Quantitation of Cannabinoids and Pesticides in Cannabis Products Using the Triple Quad™ 3500 LC-MS/MS System

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Overview

With the recent legalization of cannabis in several states, there is a growing need for robust, reliable, and cost-effective analytical methods to facilitate routine testing for potency and contaminants. Here we present an LC-MS/MS method that uses the budget-friendly SCIEX Triple Quad™ 3500 LC-MS/MS system for the simultaneous detection and quantification of cannabinoids, pesticides, and mycotoxins in cannabis products using the *Scheduled* MRM™ algorithm.

Introduction

Potency testing is performed to measure concentrations of cannabinoids, some of which are psychoactive components, while others are believed to have medicinal properties for ailments such as glaucoma, insomnia, and epilepsy.

Like any agricultural product, cannabis has the potential to be attacked by pests or pathogens resulting in the need for treatment with insecticides, acaricides, fungicides, and potentially other crop protection agents. Tolerance levels for these chemical contaminants have been established through health risk analyses.

Pesticide use in cannabis production is of concern for several reasons. While residues on the marketed product are important metrics for quality, it may be difficult to associate trace residues with human health effects, or these correlations may take years of careful medical research to identify. Perhaps more importantly, the creation of rational guidelines for pesticide use can serve to protect workers in the production system and the environment. As a high value crop, crop yields are economically vital to growers and others in the industry. As such, it becomes paramount to determine any potential risks to employees, customers, and the environment as a result of growing practices. This can be accomplished by appropriate registrations, inspection, and residue analysis. As such, sensitive, selective, accurate and economical analytical methods are needed to screen cannabis products for pesticide residues and mycotoxins.[1]

The list of contaminants grows as more states approve the use of cannabis for medicinal or recreational purposes. All states that have legalized it require testing for labeling; however few states actually specify what pesticides to measure or what the minimum residual level (MRL) should be. Currently, several contract laboratories use HPLC/UV, which can provide potency data, however, these methods do not provide sufficient specificity or sensitivity for more comprehensive analyte lists such as pesticides and mycotoxins, especially at low levels (~10 µg/kg). Using HPLC-MSMS analysis provides the sensitivity and selectivity for these low levels using MRM analysis.

Experimental

Standards and Internal Standards

Standards, internal standards (ISTDs), ACS grade ammonium acetate, formic acid and distilled-in-glass grade methanol were used as received. High purity water was produced by passing reverse osmosis water through a Barnstead NANOpure water purification system. All standard solutions and samples were stored at 5 ± 3°C and allowed to reach room temperature before analysis.

Sampling and sample preparation

Ten cannabis samples were extracted by adding 0.1 to 2 g of cannabis product to 10 mL of methanol then sonicated for 10 minutes followed by centrifugation at 16,000rpm for five minutes.

Following filtration samples were analyzed without further workup.

LC Separation

Chromatography was performed on a SCIEX ExionLC™ AC system. Chromatographic separation was achieved using a Restek 2.7 µm Raptor ARC-18 50 x 2.1 mm column heated to 40°C. The aqueous mobile phase consisted of 5 mM ammonium formate + 0.1% formic acid and the organic mobile phase was 5 mM ammonium formate + 0.1% formic acid in 98% acetonitrile, 2% water and a 400µL/min flow rate was used (Table 1).

Table 1. LC gradient

Step	Time (min)	A (%)	B (%)
0	0.0	70	30
1	0.5	70	30
2	4.0	5	95
3	5.0	5	95
4	5.1	70	30
5	7.0	70	30

MS/MS Detection

Data were acquired in positive ESI mode using a SCIEX Triple Quad™ 3500 system and Analyst® 1.6.2 software. The MS and MRM parameters are provided in Table 2. The *Scheduled* MRM™ algorithm was used to acquire the quantifier and qualifier ions for each analyte, with a total of 82 transitions monitored.

Table 2. MS parameters

Parameter	Value
Polarity and Ionization Mode	Positive ESI
Ion Spray Voltage	4000 V
Temperature (TEM)	650°C
Nebulizer Gas (GS1)	40 psi
Heater Gas (GS2)	50 psi
Collision Gas (CAD)	7
Curtain Gas (CUR)	25 psi

Data processing was done in MultiQuant™ 3.0.2 software using the MQ4 integration algorithm.

Results and Discussion

All target compounds are listed in Table 3.

Table 3. Pesticides, mycotoxins, and cannabinoids screened for in cannabis samples

Pesticides		Mycotoxins
Acequinocyl	Imazalil	Aflatoxin B1
Avermectin B1a	Imidacloprid	Aflatoxin B2
Avermectin B1b	Myclobutanil	Aflatoxin G1
Bifenazate	Paclobutrazol	Aflatoxin G2
Bifenthrin	Spinosyn A	Ochratoxin A
Chlormequat	Spinosyn D	
Cyfluthrin	Spiromesifen	
Daminozide	Spirotetramat	
Etoxazole	Trifloxystrobin	
Fenoxycarb		
Cannabinoids		
Δ-9-Tetrahydrocannabinol (THC)	Cannabigerol (CBG)	
Tetrahydrocannabidiolic acid (THCA)	Tetrahydrocannabivarin (THCV)	
Cannabidiol (CBD)	Cannabinol (CBN)	
Cannabidiolic acid (CBDA)	Cannabigerolic acid (CBGA)	
Cannabichromene (CBC)	Cannabidivarin (CBDV)	

The chromatographic method was rapid and provided baseline separation of all isobars (Figure 1).

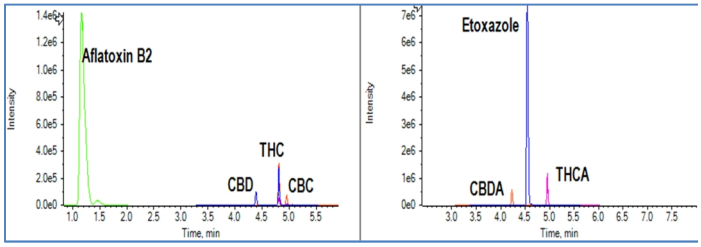

Figure 1. Chromatographic separation of isobars

Instrument sensitivity was evaluated for all compounds using external calibration from duplicate injections of solvent standards. An aliquot of the cannabis extracts were spiked with a concentration of 1 ng/mL of pesticides/mycotoxins (Figure 2),

which is representative of a plant concentration of 10 µg/kg assuming a 1 gram sample. This was done to demonstrate a limit of detection for these components in matrix. The 1 ng/mL spiked sample results demonstrate excellent precision, with %CV values < 10%.

Cannabinoids were not spiked due to their potential presence in the samples at very high concentrations.

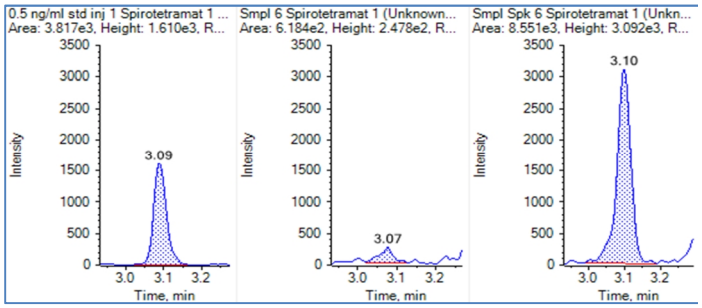

Figure 2. Representative chromatograms for Spirotetramat standard in solvent at 0.5 ng/mL (left), unspiked sample (middle), and 1ng/mL spiked sample (right) with 109% accuracy

For pesticide and mycotoxin analysis, all calibration curves were fit with linear regression and 1/x concentration weighting from 0.1 to 1,000 ng/mL (Figure 3). Linearity was demonstrated across the entire calibration range for most compounds with correlation coefficients > 0.99.

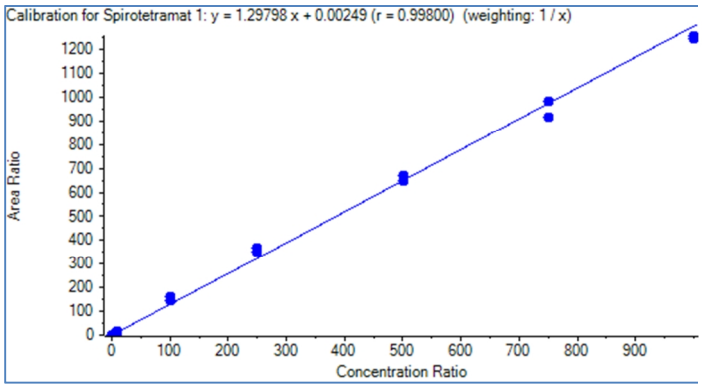

Figure 3. Representative pesticide calibration curve for Spirotetramat; linear fit with 1/x weighting

The extracts were quantified using external calibration and four samples were found to have hits above 1 µg/kg for some of the screened pesticides and mycotoxins (Table 4). Sample 1 was a flower from a cannabis plant, which may explain the positive hits for pesticides and mycotoxins.

Table 4. Pesticides and mycotoxins detected in cannabis samples above a concentration of 1µg/kg

Sample	Positive Hits above 1 µg/kg
1 (flower)	Spirotetramat, Imazalil, Imidacloprid, Spiromesifen, Aflatoxin B1 and B2, Aflatoxin G1 and G2, Ochratoxin A
2 (extract)	Etoxazole
3 (extract)	Spiromesifen
6 (raffinate)	Imidacloprid

For cannabinoids, two curves were assessed: from 0.1 to 1000 ng/mL for trace level analysis, and from 1 to 100 µg/mL for high concentration analytes such as THC and THCA (Figure 4).

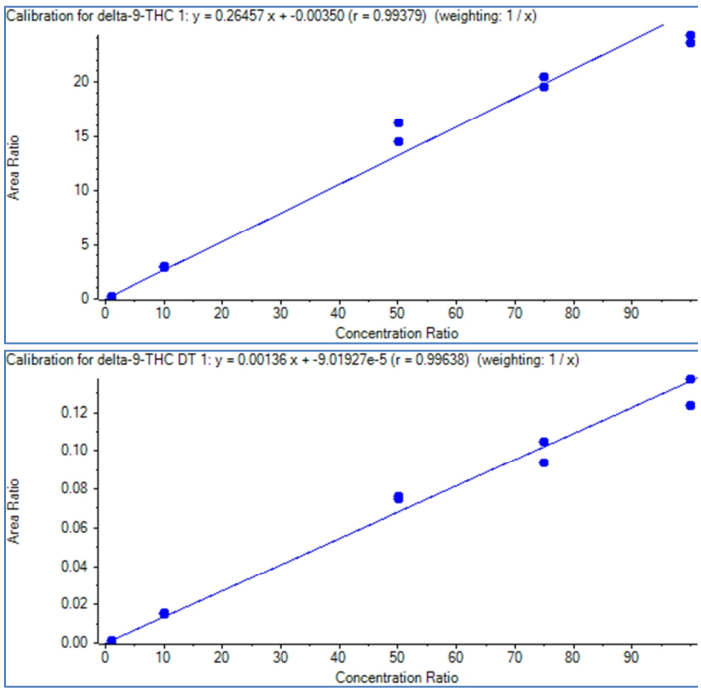

Figure 4. Representative cannabinoid calibration curves for THC (top) and "detuned" CE calibration curve for THC (bottom); 1 to 100 µg/mL; both with linear fit and 1/x weighting

Certain cannabinoids, such as THC and THCA, are present at much higher concentrations relative to others and to trace level

Table 5: Cannabinoid sample results in µg/kg

	Sample									
Compound	**1 Flower**	**2 Extract**	**3 Extract**	**4 Flower**	**5 Edible**	**6 Raffinate**	**7 Topical**	**8 Edible**	**9 Concentrate**	**10 Flower**
THC	142620	165058	360329	155437	7982	900	19777	1562	346416	167166
THCA	190912	216661	313253	206744	1805	564.6	14867	1740	319314	222017
CBD	3019	2313	15775	8.3	19.4	-	686.2	-	12127.2	2376
CBDA	-	12359	62558	5356	30.4	23.4	1837	35.9	46581	10241
CBC	19579	1717	926.4	1452	185.6	94.1	1105	-	1794	555
CBG	12833	23749	89028	4134	-	-	-	-	116397	19751
THCV	5243	6366	58734	130.0	144.7	-	603.3	-	77730	4857
CBN	35.9	7.6	8018	38.4	262.0	118.6	458.8	20.2	10370	1991
CBGA	41002	72988	221842	29991	173.0	58.6	2006	33.9	256775	79258
CBDV	4214	3669	418.8	4194	3.9	10.0	83.7	-	1568	3253

contaminants. To allow for the analysis of these high level cannabinoids in the same injection as the lower level targets in the method, transitions for THC and THCA using “detuned” collision energies were included in the acquisition method. The use of a “detuned” transition will result in much smaller dilutions being required, or no longer needing to dilute at all for potency analysis, producing more accurate and precise results.

A series of cannabis extracts were quantified using external calibration (Table 5). The extracts represented a cross-section of cannabis products including flowers, edibles, extracts, a raffinate, a concentrate, and a topical formulation.

Summary

These results demonstrate the utility and quantitative potential of this method for the routine analysis and quantification using the SCIEX Triple Quad™ 3500 system for the simultaneous analysis of cannabinoids and pesticides in cannabis products. The method demonstrates the accuracy, precision, selectivity, and sensitivity necessary for the low level analysis of pesticides, as well as the higher levels of cannabinoids.

Acknowledgement

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References

1 P. Daley, D. Lampach, S. Sguerra: ‘Testing Cannabis for Contaminants’ (2013) http://liq.wa.gov/publications/Marijuana/BOTEC%20reports/1a-Testing-for-Contaminants-Final-Revised.pdf

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