Cannabis and Hemp Testing Compendium

Volume 1

SCIEX

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SCIEX Cannabis and Hemp Testing Compendium Volume 1

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Introduction

SCIEX Cannabis and Hemp Testing Compendium Volume 1

Cannabis has been used by societies for thousands of years. Over 10,000 years ago, the hemp strains of the Cannabis sativa and Cannabis indica plants were used as a source of fiber to make garments.[1] One of the earliest written records of Cannabis sativa and Cannabis indica in their psychoactive forms is documented by Herodotus, a Greek historian, who detailed their use in steam baths inc.440 BCE.[2]

Over time, the use of cannabis in its medical and recreation forms has been restricted. While hemp is still widely used, there are strict guidelines around its classification and authentication to comply with limitations on its percentage of tetrahydrocannabinol (THC). For example, the EU classifies a product as hemp when the roots and seeds have no THC, dried stem material contains 0.3% or less and the lower leaves contain less than 1%.[3]

Recent changes have brought about the legalization of cannabis for adult recreational use in some countries. For example, in June 2018, the Canadian parliament passed the Cannabis Act (Bill C-45). This law legalized the recreational use of cannabis nationwide.[4]

Before they are sent to market and can be obtained by consumers, cannabis products— both marijuana and hemp—must be tested against strict regulations. The products are tested for analytes such as pesticides and natural toxins to protect the consumer. Marijuana and hemp are subjected to testing similar to what is conducted in the food production environment. Consumer safety should be paramount.

This compendium is a collection of application notes to assist laboratories in delivering premium testing of cannabis products to detect pesticides and natural toxins. In addition, this compendium will help in quality control efforts by presenting workflows and approaches to potency testing, terpenes testing, authenticity and cannabis strain profiling. There are also useful applications for law enforcement bodies to use when testing for driving under the influence of drugs (DUID). With increased use and availability of marijuana, there could be a rise in the number of DUID cases.

The compendium details global trends, the current and future states of analytical approaches and what technology is right for your organization.

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Enter new markets with legendary technology

Be one of the first laboratories in your region to offer comprehensive analysis in an emerging and lucrative market. SCIEX mass spectrometers deliver consistent analysis of cannabis and hemp samples. With an LC-MS/MS system from SCIEX, your lab can be ready to take advantage of opportunities for pesticide, potency, terpene and toxin testing contracts.

Cultivate your laboratory's potential with SCIEX, today.

1. Tourangeau, Wesley (2015), "Re-defining Environmental Harms: Green Criminology and the State of Canada's Hemp Industry", Canadian Journal of Criminology & Criminal Justice, 57 (4): 528–554, doi:10.3138/cjccj.2014.E11
2. Herodotus. Herodotus : The Histories. London, Eng. ; New York :Penguin Books, 1996.
3. European Monitoring Centre for Drugs and Drug Addiction (2018), Cannabis legislation in Europe: an overview, Publications Office of the European Union, Luxembourg - http://www.emcdda.europa.eu/system/files/publications/4135/TD0217210ENN.pdf.
4. An Act respecting cannabis and to amend the Controlled Drugs and Substances Act, the Criminal Code and other Acts - https://laws-lois.justice.gc.ca/PDF/C-24.5.pdf

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Current trends in cannabis and hemp analysis using LC-MS/MS

The legalization of cannabis for recreational adult use has motivated a new wave of intense method development, that focus on the analysis of cannabis plant samples and the wide variety of products manufactured from the cannabis material. Early LC-MS/MS methods were developed primarily for residue analysis of pesticides, herbicides, plant growth regulators and mycotoxins. These methods cover a wider range of compounds than GC/MS methods and offer more sensitivity, robustness and productivity.

Since then, we've seen other LC-MS/MS methodologies and applications developed that easily and accurately quantify cannabinoids. This includes high-resolution accurate mass (HRMS) techniques that prove to be useful in chemovar identification and QTRAP® technology, which offer the increased specificity of its MRM^3 scans.

Residue analysis in cannabis matrices has been complicated by the creation of target analyte lists that are different between states or countries. Besides having different compound panels across different regulated territories, each regulatory entity can have different maximum residue limits (MRL) for the compounds that are common to all of the lists. The state of Oregon rolled out the first pesticide list, and it was only possible to meet the analysis requirements using electrospray ionization. Later, other regulators added compounds such as quintozene, chlordane and endosulfan sulfate that required atmospheric pressure chemical ionization (APCI). LC-MS/MS methods that can meet the requirements for all of the current pesticide lists, including those for California and Canada, are now available.[1]

These methods require good chromatographic resolution to minimize the effect of matrix compounds, such as terpenes, cannabinoids and lipids, on ionization suppression. Efforts to perform sample cleanup to help with the matrix interferences generally suffer from low recoveries for at least a few of the required pesticides. As a result, the most common sample preparation is a solvent extraction (1 gram of cannabis flower in 10 mL of solvent, for example).

1. Mandatory cannabis testing for pesticide active ingredients - Requirements
   Cat.: H14-270/2-2018E-PDF
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   Pub.: 180314

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This approach has the advantage of not losing compounds during cleanup, and of being able to use the same extract for multiple analyses, such as those for pesticides and potency. The disadvantage of the solvent extraction method is that the extract is often diluted to minimize matrix effects. The diluted sample requires the most sensitive instruments to meet the method MRL requirements.

However, the complexity of the cannabis matrix still creates problems with residue analyses despite cleanup procedures or dilutions. The state of Massachusetts, for example, requires an MRL for cyfluthrin of 10 ppb in cannabis flower.[2] This is a challenge because isobaric interfering chemical noise from the matrix can make the detection of small peaks impossible. In some other situations, the MRL is not particularly low but an interfering peak can make accurate quantification of the target analyte impossible. These issues depend on sample type (such as different cultivars), which create the need for a more specific technique. This is where the MRM^3 scan functionality of QTRAP® instruments can help significantly improve specificity.

An MRM^3 scan takes advantage of the ability of the ion trap to isolate and store ions that are used to create another fragment from the original fragment ion. This increase in specificity removes background signals, making it possible to detect cyfluthrin at 10 ppb in cannabis flower and to accurately detect other compounds that have an interfering peak. These scan types are easy to set up and can be incorporated into a method so that both a normal MRM scan and an MRM3 scan are present for those analytes that have demonstrated interferences. QTRAP technology provides the laboratory with an important capability needed to meet the exact requirements of cannabis customers.

Cannabinoid analysis has traditionally been performed using HPLC/UV methods. HPLC is the preferred separation technique because it does not require additional sample preparation to prevent the decarboxylation of the acid forms of the cannabinoids. Most regulations require the analysis of THC, THCA, CBD, CBDA and CBN only, but cannabis researchers have been increasingly interested in many of the minor cannabinoids such as CBC, THCV and CBG. These cannabinoids, along with several other minor cannabinoids, are difficult to determine with confidence because identification relies only on retention time, and interference peaks are common.

2. Medical Use of Marijuana Program product testing https://www.mass.gov/info-details/medical-use-of-marijuana-program-product-testing

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Many compounds co-elute, further complicating the analytical results. One solution is the use of LC-MS/MS for the analysis of the cannabinoids because it is possible to get specific and accurate results for the minor compounds. The major compounds are more difficult to measure because of the high concentrations, especially for THC and CBD which are the most interesting. An elegant solution to this is to use LC-UV/MS/MS. The UV is used to quantify the five major cannabinoids and the mass spectrometer is used to quantify the minor cannabinoids. This allows for accurate quantification of all cannabinoids with unambiguous identification of the minor compounds in a single injection. LC-UV/MS/MS methods provide a highly efficient, accurate and productive workflow for potency and cannabinoid analysis

Frequently asked questions in the cannabis marketplace include: What strain is this? How do I know that this is really the expected strain?

QTOF technology can offer you true confidence in cannabis identification using a fast and easy analytical workflow. QTOF technology makes it possible to generate a full scan of the molecular ions with high enough resolution that nominally isobaric compounds can be separated by their difference in exact mass. With retention time, exact mass and signal intensity data, it is possible to use principal component analyses to create a map of each sample. Because different cultivars have different lipid profiles, different cannabinoid profiles and different terpene profiles, it is possible to distinguish one cultivar from another. It is also possible to identify which compounds were most influential in creating a unique space in the principal component analysis (PCA) plot. This is extremely valuable information for cannabis researchers.

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Top 5 analytical challenges for pesticides in cannabis

Now that cannabis is legalized in a majority of the United States and Canada, new regulations to help protect consumers lead us to an important question: What are they getting? Here, we'll help provide the answer.

Some of the main goals behind cannabis regulation are to ensure that consumers know what's in their cannabis and that the products aren't contaminated with harmful residues. One evaluation of the most readily available CBD products in the US (from online retailers) showed only 31% were accurately labeled for CBD concentration.[5] The study highlighted the need for manufacturing and testing standards and general oversight of cannabis products.

State testing regulations for pesticides in cannabis products are inconsistent. For example, California implemented mandatory testing shortly after legalization in early 2018, but Washington has prohibited the use of certain pesticides and doesn't require any testing. In California, 3,373 batches of cannabis products failed safety testing between July 1 and November 30, 2018.[6] Of those failures, 700 rejections were because of pesticides.

While the enforcement of mandatory pesticide testing remains a challenge for some, there are also analytical challenges that cannabis testing labs need to overcome.

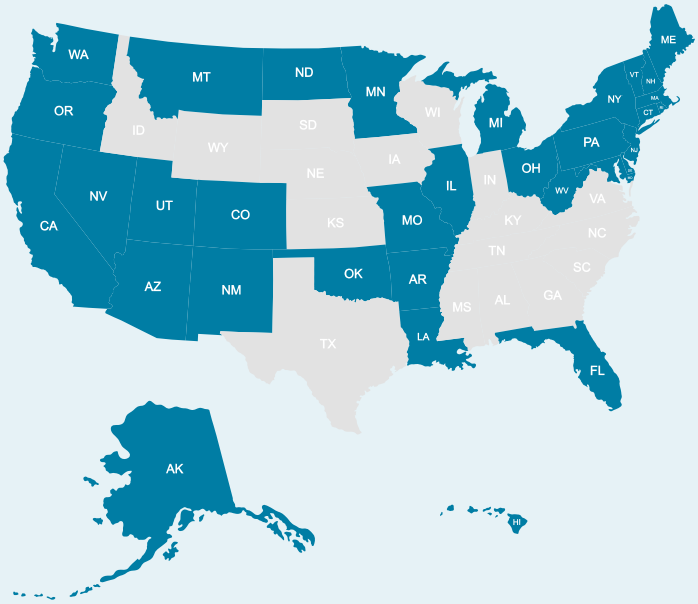
Cannabis analysis that's up to the challenge

When it comes to making your lab operations more efficient and productive, you need a high-performance instrument that's tough enough to handle routine cannabis testing and to meet the needs of ongoing regulatory changes. You need an LC-MS/MS solution that can stand up to these common testing challenges:

1. A "dirty" matrix
2. Measuring trace-level contaminants
3. Method development
4. Application support
5. Training

SCIEX offers world-class solutions designed to simplify your workflows and get you operational as quickly as possible. Explore the incredibly sensitive SCIEX Triple Quad™ 6500+ or QTRAP® 6500+ system featuring advanced SCIEX technology, combined with our vMethod™ application and our expert application support and training.

A map of current legalized states for recreation and medicinal cannabis use



5. Bonn-Miller, M. O., Loflin, M. J. E., Thomas, B. F., Marcu, J. P., Hyke, T., & Vandrey, R. (2017), "Labeling Accuracy of Cannabidiol Extracts Sold Online" Jama, 318(17), 1708. doi: 10.1001/jama.2017.11909

6. Blood, M. R. (2018, December 16). Higher percentage of California pot passing safety tests- https://apnews.com/b45648b55ec84f4ea3a43439f0d4b7e5.

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Cannabis and Hemp Application Notes

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Potency Analysis in Hemp and Cannabis Products using a Single-Dilution Combined LC-UV-MS/MS Approach

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[1]SCIEX, Redwood City, CA, USA, [2]Phenomenex, Inc., Torrance, CA, USA, [3]Phenova, Inc., Golden, CO, USA

Overview

A rapid and robust method for 11 cannabinoids using a combination of LC with UV and MS/MS detectors in a single analytical run is presented for cannabis and hemp potency testing. The method separates the psychoactive delta-9-tetrahydrocannabinol (delta-9-THC) and its isomer delta-8-tetrahydrocannabinol (delta-8-THC) in a 16 minute gradient providing accurate levels of total THC for potency labeling of cannabis products. This two detector approach covers a wide quantitation range of individual cannabinoid content from 0.05-100% by product weight. By simultaneously utilizing both UV and MS detectors, higher and lower abundant cananbinoids can be accurately detected and quantified in a single analysis with the same sample injection and dilution factor, thus increasing laboratory sample throughput.

Introduction

Based on individual state regulatory requirements in the US, the potency of commercial cannabis products must be reported as the percentage of THC and printed on cannabis product labels after being certified by a licensed cannabis testing facility. The methodology for obtaining cannibis potency values can vary based on the analytical technique and instrumentation used, which gives options for testing facilities to customize or streamline their workflows.

All analytical instruments exhibit a dynamic range of detection, and to accurately quantify the concentration of any component in a sample, that component must be diluted to a concentration within the dynamic range of the instrument. The dynamic range of an instrument is controlled by various factors, including detector performance, chromatographic efficiency, and ionization efficiency. At very high concentrations of a compound, a detector may not be able to distinguish small changes of concentration from one sample to the next and will not show a linear response of increasing detector response to analyte concentration.

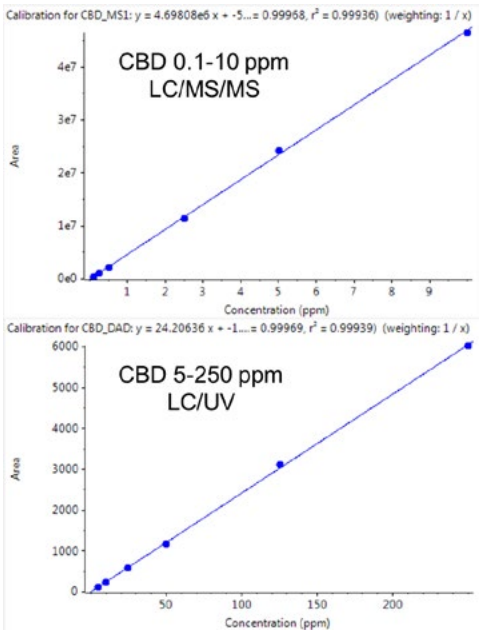

Figure 1: CBD Calibration Curves. (Top) CBD calibration curve using MS detector (0.1-10 ppm in vial; corresponds to 0.05-5% in samples) showing r2 of 0.999. (Bottom) CBD calibration curve using PDA (5-250 ppm in vial; corresponds to 2.5-125% in samples) showing r^2 of 0.999.

Key Advantages of HPLC-UV in Tandem with MS/MS Potency Analysis

- Assay panel covers 0.05-100% potency by weight allowing testing for both flower and pure distillate without any carryover or change in dilution factor
- SCIEX OS software provides custom flagging to determine whether the PDA or the MS is used as a detector automatically to generate accurate quantitative results

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The simplest approach to cannabinoid analysis is LC separation with UV detection in the 200-230 nm wavelength range. Due to limitations in the linear dynamic range of UV and photodiode array (PDA) detectors, it may be difficult to accurately quantitate a wide range of cannabinoids in a single injection using a single dilution scheme for all samples. The concentrations of highly abundant cannabinoids, such as delta-9-THC and tetrahydrocannabinolic acid A (THCA) in cannabis or cannabidiol (CBD) and cannabidiolic acid (CBDA) in hemp, may exceed 90%. However, other cannabinoids may only be present at concentrations less than 0.5%. Therefore, with UV analysis alone, a multiple dilution protocol may be necessary to analyze a wide panel of cannabinoids to ensure that the calculated concentrations fall within the linear dynamic range of the UV detector.

LC separation with MS/MS detection is another commonly used technique for cannabis potency analysis. It is capable of a larger dynamic range and more specific detection because MS/MS detection measures the response of individual fragments of each compound. Modern mass spectrometers are designed to be sensitive enough to measure compounds in the fg/mL and pg/mL range, however some cannabinoids may be present in concentrations exceeding 90% of the weight of the product. Achieving an adequately low concentration for MS/MS analysis requires diluting the original extract multiple times to achieve final dilutions of 1:250,000 to 1:2,250,000. Due to the high hydrophobicity of cannabinoids, non-specific binding of cannabinoids on plastic or glass surfaces may occur, decreasing the apparent concentration of cannabinoids in the sample.

Table 1. List of Cannabis and hemp Samples Tested.

Name	Product Type	Plant
Blue Dream	Flower	Cannabis
Lemon Kush	Flower	Cannabis
Mile High Hemp	Flower	Hemp
Phenova Hemp	Flower	Hemp
Phenova Proficiency Test Hemp	Flower	Hemp
FLO Sativa	Flower	Cannabis
Gorilla Glue	Oil	Cannabis
M.H. Hemp D	Distillate	Hemp
Wedding Cake	Wax	Cannabis
Pachymama	Wax	Cannabis
Tropical Fruit	Oil	Cannabis
CBD Distillate	Distillate	Hemp

Therefore, performing multiple serial dilutions of cannabis extracts can lead to inaccurate results.

In this study, a workflow for analyzing 11 cannabinoids in cannabis and hemp products with varying levels of potency is presented using LC-UV in tandem with a triple quadrupole mass spectrometer. The mass spectrometer provides sensitivity for low abundance cannabinoids and the HPLC-UV detector provides quantitation up to 100% THC or CBD potency by weight.

Experimental

Sample Preparation

Flower, Distillates and Concentrates

1. Homogenize flower samples, process concentrates without homogenization
2. Place 0.2 gram of sample in 10 mL of acetonitrile
3. Shake and sonicate for 30 minutes
4. Centrifuge for 5 min at 300xg
5. Filter extract with a 0.2 µm nylon syringe filter
6. Dilute filtered extract 1:100 (v/v) with acetonitrile
7. Inject 2 µL for analysis

The mass of sample extracted can be modified if necessary. For example, 0.5 g of sample may be extracted into 25 mL of acetonitrile.

Water content was not determined in this study. Therefore, the percent results represent the weight as received of each sample. Moisture content analysis must be performed separately to normalize results to the water content of each sample.

Samples

Six cannabis and hemp flower strains were tested and six concentrates of different varieties were tested (Table 1).

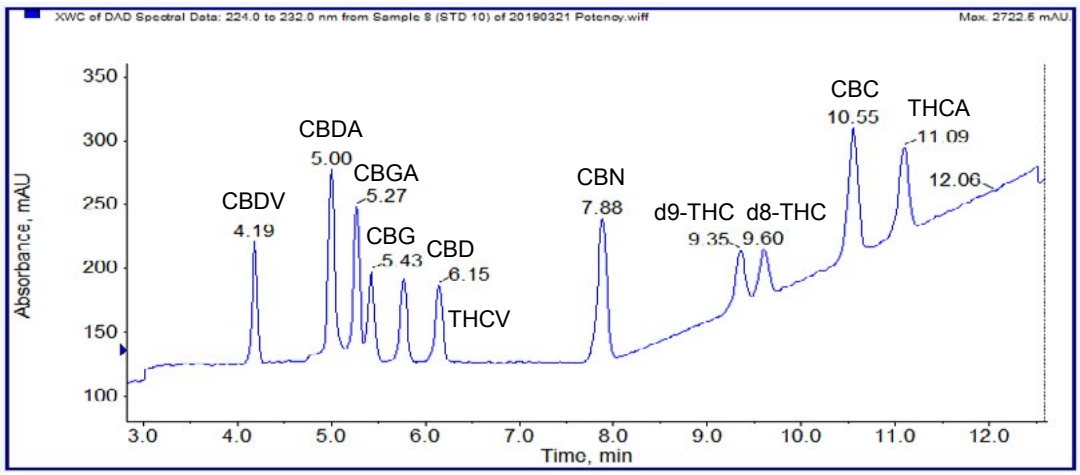

Figure 2: Cannabinoid Elution Profile of a 10 ppm Standard Showing UV trace Data.

LC Separation

A 2 µL volume of sample was injected using an ExionLC™ AD system with a PDA (photodiode array) detector coupled to a QTRAP® 6500+ system. Separation was performed using a Phenomenex Luna Omega Polar C18 (150×4.6 mm, 3 um) analytical column. The LC mobile phases consisted of 0.1% formic acid in water (A) and 0.1% formic acid in 96% acetonitrile and 4% water (B) at a flow-rate of 1 mL/min and column temperature of 25°C.

Table 2: Gradient Conditions Used for the LC Separation. Flow rate of 1 mL/min was used.

Time (min)	B (%)
0	75
0.5	82
6	82
12	90
12.5	100
14	100
14.1	75
16	End

Acquisition Method

Analysis was performed using the ExionLC system with integrated PDA UV detector and LC-MS/MS operated in both positive and negative polarity modes. The PDA detector was set to collect absorbance from a wavelength range of 210-230 nm. The following MS source conditions were used: CUR=40 psi, CAD=11, IS =5500/-4500 V, TEM=500°C, and GS1= 60 psi and GS2= 60 psi.

Data Processing

Data were processed using SCIEX OS-MQ Software 1.5. For the top 4 commonly detected cannabinoids (THC, THCA, CBD, CBDA), a high calibration range curve was generated using the PDA detector, and a low calibration range curve was generated using MS/MS on the MS detector. For the remaning cannabinoids, only an MS curve was analyzed because concentrations of these rarely exceed the maximum concentration of approximately 4% quantifiable by LC-MS/MS in this method. Once the curves were established, custom calculations were developed in SCIEX OS-MQ processing software to automatically convert the calculated concentrations to percent by weight of the plant using the mass extracted, volume extracted, and dilution factor, which were entered into Analyst® software when the samples were submitted for analysis.

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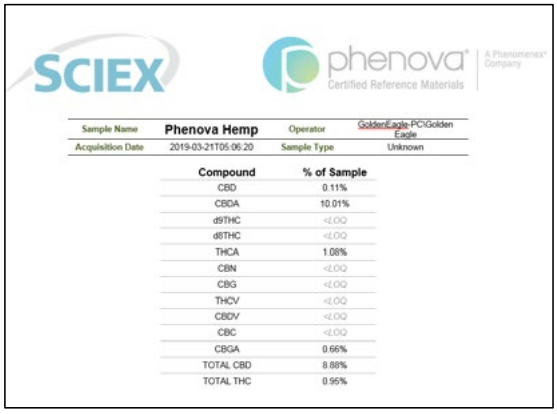
Result and Discussion

An ExionLC system with integrated PDA detector and a SCIEX Triple Quad 6500+ mass spectrometer were used together in a single injection with a single dilution scheme to quantitate 11 cannabinoids in cannabis and hemp samples ranging from 0.05-100% total weight. At the low end of this range, sufficient signal was present using the MS/MS system to calibrate even lower than the limit used in this study (approximately 0.005%). This extra sensitivity could be important when analyzing low abundance cannabinoids or small sample masses for research purposes. The PDA detected the high end of the potency range for the abundant cannabinoids at 2.5-100% by weight without detector saturation at the highest point in the calibration curve. An example of the two overlapping calibrations curves from two different detectors is shown in Figure 1.

Using the custom flagging features in SCIEX OS-MQ, the software automatically determined whether the calculated value for the MS/MS or the PDA was to be reported. SCIEX OS-MQ also automatically converted the results to a percentage using the extracted sample mass entered into the batch and the total dilution factors. Finally, the software calculated the total percentage of CBD and THC by adding the acid and neutral forms of each (CBD+CBDA and THC+THCA) after applying a 0.877x molar correction factor to the acids, due to the extra molecular weight of the acid before decarboxylation. A customizable report template was then used to generate a report as shown in Figure 2.


SCIEX
phenova
Certified Reference Materials

Sample Name	Phenova Hemp	Operator	GoldenEagle-PC\Golden Eagle
Acquisition Date	2019-03-21T05:06:20	Sample Type	Unknown

Compound	% of Sample
CBD	0.11%
CBDA	10.01%
d9THC	<LOQ
d8THC	<LOQ
THCA	1.08%
CBN	<LOQ
CBG	<LOQ
THCV	<LOQ
CBDV	<LOQ
CBC	<LOQ
CBGA	0.66%
TOTAL CBD	8.88%
TOTAL THC	0.95%

Figure 2: Custom Report Template Exported from Results Quantifiying Potency of Cannabinoids in Hemp Provided by Phenova.

In addition to an outstanding linear dynamic range, the method also exhibited good reproducibility, likely due to the single 1:5,000 dilution used during sample preparation coupled with a 2 µL injection. Continuing calibration verifications (CCVs) were analyzed every 10 samples, and their responses were consistent over the course of the batch. Table 3 shows good reproducibility of THCA in a 0.5 ppm MS/MS CCV and a 25 ppm PDA CCV with RSDs of 1.6% and 2.0%, respectively. The calculated concentrations of the CCVs were within the desired 25% of the expected concentration throughout the course of the run, which included approximately 60 injections of cannabis flower, hemp flower, and concentrate samples.

Concentrates were also quantified using the same workflow, including the same dilution factor, injection volume, and calibration standards. In Figure 3, CBD results are shown using the PDA curve or the MS curve. Because the concentration was higher than the linear dynamic range of the MS, the calculated result of 30% by weight CBD is inaccurate. However, the PDA detector, which can accurately quantify up to 100% by weight, showed that the CBD concentration in the wax was 70.2%. The automatic flagging rules used in SCIEX OS-MQ software reported the 70.2% CBD value to the report and ignored the inaccurate 30.7% MS/MS calculated value.

Table 3: Reproducibility of CCV Standards Analyzed Throughout the 60 Sample Batch.

Sample	Expected Concentration THCA (ppm)	Calculated Concentration THCA (ppm)	Accuracy
MS QC1	0.5	0.538	108%
MS QC2	0.5	0.533	107%
MS QC3	0.5	0.555	111%
MS QC4	0.5	0.547	109%
MS QC5	0.5	0.532	106%
MS QC6	0.5	0.540	108%
MS QC Summary		RSD=1.6%	
UV QC1	25	24.9	100%
UV QC2	25	23.8	95%
UV QC3	25	24.9	100%
UV QC4	25	24.7	99%
UV QC5	25	24.8	99%
UV QC6	25	25.3	101%
UV QC Summary		RSD=2.0%	

The results of 4 cannabis flower samples, 4 cannabis concentrates, 3 hemp flower samples, and 1 hemp concentrate are shown in Table 5. All 11 cannabinoids were detected in at least 1 sample. Because the moisture content was not analyzed for these samples, the values represent the percentage of each cannabinoid in the the entire sample and were therefore not directly comparable to reported label values. All 12 samples were prepared using the protocol described in the sample preparation section without modification based on sample type. The advantage of this workflow is this ability to accurately analyze this diverse set of samples without changing the mass of sample extracted, dilution factor, injection volume, or any other parameter.

Conclusions

The feasibility of using a dual detector approach to analyze 11 cannabinoids for potency reproducibly with a 1:5000 fold sample dilution is shown to be possible with very small replicate deviation. The method was tested on both hemp and cannabis matrices for flowers and concentrates that cover the entire potency range. Sample preparation no longer requires a multiple injection or multiple dilution sample method to monitor both the low- and high-abundant cannabinoids.

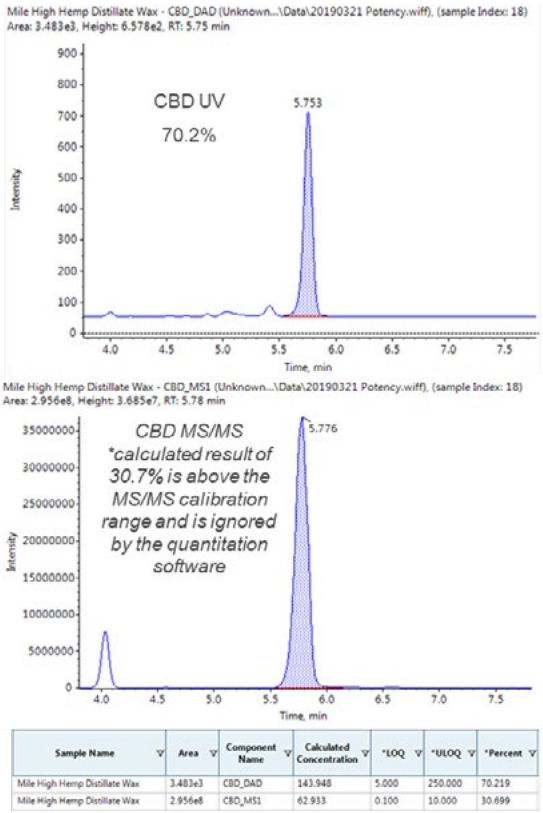


Figure 3: Quantitative Results for CBD in Hemp Wax. In this sample, SCIEX OS reported the UV value (top) because the MS/MS value was too high for the MS/MS calibration curve (bottom).

Table 4. MRM Parameters.

Name	Q1 m/z	Q3 m/z	DP	CE
CBG_1	317	193	200	10
CBG_2	317	123	100	30
THCV_1	287.1	165	125	30
THCV_2	287.1	231.3	125	24
CBDV_1	287.1	165.3	150	32
CBDV_2	287.1	123.1	150	41
CBC_1	315	193	94	27
CBC_2	315	81.2	94	17
THC_1	315	193.1	150	25
THC_2	315	135	150	25
CBN_1	311.2	223	50	15
CBN_2	311.2	241	50	15
CBD_1	315	259	200	27
CBD_2	315	193	150	27
CBGA_1	359	191.1	-200	-45
CBGA_2	359	315.3	-200	-30
CBDA_1	357	245.3	-200	-39
CBDA_2	357	179.1	-200	-32
THCA_1	357	313.4	-100	-34
THCA_1	357	191.2	-100	-42

Table 5. Summary Table of Cannabinoid Concentrations for all Samples Analyzed in this Study. *Total CBD and THC concentrations assume 100% decarboxylation of CBDA and THCA to CBD and THC, respectively, on a molar basis.

Sample Name	CBD	CBDA	d9THC	d8THC	THCA	CBN	CBG	THCV	CBDV	CBC	CBGA	Total CBD*	Total THC*
Blue Dream Cannabis Flower	<LOQ	0.06%	0.14%	<LOQ	18.38%	<LOQ	0.07%	<LOQ	<LOQ	<LOQ	0.19%	0.05%	16.26%
FLO Cannabis Flower	<LOQ	<LOQ	<LOQ	<LOQ	12.67%	<LOQ	<LOQ	<LOQ	<LOQ	0.05%	0.18%	0%	11.11%
Lemon Kush Cannabis Flower	<LOQ	0.06%	0.83%	<LOQ	17.48%	<LOQ	0.12%	<LOQ	<LOQ	<LOQ	0.91%	0.05%	16.16%
Phenova Cannabis Flower	3.37%	3.90%	<LOQ	<LOQ	2.31%	0.14%	0.15%	0.19%	<LOQ	0.27%	0.15%	6.79%	2.02%
Pachamama Sugar Wax	0.38%	3.25%	9.25%	<LOQ	59.88%	<LOQ	0.39%	0.45%	<LOQ	0.21%	1.05%	3.23%	61.76%
Wedding Cake Sugar Wax	<LOQ	0.22%	4.90%	<LOQ	69.83%	<LOQ	0.27%	<LOQ	<LOQ	0.11%	2.13%	0.19%	66.14%
Evolabs Tropical CO2 Oil	3.77%	<LOQ	72.45%	<LOQ	<LOQ	0.76%	1.78%	0.66%	<LOQ	1.18%	<LOQ	3.77%	72.45%
Gorilla Glue CO2 Oil	0.16%	0.25%	41.08%	<LOQ	13.02%	1.02%	1.58%	0.37%	0.12%	1.14%	1.17%	0.38%	52.50%
Phenova Hemp Flower 1	0.12%	12.27%	<LOQ	<LOQ	1.15%	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.68%	10.9%	1.01%
Phenova Hemp Flower 2	4.13%	5.70%	<LOQ	<LOQ	0.58%	<LOQ	0.22%	<LOQ	<LOQ	0.25%	0.36%	9.12%	0.50%
Mile High Hemp Flower	1.62%	4.92%	0.07%	<LOQ	0.10%	<LOQ	<LOQ	<LOQ	<LOQ	0.11%	0.06%	5.93%	0.15%
Mile High Hemp Distillate	69.97%	<LOQ	3.76%	<LOQ	<LOQ	0.39%	3.53%	<LOQ	0.14%	4.45%	<LOQ	69.97%	3.76%

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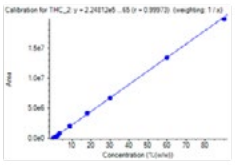
Comprehensive Cannabis Analysis: Pesticides, Aflatoxins, Terpenes, and High Linear Dynamic Range Potency from One Extract Using One Column and One Solvent System

Robert Di Lorenzo[1], Diana Tran[2], KC Hyland[2], Simon Roberts[2], Scott Krepich[3], Paul Winkler[2], Craig Butt[2], April Quinn-Paquet[2] and Christopher Borton[2]
[1]SCIEX, Canada, [2]SCIEX, USA, [3]Phenomenex, USA

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[1]. 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.

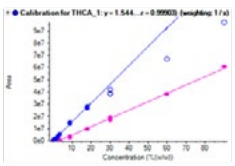
The SCIEX vMethod™ Application demonstrates the capability of the SCIEX Triple Quad™ or QTRAP® 6500+ system in meeting the maximum residual levels (MRLs) for the full suite of pesticides comprising the Oregon Pesticide List in *Cannabis* flower matrix, and typical potency assessment through cannabinoid quantitation. In order to perform comprehensive testing of *Cannabis* products, four compounds classes (pesticides, cannabinoids, aflatoxins and terpenes) were measured using a novel high LDR potency analysis strategy (Figure 1) in flower samples, using a single sample preparation protocol and two sample injections.

Case 1: THC – Full Calibration with one transition



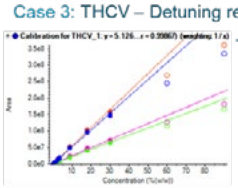
[Analyte]	%CV MRM 1	%CV MRM 2	Accuracy MRM 1	Accuracy MRM 2
0.03	6.32	--	101.33	--
0.15	3.59	--	95.15	--
0.60	1.99	--	93.83	--
1.50	3.77	--	96.67	--
2.40	2.18	--	100.11	--
3.60	1.27	--	98.87	--
9.00	0.78	--	104.06	--
18.0	1.64	--	106.45	--
30.0	0.48	--	102.26	--
60.0	--	--	101.36	--
90.0	--	--	100.23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

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	100.30
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 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.

Key Advantages of Comprehensive Cannabis Analysis

- The SCIEX vMethod application for Quantitation of Pesticide Residues in *Cannabis* Matrices presents a simplified sample preparation protocol complete with analysis of all 59 compounds using electrospray ionization (ESI) and LC-MS/MS[2]. A 16 minute gradient maximizes separation of endogenous isobaric matrix interferences for pesticide and aflatoxin analyses.
- Additionally, the method can be used to analyze ten cannabinoids and six terpenes from the same sample extract using a seven minute acquisition method utilizing atmospheric pressure chemical ionization (APCI. This single method can be used to determine potency from product cannabinoid concentrations between 0.03-90%, provide baseline separation of all isobaric cannabinoids and separate terpene isomers to assess the *Cannabis* flavor profile.

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Experimental

Extraction: Samples were extracted into acetonitrile according to the modified vMethod™ protocol (Figure 2)[2]. No further sample cleanup was performed, although additional dilution was used for potency and terpene analysis.

HPLC Conditions: Analytes from all compound classes were separated on a Phenomenex Kinetex 2.6 µm Biphenyl LC Column (150 x 4.6 mm) using a SCIEX ExionLC™ AD system, with mobile phases consisting of A) Water + 5 mM ammonium acetate + 0.1% formic acid and B) Methanol:Water (98:2) + 5 mM ammonium acetate. Pesticides and aflatoxins can be separated concurrently in a 16 minute gradient, while cannabinoids and terpenes can be separated concurrently in a seven minute gradient.

MS Conditions: All compounds were analyzed using a SCIEX QTRAP® 6500+ system with *Scheduled* MRM™ Algorithm (Analyst® software 1.6.3). Pesticides and aflatoxins were analyzed using electrospray ionization (ESI) in positive polarity with the following source settings: ISV = 5500 V, TEM = 450 ºC, CUR = 35 psi, CAD = 11, GS1 = 80 psi, GS2 = 70 psi. Terpenes and cannabinoids were analyzed using atmospheric pressure chemical ionization (APCI) in positive polarity with the following source settings: NC = 1 µA, TEM = 625 ºC, CUR = 35 psi, CAD = 11, GS1 = 37 psi.

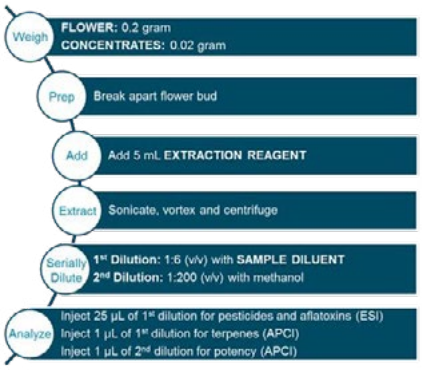

Figure 2. Simplified Sample Preparation. A simplified extraction procedure is outlined by the SCIEX vMethod Application for Quantitation of Pesticide Residues in *Cannabis* Matrices which is also employed for the analysis of terpenes and aflatoxins.

Pesticides and Aflatoxins by ESI(+)

The 59 OR list pesticides include multiple highly polar compounds which can be difficult to retain using C18 column chemistry. The Kinetex biphenyl column improves retention of such compounds (eg. acephate, daminozide) while also providing improved separation of target analytes from isobaric matrix interferences (Figure 3). 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[2].

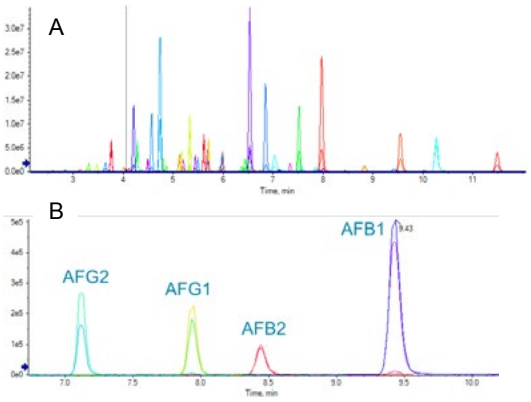

Figure 3: Improved Chromatographic Separation. A.) OR list pesticides analyzed in ESI+ mode. Chromatography achieved using a Kinetex biphenyl column. Elution profile is shown for a calibration standard. B.) Separation of four aflatoxins was achieved in conjunction with the pesticides using aKinetex biphenyl column and a 16 minute gradient.

Some states, including California, 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 2ppb in plant correspond to 0.0133ppb in the injected sample. Chromatographic peaks at LOQs below this concentration (at 0.0125ppb) are clearly detectable (Figure 4).

Contents

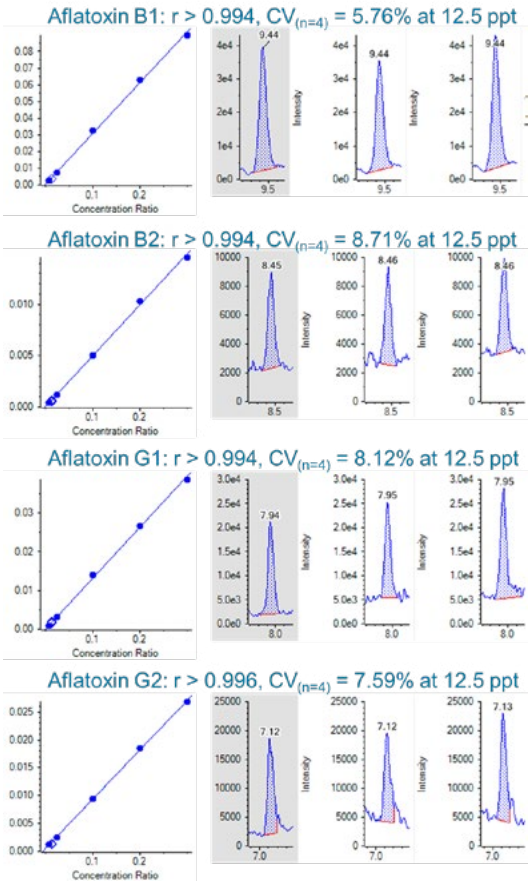

Figure 4. Monitoring Aflatoxins. Calibration linearity, as well and precision and replicate (n=4) chromatographic peaks for aflatoxins at LOQ concentrations of 12.5ppt.

High Linear Dynamic Range (LDR) Potency Analysis by APCI(+)

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 10ppb standard becomes equivalent to 0.03% concentration in extract, achieving quantitation at the low end. Additional calibration standards up to 33ppm (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 above, 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 5) 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.

Contents

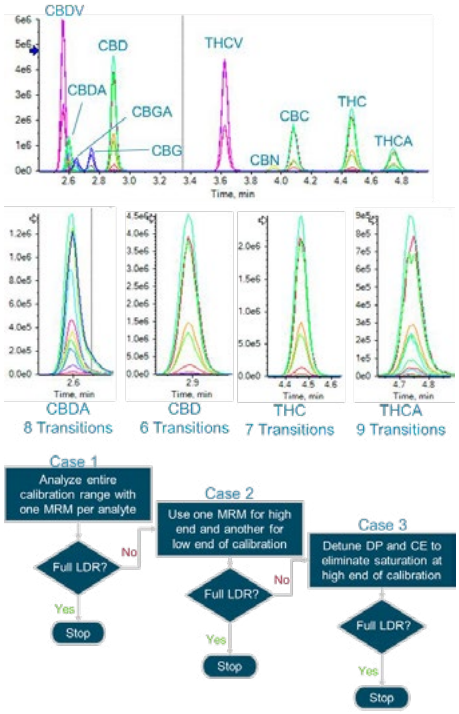

Figure 5. Multiple MRMs Identified for Cannabinoids Allow Options for Choosing Alternative Transitions Appropriate for High or Low Concentration Ranges. A decision tree to extend linear dynamic range is employed in the data processing of cannabis samples with widely variant potency profiles, without need for re-injection of samples.

Terpene Analysis by APCI(+)

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 the majority of relevant terpenes are structural isomers which produce identical MRM transitions. Separation and quantitation of six cannabis-relevant terpenes was achieved on the biphenyl column over 7 minutes, in the same acquisition as the cannabinoid analysis (Figure 6).

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*	

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%).

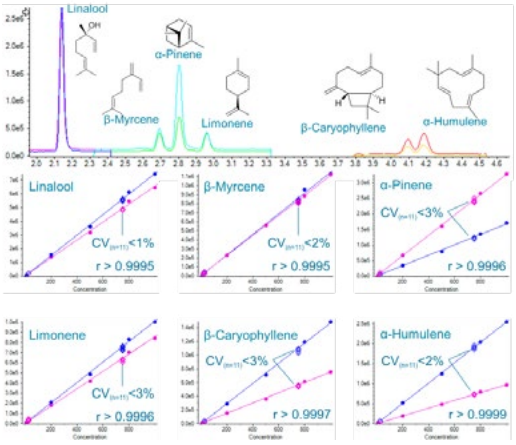

Figure 6. APCI Analysis of Terpenes. Separation, calibration, and precision for six *Cannabis*-relevant terpenes.

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Summary

The SCIEX vMethod is verified for extraction of *Cannabis* flower and concentrate and subsequent analysis for Oregon mandated pesticides and potency[2]. 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. 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.

References

1. Farrer DG. Technical report: Oregon Health Authority's process to decide which types of contaminants to test for in cannabis. Oregon Health Authority. (2015)
2. Tran, D., Hyland, K., Roberts, S., Krepich, S., Winkler, P., Butt, C. Quinn-Paquet, A., and C. Borton. *Quantitation of Oregon List of Pesticides and Cannabinoids in Cannabis Matrices by LC-MS/MS.* SCIEX Application Note. (2016)

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Analysis of the Canadian Cannabis Pesticides List Using Both ESI and APCI Techniques

Robert Di Lorenzo[1], Diana Tran[2], Craig Butt[2], Paul Winkler[2], Scott Krepich[3], Christopher Borton[2]
[1]SCIEX, Canada; [2]SCIEX, USA; [3]Phenomenex, USA

Federal legalization of adult-use Cannabis in Canada substantiates the need for robust and reproducible methods for analysis of Cannabis and derivative products for consumer health and safety. Target maximum residue limits (MRLs) for stringent regulations in the United States, including those imposed by Oregon[1] and California[2] have been demonstrated. These states monitor for 59 and 65 pesticides, respectively, at levels down to 100 ppb in the product. The pesticides represent a wide range of chemical classes and properties.

In the United States, pesticide regulations in Cannabis products are governed by individual state legislation, whereas Health Canada regulates pesticide screening at a federal level. Health Canada has currently proposed regulations for 96 pesticides, with tolerance levels between 10 to 500 fold lower than those regulated by California or Oregon, with many analytes regulated at 10 ppb in product[3]. Health Canada has assigned individual MRLs for dried Cannabis flower, Cannabis oil and fresh Cannabis flower and plants, the last of which has not been regulated elsewhere. Alternative Cannabis products, such as edibles and topicals, are yet to have defined regulations. Although testing for these 96 pesticides in the three matrices has been mandated, only 64% of the MRLs have been defined by Health Canada as of March, 2019[3]. Federal legalization implies unification of regulations across the country, however these incomplete lists necessitate method flexibility, performance and robustness as regulations continue to evolve.

As with the California and Oregon lists, several Canadian list pesticides have been historically analyzed by GC-MS; necessitating complicated sample preparation, derivatization, and long sample run times. Compounds on the Health Canada list such as endosulfans and pentachloronitrobenzene (quintozene) do not have functional groups traditionally ionizable by electrospray ionization (ESI). Typically, multiple analysis techniques and multiple instruments would be required. Alternatively, it is demonstrated that the sensitivity afforded by ESI can be employed to reach the stringent Health Canada limits for the majority of mandated pesticides, while additionally leveraging the flexibility of atmospheric pressure chemical ionization (APCI) to analyze those compounds that are either not ionizable by ESI, or show better sensitivity by this mechanism. APCI has the advantages of being more robust against matrix effects compared to ESI and, particularly in negative mode, being more selective. This leads to a method that is more robust, which is extremely important in a sample with as much potential for variability, ion suppression and isobaric interferences as Cannabis. This also allows for sample prep to be minimized and streamlined to increase throughput and decrease analysis cost.

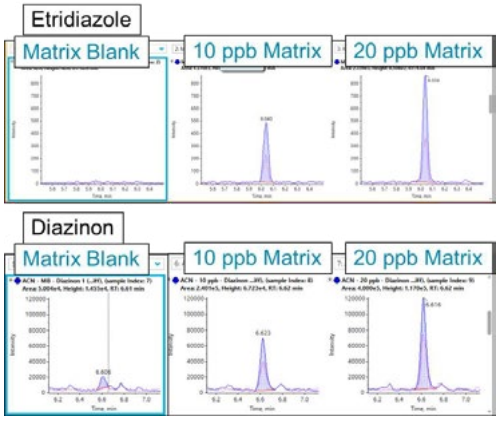


Figure 1. Representative matrix spike data for two compounds traditionally analyzed by gas chromatography. (Top) Etridizaole and (bottom) Diazinon are regulated down to 10 ppb in *Cannabis* matrices and can be analyzed down to these levels using a SCIEX QTRAP® 6500+ System using APCI and ESI techniques, respectively.

Key Advantages

- Leveraging both sensitivity of ESI and flexibility of APCI to meet demanding LOQ criteria and ionize compounds traditionally analyzed by GC-MS
- Solvent extraction and dilution of samples streamlines workflow, maximizes extraction efficiency, and minimizes cost
- Efficient desolvation in the IonDrive™ Turbo V source allows for improved ionization of temperature sensitive analytes
- Optimized Curtain Gas™ interface, active source exhaust, QJet® ion guide, and Scheduled Ionization feature of Analyst® 1.7 software provides protection from matrix components to maximize instrument uptime, even in a challenging matrix.

Contents

Experimental

Sample Preparation: Analytical standards mixtures were purchased from SPEX CertiPrep® (Metuchen, NJ, USA) and individual standards for optimization were purchased from AccuStandard (New Haven, CT, USA). Dried *Cannabis* flower samples were extracted into acetonitrile according to the protocol below.

1. 1 gram of homogenized dried flower was weighed into a 15 mL plastic centrifuge tube and 5 mL of acetonitrile added
2. Sample was vortexed for 30 seconds
3. Sample was sonicated for 15 minutes
4. Acetonitrile extract was decanted to separate vial
5. Steps 1 through 3 were repeated on the same sample and extracts were combined to yield a final extract ratio of 1 gram homogenized flower to 10 mL of acetonitrile
6. Extracts were winterized for at least 2 hours in a -20ºC freezer or colder
7. Supernatant was transferred to another vial and winterized again for 2 hours
8. Winterized extracts were centrifuged at 4000 rpm and passed through a 0.2 µm syringe filter
9. A 500 µL aliquot was diluted 1:1 with methanol
10. For analysis, an injection volume of 1 µL was used for ESI and 4 µL for APCI.

HPLC Conditions: Analytes were separated on a Phenomenex Luna Omega Polar C18, 3 µm LC column (150 x 3 mm) using a SCIEX ExionLC™ AD system with a 20 µL solvent mixer. Separation was performed at a flow rate of 420 µL/min with a column temperature of 30ºC and an autosampler sample storage temperature of 10ºC. For ESI analysis, mobile phase solvents were (A) water + 0.1% formic acid + 5 mM ammonium formate and (B) methanol + 0.1% formic acid + 5 mM ammonium formate (B) with a gradient program listed in Table 1. For APCI analysis, mobile phase solvents were (A) water and (B) methanol without modifiers with a gradient program listed in Table 2 and example chromatography in Figure 2.

Mass Spectrometry Conditions: All compounds were analyzed using a SCIEX QTRAP® 6500+ system with *Scheduled*MRM™ Pro Algorithm. The target scan time for both positive and negative polarity experiments was optimized to obtain at least 12 scans across each peak. Pesticides analyzed by ESI were acquired with the following source settings: CUR = 50 psi, CAD = HIGH (12), ISV = +3500 / -4500 V, TEM = 350ºC, GS1 = 80 psi, GS2 = 60 psi. Pesticides analyzed by APCI were acquired with the following source settings CUR = 50 psi, CAD = HIGH (12), NC = -3 µA, TEM = 400ºC, GS1 = 50 psi.

Table 1. LC gradient program for ESI panel

Time (min)	A (%)	B (%)
0	100	0
0.75	100	0
1	75	25
5	20	80
16	0	100
18	0	100
18.01	100	0
20	100	0

Table 2. LC gradient program for APCI panel

Time (min)	A (%)	B (%)
0	15	85
0.5	15	85
2	0	100
4	0	100
4.01	15	85
6	15	85

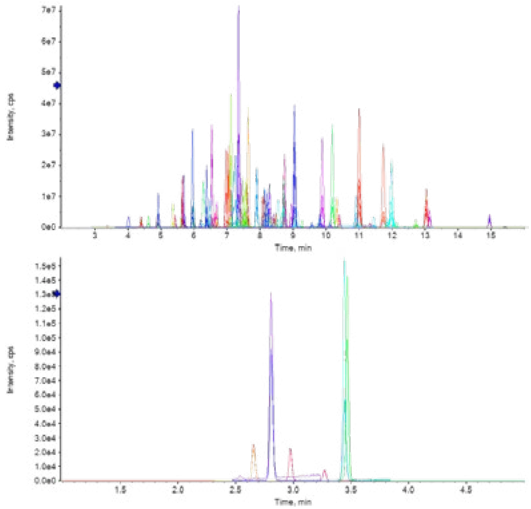


Figure 2. Example chromatography of (top) ESI panel and (bottom) APCI panel.

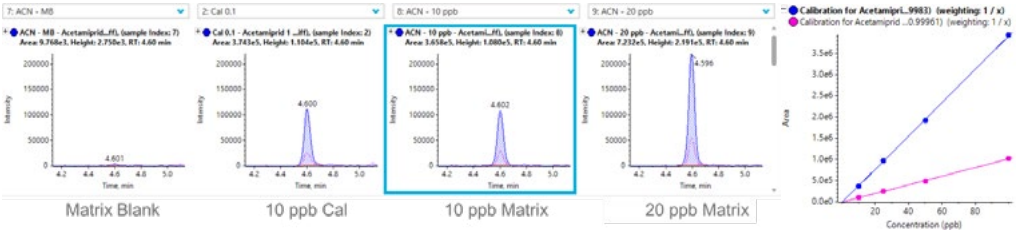
Results and Discussion

Testing requirements set forth by Health Canada represent analytically challenging demands for pesticide detection and quantitation in *Cannabis*. These requirements necessitate an approach utilizing two injections; one using ESI and one using APCI.

The MRLs set by Health Canada demand the sensitivity afforded by ESI for the majority of the pesticide panel, especially those with LOQ requirements at 10 ppb. The compounds highlighted in Figure 3 show ample signal to noise and excellent linearity for compounds traditionally analyzed by LC-MS/MS. With large-panel multiresidue analyses, it is the compounds that show poor ionization efficiency that afford the greatest challenge. Figure 4 highlights compounds that are traditionally analyzed using gas chromatographic (GC) techniques, but show quantitative performance that meets, and in most cases exceeds, the requirements set forth by Health Canada using ESI. Additionally, there are certain compounds that simply do not ionize under traditional electrospray mechanisms, such as quintozene, etridiazole and endosulfan. These are also compounds traditionally analyzed by GC. For this reason, it is necessary to employ alternative ionization techniques, namely APCI. This also allows for compounds that show improved ionization efficiency by APCI to be analyzed by their more preferred mechanism. Figure 5 highlights the performance of the APCI portion of this method to meet the Health Canada requirements. Together, these two methods have a combined run time under 30 minutes and the same extract is used for both analyses. Switching between ESI and APCI probes takes less than one minute to perform, with no software changes necessary.

Solvent extraction and winterization have been used to prepare all samples for analysis of the pesticide panel from a single extract. No difference in performance was observed between acetonitrile and acidified acetonitrile extracts, and showed improved performance over acetonitrile with QuEChERS salts, so neat acetonitrile was chosen as the extraction solvent for this method. Winterization reduces the solubility of all components in the extract, but only the highest concentration components (i.e matrix components) will precipitate out of solution, while target pesticides at low concentrations remain in solution for analysis. Since fresh Cannabis could not be obtained, only dried *Cannabis* matrices were tested. Interferences between the two matrices are expected to be similar, but since fresh *Cannabis* is roughly 50% water, it is anticipated to demonstrate less suppressive character. For Cannabis oil, it may be worthwhile to employ a lipid removal technique as recommended by Health Canada[4]. Lipid removal sorbents, either in the form of dSPE or SPE-pass-through cartridges, in combination with winterization, might be expected to remove the majority of the hydrophobic matrix, but compound losses, specifically of daminozide, may still occur.

Acetamiprid – Health Canada LOQ = 50 ppb (Fresh *Cannabis*), 100 ppb (Dried *Cannabis*)



Bifenazate – Health Canada LOQ = Under Development (Fresh *Cannabis*), 20 ppb (Dried *Cannabis*)

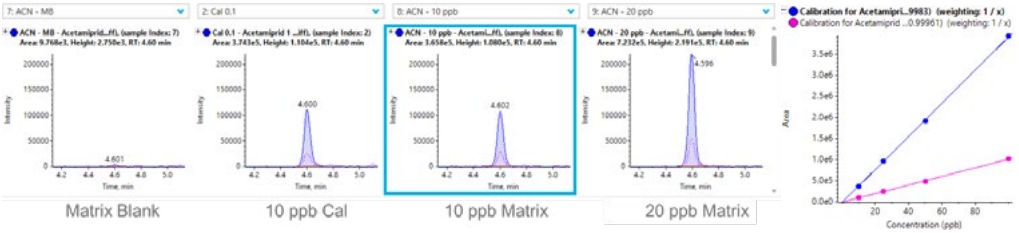


Figure 3: Example data from pesticides traditionally analyzed by LC-MS/MS monitored with ESI. (Top) Acetamiprid data in solvent and in dried *Cannabis* flower extract. (Bottom) Bifenazate data in solvent and in dried *Cannabis* flower extract . In both cases, LOQs are exceeding Health Canada requirements, and, in the case of Bifenazate, showing success where the LOQ is still under development.

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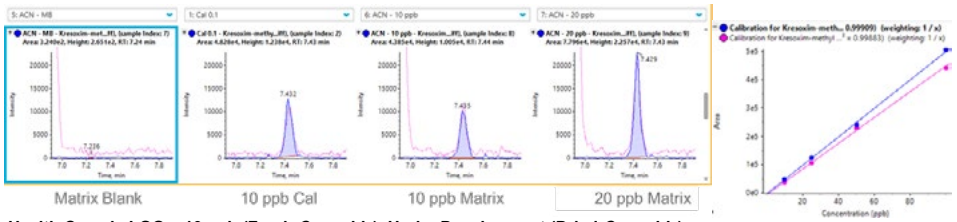
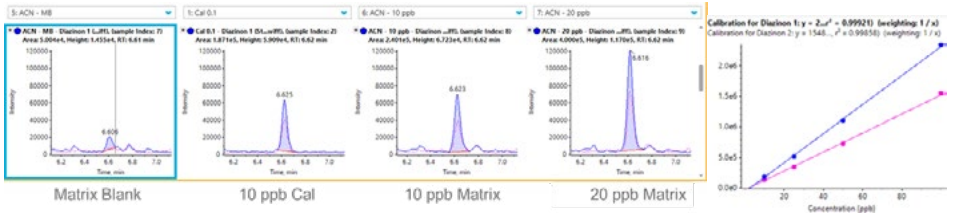
Kresoxym-Methyl – Health Canada LOQ = 10 ppb (Fresh *Cannabis*), Under Development (Dried *Cannabis*)



Diazinon – Health Canada LOQ = 10 ppb (Fresh *Cannabis*), Under Development (Dried *Cannabis*)



Endosulfan Sulfate – Health Canada LOQ = 500 ppb (Fresh *Cannabis*), Under Development (Dried *Cannabis*)

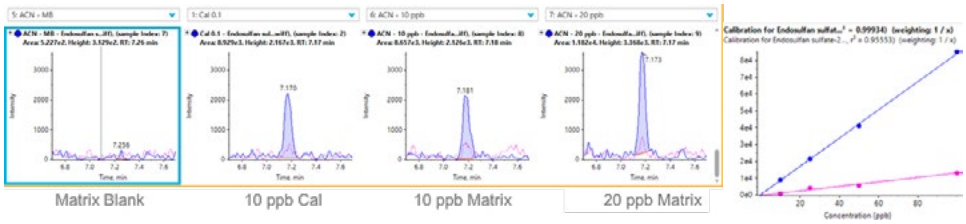


Figure 4: Example data from pesticides traditionally analyzed by GC-MS(/MS) monitored with ESI. (Top) Kresoxym-methyl, (middle) Diazinon, and (bottom) Endosulfan sulfate data in solvent and in dried Cannabis flower extract. In all cases, quantitative performance is achieved down to 10 ppb in cases where dried *Cannabis* LOQs are still under development by Health Canada.

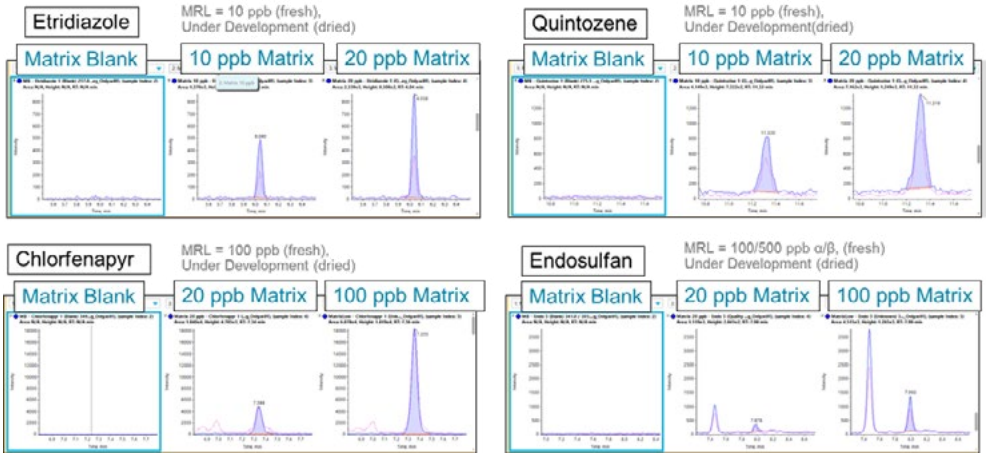


Figure 5: Example data from pesticides monitored with APCI. (Clockwise from top left) Etridiazole, quintozene, chlorfenapyr and endosulfan α/β in dried C*annabis* flower extract. In all cases, fresh *Cannabis* LOQs are achieved in dried *Cannabis* matrix, where dried *Cannabis* LOQs are still under development by Health Canada.

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Table 3. Health Canada mandated LOQs for fresh and dried *Cannabis*. All LOQs can be achieved with the exception of Kinoprene. In cases where the LOQ is under development, the respective pesticides can be quantitatively detected.

	Health Canada LOQ				Health Canada LOQ				Health Canada LOQ		
	Fresh (µg/g)	Dried (µg/g)	Meet LOQ?		Fresh (µg/g)	Dried (µg/g)	Meet LOQ?		Fresh (µg/g)	Dried (µg/g)	Meet LOQ?
Abamectin	0.25	*	✓	**Dodemorph**	0.05	*	✓	**Naled**	*	*	✓
Acephate	*	0.02	✓	**Endosulfan-alpha**	0.1	*	✓	**Novaluron**	0.025	0.05	✓
Acetamiprid	0.05	0.1	✓	**Endosulfan-beta**	0.5	*	✓	**Oxamyl**	1.5	3	✓
Acequinocyl	*	*	✓	**Endosulfan sulfate**	0.5	*	✓	**Paclobutrazol**	0.01	0.02	✓
Aldicarb	0.5	1	✓	**Ethoprophos**	0.01	0.02	✓	**Permethrin**	0.5	*	✓
Allethrin	0.1	0.2	✓	**Etofenprox**	*	*	✓	**Phenothrin**	0.025	0.05	✓
Azadirachtin	0.5	1	✓	**Etoxazole**	0.01	0.02	✓	**Phosmet**	*	*	✓
Azoxystrobin	0.01	0.02	✓	**Etridiazol**	0.01	*	✓	**Piperonyl butoxide**	0.25	*	✓
Benzovindiflupyr	0.01	0.02	✓	**Fenoxycarb**	0.01	0.02	✓	**Pirimicarb**	0.01	0.02	✓
Bifenazate	*	0.02	✓	**Fenpyroximate**	*	0.02	✓	**Prallethrin**	*	*	✓
Bifenthrin	0.1	*	✓	**Fensulfothion**	0.01	0.02	✓	**Propiconazole**	0.01	*	✓
Boscalid	0.01	0.02	✓	**Fenthion**	0.01	*	✓	**Propoxur**	0.01	0.02	✓
Buprofezin	0.01	0.02	✓	**Fenvalerate**	*	*	✓	**Pyraclostrobin**	0.01	0.02	✓
Carbaryl	0.025	0.05	✓	**Fipronil**	0.01	0.06	✓	**Pyrethrins**	0.025	0.05	✓
Carbofuran	0.01	0.02	✓	**Flonicamid**	0.025	0.05	✓	**Pyridaben**	0.025	0.05	✓
Chlorantraniliprole	*	*	✓	**Fludioxonil**	0.01	0.02	✓	**Quintozene**	0.01	*	✓
Chlorphenapyr	0.1	*	✓	**Fluopyram**	0.01	0.02	✓	**Resmethrin**	*	0.1	✓
Chlorpyrifos	0.01	*	✓	**Hexythiazox**	*	*	✓	**Spinetoram**	*	*	✓
Clofentezine	0.01	0.02	✓	**Imazalil**	*	*	✓	**Spinosad**	*	*	✓
Clothianidin	0.025	0.05	✓	**Imidacloprid**	0.01	0.02	✓	**Spirodiclofen**	*	*	✓
Coumaphos	0.01	0.02	✓	**Iprodione**	0.5	1	✓	**Spiromesifen**	*	3	✓
Cyantranilipole	0.01	*	✓	**Kinoprene**	0.05	*		**Spirotetramat**	*	0.02	✓
Cyfluthrin	*	*	✓	**Kresoxim-methyl**	0.01	*	✓	**Spiroxamine**	*	*	✓
Cypermethrin	*	*	✓	**Malathion**	0.01	0.02	✓	**Tebuconazole**	*	*	✓
Cyprodinil	*	*	✓	**Metalaxyl**	0.01	0.02	✓	**Tebufenozide**	0.01	0.02	✓
Daminozide	*	*	✓	**Methiocarb**	0.01	0.02	✓	**Teflubenzuron**	0.025	0.05	✓
Deltamethrin	*	*	✓	**Methomyl**	*	0.05	✓	**Tetrachlorvinphos**	0.01	0.02	✓
Diazinon	0.01	*	✓	**Methoprene**	1	*	✓	**Tetramethrin**	0.05	0.1	✓
Dichlorvos	0.05	0.1	✓	**Methyl parathion**	*	*	✓	**Thiacloprid**	0.01	0.02	✓
Dimethoate	0.01	0.02	✓	**Mevinphos**	0.025	0.05	✓	**Thiamethoxam**	0.01	0.02	✓
Dimethomorph	*	*	✓	**MGK-264**	*	*	✓	**Thiophanate-methyl**	*	0.05	✓
Dinotefuran	0.05	0.1	✓	**Myclobutanil**	0.01	0.02	✓	**Trifloxystrobin**	0.01	0.02	✓

*- LOQ under development by Health Canada

Contents

Conclusions

All Health Canada regulated pesticides were ionized, detected and quantitatively analyzed by LC-MS/MS, using ESI or APCI techniques. Dried *Cannabis* flower was used as representative matrix, and matrix spikes at the mandated LOQs showed method performance meeting or exceeding LOQ requirements for all but one of the 96 target panel. For kinoprene, the mandated LOQ in fresh *Cannabis* may able to be achieved using a larger volume injection, as the dried LOQ is still under development, and fresh *Cannabis* represents a less challenging matrix.

A simplified extraction protocol can be used by leveraging the sensitivity and robustness of the SCIEX QTRAP® 6500+ system with IonDrive™ Turbo V source, to streamline sample prep by reducing the need for complex and costly cleanup techniques to maintain instrument performance, and analyze the entire pesticide panel together. This workflow also retains the flexibility to add additional components, such as mycotoxins, to further increase productivity in testing labs. This comprehensive approach reduces the need for gas chromatographic techniques, and the frequent maintenance they require when analyzing dirty matrices.

References

(1) Quantitation of Oregon List of Pesticides and Cannabinoids in Cannabis Matrices by LC-MS/MS. SCIEX Technical Note RUO-MKT-02-6729-B.

(2) Achieving the California Pesticide Regulations in *Cannabis* Using Optimized APCI and ESI Techniques. SCIEX Technical Note RUO-MKT-02-8859-A.

(3) Health Canada. Mandatory Cannabis Testing For Pesticide Active Ingredients – Lists and Limits. Published online November 8, 2018. https://www.canada.ca/en/public-health/services/publications/drugs-health-products/cannabis-testing-pesticide-list-limits.html

(4) Moulins, J.R., et al., Multiresidue Method of Analysis of Pesticides in Medical *Cannabis*. **J AOAC Int,** 101(6):1948-1960, 2018.

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Chemovar Typing of *Cannabis* Strains with MarkerView® and SCIEX X500R QTOF System

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[1]*SCIEX, Redwood City, CA USA*

Consumers of *Cannabis* have long asserted the differences between cultivars, or strains, for user experience. The historical classification scheme of Indica, Sativa, and Hybrid has prevailed as a popular and simplified way to group together *Cannabis* cultivars of perceived properties and ancestral origin, allowing the consumer to select desired products based on a class-based assumption of its qualities. However, modern approaches to analytical testing of *Cannabis*, spurred largely by a rapidly-expanding legal market and new regulations on the industry, have shown that these historical classifications no longer explain the differences between or properties of the hundreds of commercially available *Cannabis* strains[1]. More relevant descriptions and explanations of strain-specific chemical profiles are increasingly discussed topics, with significant implications for patient treatment, intellectual property development, metabolomic profiling, and economically motivated adulteration. The concept of chemovars – a chemistry-based, phenotypical fingerprint rather than a horticultural cultivar – has been proposed and gained popularity in the scientific community.

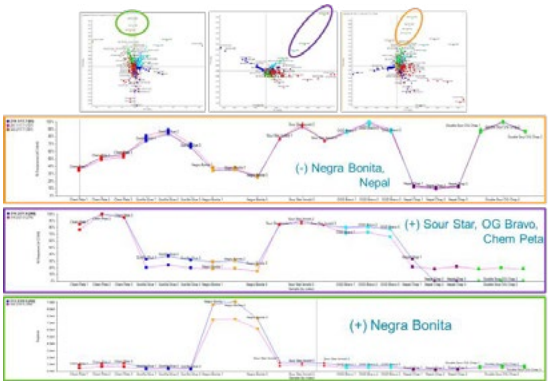

Figure 1. Principle Component Analysis of seven *Cannabis* strains in MarkerView™ software shows distinct clustering of the sample groups (strains) in several combinations of Principle Component (PC) combinations as well as with and without data normalization. Extracting features most distinctive along the PCA groups reveals the profile of those features for the different sample groups, and the Peaks of Interest features can be classified as being upregulated (+) or downregulated (-) in different strains.

A nontargeted approach is described which does not presume to know the differences in chemical signatures between strains, but instead uses PCA statistics and suspect screening to identify those differences. A targeted method, like those employed historically, operates on the assumption that differences can be identified using a set list of cannabinoids and terpenes.

Key Advantages of Chemovar Classification Approach

- The nontargeted approach does not try to target a "short-list" of cannabinoids or terpenes, and is therefore more able to identify novel biochemical markers which may explain the differences between sample groups,
- Generic mass spectrometric data acquisition in the form of SWATH® for collection of HRAM MS and MS/MS information means that minimal method development is required to run this workflow.
- Atmospheric pressure chemical ionization (APCI) allows for robust ionization of endogenous compounds found in the *Cannabis* plant with minimal ion suppression.
- Ability to use MarkerViewTM statistical tools to build up a "Peaks of Interest" list means that the workflow does not depend on processing data for potentially thousands of detected features resulting from a simple screening workflow.
- SCIEX OS v1.4 software platform allows easy interfacing of acquired data with tools for compound ID, such as FormulaFinder and the ChemSpider database.

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Experimental

Sample Collection and Extraction: Seven different strains were sampled to test the workflow and the concept of statistical classification of chemovars based on chemical signatures. Triplicate samples of each strain were extracted into acetonitrile according to the modified vMethod™ protocol, followed by a 200x dilution in methanol[2,3].

HPLC Conditions: Analytes from all compound classes were separated on a Phenomenex Kinetex 2.6 µm Biphenyl LC Column (150 x 4.6 mm) held at 30° C using a SCIEX ExionLC™ AD system, with mobile phases consisting of A) Water + 5 mM ammonium acetate + 0.1% formic acid and B) Methanol:Water (98:2) + 5 mM ammonium acetate. The gradient and separation conditions had been previously optimized for the separation of terpene and cannabinoid isomers, and as such were deemed the most appropriate method for this application[3]. The flow rate was 0.8 mL/min and the gradient program can be seen in Figure 2.

MS Conditions: SCIEX X500R QTOF system with Turbo V™ source outfitted with probe for Atmospheric pressure chemical ionization (APCI) was operated in positive ionization mode. TOF MS scan conducted from 50-1000 m/z. Variable Window SWATH® Acquisition was employed to collect all MS/MS information for potential marker compounds in the highly complex *Cannabis* matrix. The variable window precursor ion widths were designated for maximum MS/MS specificity by using the Variable Window Optimization Tool[5]. APCI utilized the following source settings: NC = 1 µA, TEM = 625 ºC, CUR = 35 psi, CAD = 11, GS1 = 37 psi.

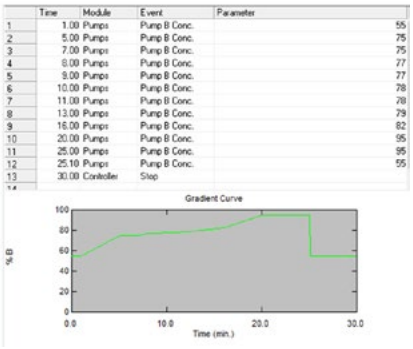

Figure 2. LC gradient time program. The total gradient run time was 30 minutes and flow rate 0.8 mL/min.

A Nontargeted Approach

Workflow

This workflow is considered “nontargeted” for two main reasons. First, the experimental data collection is a SWATH® method with no defined target analytes. Second, the statistical data processing in MarkerView does not designate a target list of predetermined features on which to build the PCA model. It should be noted that certain method parameters (i.e. sample preparation conditions, LC separation conditions, ionization source conditions, and defined variable SWATH windows) cannot be considered truly “nontargeted” techniques, as these were optimized for the detection of the endogenous *Cannabis* terpene and cannabinoid content. Since the goal of this work is to evaluate the chemical differences between *Cannabis* strains, such parameter selection is deemed appropriate.

The workflow used for processing the acquired data is outlined in Figure 3. Following the loading of the data for all samples and sample groups into MarkerView, the PCA modeling of the features and t-Test comparison of sample groups was first tested. Using these functions is a rapid way to pinpoint important chemical features before performing a compound identification. Searching MS/MS databases represents the “first pass” at identifying the Peaks of Interest, and the All-in-One with NIST library is a key tool for maximizing coverage of natural products and other potential marker compounds. If the library search produces no spectral match for a Peak of Interest, the second portion of the workflow is triggered. In this procedure, the accurate mass and isotope ratio data from precursor ions allows the FormulaFinder algorithm to provide an empirical formula for the target feature. There may be more than one proposed formula, in which case the best match or most likely formula should be selected. The formula can be searched against the ChemSpider database within the SCIEX OS software to achieve a list of structures in the database corresponding to the proposed formula. This list of structures can be narrowed down to a selection of potential candidate structures by comparing the experimental MS/MS data to in-silico predicted fragmentation of the database structures and selecting the structures demonstrating the closest match.

Statistical Analyses in Markerview Software

Figure 4 highlights the clustering of the seven strains analyzed in PCA space. The unsupervised statistical differentiation clustered together the replicate extractions within the same *Cannabis* strain giving confidence to the chemical differences observed between strains. The proximity of the strains in this PCA space can give insight into the chemical similarity between the strains, while the PCA loadings plot gives insight into the specific

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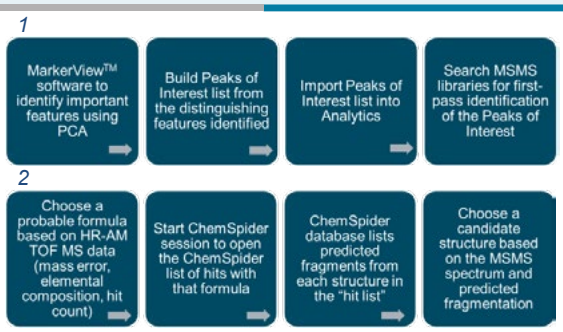

Figure 3: Detailed Data Processing Workflow for Nontargeted Chemotyping. 1.) Load acquired data into MarkerView program and utilize available statistical tools to build a list of potential marker compounds which describe or distinguish the chemovar differences between the sample groups. Use SCIEX OS Analytics module to screen this target list against MS/MS libraries for candidate identification. 2.) If the library search produces no spectral match for a Peak of Interest, this portion of the workflow is triggered. Empirical formula was achieved using HRAM MS data and searched against the ChemSpider to produce structures corresponding to the formula. This list was narrowed down to a selection of candidates by matching the experimental MS/MS data to predicted fragmentation of the database structures.

chemical features causing the most differentiation; features existing further away from the center of the PCA loadings plot are responsible for the most differentiation, whereas features towards the center of the loadings plot are the most similar between the samples.

T-test analysis also provides statistical information when comparing one strain to another, or one strain to the rest of the samples. This can be further used to identify features that are uniquely upregulated or downregulated in a sample. One example t-test is shown in Figure 5, demonstrating the functionality of the MarkerView software to take the t-test results and construct the volcano plot, in the example, for the Negro Bonita strain versus all other strains. This plot displays the statistical significance against the causing differentiation between strains. Here, features at the extremes of the x-axis show the most difference between chosen samples, whereas those lowest on the y-axis exhibit the most statistical certainty of that difference, hence features in the bottom left and right corners are the most distinguishing features and make reasonable sense to first probe for structural identification. Features can be highlighted in this plot, and their area distribution across the samples and replicates can be automatically be displayed as a profile, as shown in the Figure.

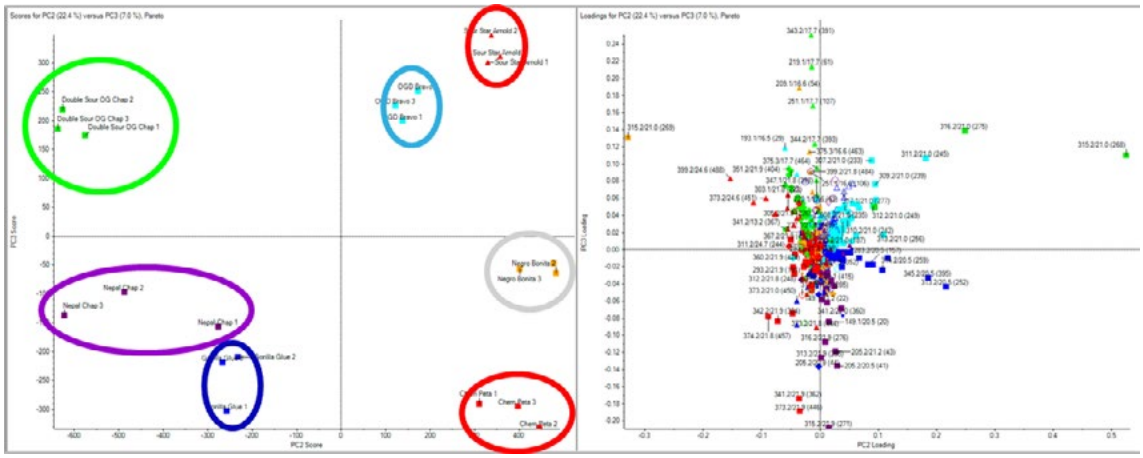

Figure 4: (Left) PCA Score distribution and (Right) PCA loadings plot for the seven cannabis strains analyzed. This data visualization tool designed for scientists who wish to visualize their data in terms of sample groupings and apply statistics in order to gain valuable insight into any trends within their mass spectral data. MarkerView is unique in that SCIEX users can explore statistical correlations with direct connections back to the raw data. This allows users to find meaningful relationships quickly.

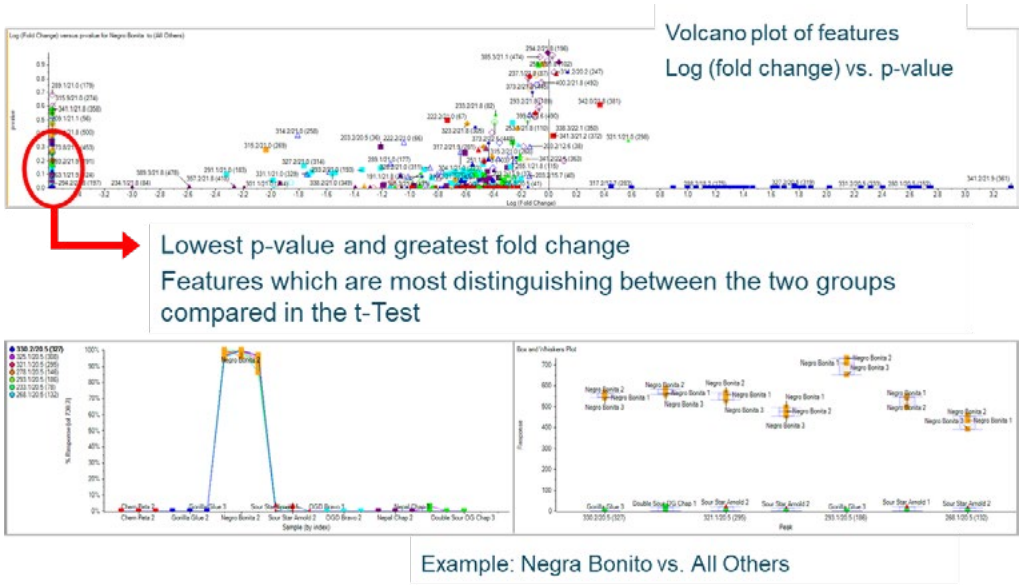

Figure 5: T-test analysis comparing features in Negro Bonita strain against all other *Cannabis* samples. (Top) volcano plot highlighting features with the greatest fold change and statistical significance. (bottom) Area distribution of selected features as a function of (left) sample and (right) feature. Here, it is easy to determine that the selected features are up-regulated in the Negro Bonita strain.

Peaks of Interest: Identifying the Unique Features

These tools can be used to create a target list of compounds to identify using the Analytics tools (examples in Table 1). This is an advantageous approach, as only the features that differ between samples are processed for identification, rather than attempting to process, search, and identify thousands of features, most of which do not represent differences or unique characteristics between the strains.

Utilizing the MS/MS spectral library and the Analytics tools in SCIEX OS, some tentative structural identifications are proposed for some of the unique components of different *Cannabis* strains (Figure 6). Several factors can be assessed for the quality of the spectral match, including mass error of the MS1 data versus the accurate mass of the candidate structure and fit score of the empirical MS/MS spectrum to that in the database. Qualitative analysis rules can be set by the user in the SCIEX OS software in order to automatically display the best potential matches.

In those instances where the MS/MS library does not produce a candidate match, the candidate empirical formula (produced by the FormulaFinder using the experimentally collected accurate mass of the precursor ion) can be searched against the ChemSpider database, which will produce a list of candidate structures in the database which match that formula. *In silico* MS/MS spectra overlaid with the experimentally collected spectrum can suggest some potential candidate identities. These functionalities are all accessible within the Analytics module of SCIEX OS and represent the final stage of the nontargeted workflow. Figure 7 shows an example of an *m/z* feature with an experimentally collected MS/MS spectrum that did not produce any library hits; however, the likely empirical formula ($C_{22}H_{30}O_3$) has many structures in the ChemSpider database and one of them (Myrsinoic Acid) is a potential candidate based on the predicted MS/MS fragmentation pattern. It is important to note that without an analytical reference standard, it is impossible to confidently confirm any structural identification.

Summary

A nontargeted approach is described which utilizes advanced software and statistical data processing of nontargeted high resolution accurate mass spectrometric data in order to asses chemical signature differences between unknown *Cannabis* strains. Some potential feature markers for different strains can be identified using high resolution mass spectra, MS/MS libraries, and SCIEX OS software tools.

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Table 1. Some example Peaks of Interest. Features (*m/z* and RT pairs) are identified as uniquely upregulated (+) or downregulated (-) in different *Cannabis* strains. This Peaks of Interest table can be built up by probing the MarkerView statistical analyses, then imported to Analytics for searching against spectral libraries.

Peak of Interest	*m/z*	RT
(-) Negro Bonita_1	*341.2107*	*21.86*
(-) Nepal_1	*219.1014*	*17.67*
(+) Chem Peta + Sour Star + Bravo_1	*311.2002*	*21*
(+) Negro Bonita_1	*313.1794*	*20.52*
(+) Sour Star + Double Sour_1	*375.2528*	*16.56*

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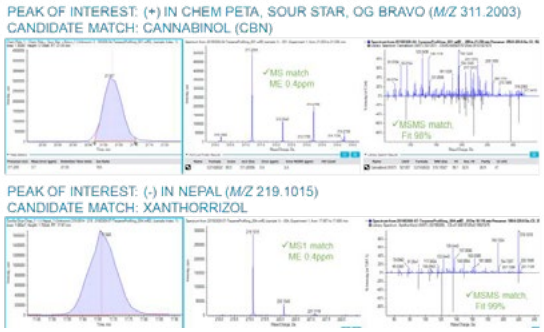

Figure 6. Candidate structure identifications for some Peaks of Interest. Cannabinol, a minor, nonpsychoactive cannabinoid, was tentatively identified as being upregulate in Chem Peta, Sour Star, and OG Bravo versus the other strains. Xanthorrizol, a sesquiterpenoid, was tentatively identified as being a unique feature in the Nepal strain.

References

1. P. Henry. Cannabis chemovar classification: terpenes hyper-classes and targeted genetic markers for accurate discrimination of flavours and effects. PeerJ Preprints. (2017)
2. Tran, D., Hyland, K., Roberts, S., Krepich, S., Winkler, P., Butt, C. Quinn-Paquet, A., and C. Borton. *Quantitation of Oregon List of Pesticides and Cannabinoids in Cannabis Matrices by LC-MS/MS.* SCIEX Application Note. (2016)
3. Di Lorenzo, R., Tran, D., Hyland, K., Roberts, S., Krepich, S., Winkler, P., Butt, C. Quinn-Paquet, A., and C. Borton. *Comprehensive Cannabis Analysis: Pesticides, Aflatoxins, Terpenes, and High Linear Dynamic Range Potency from One Extract Using One Column and One Solvent System.* SCIEX Application Note. (2018)
4. Elzinga S., Fischedick J , Podkolinski R and J. Raber *Cannabinoids and Terpenes as Chemotaxonomic Markers in Cannabis*. Natural Products Chemistry & Research (2015)
5. Improved Data Quality Using Variable Q1 Window Widths in SWATH® Acquisition. SCIEX Application Note. (2017)
6. A. Schreiber, N. Pace. *Identifying Unexpected Environmental Contaminants with High-Resolution, Accurate Mass LC–MS-MS.* LCGC Chromatography Online (2010)

Contents

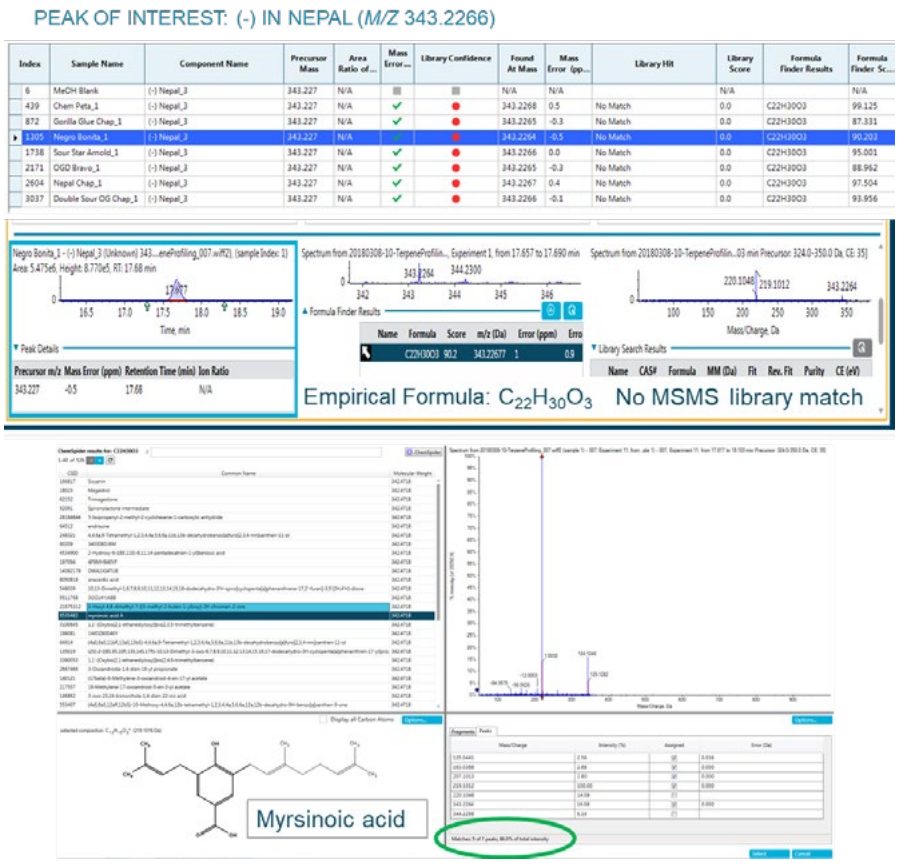

Figure 7. One of the Peaks of Interest, when imported into the SCIEX OS Analytics module as a targeted component, did not have an MS/MS spectral match in the library. However, the *m/z* value did produce a candidate formula, which could be searched against the ChemSpider database from within the software interface. One structure in ChemSpider with this formula had a predicted fragmentation pattern which closely matched the acquired MS/MS spectrum. This potential candidate ID of Myrsinoic Acid remains unconfirmed without a reference standard for MS/MS fragmentation as well as retention time confirmation. This feature was uniquely downregulated in Nepal versus the other strains.

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Achieving the California Pesticide Regulations in *Cannabis* Using Optimized APCI and ESI Techniques

Diana Tran[1], Karl Oetjen[1], Robert Di Lorenzo[2], Paul Winkler[1], Scott Krepich[3], KC Hyland[1], Christopher Borton[1]
[1]SCIEX, USA, [2]SCIEX, Canada, [3]Phenomenex, USA

Cannabis testing regulations in the USA are currently defined at the state level, with each state outlining which pesticides to monitor and the acceptable maximum residue limits (MRL) for each pesticide. California legalized adult usage of *Cannabis* in 2018 and its state-specific regulations for cannabis testing are still developing. Prior to California legalization, Oregon had one of the most comprehensive pesticide testing panels in the United States. The adoption of the current California testing regulations, however, make it the largest pesticide panel for cannabis-specific testing in the United States, with generally lower MRL's than Oregon.

Currently, the California List is divided in two categories. The Category I pesticides contain 21 residues that must be reported as "Pass" or "Fail," dependent on whether the residue exceeds a limit of detection (LOD) of 0.1 ppm in all Cannabis products. The Category II residues list 45 compounds with MRL's in "Inhalable *Cannabis* Goods" or "Other *Cannabis* Products." The Category II pesticides also have limits of quantitation (LOQ) at variable MRL's for inhalables or "Other *Cannabis* Goods." Generally, inhalables have the lowest action limits at 0.1 ppm. Of the six California List compounds not currently on the Oregon List, three are considered extremely difficult to analyze by LC-MS/MS: (1) Captan, (2) Chlordane and (3) Pentachloronitrobeneze (PCNB). Historically, these have been analyzed by GC-MS. Captan, however, is challenging to analyze by GC-MS due to its temperature sensitive nature and tendency to degrade during analysis.

The variability and diversity of tested matrices make high throughput pesticide residue testing for cannabis particularly difficult. Additionally, the abundance of cannabinoids and terpenes often suppress chemical response in electrospray ionization (ESI) analysis. This suppression can lead to inaccuracies in quantitation and potentially cause reported pesticide values to be lower than actual concentrations. The method presented here was created by SCIEX to optimize pesticide residue testing and to meet the entire California List regulatory requirements. This method uses atmospheric pressure chemical ionization (APCI) for the majority of the panel, as it is less prone to both ion source saturation and ion suppression. While a smaller subsect of the panel is analyzed using ESI.

This two-injection method, utilizing ESI and APCI, allows for the entire pesticide suite on the California List to be analyzed by LC-MS/MS.

Key Advantages of APCI and ESI Ionization

- The entire California pesticide suite can be accomplished using LC-MS/MS on a single instrument
- Analytes analyzed in APCI are less prone to ion suppression, therefore a smaller variety of internal standards are needed to correct for matrix effects
- Noise enhancement of the baseline in dirty matrices, such as *Cannabis*, is highly mitigated in APCI when compared to traditional ESI
- Greater sensitivity for Chlorfenapyr and Methyl Parathion in APCI compared to ESI
- Matrix data at the action limits and recovery against a solvent calibration curve was collected on a SCIEX QTRAP® 6500+ system.

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Experimental

Sample Preparation: Analytical standards were purchased from RESTEK (State College, PA) and Sigma Aldrich (St. Louis, MO). Chlordane analysis was spiked with purified cis-chlordane purchased from Supelco. During analysis, it was discovered that technical Chlordane standards from multiple vendors showed varying concentrations of cis- or trans-chlordane at 8-10% purity compared to a purified cis-chlordane analytical standard. Extreme variability was also observed from commercial mixes that contained Chlordane and Captan. Due to concerns about standard stability and purity of cis or trans chlordane, individual purified standards were purchased, and a spiking pesticide mix was created in house.

Samples were extracted into acetonitrile according to the modified vMethod protocol.

- 1 gram of homogenized flower was extracted in 10 mL of acetonitrile
- Sample was vortexed for 30 seconds
- Sonicated for 15 minutes
- Extracts were winterized for at least 2 hours in a -20ºC freezer or colder
- Supernatant was transferred to another vial and winterized again for 2 hours
- Centrifuged at 4000 rpm and passed through a 0.2 µm nylon syringe filter
- Injected 2 µL for ESI analysis and 5 µL for APCI analysis

HPLC Conditions***:*** Analytes from all compound classes were separated on a Phenomenex Luna Omega Polar C18, 3 µm LC Column (150 x 4.6 mm) using a SCIEX ExionLC™ AD system

Figure 1: Variability in Matrix. Flower extract after winterization (left). Flower extract after two rounds of winterization at -20ºC (right).

Table 1. LC Gradient Conditions for ESI Pesticide Panel.

Time	% B Concentration
1.5	*70*
2.0	*80*
6.0	*100*
8.0	*100*
8.1	*70*

Mobile Phase A: 0.1 % Formic Acid (5mM Ammonium Formate in H_2O)
Mobile Phase B: 0.1 % Formic Acid (5mM Ammonium Formate in MeOH)
Column Oven: 30ºC
Flow Rate: 0.8 mL/min

with a 20 µL solvent mixer. Any changes to the LC hardware have been observed to change analyte elution profile and areas of ion suppression in flower samples.

Mass Spectrometry Conditions: All compounds were analyzed using a QTRAP 6500+ system with *Scheduled* MRM™ Pro Algorithm (SCIEX). The Target Scan Time for both positive and negative polarity experiments were optimized to obtain at least 10 scans across each peak. Pesticides analyzed in positive polarity with the following source settings: NC = 5 V, TEM = 350ºC, CUR = 50 psi, CAD = 11, GS1 = 80 psi, GS2 = 60 psi. Pesticides analyzed in negative polarity with the following source settings: NC = -5 V, TEM = 700ºC, CUR = 50 psi, CAD = 11, GS1 = 40 psi.

Table 2. LC Gradient Conditions for APCI Pesticide Panel.

Time	% B Concentration
1.5	*5*
2.75	*65*
3	*65*
7	*70*
9	*85*
15	*95*
16.5	*100*
18	*100*
18.1	*5*

Mobile Phase A: Water
Mobile Phase B: Methanol
Column Oven: 30ºC
Flow Rate: 0.8 mL/min

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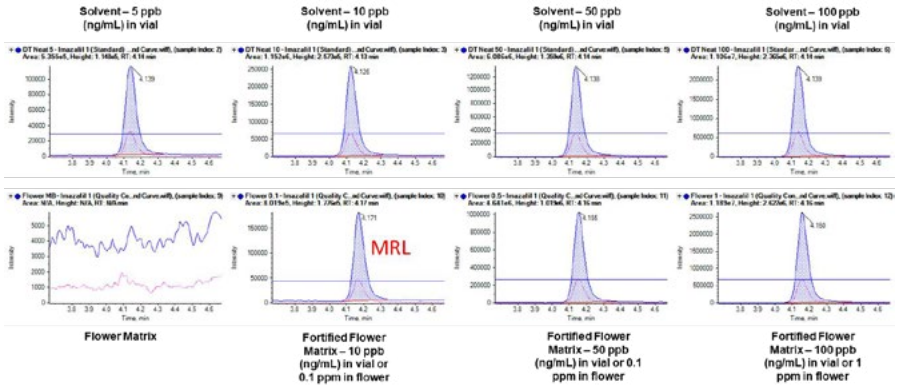
This method completes the entire California pesticide panel by two separate injections in the same instrument platform. The first injection is analyzed by ESI on the IonDrive™ Turbo V source and the second injection is by APCI. Example data is shown in *Cannabis* flower extract fortified with pesticide standards at the state designated limits for inhalable product, as well as solvent blank for the ESI method (Figure 2) and the APCI method (Figure 3). Example compounds are shown for the unspiked flower matrix and flower matrix spiked with increasing pesticide concentrations. Each increasing spike concentration is shown as two values: the concentration "in-vial," which is calculated by external calibration regression, and the concentration of the original flower sample.

Table 3. Pesticides Analyzed by ESI Method.

Abamectin	*Permethrin*
Acequinocyl	*Phosmet*
Aldicarb	*Piperonyl Butoxide*
Bifenthrin	*Spinetoram*
Captan	*Spinosad*
Cyfluthrin	*Spiromesifen*
Cypermethrin	*Spiroxamine*
Imazalil	*Thiamethoxam*
Methomyl	

Imazalil



Spinosad

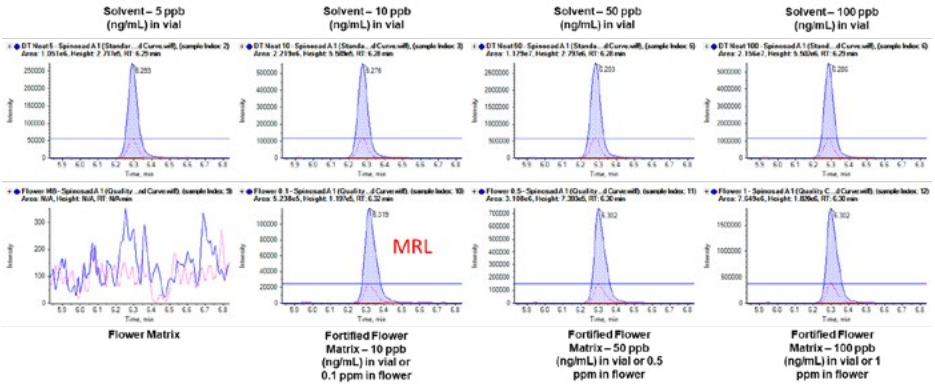


Figure 2: Example Data from Pesticides Monitored in ESI Method. (Top) Imazalil data in solvent and in cannabis flower extract. (Bottom) Spinosad data in solvent and in cannabis flower extract.

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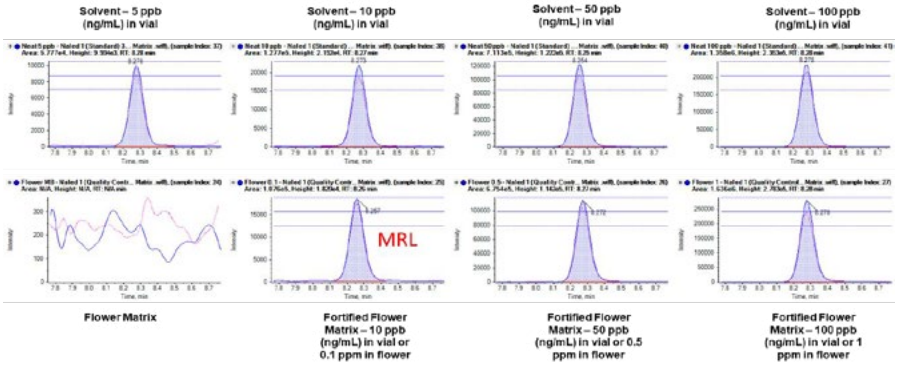
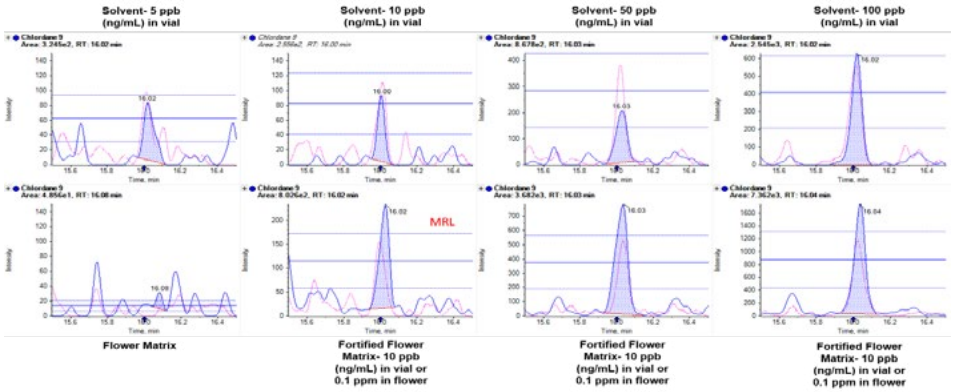
Naled



Chlordane



Quintozine (PCNB)

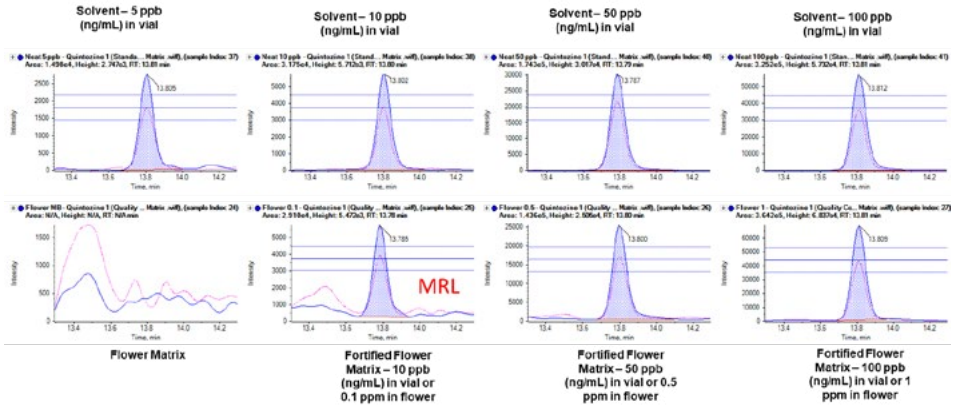


Figure 3: Example Data from Pesticides Monitored in APCI Method. (Top) Naled data in solvent and in cannabis flower extract. (Middle Chlordane data in solvent and in cannabis flower extract. (Bottom) Pentachloronitrobenzene (PCNB, Quintozine) data in solvent and in can flower extract.

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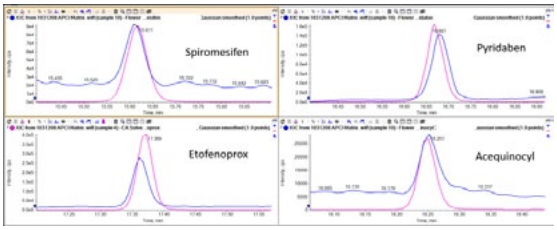

Figure 4: Extracted Ion Chromatogram (XICs) of 4 of the Most Hydrophobic Pesticides. The latest eluting pesticides on a reverse phase column chemistry showing a pesticide solvent standard (pink trace) overlaid with pesticide spiked into cannabis flower extract (blue trace) at the same concentration in vial. Spiromesifen, Pyridaben, and Acequinocyl shows recovery 80-120% as allowed by California. Etofenoprox shows 1.7-fold suppression in cannabis flower extract and will need correction with a deuterated internal standard.

Decreased ion suppression is observed in APCI when compared to ESI due to the differences in ionization mechanism. Therefore, a smaller variety of internal standards is needed to correct for matrix effects (Figure 4). The difference in ionization is key for analysis of complex matrices, such as *Cannabis*, because the abundant cannabinoid (~mg/g) concentrations are not out-competing pesticides for ionization.

Cannabis flower extract was fortified with pesticide analytical standards and back-calculated against a solvent calibration curve (Figure 5) to show matrix spike and recovery. The solvent standards were set as “standards,” while the pesticide-fortified flower extracts were designated as “quality controls” to analyze for %recovery.

Component Name	Sample Name	Num. Values	Mean	Standard De..	Percent CV	Value #1	Value #2	Value #3
Dichlorvos 1	Flower ESI 0.1 ppm	3 of 3	0.10	0.00	4.75	0.09	0.10	0.10
Dichlorvos 1	Flower ESI 0.5 ppm	3 of 3	0.38	0.02	5.75	0.37	0.41	0.37
Dichlorvos 1	Flower ESI 1 ppm	3 of 3	0.99	0.03	2.70	1.00	0.96	1.02

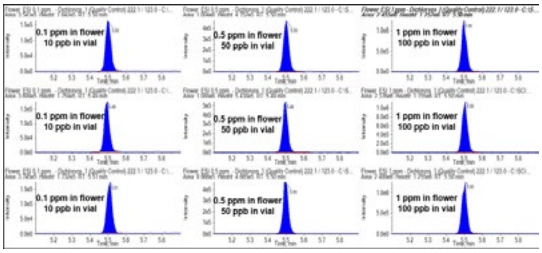

Figure 5: Quantitation of Dichlorvos. (Top) Statistics of back-calculated pesticide spiked cannabis flower against a solvent calibration curve without internal standard. Dichlorvos spiked at 3 different calibration levels and showed % Recovery of 76-100% and %CV of 4.75% at the MRL of 0.1 ppm. (Bottom) XIC’s of Dichlorvos spiked at 3 different calibration levels and showed % Recovery of 76-100% and %CV of 4.75% at the MRL of 0.1 ppm (n=3).

Table 4: Category I Pesticides. This table highlights the ability to analyze in matrix at the MRL on a QTRAP 6500+ system.

Category I Residual Pesticide	Maximum Residue Limit (ppm)	MRL in Matrix
Aldicarb	*0.1*	√
Carbofuran	*0.1*	√
Chlordane	*0.1*	√
Chlorfenapyr	*0.1*	√
Chlorpyrifos	*0.1*	√
Coumaphos	*0.1*	√
Daminozide	*0.1*	√
Dichlorvos	*0.1*	√
Dimethoate	*0.1*	√
Ethoprophos	*0.1*	√
Etofenoprox	*0.1*	√
Fenoxycarb	*0.1*	√
Fipronil	*0.1*	√
Imazalil	*0.1*	√
Methiocarb	*0.1*	√
Methyl Parathion	*0.1*	√
Mevinphos	*0.1*	√
Paclobutrazol	*0.1*	√
Propoxur	*0.1*	√
Spiroxamine	*0.1*	√
Thiacloprid	*0.1*	√

Summary

The two-injection application for the California List is an expansion on the SCIEX vMethod™ Application[2] for Quantitation of Pesticide Residues in *Cannabis* Matrices. Ongoing testing will be conducted in more flower strains and *Cannabis* products to fully address the needs for routine commercial analysis.

All 66 pesticides were ionized using the IonDrive Ion Source, including pesticides that were historically analyzed via GC-MS. The data presented indicates that this method, coupled with the SCIEX 6500+ QTRAP, meets and exceeds the MRLs for *Cannabis* flower defined by the California List (Table 4 and 5).

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Table 5. Category I Pesticides. This table highlights the ability to analyze in matrix at the MRL on a QTRAP 6500+ system.

Category II Residual Pesticide	MRL (ppm) Inhalable Goods	MRL in Matrix	Category II Residual Pesticide	MRL (ppm) Inhalable Goods	MRL in Matrix
Abamectin	0.1	√	*Krexosim-methyl*	0.1	√
Acephate	0.1	√	*Malathion*	0.5	√
Acequinocyl	0.1	√	*Metalaxyl*	2	√
Acetamiprid	0.1	√	*Methomyl*	1	√
Azoxystrobin	0.1	√	*Myclobutanil*	0.1	√
Bifenazate	0.1	√	*Naled*	0.1	√
Bifenthrin	3	√	*Oxamyl*	0.5	√
Boscalid	0.1	√	*PCNB*	0.1	√
Captan	0.7	√	*Permethrin*	0.5	√
Carbaryl	0.5	√	*Phosmet*	0.1	√
Chlorantraniliprole	10	√	*Piperonyl Butoxide*	3	√
Clofentezine	0.1	√	*Prallethrin*	0.1	√
Cyfluthrin	2	√	*Propiconazole*	0.1	√
Cypermethrin	1	√	*Pyrethrins*	0.5	√
Diazinon	0.1	√	*Pyridaben*	0.1	√
Dimethomorph	2	√	*Spinetoram*	0.1	√
Etoxazole	0.1	√	*Spinosad*	0.1	√
Fenhexamid	0.1	√	*Spiromesifen*	0.1	√
Fenpyroximate	0.1	√	*Spiroteramat*	0.1	√
Flonicamid	0.1	√	*Tebuconazole*	0.1	√
Fludioxonil	0.1	√	*Thiamethoxam*	5	√
Hexythiazox	0.1	√	*Trifloxystrobin*	0.1	√
Imidacloprid	5	√			

References

1. Farrer DG. Technical report: Oregon Health Authority's process to decide which types of contaminants to test for in cannabis. Oregon Health Authority (2015).
2. Quantitation of Oregon List of Pesticides and Cannabinoids in Cannabis Matrices by LC-MS/MS. SCIEX Technical Note RUO-MKT-02-6729-B.
3. United States. BUREAU OF CANNABIS CONTROL PROPOSED TEXT OF REGULATIONS. *California Code of Regulations Title 16. Division 42. Bureau of Cannabis Control Emergency Regulation Text*. Web. 2 November 2017.http://www.bcc.ca.gov/law_regs/bcc_prop_text_reg.pdf

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Contents

Analysis of Vitamin E and Vitamin E Acetate in Vape Oils

Triple Quadrupole Analysis of Vape Oils Produces High Quality Quantitative Results

Ritu Arora, Swetha Kaul[1]; Paul Winkler[2], Phil Taylor[3]
[1]Cannalysis, Santa Ana, CA, USA; [2]SCIEX, Framingham, USA, [3]SCIEX, Warrington, UK

Recently, a drastic increase in vaping related lung illnesses has been observed.[1] The cause of this unprecedented number of people gravely affected from vaping is of urgent concern to the CDC and FDA.[2] Vitamin E acetate, a compound used to thicken vaping liquids, has been implicated in the rise in the rate of observed lung illnesses.[3] While the link between lung illness and Vitamin E Acetate is not certain[1], there has been an increase in requests to have products tested for the presence of Vitamin E and Vitamin E Acetate.[4] LC-UV methods have historically been employed for these analytes, but the variability of the relevant matrices and the possibility of co-eluting interferences in a non-specific method demands that a more specific and reliable analytical approach be used to assure product safety. Increased specificity is a hallmark of Multiple Reaction Monitoring (MRM) analysis on a triple quadrupole mass spectrometer. The mass spectrometric approach using two MRM transitions for each analyte as well as an isotopically-labelled internal standard, ensures that the detected signal for the vitamin E and vitamin E acetate can indeed be attributed to the presence of these species and are not the artifact of complex matrix interferences.

This application note details a workflow for accurate and precise analysis of Vitamin E and Vitamin E Acetate in vape oils. The SCIEX Triple Quad™ 3500 LC-MS/MS system was leveraged to produce quantitative results which are of high quality, robust, and time efficient.

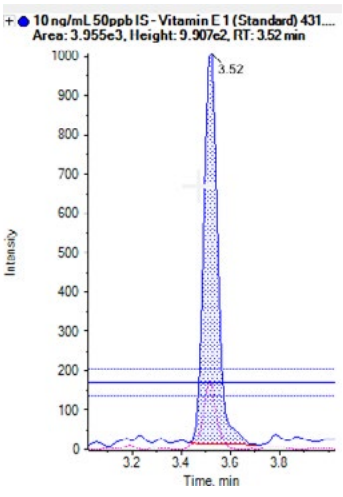

Figure 1. Vitamin E Acetate at 10ng/mL, showing acceptable ion ratio confirmation.

Key Advantages of the Vitamin E Method

- Quantitative method combines analyses for Vitamin E and Vitamin E Acetate
- MRM analysis for high specificity of detection
- Highly simplified sample preparation: “dilute-and-shoot”
- Fast 7-minute analysis
- Linear response for quantitation from 10ppb up to 500ppb with excellent precision (5%CV) for both analytes

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Experimental

Sample Preparation: Samples were prepared for analysis by dissolving 500mg of sample in 40mL of methanol. The injection solvent contained Vitamin E d6 at a concentration of 50ppb. This was used as the internal standard for quantitation. The diluted sample was analyzed without any further processing.

Chromatography: Chromatographic separation was achieved using an Agilent Poroshell 120 EC-C18, 2.7µm, 100 X 4.6mm column with a solvent flow rate of 1.2 mL/min. The column oven was set to 50°C. 5 µL injection volume was used. The chromatographic gradient and mobile phases are outlined in Table 1.

Table 1. Gradient for Vitamin E Separation.

Time (min)	Mobile Phase B (%)
0.0	95.0
0.5	100.0
5.0	100.0
5.1	95.0
7.0	95.0

Mobile phase A: Water with with 5mM ammonium formate, 0.3% formic acid
Mobile phase B: Methanol with with 5mM ammonium formate, 0.3% formic acid

Mass Spectrometry: Analysis was performed on the SCIEX Triple Quad 3500 System with a Turbo V™ source using electrospray ionization (ESI) in the positive ion mode. Data were collected using the conditions shown in Table 2. Ion source and collision gas conditions were as follows: GS1 = 30, GS2 = 30, CUR = 35, CAD = 11, TEM = 300°C.

Table 2. Compound-Specific Acquisition and Data Processing Parameters.

	Precursor	Fragment	DP (V)	CE (V)	RT (min)
Vitamin E 1	431.1	165.1	121	37	3.53
Vitamin E 2	431.1	137.1	111	59	3.53
Vitamin E Acetate 1	473.2	207.1	176	25	4.38
Vitamin E Acetate 2	473.1	165.1	176	55	4.38
Vitamin Ed6	437.1	171.1	106	37	3.54

Results and Discussion

Linearity, Precision and Sensitivity

Calibration curves for Vitamin E and Vitamin E Acetate were acquired from 10ppb to 500ppb. An example curve is shown in Figure 2. The top trace is the calibration curve for the primary MRM transition of Vitamin E and the bottom calibration is the primary MRM transition for Vitamin E Acetate. Both compounds exhibit excellent linearity over this range (r-value >0.98).

The calibration was run 5 consecutive times to demonstrate the precision and stability of the method. Very good reproducibility was obtained and is shown in Table 3. The percent CV for the 5 injections was 6% except for Vitamin E at 10ppb, which had a percent CV of 9%. The measured accuracy ranged from 83% to 118% and was generally within 10% of the expected value. These data demonstrate that highly reproducible analytical results are observed using this method. These values indicate that the accuracy expected to be obtained with this method will meet analytical requirements, based on the regulation in place for residues testing. Figure 3 shows the MRM group for Vitamin E Acetate for each of the five 10ppb injections. The ion ratios (ratio of primary MRM signal to secondary MRM signal) for each of the injections demonstrates that reliable ion ratios are consistently obtained even at low concentration.

Sample Results

Thirty-three vape oils from a wide variety of sources were analyzed using the method. Typical results from a subset of these samples are shown in Table 4. These representative results show that Vitamin E Acetate is detectable in all the products, while Vitamin E is not detected in any of the products. The data also demonstrate that there is no correlation between the concentration of Vitamin E and Vitamin E Acetate.

Table 4: Typical Values for Vitamin E and Vitamin E Acetate from Range of Vape Oils (Subset of 33 Samples Analyzed).

Sample	Vitamin E (ppb)	Vitamin E Acetate (ppb)
1	<0	0.415
2	74.1	0.461
3	76.4	0.383
4	<0	0.532
5	<0	2.32
6	<0	2.02
7	106.7	0.594
8	72.7	0.524

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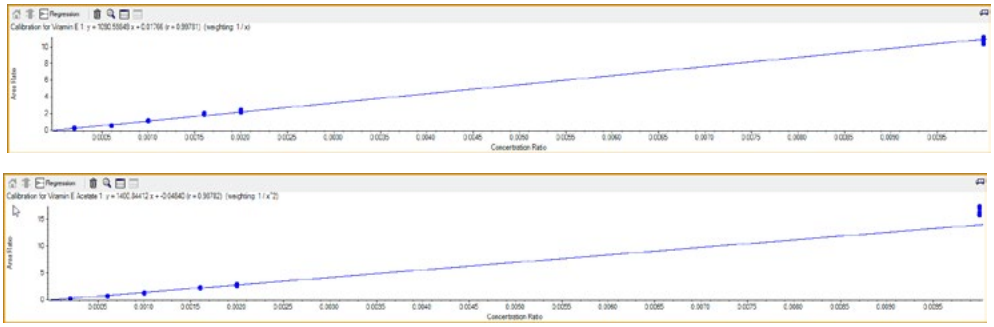

Figure 2: Example Calibration Curves. (Top) Calibration curve for Vitamin E from 10 to 500ppb. (Bottom) Calibration curve for Vitamin E Acetate from 10 to 500ppb.

Table 3. Precision and Accuracy for Five Consecutively Analyzed Calibration Curves.

Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy
1	Vitamin E 1	0.010	5 of 5	0.0	0.0	8.85	99.99
2	Vitamin E 1	0.030	5 of 5	0.0	0.0	5.22	85.57
3	Vitamin E 1	0.050	5 of 5	0.1	0.0	3.64	101.63
4	Vitamin E 1	0.080	5 of 5	0.1	0.0	4.15	109.59
5	Vitamin E 1	0.100	5 of 5	0.1	0.0	3.71	105.08
6	Vitamin E 1	0.500	5 of 5	0.5	0.0	2.67	98.15

Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy
1	Vitamin E Acetate 1	0.010	5 of 5	0.0	0.0	5.62	106.76
2	Vitamin E Acetate 1	0.030	5 of 5	0.0	0.0	5.54	83.03
3	Vitamin E Acetate 1	0.050	5 of 5	0.0	0.0	5.43	92.54
4	Vitamin E Acetate 1	0.080	5 of 5	0.1	0.0	2.90	101.75
5	Vitamin E Acetate 1	0.100	5 of 5	0.1	0.0	4.58	98.14
6	Vitamin E Acetate 1	0.500	5 of 5	0.6	0.0	4.40	117.78

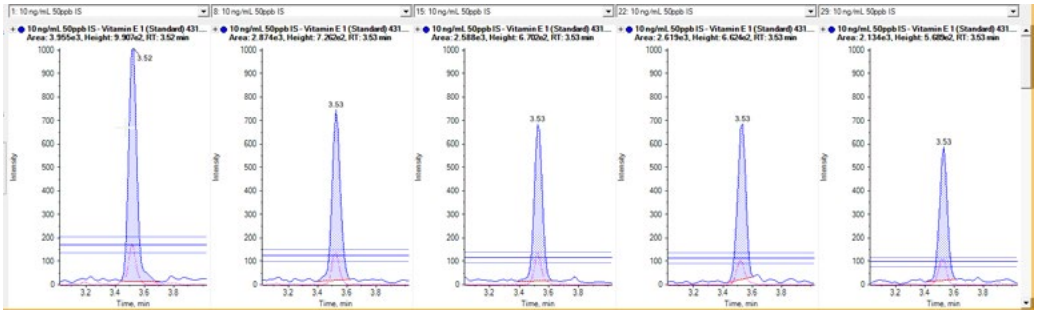

Figure 3: Example Chromatography. Peaks for the primary and secondary ions overlaid at 10ppb Vitamin E Acetate showing acceptable ion ratios for each injection.

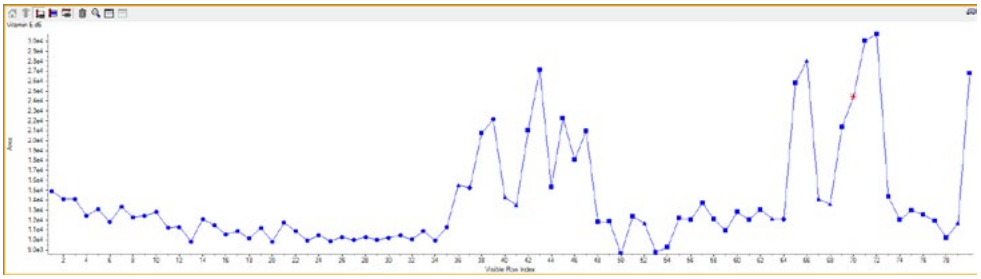

Figure 4: Peak Areas for the Internal Standard, Vitamin E d6. Circles represent calibration standards, Squares represent unknown samples, and triangles represent CCV standards.

The peak areas for the internal standard, Vitamin E d6 are shown in Figure 4. The plot shows area values for the injected samples of a single batch. The standards (represented by closed circles) run at the start of the batch show a very stable response during the analysis of the calibration solutions with an RSD of 12%. The IS areas for the samples (represented as squares) show elevated areas for some of the standards resulting in an RSD for the sequence of 37%. Elevated areas for the internal standard were not observed for all samples and appear to be related to those samples that had high concentrations of Vitamin E. The areas do, however, demonstrate the need for using an internal standard to achieve accurate quantitation.

The performance of the method was monitored during the run with the analysis of Continuing Calibration Verification standards (CCV). These samples were spiked with Vitamin E Acetate. The results for the method QC are shown in Table 5. The CCVs were stable during the sequence of injections with recoveries from 86 to 108%, which is within general acceptance criteria required for residue analysis methods. The CCVs were acquired with different concentrations throughout the sequence and further demonstrate that the method provides accurate quantitation across the calibration concentration range during sample analysis.

Conclusions

A method has been developed for the analysis of Vitamin E and Vitamin E Acetate that is suitable for the quantitative determination of these compounds in vaping oils from 0.01% to 0.5%. The method has been demonstrated to provide accurate and precise results during an extensive analysis of several actual samples of vaping oil.

Table 5: Continuing Calibration Verification Results

Sample Type	Spike Conc (ppm)	Calc Conc (ppm)	Accuracy (%)
CCV 1	*0.050*	*0.054*	*108*
CCV 2	*0.100*	*0.101*	*101*
CCV 3	*0.050*	*0.043*	*87*
CCV 4	*0.100*	*0.101*	*101*

Contents

References

1. U.S. Food and Drug Administration. https://www.fda.gov/consumers/consumer-updates/vaping-illnesses-consumers-can-help-protect-themselves-avoiding-tetrahydrocannabinol-thc-containing (accessed September 29, 2019).
2. Centers for Disease Control and Prevention, Smoking and Tobacco Use. https://www.cdc.gov/tobacco/basic_information/e-cigarettes/severe-lung-disease.html (accessed September 29, 2019).
3. David Downs. Amid Vape Pen Lung Disease Deaths: What Exactly Is Vitamin E Oil? https://www.leafly.com/news/health/vape-pen-lung-disease-vitamin-e-oil-explained (Accessed September 29, 2019).
4. Swetha Kaul, Cannalysis Laboratories, Santa Ana, CA (September 26, 2019)

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Advancing Forensic DUID Screening with Mass Spectrometry

Optimized Evolution of a Toxicology Laboratory from Immunoassay to the SCIEX X500R QTOF System

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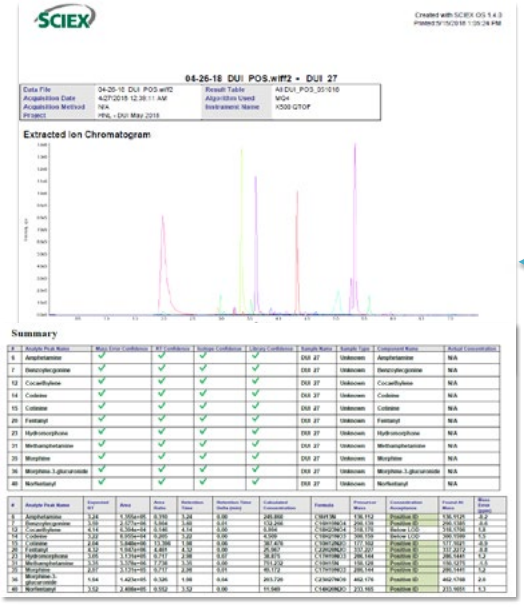
Over the past decade, the National Safety Council's Alcohol, Drugs and Impairment Division (NSC-ADID) started an initiative to standardize forensic toxicology laboratory testing for cases involving driving under the influence of drugs (DUID).

Target forensic compounds of interest were divided into two tiers: Tier I drugs include the most frequently encountered drugs found in DUID casework, and those which could be screened and confirmed with commercially available immunoassay and GC-MS instrumentation. Tier II analytes were those that had limited occurrence or required more advanced instrumentation such as LC-MS/MS, which is typically not readily available in every forensic laboratory.

More recently, the NSC-ADID made further changes on the list of target analytes for impaired driving and motor vehicle fatality forensic testing, due to recent advances in analytical technology and rapidly growing of novel psychoactive substances (NPS), like synthetic cannabinoids, bath salts and novel opioid analogs.[1]

In this technical note, a comprehensive drug screening workflow for the analysis of forensic DUID blood samples is described. The methodology was developed using a simplified sample preparation approach in combination with the SCIEX X500R QTOF System following the new NSC-ADID recommendations for forensic testing in DUID and motor vehicle fatality cases.



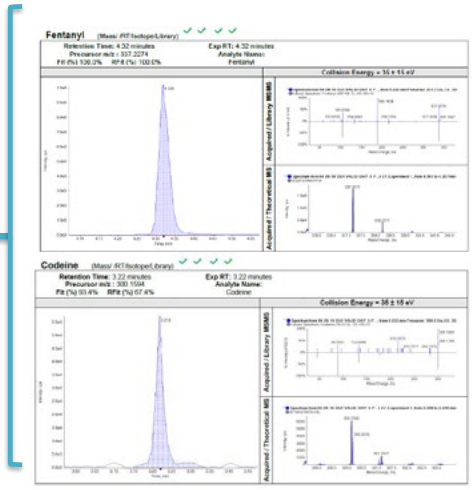


Figure 1. Confidently Identify All Analytes Present Within a Forensic DUID Case Sample. Obtain a simplified sample report showing all positively identified compounds present in a case sample. (Top) Chromatogram and results table showing all target compounds identified in the blood sample based on difference acceptance criteria. (Right) Detailed XIC, TOF MS and MS/MS spectral library identification of fentanyl and codeine present in the screened sample.

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Experimental Details

Sample Preparation: Control whole blood samples were spiked with a stock standard solution mixture containing all the different drugs for initial method development. A detailed list of the forensic compounds targeted, including accurate mass information and limits of detection (LOD) used for this screening are detailed on Supplement A of this technical document. Forensic DUID case samples and controls were extracted for LC-MS screening using the protocol in Figure 2.[2]

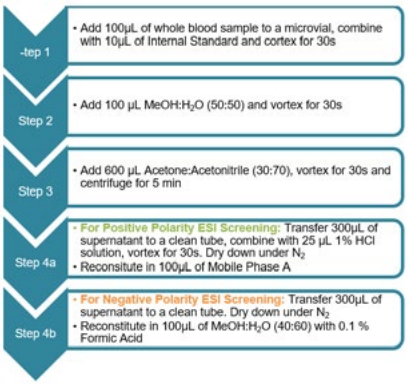
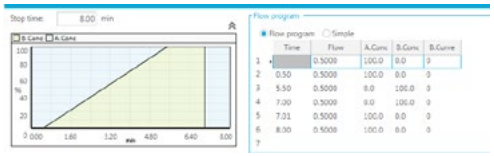


Figure 2. Sample Preparation Protocol.

Liquid Chromatography: HPLC separation was performed at 30 °C on a Phenomenex Kinetex Phenyl-Hexyl column (50 × 2.1 mm, 2.6µm) on the SCIEX ExionLC™ AC system using the following conditions: Mobile Phase A: 10 mM Ammonium Acetate in H_2O:ACN (90:10). Mobile Phase B: 10 mM Ammonium Acetate in ACN:H_2O (90:10) plus 0.1% Formic Acid. LC separation conditions are detailed in Figure 3.

ESI+ Screen: LC Runtime: 8 min, Flow Rate: 0.5 mL/min



ESI- Screen: LC Runtime: 5.1 min, Flow Rate: 0.5 mL/min

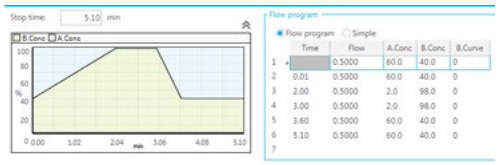
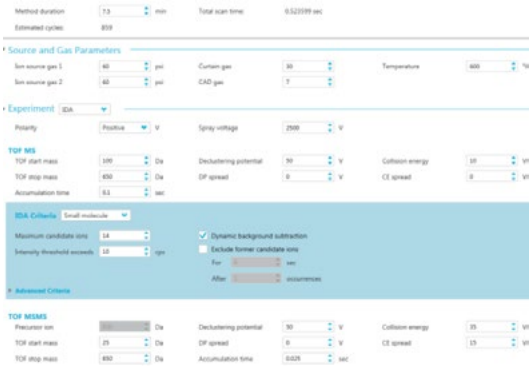


Figure 3. Chromatography Conditions.

Mass Spectrometry and Data Analysis: MS and MS/MS data were collected using the SCIEX X500R QTOF System. For all positive ionizable compounds, an Information Dependent Acquisition (IDA) approach was used. For the negative ionizable target compounds, the MRM^{HR} workflow with the *Apply TOF start/stop* mass feature was used. Both screening strategies included a TOF MS experiment in each cycle. Detailed acquisition parameters are shown in Figure 4.

ESI+ Screen: Information Dependent Acquisition (IDA)

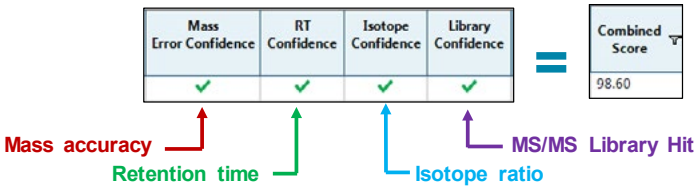


ESI- Screen: MRM^{HR} Workflow (High Resolution MRM)

Figure 4. MS Conditions.

Targeted data processing was performed using SCIEX OS Software for positive analyte identification based on previously determined criteria. Four main confidence criteria were used including mass error, retention time, isotope ratio difference, and library score. Subsequently, a combined score was computed based on these four confidence categories with custom weightings.

Table 1. Inter-Day Sverage Combined Scores (n=9) for 60 Compounds Screened in Forensic DUID Samples at the LOD using the SCIEX X500R QTOF System.



6-MAM	*90.0 %*	*Cotinine*	*89.1 %*	*Methadone*	*96.4 %*	*Noroxycodone*	*95.8 %*	*Secobarbital*	*97.1 %*
7-Aminoclonazepam	*90.6 %*	*Diazepam*	*97.6 %*	*Methamphetamine*	*96.7 %*	*O-Desmethyl tramadol*	*97.4 %*	*Butalbital*	*97.2 %*
Alphahydroxyalprazolam	*87.1 %*	*EDDP*	*97.2 %*	*Methylphenidate*	*97.2 %*	*Oxazepam*	*97.2 %*	*Pentobarbital*	*96.9 %*
Alphahydroxymidazolam	*92.2 %*	*Etizolam*	*95.1 %*	*Midazolam*	*96.4 %*	*Oxycodone*	*94.6 %*	*Phenobarbital*	*96.9 %*
Alprazolam	*97.7 %*	*Fentanyl*	*97.2 %*	*Mitragynine*	*95.8 %*	*Oxymorphone*	*90.3 %*	*THC-COOH*	*70.1 %*
Amphetamine	*95.9 %*	*Gabapentin*	*96.8 %*	*Morphine*	*93.6 %*	*Phenazepam*	*94.3 %*		
Benzoylecgonine	*95.2 %*	*Hydrocodone*	*95.5 %*	*Morphine-3-beta-glucuronide*	*93.5 %*	*Phencyclidine*	*98.1 %*		
Beta-Naltrexol	*95.2 %*	*Hydromorphone*	*94.8 %*	*Naltrexone*	*94.0 %*	*Pregabalin*	*84.5 %*		
Buprenorphine	*97.0 %*	*Ketamine*	*97.7 %*	*Norbuprenorphine*	*95.7 %*	*Ritalinic Acid*	*97.8 %*		
Carboxyzolpidem	*97.5 %*	*Lorazepam*	*96.4 %*	*Nordiazepam*	*96.0 %*	*Tapentadol*	*98.4 %*		
Carisoprodol	*97.7 %*	*MDA*	*96.4 %*	*Norfentanyl*	*81.8 %*	*Temazepam*	*97.0 %*		
Cocaethylene	*97.9 %*	*MDMA*	*97.4 %*	*Norhydrocodone*	*97.1 %*	*Tramadol*	*98.4 %*		
Cocaine	*96.0 %*	*Meperidine*	*96.8 %*	*Norketamine*	*96.4 %*	*Zolpidem*	*98.5 %*		
Codeine	*96.8 %*	*Meprobamate*	*97.7 %*	*Normeperidine*	*95.9 %*				

Using a vMethod™ to Develop a Comprehensive Screening Workflow Applied to Forensic DUID Blood Samples

The vMethod™ Application for 664 forensic compounds[3] was initially used to obtain retention times and MS/MS spectra quality to build a data analysis processing method for the 60 target forensic compounds of interest. Two different acquisition strategies were utilized to streamline the screening workflow. For all positive ionizable compounds IDA was chosen as the acquisition mode, as it enabled the acquisition of MS/MS spectra on many precursors, in an intensity dependent manner. Subsequently, resulting MS/MS spectra is the used to match to potential analytes using MS/MS library spectral matching.

For the 5 target compounds (barbiturates and THC-COOH) that favor negative electrospray ionization, MRM^{HR} workflow was used as targeted acquisition strategy. MRM^{HR} workflow was performed using full scan MS/MS acquisition; by

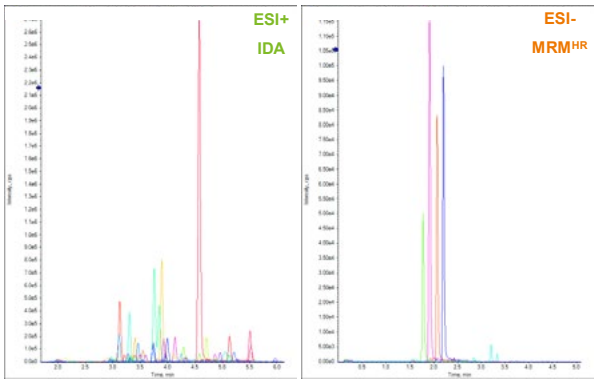


Figure 5. Obtaining Fast and Confident Identification of Forensic Compounds of Interest in Biological Matrices. (Left) Extracted Ion Chromatogram shows a rapid LC separation (6 min) and identification of 55 forensic compounds of interest spiked in whole blood at LOD concentrations using IDA-MS/MS.(Right) Extracted Ion Chromatogram shows the rapid identification of barbiturates and THC-COOH spiked in whole blood at LOD concentrations using MRM^{HR} workflow.

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defining the m/z range desired using the *Apply TOF start/stop mass feature*. This mode was beneficial as it enhanced compound identification at the LOD when performing MS/MS spectral library matching.

Figure 5 displays XIC chromatograms showing the detection of all target compounds analyzed with both positive and negative electrospray ionization modes in control blood samples spiked at the LODs, based on the latest NSC-ADID recommendations.[1]

Throughout the method development process, it was important to obtain high combined scores for all compounds based on the four main confidence criteria defined in the processing method. Additional qualification criteria were implemented by setting an analyte concentration threshold based on the LODs to minimize false positives and/or false negative hits. Figure 6 shows the successful detection of 6-MAM and Fentanyl at their corresponding LODs, with mass errors less than 2ppm and MS/MS scores over 90%.

Table 1 shows the average (n=9) combined scores obtained for all 55 target compounds, in control blood samples spiked at the LOD analyzed over the course of 3 days. Inter-day reproducibility resulted in %RSDs ranging between 1-10% for the target analytes.

It was found that THC-COOH had sufficient S/N ratios (> 200) and mass error less than 1 ppm at the LOD (10 ng/mL) for positive identification. However, low-abundance MS/MS spectra were obtained at that concentration level, subsequently resulting in an average combined score of 70%. Further optimization on the sample extraction protocol is recommended to enhance THC-COOH sensitivity and MS/MS fragmentation.

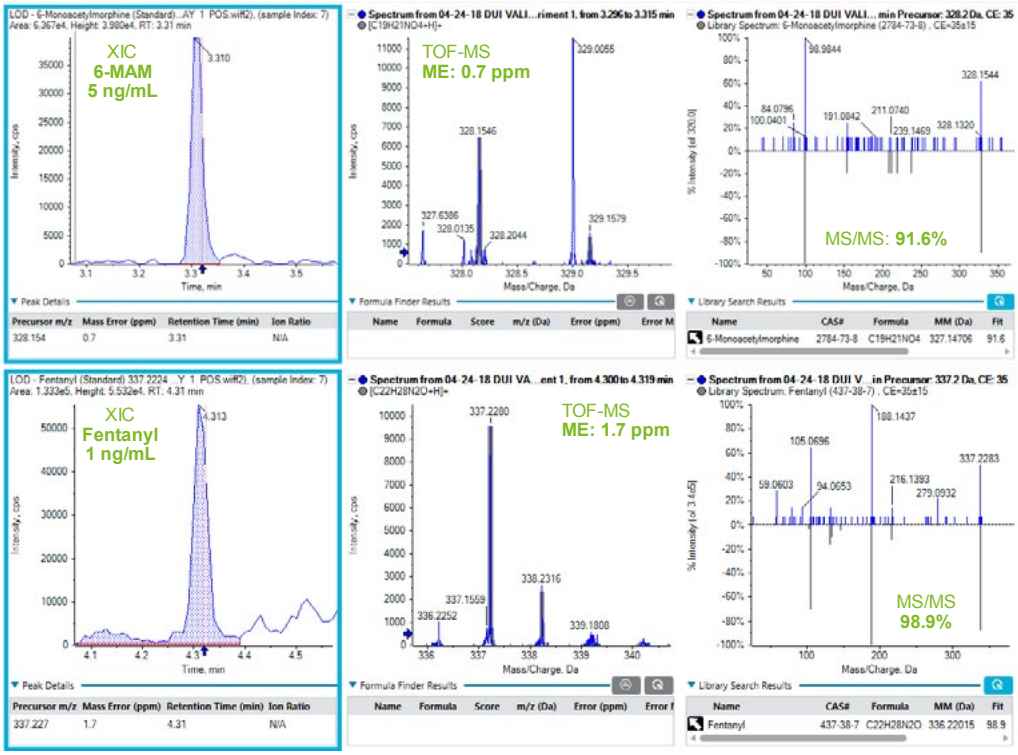

Figure 6. Successful Application of the SCIEX X500R QTOF System for Enhanced Compound Identification at Trace Concentration Levels. XICs, TOF MS and MS/MS spectra obtained showing confident and detailed identification of 6-MAM (Top) and fentanyl (bottom) spiked in whole blood at low ng/mL levels.

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Figure 7. Minimize False Positives/Negatives by Streamlining Accurate Mass Data Processing of all Compounds of Interest Present in a Forensic DUID Case Sample using SCIEX OS Software. Using multiple acceptance criteria enabled the accurate identification of target analytes present in a forensic blood sample. (Top) Sample list of all compounds passing the acceptance criteria (green traffic lights) and concentration thresholds (cells highlighted in red) set within the processing method. (Bottom) XICs of all compounds identified in the sample, showcasing TOF MS and MS/MS spectral library identification details.

Enhanced Forensic Compound Identification using the SCIEX X500R QTOF System

One of the principal goals of developing this comprehensive analysis workflow was to successfully migrate the current immunoassay approach to the SCIEX X500R QTOF System. The current immunoassay sample preparation and analysis workflow utilizes 1mL of forensic blood sample and 2mL of acetonitrile for extraction, whereas with the QTOF MS strategy the laboratory was able to reduce the sample size to 100 µL while still meeting the NSC-ADID recommended cutoffs.

The ability of meeting these cutoffs with minimal sample is ideal, as often forensic case samples are limited in volume. Additionally, it eliminates the laboratory's need for using multiple reagent kits (9 kits currently utilized) as the QTOF MS approach provides the enhanced selectivity and sensitivity to streamline the detection of Tier I and Tier II compounds.

As part of the implementation plan, 30 forensic DUID case samples were screened with both immunoassay and QTOF MS for results comparison.

Table 2 shows all compounds detected in the 30 forensic DUID samples examined with both immunoassay analyzer and the SCIEX X500R QTOF system. Compounds highlighted in green were specifically detected using QTOF MS but missed or classified as a single compound class (e.g., *OPI* for Opiates and metabolites) by the immunoassay approach.

Figures 1 and 7 show the detailed analysis of two different DUID samples in the study. In reference to the sample displayed on Figure 7, the immunoassay analyzer detected THC-COOH exclusively.

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Table 2. List of Compounds Identified in Forensic DUID Samples using Immunoassay Analyzer and the SCIEX X500R QTOF System.

ImmunoAssay Results		Mass Spectrometry Results								
DUI 1	COKE	Benzoylecgonine	Cocaethylene	Cocaine	Cotinine	Nordiazepam				
DUI 2	THC	Cotinine	THC-COOH							
DUI 3	NEG	NEG								
DUI 4	THC	Cotinine	Fentanyl	Norfentanyl	THC-COOH					
DUI 5	THC COKE	Benzoylecgonine	Cocaine	Cotinine	THC-COOH					
DUI 6	THC	Cotinine	THC-COOH							
DUI 7	AMPH	Amphetamine	Cotinine	Methamphetamine						
DUI 8	NEG	Cotinine	Lorazepam							
DUI 9	PCP THC	Cotinine	PCP	THC-COOH						
DUI 10	AMPH	Amphetamine	Cotinine	Ritalinic Acid	Methamphetamine					
DUI 11	THC BENZO	Cotinine	Diazepam	THC-COOH	Nordiazepam					
DUI 12	THC	Fentanyl	Norfentanyl	THC-COOH						
DUI 13	THC	Cotinine	THC-COOH							
DUI 14	THC	Cotinine	THC-COOH							
DUI 15	THC	Cotinine	THC-COOH							
DUI 16	THC	Cotinine	THC-COOH							
DUI 17	THC	Cotinine	THC-COOH							
DUI 18	THC	Cotinine	THC-COOH							
DUI 19	THC	Cotinine	THC-COOH							
DUI 20	THC	Cotinine	THC-COOH							
DUI 21	THC	Cotinine	THC-COOH							
DUI 22	THC	Cotinine	THC-COOH							
DUI 23	THC	Cotinine	THC-COOH							
DUI 24	COKE OPI	Benzoylecgonine	Buprenorphine	Cocaine	Codeine	Cotinine	Hydromorphone	Morphine	Metamphetamine	Morphine-3-Glucuronide
DUI 25	COKE	Benzoylecgonine	Cocaethylene	Cocaine	Cotinine					
DUI 26	OPI	Codeine	Cotinine	Fentanyl	Morphine	Morphine-3-Glucuronide	Hydromorphone			
DUI 27	AMPH COKE OPI	Amphetamine	Benzoylecognine	Cotinine	Fentanyl	Hydromorphone	Metamphetamine	Morphine	Morphine-3-Glucuronide	Norfentanyl
DUI 28	OPI	Benzoylecgonine	Codeine	Cotinine	Fentanyl	Hydromorphone	Morphine	Morphine-3-Glucuronide		
DUI 29	AMPH	Amphetamine	Cotinine	Fentanyl	Norfentanyl	Metamphetamine				
DUI 30	THC	Cotinine	THC-COOH							

*Compounds highlighted in **GREEN** are not screened for using the immunoassay analyzer operating in the laboratory but were detected in the MS assay.*

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However, when analyzed with the SCIEX X500R QTOF System, the same compound was identified but also three compounds of interest, which were not tested by immunoassay were detected:

- Cotinine (~ 482.32 ng/mL) Combined Score 100%
- Fentanyl (~2.1 ng/mL) Combined Score 98.3%
- Norfentanyl (~1.32 ng/mL) Combined Score 53.9%
- THC-COOH (~92.52 ng/mL) Combined Score: 97%

It is important to highlight that norfentanyl was considered a positive hit although obtaining a combined score of 53.9%. Analyte review based on the acceptance criteria like retention time, mass error on the TOF MS scan, concentration threshold (> 1 ng/mL) as well as parent drug metabolism pathway knowledge, were supporting evidence of compound presence in the forensic DUID sample.

Conclusions

A comprehensive drug screening workflow for the analysis of forensic DUID blood samples has been successfully developed using the SCIEX X500R QTOF System based on the new NSC-ADID recommendations.

- The vMethod™ Application for forensic compound screening was successfully used to obtain retention times and MS/MS spectra necessary to build a targeted analysis workflow for the 60 forensic compounds of interest in DUID case samples.
- Average combined scores based on multiple acceptance criteria (Ret. Time, Mass error, Isotope ratio, MS/MS library hit and concentration) ranged between 70-98% for all target analytes, resulting in successful compound identification.
- The developed QTOF MS screening approach enabled the identification of multiple number of the targeted compounds present in authentic forensic DUID case samples in comparison to immunoassay based screening.
- The adaptation of QTOF MS technology enabled the use of microliter volumes of forensic blood samples, while meeting NSC-ADID cutoff recommendations. Thus, eliminating the use of multiple immunoassay reagent kits used for screening.

References

1. Logan B. et al., Recommendations for Toxicological Investigation of Drug-Impaired Driving and Motor Vehicle Fatalities-2017 Update. J Anal Toxicol 2018, 42 (2), 63-68. https://doi.org/10.1093/jat/bkx082
2. Desharnais B. et al., Protein precipitation of whole blood for quantification of 58 different analytes by LC-MS/MS: method development challenges, https://goo.gl/2Ma9RU
3. vMethod™ Application - Single-Injection Screening of 664 Forensic Toxicology Compounds on a SCIEX X500R QTOF System.

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Supplement A. List of Target Forensic DUID Compounds.

Component Name	Retention Time	Formula	Precursor (Q1) Mass (Da)	Adduct & Charge	LOD (ng/mL)
6-Monoacetylmorphine	3.32	$C_{19}H_{21}NO_4$	328.1543	[M+H]+	5
7-Aminoclonazepam	3.97	$C_{15}H_{12}ClN_3O$	286.0742	[M+H]+	10
Alpha-hydroxyalprazolam	4.71	$C_{17}H_{13}ClN_4O$	325.0851	[M+H]+	10
Alpha-hydroxymidazolam	4.36	$C_{18}H_{13}ClFN_3O$	342.0804	[M+H]+	10
Alprazolam	4.95	$C_{17}H_{13}ClN_4$	309.0902	[M+H]+	10
Amphetamine	3.24	$C_9H_{13}N$	136.1121	[M+H]+	20
Benzoylecgonine	3.59	$C_{16}H_{19}NO_4$	290.1387	[M+H]+	5
6-Beta-Naltrexol	3.28	$C_{20}H_{25}NO_4$	344.1856	[M+H]+	10
Buprenorphine	4.39	$C_{29}H_{41}NO_4$	468.3108	[M+H]+	1
Zolpidem Phenyl-4-carboxylic acid	3.5	$C_{19}H_{19}N3O3$	338.1499	[M+H]+	5
Carisoprodol	4.86	$C_{12}H_{24}N2O_4$	261.1809	[M+H]+	50
Cocaethylene	4.14	$C_{18}H_{23}NO_4$	318.1700	[M+H]+	5
Cocaine	3.93	$C_{17}H_{21}NO_4$	304.1543	[M+H]+	5
Codeine	3.22	$C_{18}H_{21}NO_3$	300.1594	[M+H]+	5
Cotinine	2.04	$C_{10}H_{12}N_2O$	177.1022	[M+H]+	5
Delorazepam	5.2	$C_{15}H_{10}Cl_2N_2O$	305.0243	[M+H]+	10
Diazepam	5.53	$C_{16}H_{13}ClN_2O$	285.0789	[M+H]+	10
EDDP	4.59	$C_{20}H_{23}N$	278.1903	[M+H]+	50
Etizolam	5.12	$C_{17}H_{15}ClN_4S$	343.0779	[M+H]+	10
Fentanyl	4.32	$C_{22}H_{28}N_2O$	337.2274	[M+H]+	1
Gabapentin	3.12	$C_9H_{17}NO_2$	172.1332	[M+H]+	250
Hydrocodone	3.41	$C_{18}H_{21}NO_3$	300.1594	[M+H]+	5
Hydromorphone	3.05	$C_{17}H_{19}NO_3$	286.1438	[M+H]+	5
Ketamine	3.55	$C_{13}H_{16}ClNO$	238.0993	[M+H]+	5
Lorazepam	4.9	$C_{15}H_{10}Cl_2N_2O_2$	321.0192	[M+H]+	10
MDA	3.3	$C_{10}H_{13}NO_2$	180.1019	[M+H]+	20
MDMA	3.4	$C_{11}H_{15}NO_2$	194.1176	[M+H]+	20
Meperidine	3.89	$C_{15}H_{21}NO_2$	248.1645	[M+H]+	25
Meprobamate	4.11	$C_9H_{18}N_2O_4$	219.1339	[M+H]+	500
Methadone	4.71	$C_{21}H_{27}NO$	310.2165	[M+H]+	5
Methamphetamine	3.35	$C_{10}H_{15}N$	150.1277	[M+H]+	20

Contents

Component Name	Retention Time	Formula	Precursor (Q1) Mass (Da)	Adduct & Charge	LOD (ng/mL)
Methylphenidate	3.77	$C_{14}H_{19}NO_2$	234.1489	[M+H]+	25
Midazolam	4.3	$C_{18}H_{13}ClFN_3$	326.0855	[M+H]+	10
Mitragynine	4.59	$C_{23}H_{30}N_2O_4$	399.2278	[M+H]+	2.5
Morphine	2.97	$C_{17}H_{19}NO_3$	286.1438	[M+H]+	10
Morphine-3-glucuronide	1.94	$C_{23}H_{27}NO_9$	462.1759	[M+H]+	49.4
Naltrexone	3.32	$C_{20}H_{23}NO_4$	342.1700	[M+H]+	10
Norbuprenorphine	3.95	$C_{25}H_{35}NO_4$	414.2639	[M+H]+	2.5
Nordiazepam	5.12	$C_{15}H_{11}ClN_2O$	271.0633	[M+H]+	10
Norfentanyl	3.52	$C_{14}H_{20}N_2O$	233.1648	[M+H]+	1
Norhydrocodone	3.34	$C_{17}H_{19}NO_3$	286.1438	[M+H]+	25
Norketamine	3.46	$C_{12}H_{14}ClNO$	224.0837	[M+H]+	5
Normeperidine	3.85	$C_{14}H_{19}NO_2$	234.1489	[M+H]+	25
Noroxycodone	3.28	$C_{17}H_{19}NO_4$	302.1387	[M+H]+	10
O-Desmethyl-cis-tramadol	3.31	$C_{15}H_{23}NO_2$	250.1802	[M+H]+	25
Oxazepam	4.84	$C_{15}H_{11}ClN_2O_2$	287.0582	[M+H]+	10
Oxycodone	3.34	$C_{18}H_{21}NO_4$	316.1543	[M+H]+	5
Oxymorphone	3	$C_{17}H_{19}NO_4$	302.1387	[M+H]+	5
Phenazepam	5.28	$C_{15}H_{10}N_2OBrCl$	348.9738	[M+H]+	10
Phencyclidine	4.25	$C_{17}H_{25}N$	244.2060	[M+H]+	5
Pregabalin	3.11	$C_8H_{17}NO_2$	160.1332	[M+H]+	250
Ritalinic acid	3.46	$C_{13}H_{17}NO_2$	220.1332	[M+H]+	25
Tapentadol	3.74	$C_{14}H_{23}NO$	222.1852	[M+H]+	5
Temazepam	5.21	$C_{16}H_{13}ClN_2O_2$	301.0738	[M+H]+	10
Tramadol	3.74	$C_{16}H25NO_2$	264.1958	[M+H]+	5
Zolpidem	4	$C_{19}H_{21}N_3O$	308.1757	[M+H]+	5
Secobarbital	2.19	$C_{12}H_{18}N_2O_3$	237.1245	[M-H]-	250
Butalbital	1.9	$C_{11}H_{16}N_2O_3$	223.1088	[M-H]-	250
Pentobarbital	2.05	$C_{11}H_{18}N_2O_3$	225.1245	[M-H]-	250
Phenobarbital	1.76	$C_{12}H_{12}N_2O_3$	231.0775	[M-H]-	250
THC-COOH	3.19	$C_{21}H_{28}O_4$	343.1915	[M-H]-	10

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Efficiently Designed Workflows Provide Accurate Results in Forensic Analysis of THC-COOH in Hair Samples

Sensitive Detection of the Marijuana Metabolite with the SCIEX Triple Quad™ 4500 LC-MS/MS System

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Marijuana is one of the most popular recreational drugs abused worldwide. Detection of its use can be done in many biological matrices, such as urine, blood, oral fluid and hair. While urine and oral fluid are very useful for determining marijuana use in short term, hair samples are extremely valuable in testing the long-term use. Additional benefits of hair testing include but are not limited to (1) sample collection being non-invasive; and (2) little risk of sample adulteration.

Presence of the main marijuana metabolite (i.e. THC-COOH) in hair indicates active drug usage. However, there are two major analytical challenges associated with detecting THC-COOH in hair samples: The concentration of THC-COOH in hair samples is very low and the high abundance of matrix interferences associated within hair samples that specifically interfere with the detection of THC-COOH.

Previously, the successful applicability of the QTRAP® 5500 or 6500+ systems for THC-COOH analysis in hair was demonstrated. Using MS/MS/MS or MRM^3, very low level of THC-COOH (~ 0.1 pg/mg) in hair samples can be detected without exhaustive sample preparation.

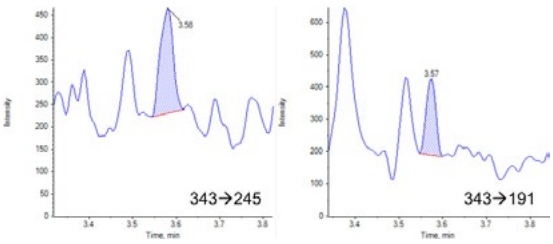


Figure 1. Detect THC-COOH in Hair Down to 0.2 pg/mg Trace Concentration Levels with an Efficient SPE Sample Preparation Procedure. Using fast MRM cycle times (~100 msec) allowed the accurate quantitation (Quantifier Ion shown on left) and confirmation (Qualifier ion shown on right) of THC-COOH extracted from 25 mg of a hair sample.

In the absence of a QTRAP System for higher specificity scans, forensic laboratories must efficiently design analysis approaches for the accurate detection of marijuana metabolite present in hair samples. In this technical note, a workflow is presented that combines the Triple Quad 4500 LC-MS/MS System with a solid phase extraction procedure that allows the reliable and sensitive detection of trace levels of THC-COOH in hair matrix.

Features of the Triple Quad 4500 LC-MS/MS System for Forensic Hair Analysis

- Turbo V™ source and Curtain Gas™ interfaces delivers highly efficient desolvation for stable and sensitive performance while analyzing complex biological matrices.
- Ultra-fast MRM cycle times (With minimum dwell times of 1 msec for MRM acquisition) increasing sample throughput and data quality for trace levels of THC-COOH.
- Achieve up to 5 orders of dynamic range for high performance quantitation, reducing the needs for repeat analyses for increased productivity.
- MultiQuant™ Software allows fast data processing, with less manual intervention and quick flagging of outliers, so forensic laboratories can release results faster.

Contents

Methods

***Hair Sample Preparation and Digestion*:** Hair samples were washed according to accepted laboratory procedure, dried and cut into segments of ~ 2 mm lengths. Approximately 25 mg of each hair sample was transferred into suitable and sealable container with cap. 20 µL THC-COOH-d9 internal standard solution in methanol and ~ 1.1 mL 1N potassium hydroxide solution was added, and the container was capped and gently agitated to suspend the hair segments in the solution. The containers were placed at 70ºC for 1 hour with gentle agitation every 20 min (to keep hair segments fully suspended in digestion solution) for complete digestion of the hair samples. The containers were allowed to cool to room temperature. Contents of the containers were transferred to 2-mL microcentrifuge tubes for ultra-centrifugation at 15,000 rpm for 5 min.

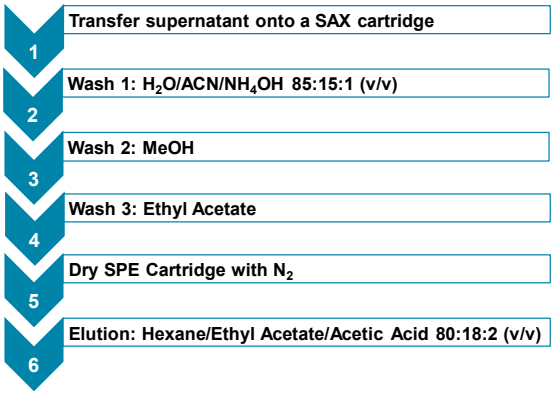


Figure 2. Strong Anion Exchange (SAX) Solid Phase Extraction workflow. A 6-step extraction protocol can be rapidly implemented and optimized for selectively extracting THC-COOH from hair samples for analysis with the Triple Quad 4500 LC-MS/MS System.

LC Conditions: HPLC separation was performed on Phenomenex Kinetex Phenyl-hexyl column (50 × 3 mm, 2.6 µm, 00B-4495-E0) on the SCIEX ExionLC™ AC system. Mobile phase A (MPA) and mobile phase B (MPB) were 0.01% acetic acid in water and methanol, respectively. The LC flowrate was 0.75 mL/min, column temperature was held at 40ºC, and the total LC runtime was 5.5 min.

MS and MS/MS Conditions: Source conditions were in Table 1 and MRM conditions were listed in Table 2.

***Data Processing*:** Data was acquired with Analyst® Software 1.6.3 and processed with MultiQuant Software 3.0. Linear dynamic range was evaluated through calibration curves with analyte concentrations ranging from 0.2 – 2 pg/mg.

Table 1: Source Parameters.

Curtain gas	*25*
CAD	*10*
Spray voltage (V)	*-4500*
Temperature (C)	*650*
GS 1	*60*
GS 2	*60*

Table 2: MRM Transitions Used.

Analyte	Q1	Q3	DP	CE
THC-COOH (1)	*342.9*	*245.1*	*-100*	*-39*
THC-COOH (2)	*342.9*	*191.0*	*-100*	*-45*
THC-COOH-d9 (1)	*351.9*	*254.1*	*-100*	*-39*
THC-COOH-d9 (2)	*351.9*	*194.0*	*-100*	*-45*

Designing an Efficient Sample Preparation Workflow to Maximize THC-COOH Recovery

One of the biggest challenges in getting the clean extracts of THC-COOH from hair for detection was the presence of complex matrix contents, some of which were structurally similar to THC-COOH. To remove these interferences, a SAX SPE procedure was suggested and tested.

Usually in the SAX procedure, the sample need to be basified first before being applied to the SPE cartridge, so the target analytes can bind to the oppositely charged SAX stationary phase strongly. Because hair samples usually were digested in highly alkaline solutions for more complete release of analytes from the hair samples into the extraction media, the sample solutions were already basified and can be, in theory, directly applied onto the SPE cartridge.

It was discovered that an extra ultra-centrifugation step was needed before the samples were applied to the SPE cartridge, mainly to remove the insoluble particulates in the digested hair samples. Failure to remove these particulates rendered a very long sample application step as the SPE cartridge would be clogged or partially clogged during this process.

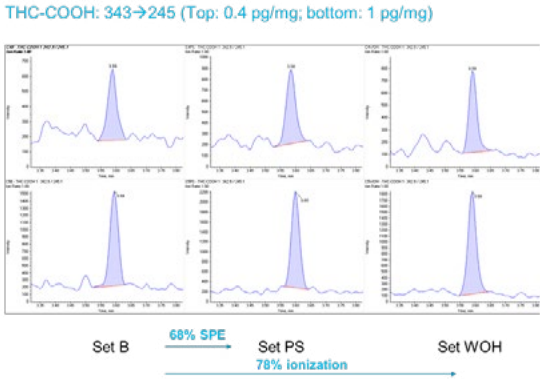


Figure 3. Obtain Maximized Analyte Recovery Performance and Reduced Matrix Effects. The combination of SPE, LC separation and highly efficient ionization through the Turbo V source delivered high analyte recovery, allowing consistent quantitation of THC-COOH at low picogram levels.

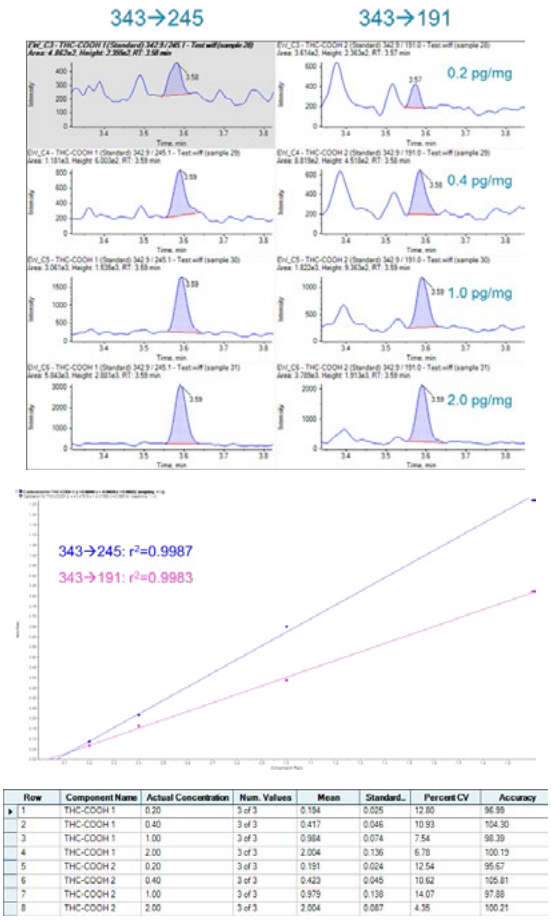
To test the sample preparation recovery and matrix effects, three sets of samples were prepared:

- Set B was spiked hair samples with 0.4 and 1 pg/mg THC-COOH processed with SCX SPE.
- Set PS was similar to B but THC-COOH was not spiked before SPE step was completed, so the sample preparation recovery could be measured.
- Set WOH was also similar to B but the hair samples (in solution) were replaced with 1 N potassium hydroxide, so the signal difference between B and WOH solely indicated ion suppression or enhancement.

It was observed that the sample preparation recovery was at 68% and the matric effects showed 22% loss of signal (or 78% signal recovery due to ion suppression, Figure 3). This allows the reliable quantitation of THC-COOH at low picogram concentration levels, which is only possible through the implementation of the SAX SPE procedure designed.

Evaluating the Analytical Sensitivity of the Triple Quad™ 4500 LC-MS/MS System

Six levels of calibrators were prepared at 0.04, 0.1, 0.2, 0.4, 1 and 2 pg/mg for THC-COOH in hair. Figure 3 showed both the quantifier (343→245) and qualifier (343→191) transitions for samples from 0.2 to 2 pg/mg samples.



Row	Component Name	Actual Concentration	Num. Values	Mean	Standard...	Percent CV	Accuracy
1	THC-COOH 1	0.20	3 of 3	0.194	0.025	12.80	96.99
2	THC-COOH 1	0.40	3 of 3	0.417	0.046	10.93	104.30
3	THC-COOH 1	1.00	3 of 3	0.984	0.074	7.54	98.39
4	THC-COOH 1	2.00	3 of 3	2.004	0.136	6.78	100.19
5	THC-COOH 2	0.20	3 of 3	0.191	0.024	12.54	95.67
6	THC-COOH 2	0.40	3 of 3	0.423	0.045	10.62	105.81
7	THC-COOH 2	1.00	3 of 3	0.979	0.138	14.07	97.88
8	THC-COOH 2	2.00	3 of 3	2.004	0.087	4.35	100.21

Figure 4. Good Linear Dynamic Range, Accuracy and Precision was Achieved for THC-COOH in Hair. Calibration curves are shown as well a few representative XIC traces to demonstrate reliable quantitation from 0.2 to 2 pg/mg.

An LOQ of 0.2 pg/mg for THC-COOH in hair samples was determined. The assay showed excellent accuracy (>95%) and precision (< 15%), and the R^2 values for quantifier and qualifier were 0.9987 and 0.9983, respectively.

All quantitation results were processed with MultiQuant Software 3.0, designed for easy, quick, versatile and streamlined data processing with accurate and reliable quantitation.

Contents

Conclusions

The combination of a solid phase extraction procedure with the Triple Quad 4500 LC-MS/MS System allowed the efficient and sensitive detection of trace levels of THC-COOH (0.2 pg/mg) in hair samples, making the workflow to be readily adaptable into a forensic toxicology laboratory.

- A 6-step extraction protocol using SAX SPE can be rapidly implemented and optimized for selective analysis of THC-COOH.
- The design of the hair analysis workflow resulted in efficient ionization through the Turbo V source delivered high analyte recovery for stable and sensitive performance.
- Successful quantitation of THC-COOH was performed using MultiQuant Software 3.0 allowing streamlined and accurate data processing of trace level concentrations (0.2 to 2 pg/mg).

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