Analysis of the Massachusetts Cannabis Pesticides List Using the SCIEX QTRAP® 6500+ System

Evoking the Power of MRM³ to Reduce Matrix Background in Cannabis Extracts

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The evolving landscape of cannabis legalization in the United States demands highly robust and sensitive analytical methods. Currently, pesticide levels are regulated by the individual states and thus the analyte scope and action levels are unique to each state. The State of Massachusetts regulates 9 pesticides (Bifenazate, Bifenthrin, Cyfluthrin, Etoxazole, Imazalil, Imidacloprid, Myclobutanil, Spiromesifen, Trifloxystrobin) at 10 ppb in flower, amongst the lowest in the United States. These strict action levels necessitate a highly sensitive mass spectrometer, such as the SCIEX 6500+ QTRAP® system.

In addition, cannabis extracts are very dirty, frequently resulting in high background interferences. These matrix inferences can overwhelm the analyte signal, negatively impacting the LOQ. The linear ion trap (LIT) functionality of the QTRAP system, with the ability to perform MS3, can greatly reduce background noise in complex matrices. The QTRAP system operates by isolating first-generation product ions in the LIT (i.e. produced in the collision cell), and then resonating the product ion to make second-generation fragments (Figure 1). These fragments are scanned out of the LIT and individual ions are extracted during data processing. The technique of monitoring "fragments of fragments" (termed MRM³) results in highly selective analysis, significantly reducing the matrix background signal.[1]

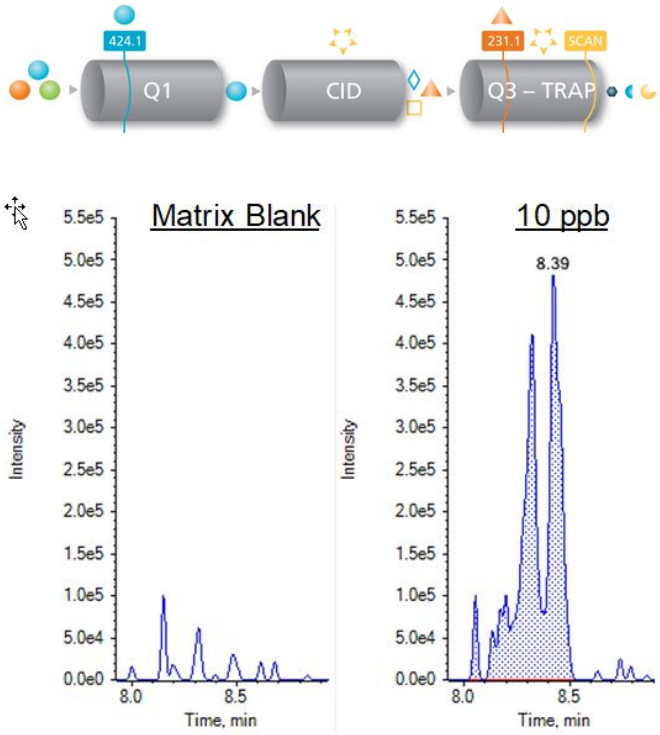

Figure 1. High Specificity MRM³ Workflow for Enhanced Specificity. (Top) MRM3 scan mode. (Bottom) Chromatograms for cyfluthrin in matrix blank and 10 ppb (in flower) are shown.

Quantitation of 9 pesticides is demonstrated in *Cannabis* extract using the SCIEX QTRAP® 6500+ with IonDrive™ Turbo V source in positive mode electrospray ionization (ESI).

Key Feature of QTRAP Technology for Cannabis Analysis

- High sensitivity for detection of low level pesticides with QTRAP 6500+ system using a 20 min gradient.
- MRM³ was employed to reduce the matrix background for cyfluthrin (Figure 1).
- LOQ values were <10 ppb (in flower) for all compounds with excellent accuracy and precision, successfully attaining the stringent Massachusetts action levels.

Methods

Sample Preparation: Extracts were prepared following the procedures outlined in "Quantitation of Pesticide Residues and Cannabinoids in Cannabis Matrices" (Figure 2). Briefly, 0.2 g of flower was ground and homogenized, and 5 mL of acetonitrile was added. Samples were sonicated for 15 min, vortexed for 30 seconds and left overnight at -20 oC for "winterization". The winterization step was essential to reduce matrix background signal in the MRM chromatograms. Immediately prior to analysis the extract was removed from the freezer and diluted 1:6 (v/v) with 75:25 methanol:water.

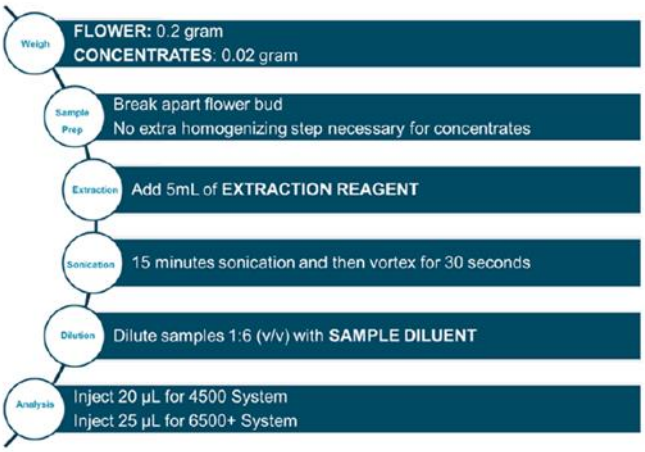

Figure 2. Pesticide Analysis Sample Preparation Flowchart.

Chromatography: The SCIEX ExionLC™ system was used as the LC system and chromatographic separation was achieved under gradient conditions using a Phenomenex Kinetex Biphenyl column (100 Å, 150 x 4.6 mm, 2.6 µm particle size). The mobile phases were water ("A", modified with 5 mM ammonium formate and 0.1% formic acid), and 98:2 methanol: water ("B", modified with 5 mM ammonium formate) with a flow rate of 1 mL/min (Table 1). The column oven was 40oC and the injection volume was 25 µL. The gradient conditions were slightly different than the original vMethod to allow for a longer column wash and equilibration.

Mass Spectrometry: Analysis was performed on a SCIEX QTRAP 6500+ system with the Ion Drive™ Turbo V source using the electrospray ionization (ESI) probe in positive ion mode. Compound specific and ion-source parameters were initially taken from the vMethod with additional optimization performed (Table 2). Two MRMs per compound were monitored except for cyfluthrin in which monitored by MS/MS/MS (MS3) using manually optimized parameters (Table 3). For the cyfluthrin MS3 optimization, the excitation energy (AF2) was ramped to identify the major secondary fragment ion and optimized AF2 value. The chromatographic run was separated into periods to optimize cycle time and maximize sensitivity.

Table 1. LC Gradient Program. 20 minute method using a flow rate of 1.0 mL/min, and injection volume of 25 µL.

Step	Time (min)	A (%)	B (%)
0	0.0	95	5
1	0.75	95	5
2	1.0	50	50
3	1.5	40	60
4	2.5	22	78
5	4.0	12	88
6	10.0	8	92
7	12.0	0	100
8	15.8	0	100
9	15.9	95	5
End	20.0		

Data Processing: Data was processed using SCIEX OS software 1.4.

Table 2. Source, Gas and Temperature Conditions.

Parameter	Value
Curtain Gas (CUR)	35 psi
Collision Gas (CAD)	12
IonSpray Voltage (IS)	*3500 V*
Temperature (TEM)	*225°C*
Nebulizer Gas (GS1)	*80 psi*
Heater Gas (GS2)	*60 psi*

Table 3. MRM and MRM^3 Masses and Compound-Specific MS Parameters for QTRAP 6500+ System.

Compound	Period	Q1	Q3	EP (V)	DP (V)	CE (V)	CXP (V)
Imidacloprid 1	1	256.1	*209.0*	*10*	*89*	*23*	*4*
Imidacloprid 2	1	*256.1*	*175.0*	*10*	*89*	*19*	*4*
Imazalil 1	*2*	*297.2*	*41.1*	*10*	*40*	*57*	*4*
Imazalil 2	*2*	*297.2*	*159.1*	*10*	*56*	*31*	*4*
Myclobutanil 1	*2*	*289.1*	*70.1*	*10*	*69*	*33*	*4*
Myclobutanil 2	*2*	*289.1*	*125.2*	*10*	*69*	*39*	*4*
Bifenazate 1	*2*	*301.1*	*198.1*	*10*	*40*	*14*	*4*
Bifenazate 2	*2*	*301.1*	*170.2*	*10*	*61*	*29*	*4*
Trifloxystrobin 1	*3*	*409.1*	*186.1*	*10*	*59*	*23*	*4*
Trifloxystrobin 2	*3*	*409.1*	*116.1*	*10*	*116*	*25*	*4*
Spiromesifen NH4 1	*3*	*388.0*	*273.1*	*10*	*61*	*17*	*4*
Spiromesifen NH4 2	*3*	*388.0*	*255.2*	*10*	*61*	*27*	*4*
Etoxazole 1	*3*	*360.2*	*141.0*	*10*	*76*	*37*	*4*
Etoxazole 2	*3*	*360.2*	*177.2*	*10*	*71*	*29*	*4*
Cyfluthrin 1 [a]	*4*	*451.0*	*434.0*	*10*	*30*	*12*	*n/a*
Bifenthrin 1	*5*	*440.2*	*181.0*	*10*	*46*	*19*	*4*
Bifenthrin 2	*5*	*440.2*	*166.1*	*10*	*46*	*59*	*4*

Notes: [a] Excitation energy (AF2) = 0.07, 2nd product fragment m/z = 190.8-191.3

Results

Cyfluthrin is challenging to analyze in cannabis extracts because 1) the compound inherently ionizes poorly, 2) the signal is split across multiple isomers and 3) the MRM background signal overwhelms the analyte signal at low to moderate levels. For example, Figure 3 shows the cyfluthrin MRM (m/z 451.1>191.0) chromatogram in a) matrix blank and b) 10 ppb in flower spike. Attempts to chromatographically separate the matrix interference were not successful. Therefore, MRM techniques for cyfluthrin yielded a LOQ value of ~150 ppb in flower which was unsatisfactory for the Massachusetts action levels.

Using QTRAP system, MRM^3 workflow was successful in sufficiently reducing the matrix background for cyfluthrin and achieving the 10 ppb, in flower, action level (Figure 1). MRM3 allows for increased selectivity above that typically provided by MRM. The NH4+ adduct of cyfluthrin was isolated in Q1 and the fragment corresponding to ammonia loss was isolated in the LIT. The second generation fragment ions were scanned out of the LIT and the optimized ion (m/z 190.8-191.3) was extracted during data processing.

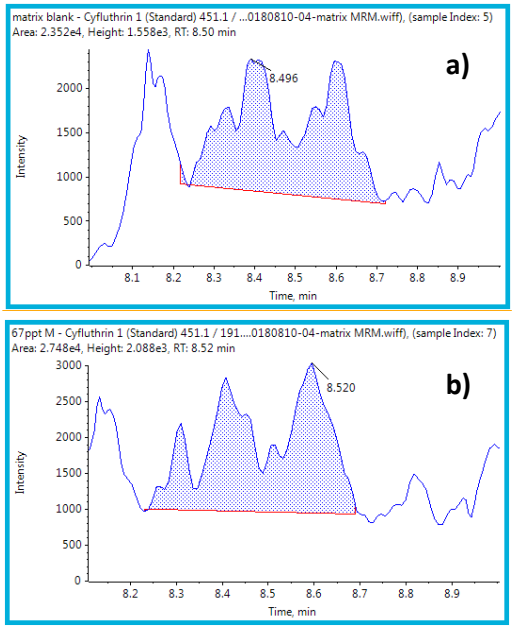

Figure 3. Cyfluthrin MRM Chromatograms. High background was observed for cyflurthrin (m/z 451.1>191.0) for a) cannabis matrix blank and b) 10 ppb in flower spike. However MRM3 analysis (Figure 1) showed significant reduction in background.

Sensitivity, Linearity and Precision

All analytes showed LOQ values of 5 ppb in flower for the quantifying MRM transition except for Cyfluthrin which had an LOQ of 10 ppb (Table 4). The actual LOQs were presumably <5 ppb as evidence by the high S/N values, but the lowest concentration evaluated was 5 ppb. Further, most analytes also had a LOQ of 5 ppb for the qualifying MRM transition except for Imazalil (LOQ=10 ppb, m/z 297.2>41.1) and Spiromesifen (LOQ=37.5 ppb, 388.0>255.2). Both Imazalil and Spiromesifen had high matrix background peaks which negatively impacted the S/N. In the case of Spiromesifen, monitoring the NH4 adduct results in a cleaner background as compared to the [M+H]+ transition. The extraction process does not include a clean-up step (e.g. SPE, QuEChERS), however, the overnight "winterization" step aided in reducing the matrix background. Although not tested in this study, monitoring alternative product ions may yield cleaner backgrounds. MRM chromatograms for the LOQ samples are shown in Figure 4.

The matrix-spiked LOQ samples (n=3) showed excellent data quality with accuracy typically within 5% and CVs <5%. These values represent very strong data quality at levels below or near the Massachusetts action limits.

All analytes showed very strong linearly throughout the calibration range with r2 values that were greater than 0.99. However, the calibration range was fairly narrow since the experimental design focused on levels near the 10 ppb action limits. In general, the QTRAP 6500+ system is capable of approximate 5 orders of linear dynamic range.

Table 4. Method Performance Parameters for Matrix-Spiked Samples. Here the performance of the method for sensitivity, linear range, LOQ accuracy and precision, signal-to-noise is shown. Peak-to-peak S/N was calculated using the Explorer module in SCIEX OS software 1.4.

Compound	Calibration Range (ppb)	Linear Correlation (r^2)	LOQ (ppb)	Accuracy of LOQ std. (%)	Precision of LOQ std (%)	Peak-to-Peak S/N at LOQ
Bifenazate 1	5-150	0.992	5	88	1.2	138
Bifenazate 2	5-150	0.991	5	86	3.0	46
Bifenthrin 1	5-150	0.999	5	98	6.7	43
Bifenthrin 2	5-150	0.999	5	101	2.6	57
Cyfluthrin 1	5-150	0.996	10	100	12	10
Etoxazole 1	5-150	0.998	5	95	0.39	339
Etoxazole 2	5-150	0.999	5	101	2.1	27
Imazalil 1	5-150	0.999	10	97	5.1	12
Imazalil 2	5-150	0.997	5	109	9.5	14
Imidacloprid 1	5-150	0.999	5	96	1.5	328
Imidacloprid 2	5-150	0.999	5	97	0.53	41
Myclobutanil 1	5-150	0.994	5	85	19.8	24
Myclobutanil 2	5-150	0.997	5	92	12.0	30
Spiromesifen NH4 1	5-150	0.999	5	107	3.9	41
Spiromesifen NH4 2	37.5-150	0.984	37.5	n/a	n/a	43
Trifloxystrobin 1	5-150	0.999	5	93	0.17	491
Trifloxystrobin 2	5-150	0.999	5	94	0.59	107

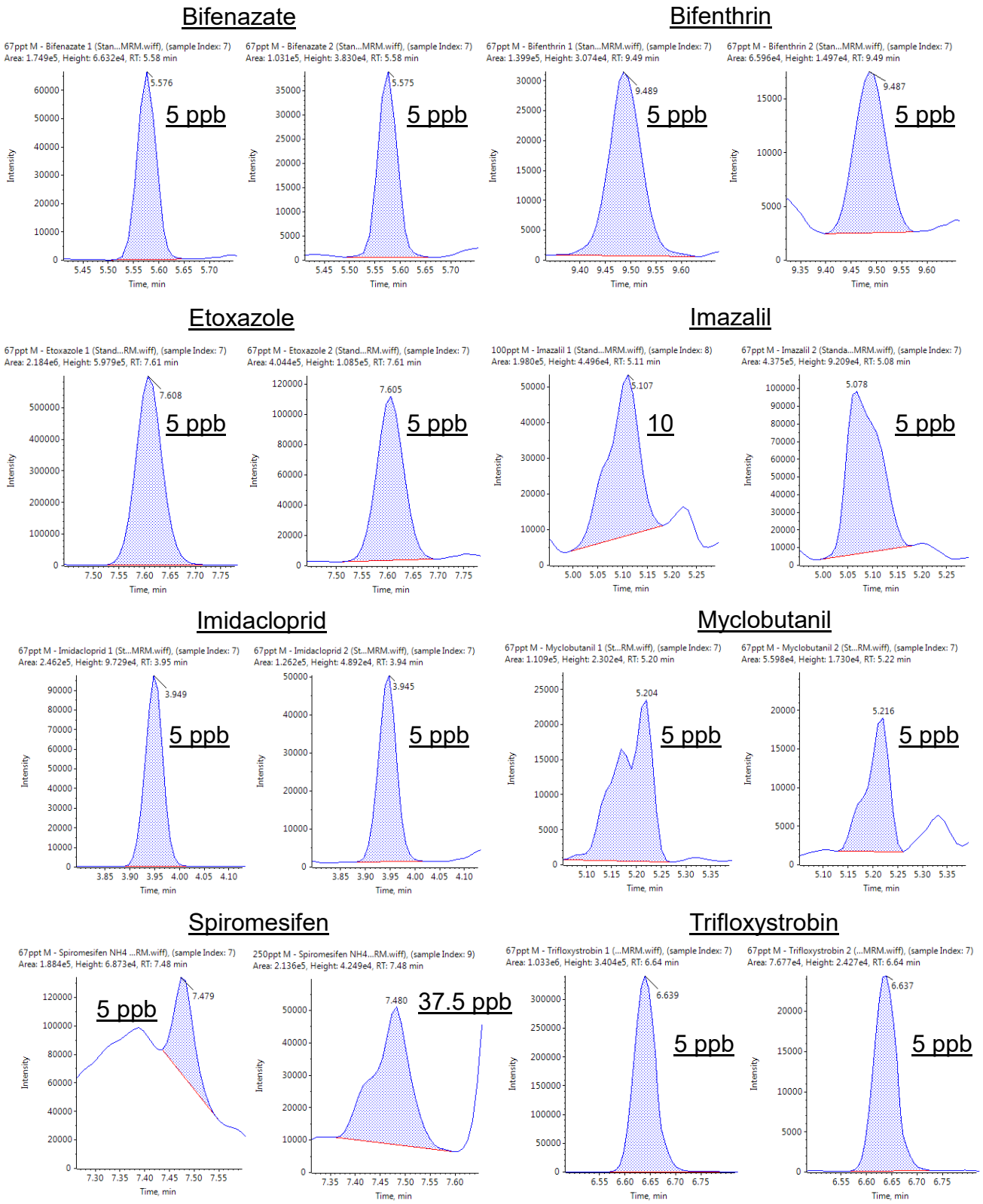

Figure 4. MRM Chromatograms for the LOQ Matrix-Spike Samples. Values represent LOQ concentration in flower.

Conclusions

Ultimately, the results showed that the Massachusetts cannabis pesticide action levels could be obtained using a combination of simplified sample preparation and the SCIEX QTRAP 6500+ system. Experiments were performed on spiked matrix extracts, representing real world samples. The QTRAP system was critical to reduce the high matrix background in the cyfluthrin chromatogram. LOQs for all analytes were below the Massachusetts action levels with strong accuracy and precision values for *matrix-spiked* samples.

References

1. MRM^3 Quantitation for Highest Selectivity in Complex Matrices. SCIEX Application Note RUO-MKT-02-2739-B.
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3. Quantitation of Oregon List of Pesticides and Cannabinoids in Cannabis Matrices by LC-MS/MS. SCIEX Application Note RUO-MKT-02-6729-B.

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