

## Colorado's Residual Pesticide Analysis using the Shimadzu Triple Quad LCMS-8060NX

### ■ Summary

The Colorado list of residual pesticides in cannabis<sup>1</sup> was analyzed using a Shimadzu LCMS-8060NX. This study demonstrated the use of ESI-LCMS to analyze the Colorado pesticide list specified by the Code of Colorado Regulations<sup>1</sup> for cannabis in one injection. Optimization was performed on fourteen pesticides and then tested in cannabis flower extract. The LOQ determined for each pesticide met or exceeded the regulatory requirements.

### ■ Background

The demand for pesticide testing of cannabis products is concurrently increasing with the approvals of medicinal and recreational cannabis legislation throughout the United States. In lieu of a current federal standard, each state is setting individual regulatory guidelines. The differing interstate regulations has resulted in variations between the number of analytes tested and the associated required action levels. This study evaluates the electrospray ionization (ESI) technique to quantitate pesticides of the Colorado (CO) list using liquid chromatography-tandem mass spectrometry (LCMS).

### ■ Method

A Shimadzu LCMS-8060NX triple quadrupole mass spectrometer with electrospray ionization source coupled with a Shimadzu Nexera X2 ultra-high pressure liquid chromatography system was used for this study. Rapid polarity switching of 5 ms and fast multiple reaction monitoring (MRM) mode enabled the acquisition of sufficient data points across each peak.

Fourteen pesticides were analyzed by ESI-LCMS. Each compound was purchased commercially as a certified neat standard or a mixture of compounds and dissolved in acetonitrile or methanol to 1 mg/mL. These stock solutions were used for dilutions during method development.

Flow injection analysis was used for the initial ionization testing and MRM optimization. Ionization evaluation consisted of Q1 and Q3 scans in both positive and negative polarity. Any viable precursors observed were further analyzed using MS/MS scans and a range of collision energies.

For each pesticide, one to three MRM transitions were acquired. On-column testing was completed using a 1- $\mu$ L injection. Established MRM transitions and final method parameters were tested in cannabis flower matrix. The resulting limits of quantification (LOQs) are shown in **Table 1**.

Separation was accomplished with a 15-minute method. LCMS method parameters are shown in **Table 2**.



**Table 1:** LOQs for pesticides compared to the CO action limit<sup>1</sup>.

Residual Pesticide	Lowest Calibration Level ng/mL	LOQ in Sample ng/g (ppb)	CO Action Limit ng/g (ppb)
Abamectin <sup>a</sup>	1	20	70
Azoxystrobin	0.25	5	20
Bifenazate	0.25	5	20
Etoazole	0.25	5	10
Imazalil	2	40	40
Imidacloprid	0.25	5	20
Malathion	0.5	10	50
Myclobutanil	0.5	10	40
Permethrins	2	40	40
Spinosyn A	0.1	2	60 <sup>b</sup>
Spinosyn D	0.25	5	60 <sup>b</sup>
Spiromesifen	0.25	5	30
Spirotetramat	1	20	20
Tebuconazole	0.25	5	10

a: The certified standard used for Abamectin was a 99:1 ratio of Avermectin B1a:B1b. Avermectin B1a was quantified for total Abamectin.

b: The action limit is set for the sum of Spinosyn A and D.

### ■ Sample Extraction

A composite sample of blank cannabis flower from all stages of production (*i.e.*, fresh, frozen, dried, and cured) was weighed out at  $0.5 \pm 0.05$  g into a 15 mL test tube. The flower sample was extracted with 10 mL of LCMS grade methanol by shaking with commercial grinder balls at 1000 rpm for 10 minutes. The extract was then centrifuged at 12000 rpm for 10 minutes to obtain the supernatant. Calibrants were prepared using post-extracted matrix by spiking 10  $\mu$ L of 500 ng/mL CO pesticides mix (diluted from CRM 1000  $\mu$ g/mL) into 490  $\mu$ L extract to produce a 10 ng/mL highest calibrant level. The highest calibrant was then serially diluted with blank flower extract to produce the calibration curve with the lowest calibrator at 0.1 ng/mL. The lowest calibration level yielding sufficient signal-to-noise and accuracy for each pesticide is listed in **Table 1** alongside its corresponding concentration in matrix and the CO action limit<sup>1</sup>.

### ■ Calibration

Matrix-matched calibration curves were prepared by serial dilution of spiked flower extract with blank flower extract and evaluated for each pesticide. The calibration set included different concentrations, ranging from 0.1 ng/mL to 10 ng/mL.

**Table 2:** LC and MS parameters for method.

Parameter	Value
Nebulizing Gas	3 L/min
Heating Gas	10 L/min
Drying Gas	8 L/min
Interface Temperature	200°C
DL Temperature	150°C
Heat Block Temperature	200°C
Flow Rate	0.5 mL/min
Column Oven Temperature	45°C

The final concentration range for each pesticide was selected and varied by the individual results. Pesticide calibration standards were analyzed in order of low to high, and each set of standards was followed by a quality control sample and a blank for performance and carryover assessment. Carbaryl-d7 was used as an internal standard for all compounds. **Figure 1** shows an example chromatogram of all compounds at 3 ng/mL, which highlights the complete separation of the pesticides below the CO action limits. Calibration curves and representative chromatograms are shown in **Figure 2**. The wide linear range and low background emphasize the superior sensitivity of this method.

### ■ Precision and Accuracy

Method precision and accuracy were determined by measuring the calibration curve standards in triplicate. Accuracy was calculated utilizing LabSolutions software by comparing the measured concentration against the theoretical concentration for each calibration standard. The LOQs were determined from the calibration curve data. The LOQ reported for each pesticide had a signal-to-noise ratio greater than 10 and %RSD value less than 20.

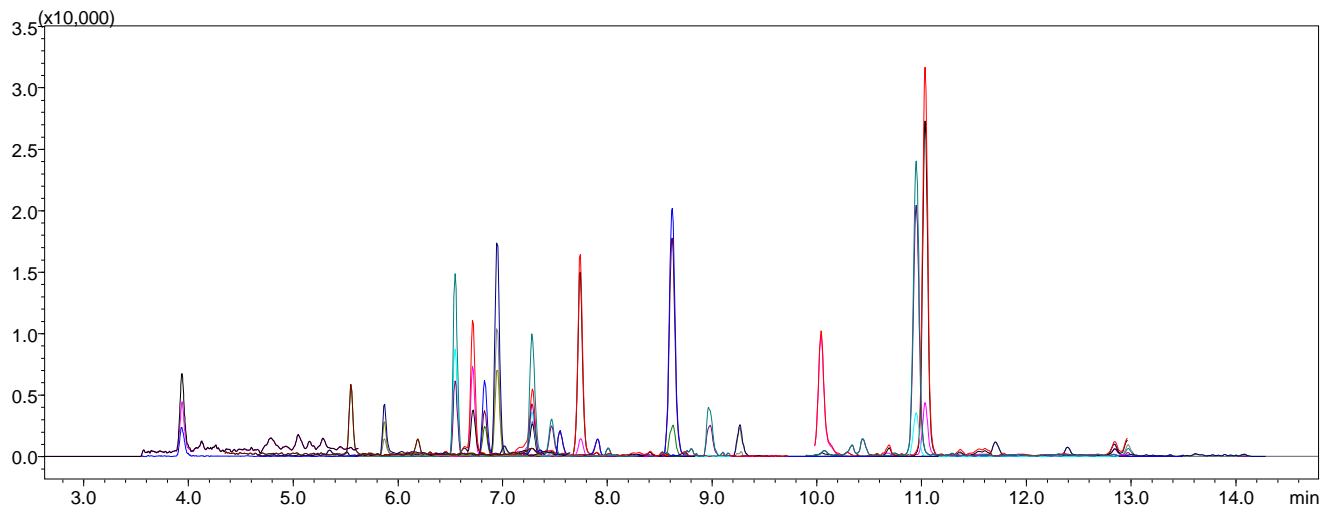


Figure 1: Example chromatogram of all compounds in flower matrix at 3 ng/mL.

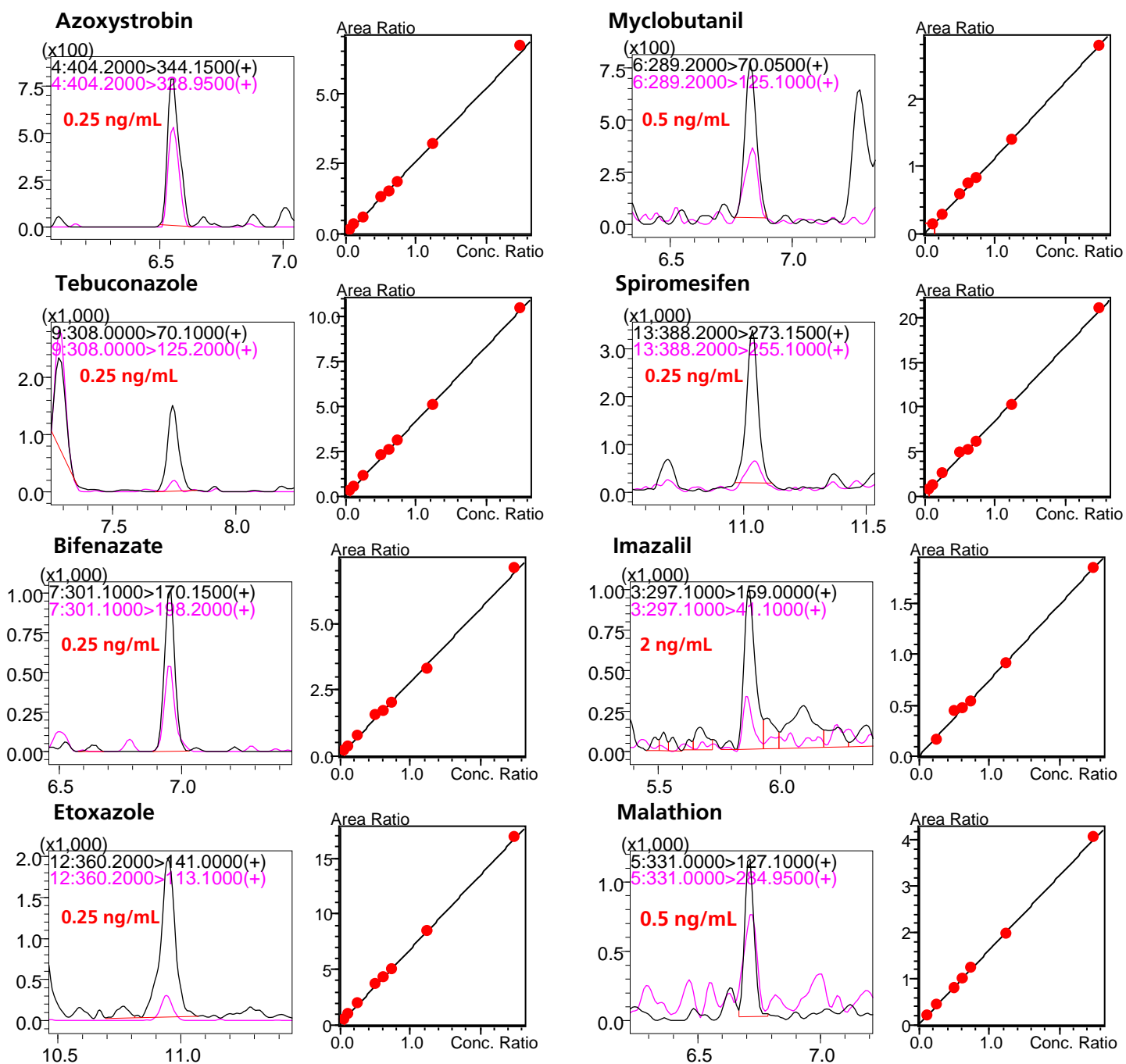


Figure 2: Calibration curves and chromatograms at LOQ in flower matrix for eight pesticides.

### ■ Results and Discussion

Fourteen Colorado residual pesticides were analyzed in a single method. The ESI-LCMS method demonstrated accurate and precise trace-level quantitation in cannabis flower for all fourteen pesticides. The LOQ for each pesticide was either less than or meeting the Colorado action levels in cannabis<sup>1</sup>, and precision and accuracy results were excellent. LOQs were determined for each pesticide using their corresponding retention time and a signal-to-noise calculation above 10:1. Linear calibration curves were prepared using spiked standards in homogenized cannabis flower extract. Calibration curves demonstrated linearity ranging from 0.1 ng/mL to 10 ng/mL on-flower concentrations. A  $1/C^2$  weighting factor was used for statistical calculations and resulted in  $R^2 > 0.97$  for all pesticides. Representative chromatograms and calibration curves can be found in **Figure 2**. Chromatographic separation of analytes from matrix interferences resulted in low signal suppression. Optimized spray voltage and low temperature interface conditions yielded strong signal intensity for several challenging analytes. The heat-assisted design of the NX source improved desolvation efficiency and increased sensitivity of analyte measurements. The addition of the ion focus unit allowed for the spray to be positioned farther away from the MS inlet, reducing contamination yet retaining sensitivity.

### ■ Conclusion

A complete LCMS solution was developed for residual pesticide testing in cannabis matrix utilizing ESI on the Shimadzu LCMS-8060NX. The developed method was optimized and tested in cannabis flower matrix for the analysis of fourteen total pesticides. The LOQs determined in this method sufficiently meet or exceed the action limits required by the Colorado Marijuana Enforcement Division<sup>1</sup>, demonstrating the viability of an LCMS total solution for cannabis testing. The improved source of the LCMS-8060NX increased sensitivity of analytes while reducing matrix contamination, leading to a more robust system. The ultrafast polarity switching capability of the LCMS-8060NX allowed for accurate and sensitive quantitation of fourteen pesticides regulated by Colorado.

### ■ Reference

1. Marijuana Enforcement Division. Code of Colorado Regulations. *CCR 212-3 2022*, 478.  
<https://www.coloradosos.gov/CCR/GenerateRulePdf.do?ruleVersionId=10319&fileName=1%20CCR%20212-3>

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SHIMADZU SCIENTIFIC INSTRUMENTS, INC.  
Applications Laboratory  
7102 Riverwood Drive, Columbia, MD 21045  
Phone: 800-477-1227 Fax: 410-381-1222  
[www.ssi.shimadzu.com](http://www.ssi.shimadzu.com)

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