Deuterated Analogues as Internal Standards in the Accuracy of Quantitative Pesticide and Mycotoxin Analysis between Differing Cannabis Matrices

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Introduction

Methods determining the presence and amount of pesticides and mycotoxins in different cannabis matrices have become important tools in qualifying the safety of patients. This importance will continue as regulation for the use of medical marijuana becomes more prevalent throughout the United States. As the industry has progressed, different methods of cannabis administration have been developed. These include smoking flower, edible products, cannabis concentrates and even balms or lotions. Analysis of these compounds is most accurately performed with the use of a UHPLC system coupled to a triple quadrupole mass spectrometer (LC-MS/MS). Methods have been created using LC-MS/MS systems that give accurate and reproducible results when analysis is performed in similar matrices. However, comparisons of these responses in differing complex matrices can lead to changes in quantitative accuracy. These issues are resolved with the use of deuterated analogues as internal standards with a Shimadzu Nexera series UHPLC system coupled to a 8060 triple quadrupole mass spectrometer. Simultaneous analysis of compounds in positive and negative mode, ultra-fast polarity switching, and ultrafast-scanning speeds are shown here to allow for accurate, quantitative, and highly selective analysis. A series of 59 pesticides, 5 mycotoxins and 24 deuterated analogues functioning as internal standards are analyzed in one method with a 16 minute total analysis time.

Analytical Method

Analytes

Abamectin B1a
Acephate
Acequinocyl
Acetamiprid
Aflatoxin B1
Aflatoxin B2
Aflatoxin G1
Aflatoxin G2
Aldicarb
Azoxystrobin
Bifenazate
Bifenthrin
Boscalid
Carbaryl
Carbofuran
Chlorantraniliprole
Chlorpyrifos
Clofentezine
Daminozide
Diazanon
Dichlorvos
Dimethoate
Dimethomorph
Ethoprophos
Etofenprox
Etoxazole
Fenhexamid
Fenoxycarb
Fenpyroximate
Fipronil
Flonicamid
Fludioxonil
Hexythiazox
Imazalil
Imidacloprid
Kresoxim-methyl
Malathion
Metalaxyl
Methiocarb
Methomyl
Mevinphos
Myclobutanil
Naled
Ochratoxin A
Oxamyl
Paclobutrazol
Permethrins
Phosmet
Piperonyl butoxide
Prallethrin
Propiconazole
Propoxur
Pyrethrins (Pyrethrin I)
Pyridaben
Spinetoram
Spinosad (Spinosyn A)
Spinosad (Spinosyn D)
Spiromesifen
Spirotetramat
Spiroxamine
Tebuconazole
Thiacloprid
Thiamethoxam
Trifloxystrobin

Sample Preparation

Calibrators and QC samples were prepared beginning with 0.5 g of each extraction matrix. A 10 µL addition of the calibration or QC spike was made followed by a 10 µL addition of a working internal standard solution containing all internal standards. Then 10.0 mL of 9:1 LCMS grade MeOH:H_2O with 0.1% glacial acetic acid was added to each sample. Each sample was agitated for 15 min and then centrifuged. 1 mL of supernatant was then transferred to an HPLC vial for analysis. Injections of 10 µL were performed for all samples.

LCMSMS

Shimadzu LC - Nexera X2 UHPLC system
Trap Column – Restek Raptor Biphenyl (3.0x30mm, 2.7 µm)
Column - Restek Raptor ARC-18 (2.1x100mm, 2.7µm)
Mobile Phase A - 0.1% Formic Acid 5 mM Ammonium formate in H_20
Mobile Phase B - Methanol

LCMS-8060

5 msec polarity switching, 30,000 u/sec scanning speed, MRM monitoring

Internal Standards

Acephate-D3	Chlorpyrifos-D10	Fipronil 13C4	Permethrin-D5
Acetamiprid-D3	Daminozide-D6	Imazalil-D5	Phosmet-D6
Aldicarb-5	Diazinon-D10	Imidacloprid-D4	Piperonyl Butoxide D9
Azoxystrobin-D4	Dimethoate-D6	Malathion-D10	Propiconazole-D3
Bifenthrin-D5	Dimethomorph-D6	Metalaxyl-D3	Propoxur-D7
Carbofuran-D3	Fenhexamid-D3	Myclobutanil-D4	Thiacloprid-D4

Results

Residues of 59 pesticides, 5 mycotoxins and 24 isotopically labeled internal standards were prepared in pure methanol, cannabis oil, gummy bear, cannabis flower and a topical cream. Calibration standards were set at 0.5, 5, 25, 100, 250, 500 and 1000 ppb in each matrix. Quality control levels were set at 1, 10 and 50 ppb in each matrix.

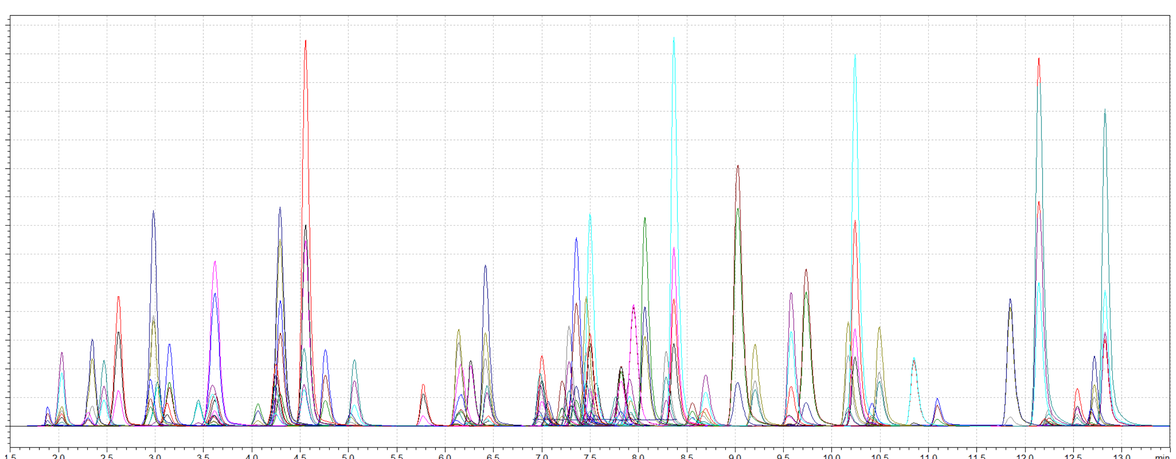


Figure 1: A complete chromatogram of all analytes and internal standards at 500 ppb

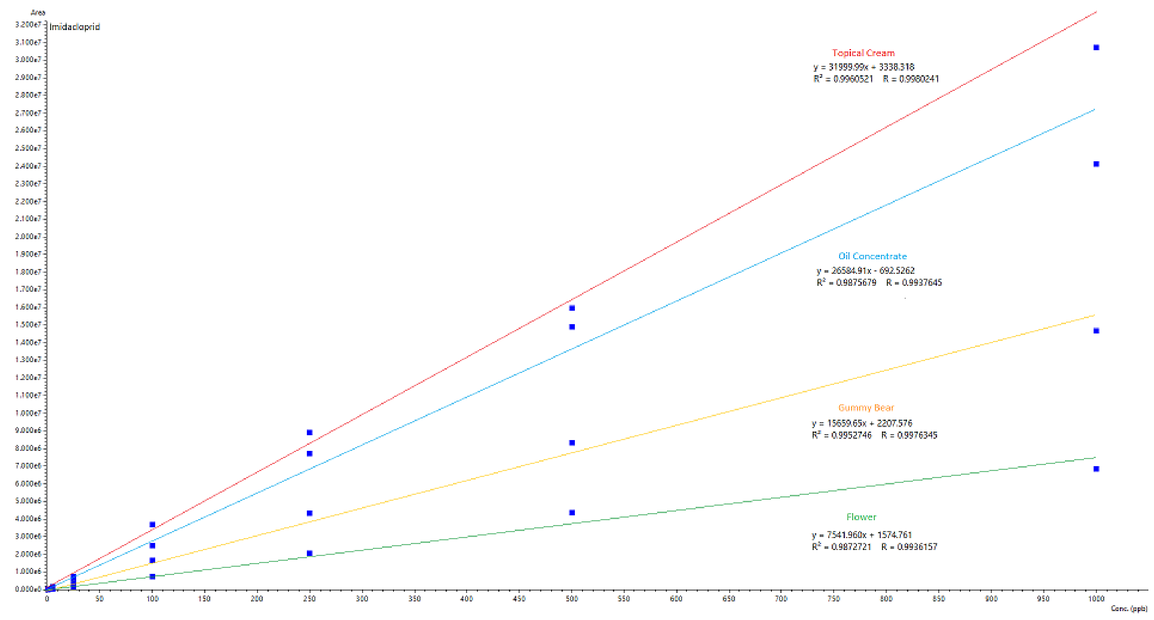


Figure 2: Calibration curves were run in each matrix. These results were then plotted against each other comparing the area under the curve of each response to the calibration concentration. This is shown for Imidacloprid above and the result was a different calibration curve for each matrix. RSD values for the same points in these different matrices reach well above 50% in some cases.

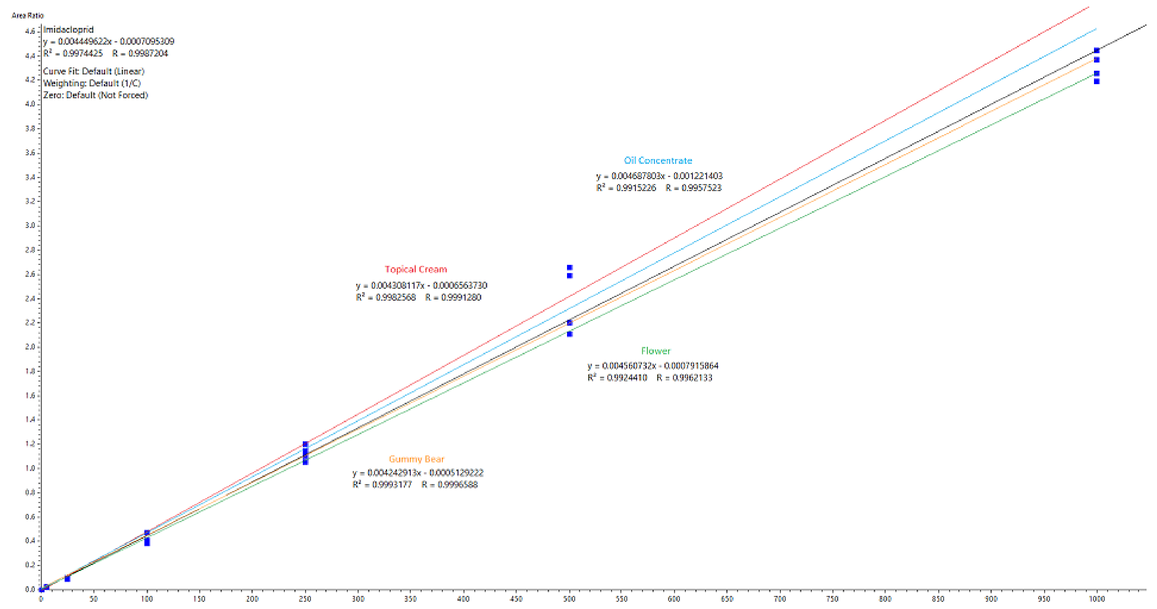


Figure 3: The relative response of Imidacloprid-D4 is used to calculate an area ratio for each calibration concentration in each matrix which is then plotted against the calibrator concentration level. This brings the % RSD between the data points generated by each curve to under 15%. Similar results are seen for all analytes paired with its isotopically labeled internal standard.

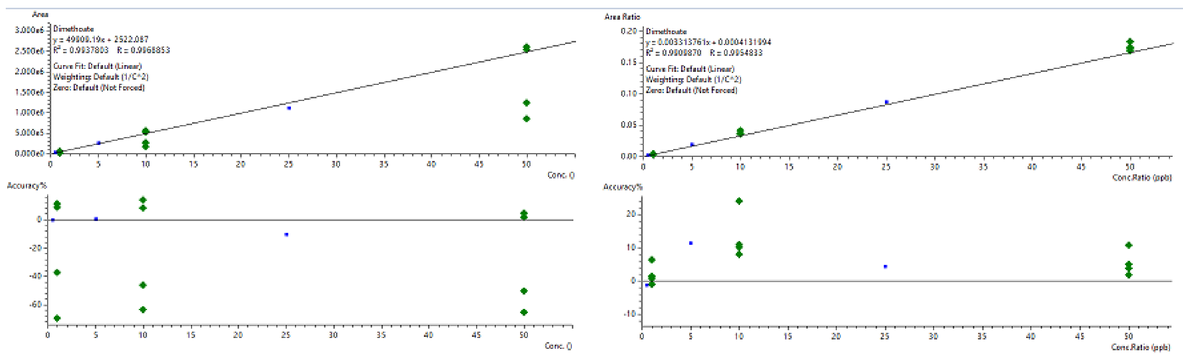
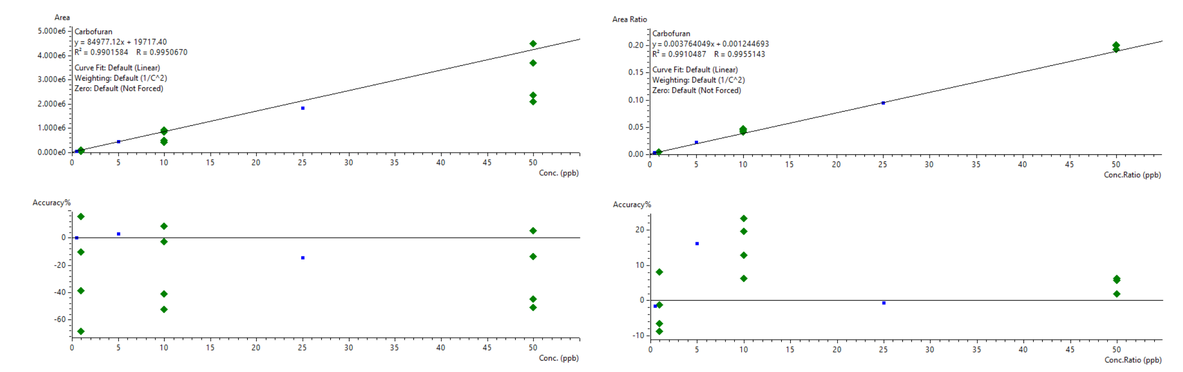


Figure 3: A calibration curve was run in pure solvent at the calibrator concentrations expected in each matrix extraction performed. This pure solvent calibration curve was then used to plot the quality control responses at three different levels (1, 10 and 50 ppb) for the four different matrices. Dimethoate is shown above and Carbofuran below. On the left, calibration curve and area responses in each matrix are plotted against the Calibration curve based on area responses only. Accuracy values differ by more that 60% for some quality controls and RSD is over 50%. On the right the data shows that when internal standard responses are measured and the area ratio compared for each analyte accuracy percent falls within 25% and RSD values drop under 20%. Similar results are seen for the other analytes coupled to their isotopically labeled analogues throughout the method.



Conclusion

The method demonstrates clearly the difficulty introduced by matrix effects in the analysis of pesticides in these different matrices. This method also demonstrates how helpful isotopically labeled internal standards can be in comparing quantitative results between these different matrices. The method is highly selective, highly automated, and achieves linearity over the extent of the dynamic range. The method is robust and reproducible. Rapid polarity switching, ultra-fast scanning speeds and extreme sensitivity of the Shimadzu LCMS-8060 allowed for the creation of very malleable method. Excess instrument time still exists with this method. This allows researchers to quickly and easily add other analytes or internal standards, significantly decrease run time or to adjust the sensitivity and dynamic range of the instrument for individual residues.

The 24 internal standards included in this method are being utilized in the quantitation of all 64 analytes. The results are comparable and limit the effect the matrix has on the quantitative value even for analytes that are not the isotopically labeled equivalent. The extreme speed of MRM analysis on the 8060 is what allows for simultaneous analysis of all of the these analytes and internal standards. The method has been developed to easily allow for more internal standards to be added as they become available.