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Comprehensive Cannabis Analysis from One Extract Using One Column and One Solvent System

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Introduction

Increased legalization of Cannabis for medical and adult use in the United States and Canada substantiates the need for robust and reproducible methods for analysis of Cannabis products for consumer health and safety. The state of Oregon released its list of pesticides and action limits required for products in 2015, with several states since adopting this or modified versions. Some pesticides on this list have been historically monitored by GC-MS requiring complicated sample preparation with derivatization and relatively long sample run times. Additionally, quantitation of aflatoxins and terpenes are increasingly demanded.

The SCIEX® vMethod™ Application demonstrates the capability of the SCIEX Triple Quad™ or QTRAP® 6500+ System in meeting the maximum residual levels (MRLs) for the full suite of pesticides comprising the Oregon Pesticide List in Cannabis flower matrix, and typical potency assessment through cannabinoid quantitation. In this technical note, four compound classes (pesticides, cannabinoids, aflatoxins and terpenes) were measured using a novel high LDR potency analysis strategy (**Figure 1**) and a single sample preparation protocol comprising two sample injections on a Kinetex 2.6 µm Biphenyl column.

Sample Preparation

Samples were extracted into acetonitrile according to the modified vMethod protocol. No further sample cleanup was performed, although additional dilution was used for potency and terpene analysis.

Step	Description
Weigh:	Flower: 0.2 gram Concentrates: 0.02 gram
Prep:	Break apart flower bud
Add:	5 mL Extraction Reagent
Extract:	Sonicate, vortex, and centrifuge
Serially Dilute:	1st Dilution: 1:6 (v/v) with Sample Diluent 2nd Dilution: 1:200 (v/v) with Methanol
Analyze:	Inject 25 µL of 1st Dilution for pesticides and aflatoxins (ESI) Inject 1 µL of 1st Dilution for terpenes (APCI) Inject 1 µL of 2nd Dilution for potency (APCI)

LC Conditions

Column: Kinetex™ 2.6 µm Biphenyl
Dimensions: 150 x 4.6 mm
Part No.: 00F-4622-E0
Mobile Phase: A: 5 mM Ammonium Acetate + 0.1 % Formic Acid in Water
B: 5 mM Ammonium Acetate + Methanol / Water (98:2, v/v)

Gradient:

Terpene/Cannabinoid		Pesticide/Aflatoxin	
Time (min)	**%B**	**Time (min)**	**%B**
0	93	0	5
3.5	93	0.75	5
5	100	1	50
6	100	1.5	60
6.1	93	2.5	78
7.5	93	4	88
		10	92
		12	100
		13.8	100
		13.9	5
		16	5

Flow Rate: 0.8 mL/min
Injection Volume: 1 µL
Temperature: Ambient – Terpene/Cannabinoid
35 °C – Pesticide/Aflatoxin
LC System: SCIEX ExionLC™
Detection: MRM
Detector: SCIEX QTRAP 6500+ with Scheduled MRM™ Algorithm

MRM Conditions

	ESI	APCI
Polarity:	Positive	Positive
Gas Temperature:	450 °C	625 °C
GS1:	80 psi	37 psi
GS2:	70 psi	-
CUR:	35 psi	35 psi
CAD:	11	11
IS:	5500 V	-
NC:	-	1 µA

Results and Discussion

The Oregon Pesticide List includes multiple highly polar compounds which can be difficult to retain using C18 column chemistry. The Kinetex Biphenyl column improves retention of such compounds (e.g., acephate, daminozide) while also providing improved separation of target analytes from isobaric matrix interferences (**Figure 2**). Cannabis flower samples, with variation observed between strains, typically exhibit an endogenous background signal for pyrethrin-like compounds, separation of which from target pyrethrins is critical for quantitation.

Some states regulate or have proposed regulation of Alflatoxin residues in Cannabis. Action levels defined for aflatoxins are well below those outlined for most pesticides and quantitation in the parts per trillion range is necessary. Four target aflatoxins were monitored in the same acquisition method as the pesticides. Two transitions of each were included in the ESI+ data collection with the pesticide suite, using the same prepared sample and solvent system. Excellent linearity and precision were demonstrated for all targets. Cannabis flower action limits of 2 ppb in plant correspond to 0.0133 ppb in the injected sample. Chromatographic peaks at LOQs below this concentration (at 0.0125 ppb) are clearly detectable (**Figure 3**).

Potency analysis involves quantitative reporting of cannabinoid compounds. Cannabinoid levels can differ vastly between cannabinoids in a single sample, but also across strain or product types, with products claiming concentrations 90+ % by weight for some compounds (i.e., THCA). High LDR Potency Analysis is a strategy to extend the range for cannabinoids quantitation from 0.05-100 % by weight in a single analysis. The strategy utilizes dilution, alternative MRM transitions, and detuned instrument voltages.

Dilution: 1:200 dilution applied to the already 1:6 diluted sample extract used for pesticide / aflatoxin analysis. A 10-ppb standard becomes equivalent to 0.03 % concentration in extract, achieving quantitation at the low end. Additional calibration standards up to 33 ppm (equivalent of 99 % in sample) extend quantitation to the high-end range.

Alternative transitions: Multiple MRM transitions can be monitored for each cannabinoid compound, and some transitions are significantly more sensitive than others (**Figure 1**). More sensitive transitions can be used for low end cannabinoid quantitation, and less sensitive transitions can be used to avoid saturation and achieve quantitation at the high end.

Detuned transitions: Declustering Potential (DP) and/or Collision Energy (CE) voltages are adjusted to non-optimized values, decreasing the sensitivity for transitions corresponding to high concentration cannabinoids in order to avoid detector saturation at the high end of calibration.

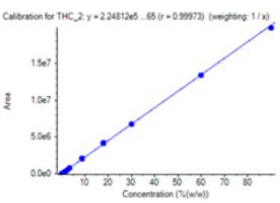
Application of these strategies to extend quantitative concentration range of cannabinoids of very different endogenous concentrations during product potency analysis was demonstrated effective. In **Figure 1**, three examples are shown: in the sample flower matrix tested, THC is shown to be measurable within the concentration range of the calibration curve for the primary, optimized MRM transition. No further adjustment to the data processing is necessary. THCA, present at a higher concentration in the sample, requires the use of an alternative (less sensitive) transition for processing in order to keep signal in the calibration range. In a third example, the high concentration of THCV necessitates further adjustment in utilization of the detuned (further decreased sensitivity) MRM transitions to achieve a signal within the calibration range (**Figure 1**).

These strategies combined with an appropriate calibration curve range spanning relevant concentration ranges allow for potency analysis with a single sample preparation and acquisition method. Including all alternative and detuned transitions in the acquisition method provides the flexibility in data processing to choose the transitions for quantitation that are suitable for the individual sample or scenario. A decision tree (**Figure 4**) outlines the process for deciding when to use each strategy during post-acquisition processing. **Table 1** details the achievable linear quantitation range for each target cannabinoid.

At least 200 terpenes have been identified in Cannabis, with unique strains presenting varying terpene profiles, which contribute to distinct flavor and aroma. Ability to quantify relevant terpenes in cannabis products is highly desirable and increasingly demanded by both growers and consumers. Challenges posed by LC-MS/MS analysis of terpenes include poor ionization by electrospray mode, which can be overcome by instead switching to APCI by easily swapping the probe on the QTRAP® 6500+ System. Chromatographic separation is also crucial, as most relevant terpenes are structural isomers which produce identical MRM transitions. Separation and quantitation of six cannabis-relevant terpenes was achieved on the Kinetex™ 2.6 µm Biphenyl column over 7 minutes, in the same acquisition as the cannabinoid analysis (**Figure 5**). Quantitation of the terpene suite was achieved over a calibration range of 10 ppb – 1 ppm in the cannabis flower matrix with excellent precision and reproducibility (%CV values <5 %).

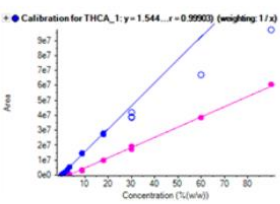
Figure 1. High LDR Potency Analysis Strategies Employed for Three Example Cannabinoids. Sample concentration is expected to be below 1 % for cannabinoids requiring detuning. All concentrations of cannabinoids are listed as effective concentrations pre-dilution. Calibration range is 10 ppb-30 ppm in vial.

Case 1: THC – Full Calibration with one transition



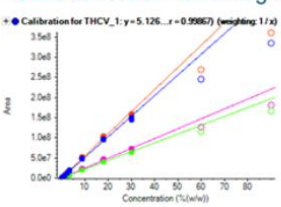
[Analyte]	%CV MRM 1	%CV MRM 2	Accuracy MRM 1	Accuracy MRM 2
0.03	6.32	--	101.33	--
0.15	3.59	--	95.15	--
0.60	1.99	--	93.83	--
1.50	3.77	--	96.67	--
2.40	2.18	--	100.11	--
3.60	1.27	--	98.87	--
9.00	0.78	--	104.06	--
18.0	1.64	--	106.45	--
30.0	0.48	--	102.26	--
60.0	--	--	101.36	--
90.0	--	--	100.23	--

Case 2: THCA – Two transitions cover full calibration



[Analyte]	%CV MRM 1	%CV MRM 2	Accuracy MRM 1	Accuracy MRM 2
0.03	3.48	--	102.35	--
0.15	2.71	--	90.68	--
0.60	0.66	--	90.42	--
1.50	2.11	--	95.95	--
2.40	4.58	--	99.17	--
3.60	2.75	--	104.87	--
9.00	2.27	2.10	110.83	98.05
18.0	1.52	0.39	105.73	100.36
30.0	--	5.34	--	101.98
60.0	--	--	--	97.38
90.0	--	--	--	99.48

Case 3: THCV – Detuning required



[Analyte]	%CV MRM 1	%CV MRM 2	Accuracy MRM 1	Accuracy MRM 2
0.03	1.05	5.67	102.83	102.53
0.15	2.56	3.85	97.05	100.01
0.60	0.87	1.11	97.74	98.24
1.50	2.72	0.24	99.06	97.37
2.40	2.71	3.77	100.36	98.42
3.60	0.94	2.71	99.51	100.32
9.00	1.84	2.73	102.86	102.48
18.0	0.74	2.85	103.29	102.79
30.0	1.07	1.22	97.98	98.37
60.0	--	--	--	--
90.0	--	--	--	--

Figure 2. Improved chromatographic separation. A.) Oregon Pesticide List analyzed in ESI+ mode. Chromatography achieved using a Kinetex™ 2.6 µm Biphenyl column. Elution profile is shown for a calibration standard. B.) Separation of four aflatoxins was achieved in conjunction with the pesticides using a Kinetex 2.6 µm Biphenyl column and a 16-minute gradient.

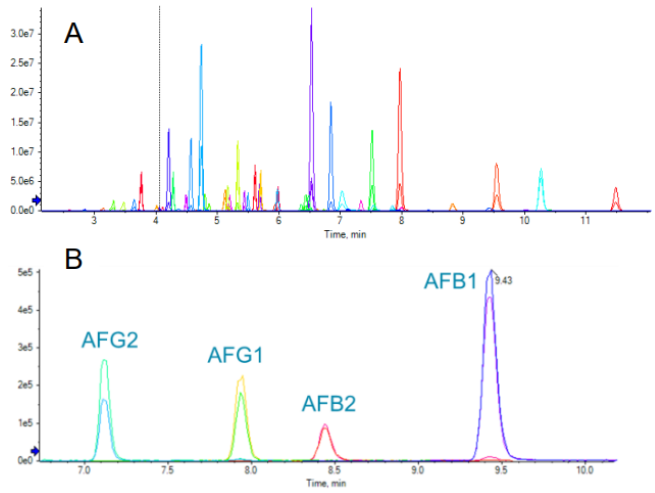


Figure 3. Monitoring Aflatoxins. Calibration Linearity, as well as Precision and Replicate (n=4) Chromatographic Peaks for Aflatoxins at LOQ Concentrations of 12.5 ppt.

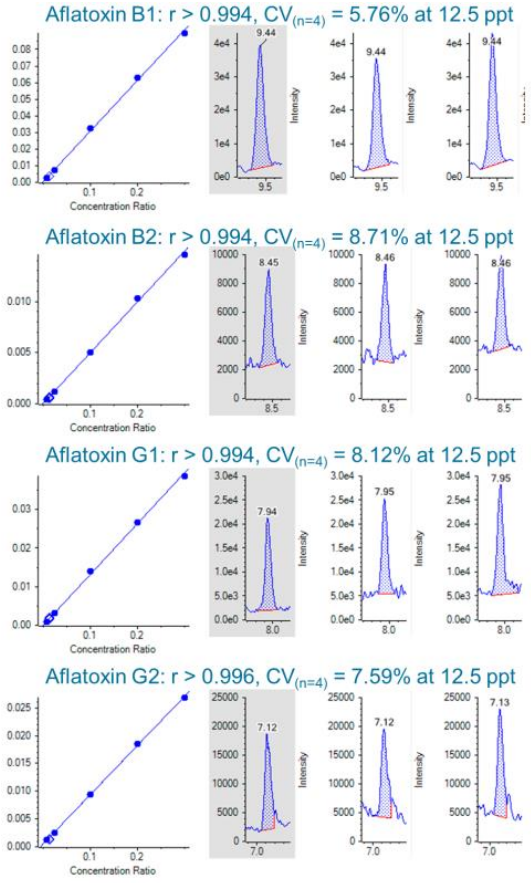


Figure 4. Multiple MRMs identified for cannabinoids allow options for choosing alternative transitions appropriate for high or low concentration ranges. A decision tree to extend linear dynamic range is employed in the data processing of cannabis samples with widely variant potency profiles, without need for re-injection of samples.

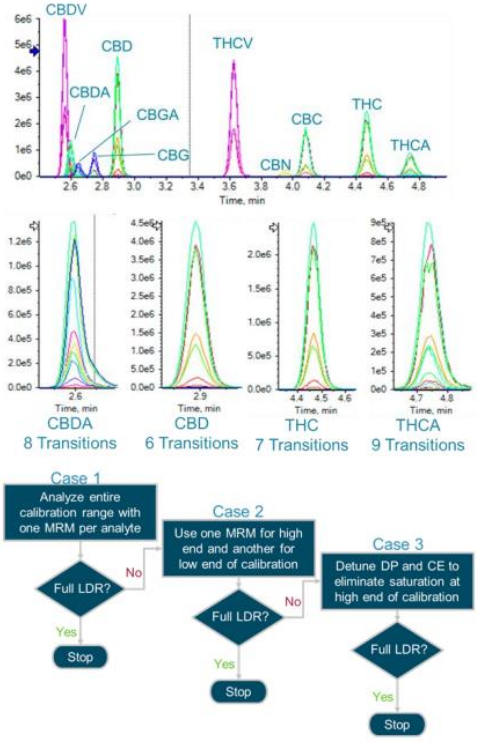
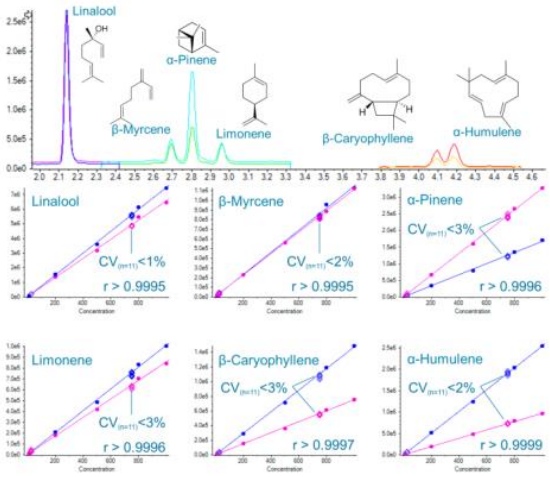


Table 1. Linearity and quantitation range achieved for individual cannabinoid compounds during assessment of product potency. Cal range 1 refers to use of diluted, optimized MRM transitions to achieve quantitation. Cal range 2 refers to use of alternative or detuned transitions to extend the quantitative concentration range.

ID	Cal Range 1	R^2	Cal Range 2
THC	0.03-90 %	0.999	
THCA	0.03-30 %	0.995	3.6-90 %
CBD	0.03-90 %	0.999	
CBDA	0.03-3.6 %	0.999	3.6-90 %
CBG	0.03-90 %	0.999	
CBGA	0.03-9 %	0.999	9-90 %
CBN	0.03-90 %	0.999	
CBC	0.03-30 %	0.998	0.15-90 %
CBDV	0.03-30 %	0.999	
THCV	0.03-30 %	0.999	

Figure 5. APCI Analysis of Terpenes. Separation, Calibration, and Precision for Six Cannabis-relevant Terpenes.



Conclusion

The SCIEX® vMethod™ is verified for extraction of Cannabis flower and concentrate and subsequent analysis for Oregon mandated pesticides and potency. Additional work is also presented showing quantitation and characterization of a comprehensive suite of residues and active ingredients including pesticides, aflatoxins, cannabinoids, and terpenes, using a single extraction protocol, mass spectrometer, and LC separation configuration using a Kinetex™ 2.6 µm Biphenyl column. These compounds can all be analyzed using two acquisition methods: one which monitors pesticides and aflatoxins, and the other monitoring terpenes and cannabinoids.

Pesticides: LOQs were established in both solvent as well as extracted cannabis flower. LOQ's in cannabis flower were achieved with ±20 %CV for all pesticides on the Oregon list. It was observed that there were many differences in the nature and extent of matrix interference between cannabis flower strains. However, during development, ten different matrix strains were analyzed, and the target transitions were found to be chromatographically separated from endogenous interferences in 9 of the tested strains.

Aflatoxins: Sensitive and precise quantitation of four commonly targeted aflatoxins is achieved to ppt levels in the same data acquisition as the pesticide method with no additional processing requirements.

Potency (cannabinoids): High linear dynamic range quantitation of the cannabinoid suite from 0.03 - 90 % concentration by weight was achieved using a combination of dilution, monitoring alternative MRM transitions, and detuning instrument voltages for MRM transitions. These plus an appropriate calibration curve range allow for potency analysis with a single sample preparation and acquisition method. These transitions were monitored in the same acquisition method as the terpenes.

Terpenes: Using APCI allows for the ionization of these flavor and aroma compounds. Chromatographic separation allows the distinction between structural isomers. Precise and accurate quantitation using the same acquisition method as the cannabinoids is demonstrated.

Kinetex Ordering Information

2.6 µm Analytical Columns (mm)							SecurityGuard™ ULTRA Cartridges*
Phases	**30 x 4.6**	**50 x 4.6**	**75 x 4.6**	**100 x 4.6**	**150 x 4.6**	**250 x 4.6**	**3/pk**
EVO C18	00A-4725-E0	00B-4725-E0	—	00D-4725-E0	00F-4725-E0	00G-4725-E0	AJ0-9296
PS C18	00A-4780-E0	00B-4780-E0	—	00D-4780-E0	00F-4780-E0	00G-4780-E0	AJ0-8949
Polar C18	00A-4759-E0	00B-4759-E0	—	00D-4759-E0	00F-4759-E0	—	AJ0-9530
Biphenyl	—	00B-4622-E0	—	00D-4622-E0	00F-4622-E0	—	AJ0-9207
XB-C18	—	00B-4496-E0	00C-4496-E0	00D-4496-E0	00F-4496-E0	—	AJ0-8768
C18	00A-4462-E0	00B-4462-E0	00C-4462-E0	00D-4462-E0	00F-4462-E0	—	AJ0-8768
C8	—	00B-4497-E0	00C-4497-E0	00D-4497-E0	00F-4497-E0	—	AJ0-8770
HILIC	—	00B-4461-E0	00C-4461-E0	00D-4461-E0	00F-4461-E0	—	AJ0-8772
Phenyl-Hexyl	—	00B-4495-E0	00C-4495-E0	00D-4495-E0	00F-4495-E0	—	AJ0-8774
F5	00A-4723-E0	00B-4723-E0	—	00D-4723-E0	00F-4723-E0	—	AJ0-9320

for 4.6 mm ID

*SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000

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