Tips and Tricks for Pesticide Residue Analysis in Cannabis

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Cannabis testing regulations in the United States are determined at the state level, with each state outlining which pesticides to monitor. Due to this, there is a wide range of pesticides currently being analyzed in cannabis. Current pesticide panels for cannabis contain a variety of compound classes, such as organochlorines, organonitrogens, carbamates, and organophosphates. Each of these compound classes, as well as the individual pesticides within each class, have unique analytical challenges, including storage requirements to ensure compound stability, ionization efficiencies, and chromatography constraints.

In this application note, the most commonly observed errors and challenges when analyzing pesticides in cannabis products are presented, as well as suggestions on how to avoid or correct these issues.

Common Challenges

Pesticide Stability

The stability of the pesticides in the calibration standards is crucial for accurate quantification. If the integrity of the calibration standards is compromised, then the reported pesticide concentration could be falsely inflated. Two pesticides that have shown to exhibit degradation, possibly due to hydrolysis, are acequinocyl and captan.[1-4] To combat the degradation of these pH sensitive pesticides, it is recommended that all pesticide stock standards be diluted and stored in 0.1% formic acid in acetonitrile.

The conversion of acequinocyl to acequinocyl-hydroxide is commonly documented in basic conditions and aqueous conditions.[1-4] Figure 1 demonstrates that by creating a basic environment, through the addition of ammonium hydroxide to an acequinocyl analytical standard, there is complete conversion of acequinocyl to acequinocyl-hydroxide (Phenova, USA).

Captan, in addition to being thermally labile, is also sensitive to both basic and aqueous conditions.[3-4] Captan degrades rapidly into its metabolites in water, however more slowly in methanol versus acetonitrile (Figure 2).[3-4] Similarly to acequinocyl, storing captan in acidified acetonitrile has been shown to diminish its degradation.

Key Advantages from the Cannabis Experts

- Data quality and accurate quantification of pesticide residues in cannabis matrices
- Detailed pitfalls to avoid and corrective solutions to address commonly observed issues in a cannabis pesticide testing facility

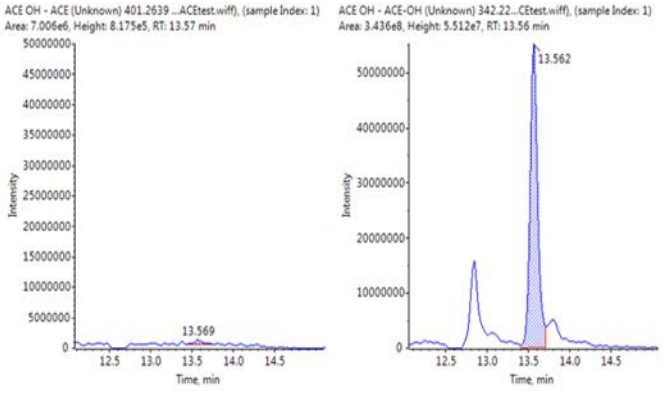

Figure 1: Conversion of Acequinocyl Under Basic Conditions. XIC of acequinocyl (left) shows that complete degradation has occurred (no acequinocyl peak detected). Following treatment with a base, XIC shows hydroxy-acequinocyl peak (right) which is the acequinocyl degradant.

Captan still appears to degrade in acetonitrile, but at a much slower rate than in methanol. Figures 2 and 3 depict the degradation of captan in the autosampler vial; Figure 2 shows the degradation difference between methanol and acetonitrile, and Figure 3 shows the improved stability with using acidified acetonitrile for storage.

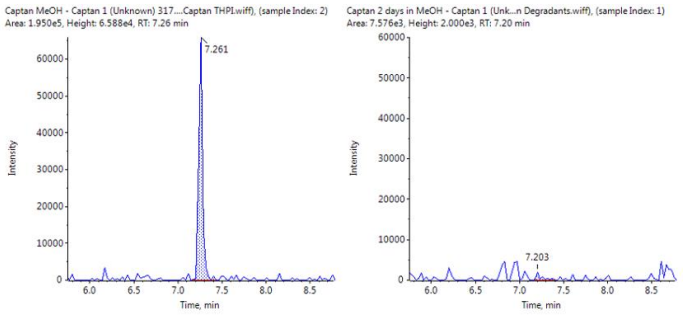

Figure 2: Stability of Captan in Organic Solution. XIC of Captan in Methanol (left). Captan has completely degraded in methanol in two days; XIC shows no peak (right).

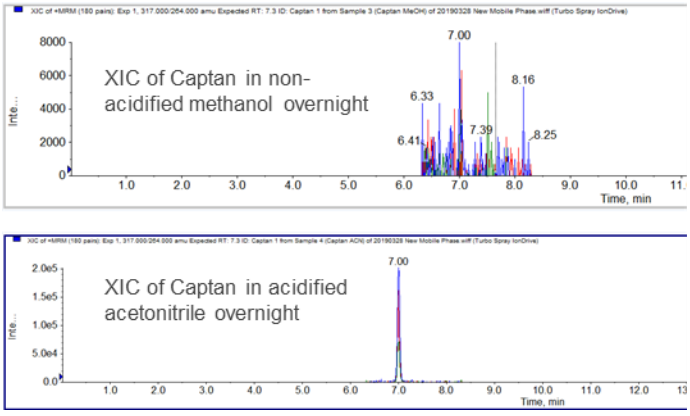

Figure 3: Stabiity of Captan in Acidified Acetonitrile. XIC of captan shows how acidified acetonitrile as a storage solvent improves the compound stability and retains the ability to see captan signal in a standard.

Chromatography

The elution profile of target analytes is achieved with chromatography and utilizing a combination of column chemistry, dimensions, flow rate, and mobile phases. Poor peak shape is typically related to the LC system and due to a some component or setting of the LC, such as the analytical column or column oven temperature. In Figures 4-6, different peak shapes are shown and the possible causes and recommended fixes are detailed.

Cause: Injection solvent is too strong for initial LC gradient conditions and how it interacts with the column chemistry; this is mainly observed in peak splitting of early eluting compounds.[5-7]

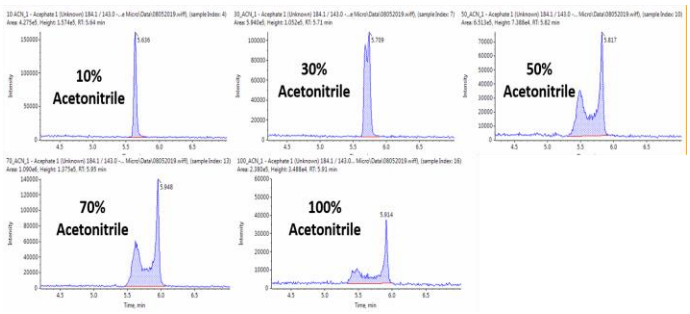

Figure 4: Peak Splitting. Example of acephate chromatographs with increasing percent by volume of acetonitrile in sample injection matrix on a 0.5 x 150 mm analytical column with an LC flow rate of 15 µL/minute.

Solution: Use a weaker injection solvent that matches the initial starting conditions of the LC gradient, as in the case of the early eluting compounds.

Cause: If peak splitting is observed in later eluting compounds or compounds of high peak signal intensity, this is likely due to overloading the column.[5-7]
Solution: Inject less material onto the column, either by decreasing injection volume or diluting the sample with solvent.

Cause: Deterioration of the analytical column due to buildup of dirty matrix after repeated injections.[5-7]
Solution: Change the analytical column.

Cause: Sample diluent is too strong for the column, hurting the specificity of the column for the target analytes.[5-7]
Solution: Dilute the sample in a weaker solvent.

Cause: There is dead volume in the fittings along the path of the sample in the LC.[5-7]
Solution: Check all fittings and ensure that there is no dead volume.

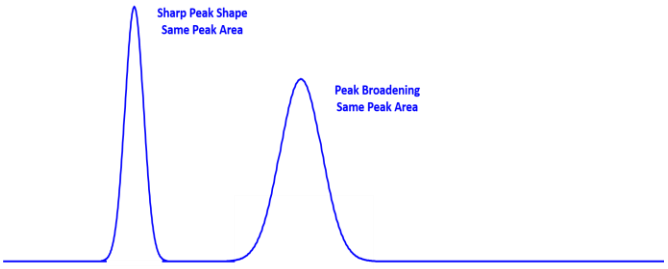

Figure 5: Peak broadening lowers peak signal intensity shown in the two peaks above with the same peak area.

Cause: There is a mismatch between flow rate, column temperature, and analytical column dimensions that is not optimized.[5-7]
Solution: Optimize the LC flow rate and column oven temperature and/or reduce the analytical column diameter.

Cause: Analyte is co-eluting with an isobaric compound either another isomer or a matrix interference.
Solution: Adjust the chromatography to separate out the coeluting peak or use a secondary ion for quantification that does not show the same interference.

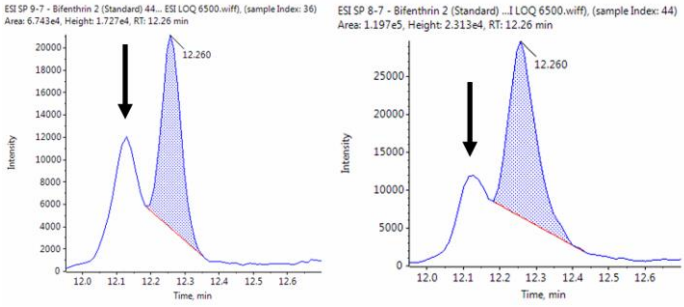

Figure 6: Separating Interferences. Chromatograms of bifenthrin showing a coeluting interference on the shoulder in cannabis matrix. The black is indicating the isobaric interference.

Internal Standards

Due to the variable nature of cannabis matrices tested, deuterated internal standards (IS) are necessary to help correct for matrix effects and to correct for variances from injection to injection. There are two ways to incorporate IS in pesticide analysis: 1) add the IS to the extraction solvent or 2) add IS post-extraction in each sample vial.

Typically deuterated internal standards or heavy isotopically labeled (C13 and N15) standards are custom synthesized. With custom sythensis also comes greater cost for manufacturing and purchasing. The addition of internal standard to the extraction solvent requires adding a larger total amount of internal standard to a large volume. However, when the internal standard is added prior to extraction it corrects for variability in top-down bottle dispensers. If part of the sample is spilled in the lab after extraction, the IS to analyte ratio should still be the same therefore the sample can still be analyzed.

For example:
Creating 1 L of 10 ppb IS extraction solvent, one must add 10 ppm of IS to the 1 L of extraction solvent. (IS addition is one step).

If spiking internal standard in autosampler post extraction, one would add a total amount of 10 ppb in 1 mL of sample. (Multiple IS addition steps; one per sample analyzed.)

To compare 10 ppm versus 10 ppb, adding IS in the extraction solvent uses more IS, and is therefore a greater cost per sample. However, the addition of IS to the extraction solvent would reduce the number of times IS is added, further decreasing sample handling variability.

Table 1: The Pros and Cons of Two Ways to Utilize Internal Standards in Pesticide Residue Analysis.

Method of IS Addition	PRO	CON
Add to the acidified acetonitrile extraction solvent	More reproducible	Uses more internal standard per sample
Add post extraction in vial	More cost effective	Does not correct for sample extraction variation

Sample Preparation

Liquid samples, such as oils and distillates, should have a corrective dilution factor for total volume when compared to flower or edible samples. If using IS in the extraction solvent, the total amount of IS for each sample will have to be corrected in liquid sample to match the total amount of IS added in solid samples. Another possibility is to create two separate extraction solvents, one for liquid samples and one for solid samples.

Example:
1 gram of flower in 10 milliliters of acidified acetonitrile
1 gram of distillate in 9 milliliters of acidified acetonitrile
Dilution factor of each sample is 1:10

Example:
1 gram of distillate in 10 milliliters of acidified acetonitrile
Dilution factor of sample is 1:11

Integration Parameters for Isomers

Many pesticide standards consist of isomers; regulations may require the sum of total isomers to be reported for pesticide residue amount. In SCIEX OS Software, default integration parameters can be set for each compound in data processing methods (Figures 7 – 9).

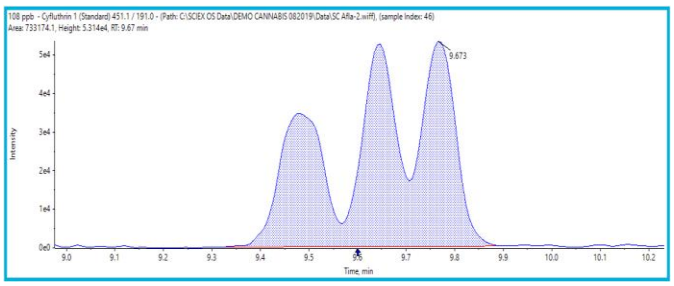

Figure 7: Cyfluthrin Isomers. Cyfluthrin consists of four isomers that are not completely baseline separated with the LC gradient. The three major isomers of cyfluthrin are evident in this characteristic elution profile and all the peaks can be integrated as one component for a total sum of cyfluthrin.

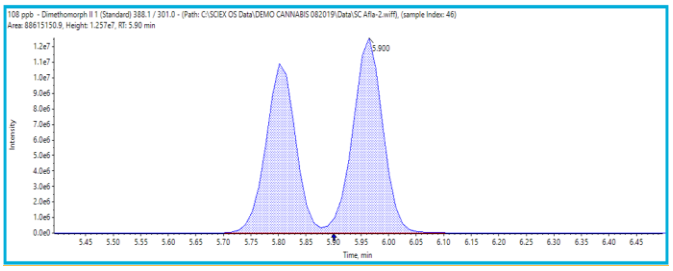

Figure 8: Dimethomorph Isomers. Dimethomorph exists as two isomers, since the two isomers are not completely separated by LC, they too can be integrated as one component of a total sum of dimethomorph.

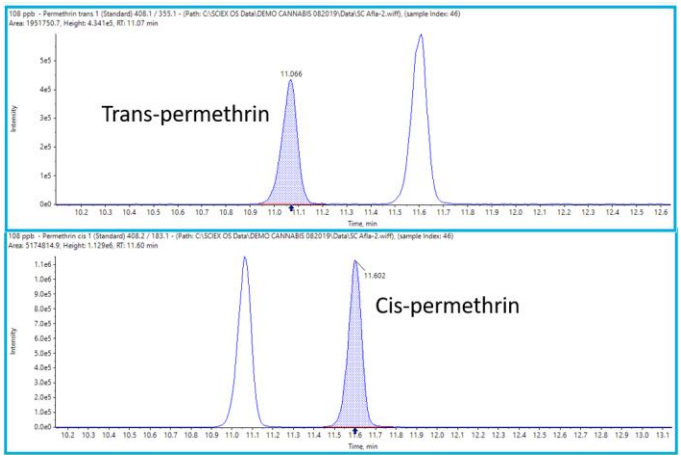

Figure 9: Permethrins Isomers. Permethrins exist as cis and tran isomers and are completely baseline separated by LC, therefore each of the isomers must be integrated separately. The total concentration of each component should be corrected by purity of standard. Example: 10 ppb standard with 60% purity of trans-permethrins yields 6 ppb corrected concentration.

Corrected Purity of Pesticide Components

Pesticide residue concentrations should always be corrected according to vendor certificate of analysis for accurate quantification of pesticide residues in cannabis matrices.

Table 2: An example of pyrethrins purity from an analytical standard as printed on the certificate of analysis. The table shows the purity and percentage of the pyrethrins standard as printed on the label.

Purity of Pyrethrins	Component Name	Percentage of Component in Standard
35 %	***Pyrethrin Pyrethrin I***	*54%*
	Pyrethrin Jasmolin I	*3%*
	Pyrethrin Cinerin I	*6%*
	Pyrethrin Pyrethrin II	*30%*
	Pyrethrin Jasmolin II	*3%*
	Pyrethrin Cinerin II	*4%*

The total purity of pyrethrins in the analytical standard is 35%; if the concentration of the standard is 1000 ppm, then the concentration of pyrethrins is 350 ppm.

To calculate the individual concentrations of each pyrethrin component, the individual purity must be taken into consideration. Individual purities are listed below.

54% Pyrethrin I: 189 ppm
30% Pyrethrin II: 105 ppm
3% Jasmolin I: 10.5 ppm
3% Jasmolin II: 10.5 ppm
6% Cinerin I: 21 ppm
4% Cinerin II: 14 ppm

Linearity and Regression

The regression type affects the calibration curve of analytes and represents the balance between *r* value and accuracy. Normally, linear regressions curves are the most ideal, however, quadratic curves can improve the *r* value at higher concentrations when detector saturation is occurring (Figure 10).

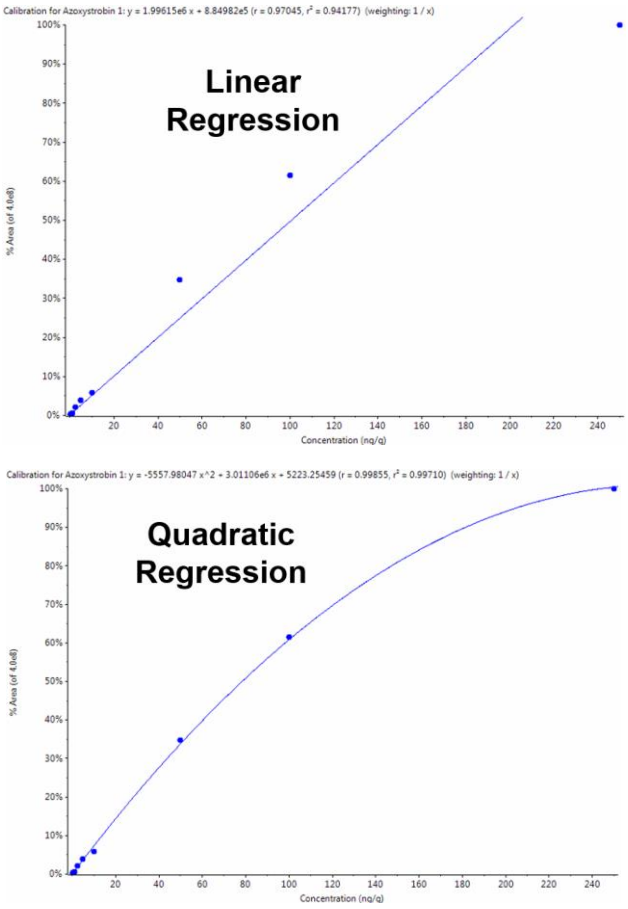

Figure 10: Calibration Curve of Axoxystrobin. Axoxystrobin ionizes extremely well in the mass spectrometer therefore the two highest concentration points are saturating the instrument detector. Quadratic fitting is helpful for a saturated compound. Although the r value is great, there will be greater error at the high end concentrations since the response is no longer linear.

Several possibilities for analyzing compounds that saturate at the high end is to 1) exclude the high concentration levels in the curve and/or 2) select another product ion to monitor that ionizes less efficiently to decrease potential for detector saturation at the high end of the curve.

It is also important to mention that quadratic regression calibration curves will concave down; if the regression curves up, opposite to Figure 10, there is non-specific binding at the low concentration levels. With pesticides, this is usually mitigated by using high organic sample diluent and glass vials for storage both in freezer and in the autosampler.

Summary

Cannabis pesticide residue analysis is extremely difficult. The suggestions and observations in this technical note are meant to aid new analysts in cannabis testing organizations to become more proficient in testing and method development, while avoiding commonly made laboratory mistakes.

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