. Examples are in Figure 4.

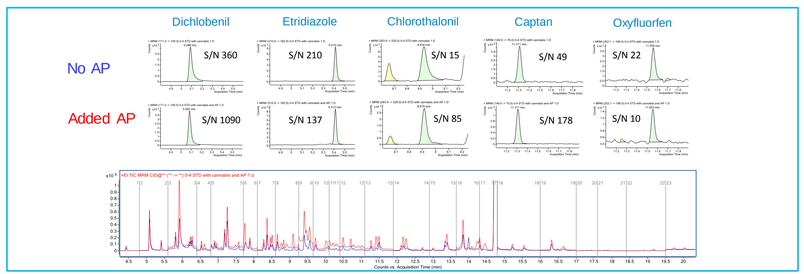

Figure 4. Top: 0.4 ppb standard spiked in cannabis matrix. MRM Quantifying transitions without and with AP (as indicated). Bottom: TIC MRM; no AP (blue trace), and with AP added (red trace).

Incurred samples quantitated at sub-ppb levels

Results for four typical samples (no added AP) demonstrated quantitation as low as sub-ppb levels (concentration injected). Concentrations given in Figure 5 are those in the autosampler vial.

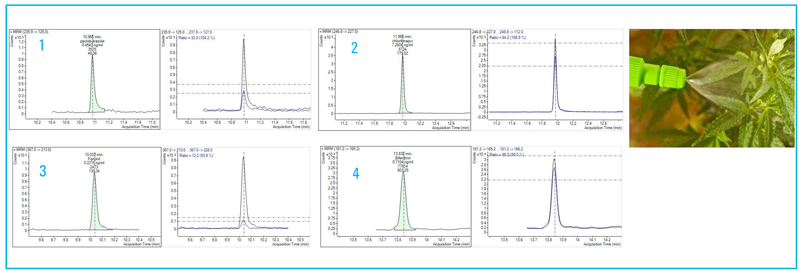

Figure 5. Incurred cannabis samples: 1) Paclobutrazole, 0.45 ng/mL; 2) Chlorfenapyr, 7.3 ng/mL; 3) Fipronil, 0.22 ng/mL; 4) Bifenthrin, 0.71 ng/mL.

Conclusions

- LOQs were 0.1 mg/kg for 85% of the target list of over 70 compounds.
- Cannabis sample dilution of up to 500-fold improved robustness. Interferences from matrix were minimized; frequent inlet maintenance and column replacement were prevented. Analyte protectants are being evaluated for use in the analysis.
- High sample dilution is made possible by using a high efficiency ion source, which creates up to 20x more ions. Injected concentrations of analytes at sub-ppb levels are part of a routine workflow.

Reference

Pesticide Analysis Reference Guide, "GC/MS/MS Pesticide Residue Analysis" Agilent Technologies publication 5991-2389EN