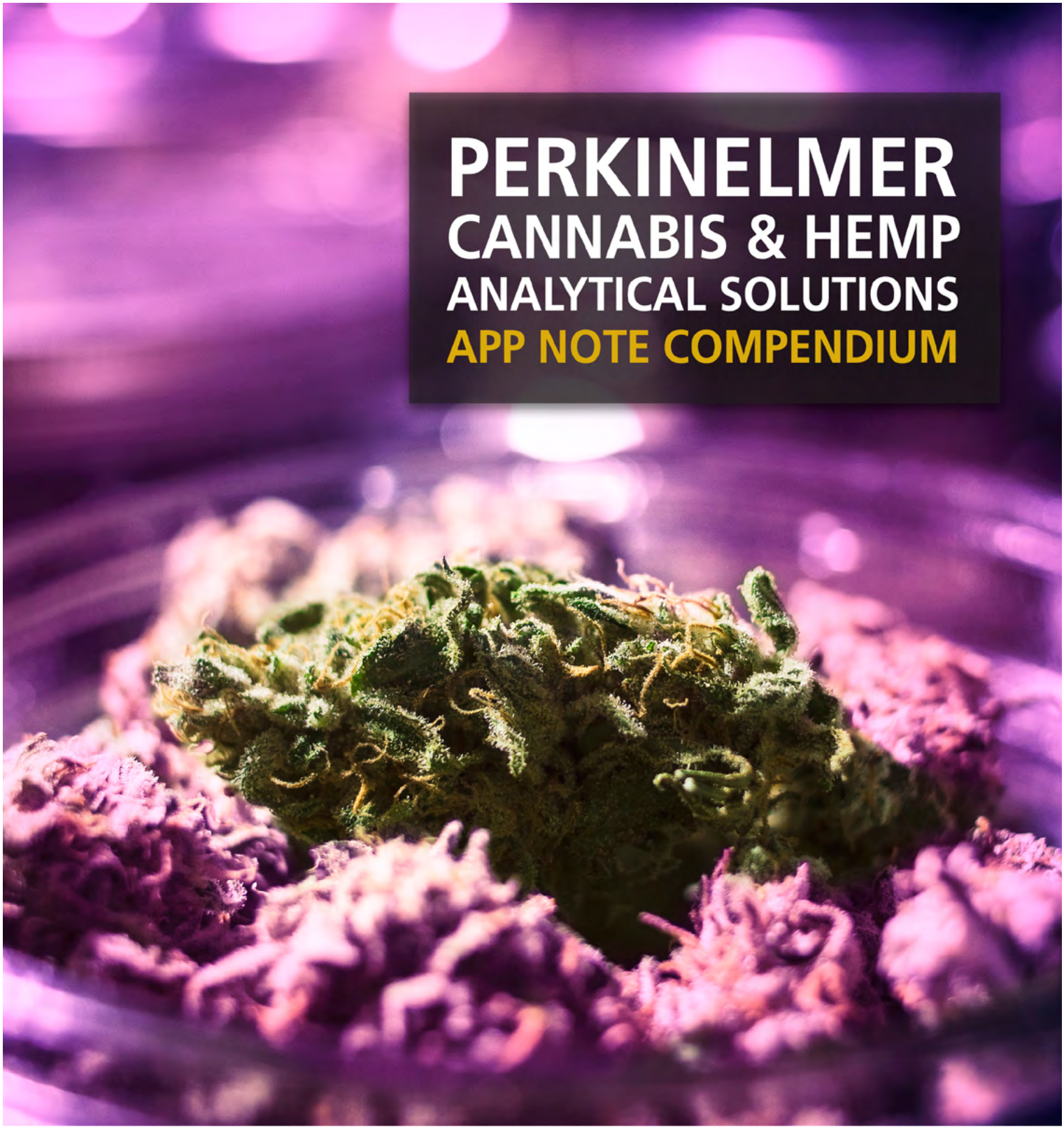

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Foreword to PerkinElmer Compendium of Application Notes

Welcome to our latest ebook, Analytical Testing of Cannabis, a compendium of key scientific facts, findings and solutions for experts working in the cannabis industry.

As it emerges from prohibition, cannabis is finally being embraced by researchers across the world. From new cannabis compounds to new medical possibilities, the almost daily dose of discoveries is enhancing our understanding of the cannabis plant more than ever before. Critically for the patients and customers who consume cannabis, research is now also beginning to shed light on the safety checks product samples undergo.

Armed with the analytical power of mass spectrometry, scientists have developed several new methods of examining cannabis. From the unique pharmacological compounds of potential benefit to patients, to the contaminants that must be identified to prevent harm, these mass spectrometry techniques are detecting cannabis analytes faster than ever before.

This compendium explores how mass spectrometry can interrogate cannabis in record time, detecting heavy metals, pesticides and residual solvents – all highly harmful to consumers in substantial amounts. In terms of desirable compounds, modified mass spectrometry techniques can also be harnessed to detect and catalog cannabinoids in a matter of minutes.

Along with methods of terpene detection and deep-dive features on decarboxylation, this collection is a must-read for anyone interested in the growing scientific field of cannabis testing.

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How To Catalogue 16 Cannabinoids in 6 Minutes

Looking at the cannabis industry from the outside, some might think its professionals only know five letters: T, H, C, B, and D. Many more would be surprised to learn that cannabis contained anything other than the two famous compounds, tetrahydrocannabinol (THC) and cannabidiol (CBD), but it certainly does. Packed with over 480 unique compounds[1], the cannabis plant has enough chemicals to keep analysts busy for days.

Around 60 of the 480 unique compounds are cannabinoids, the chemicals that interact with the central nervous system's CB1 and CB2 receptors. It is these compounds that can provide the biggest challenges for analysts, as some cannabinoids are so delicate that standard techniques can actually alter their chemical structure. For example, due to the high temperatures involved, gas chromatography techniques will often decarboxylate cannabidiolic acid (CBDA) into CBD.

Fortunately, high-pressure liquid chromatography (HPLC) – a chromatographic method that uses pressurized liquid instead of gas to separate compound mixtures – does not require the sky-high temperatures of gas chromatography. Thus, this cooler method ensures that chemicals like CBDA can be retained and measured. Many in the cannabis industry consider HPLC to be the gold standard when it comes to measuring cannabis potency. In fact, the technique is so revered that the HPLC-photodiode array method discussed in this application note recently received the Potency in Solution award from The Emerald Test, a well-regarded inter-laboratory comparison and proficiency test program for cannabis and hemp testing facilities.

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APPLICATION NOTE

Liquid Chromatography

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High Speed Chromatographic Analysis of 16 Cannabinoids by HPLC-PDA

To further validate the performance of this method for the industry, The Emerald Test Proficiency Test (PT) for Potency was conducted. The Emerald Test™ is an Inter-Laboratory Comparison and Proficiency Test (ILC/PT) program for cannabis testing labs. The results from the PT inter-laboratory samples passed; therefore, the method meets inter-laboratory reproducibility and accuracy. The method was awarded the Emerald Test Badge seen on the right.
https://pt.emeraldscientific.com/

Introduction

Current trends for the analysis of the cannabinoid content in cannabis flower extracts/concentrates and commercially available fortified foods depend on liquid chromatography for potency testing and/or ensuring the label-claim accuracy in product content descriptions.

Thereupon, this work describes a fast chromatographic method for the analysis of 16 cannabinoids commonly monitored in cannabis-centric labs. Their structures are shown in Figure 1.

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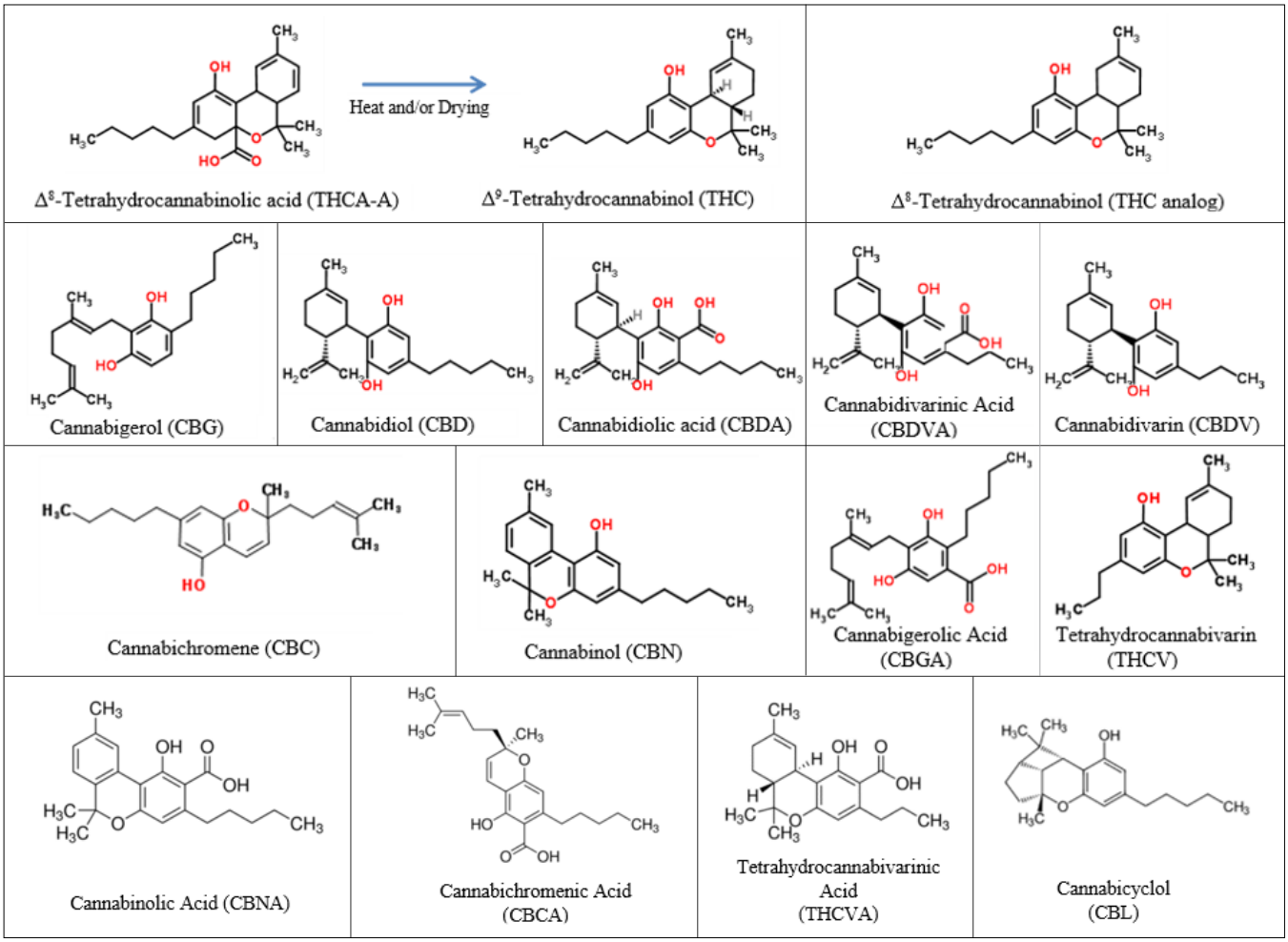

Figure 1. Chemical structures of the sixteen cannabinoids analyzed in this study.

Experimental

Hardware/Software

For the chromatographic separation, a PerkinElmer Flexar™ HPLC system was used with a PDA (photodiode array) Plus detector. Instrument control, analysis and data processing were performed using the Chromera® software platform.

Method Parameters

The LC parameters are shown in Table 1.

Solvents, Standards and Samples

All solvents and diluents used were HPLC grade. Unless otherwise specified, standard and sample extract dilutions were prepared using 80:20 methanol/water.

The sixteen 1-mg/mL cannabinoid standards were obtained from Sigma-Aldrich®, Inc (Allentown, PA). These included: Δ^9-tetrahydrocannabinol (Δ9-THC), Δ^9-tetrahydrocannabinolic acid (THCA-A), Δ^8-tetrahydrocannabinol (Δ8-THC), tetrahydrocannabivarin (THCV), cannabidivarin (CBDV), cannabidivarinic acid (CBDVA), cannabidiol (CBD), cannabidiolic acid (CBDA), cannabigerol (CBG), cannabigerolic acid (CBGA), cannabinol (CBN), cannabichromene (CBC), cannabicyclol (CBL), cannabichromenic acid (CBCA), cannabinolic acid (CBNA) and tetrahydrocannabivarinic acid (THCVA).

Table 1. LC Parameters.

Column:	PerkinElmer SPP C18, 2.7 µm, 150 x 3.0 mm (Part # N9308411)	
Mobile Phase:	**Solvent A:** Water with 0.1% formic and 5 mM ammonium formate **Solvent B:** Acetonitrile with 0.1% formic **Solvent Program:**	
Analysis Time:	6 min.	**Data Collection Rate:** 5 pts/sec (Hz)
Pressure:	4400 psi/300 bar maximum	
PDA Wavelengths:	228 nm	**PDA Flowcell:** 10 mm (standard)
Oven Temp.:	40 °C	
Injection Vol.:	10 µL	

Step	Time (min)	Flow Rate (mL/min)	%A	%B
Equil.	4.5	1.0	33	67
1	6	1.0	5	95
2	2	1.0	5	95

A 50-ppm stock standard mix solution was prepared by adding the entire contents of each standard into a 20-mL volumetric flask and filling to mark with water. This also served as the L10 calibration standard.

Additional calibrants were prepared by serially diluting the standard mix to concentration levels of 25, 10, 5, 2.5, 1.0, 0.5, 0.25, 0.10 and 0.05 µg/mL (ppm), providing a 10-level calibration set. Depending on the response, the lowest level (0.05 µg/mL) was not used for some analytes.

Results and Discussion

The chromatogram of the 50-µg/mL standard is shown in Figure 2, with all 16 cannabinoids eluting in under five minutes.

A 10-replicate chromatographic overlay of the 10-µg/mL cannabinoid standard is shown in Figure 3, highlighting very good repeatability.

The linearity plots for three representative cannabinoids are shown in Figure 4. The R^2 values for all 16 cannabinoids were above 0.999.

The chromatogram of the low-level 0.1-µg/mL cannabinoid standard is shown in Figure 5.

The chromatogram of an 80:20 methanol/water blank injection is shown in Figure 6, run right after the high-level calibrant set, showing no carryover or interferences.

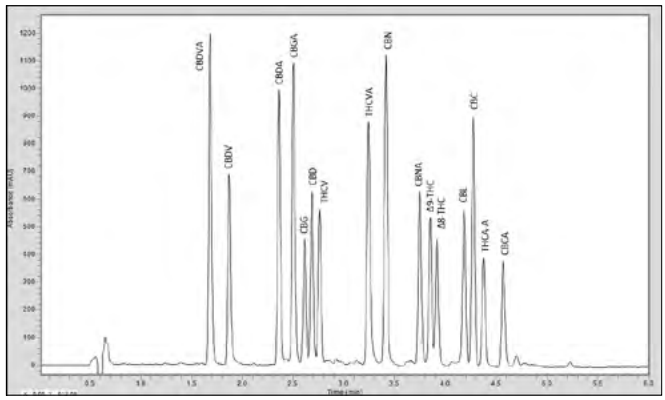

Figure 2. Chromatogram of the the 50-µg/mL cannabinoid standard.

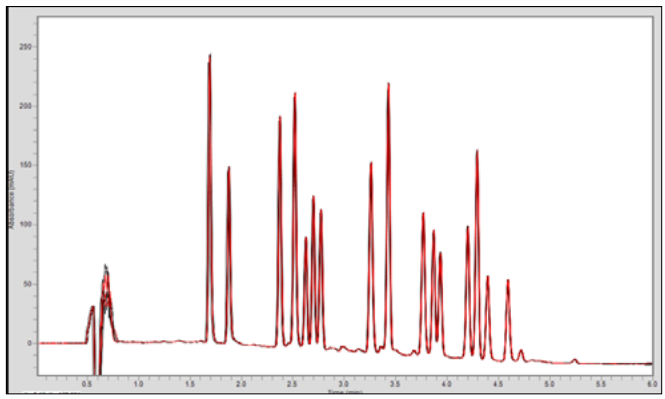

Figure 3. Chromatographic overlay of the 10-replicate injections of the 10-µg/mL cannabinoid standard.

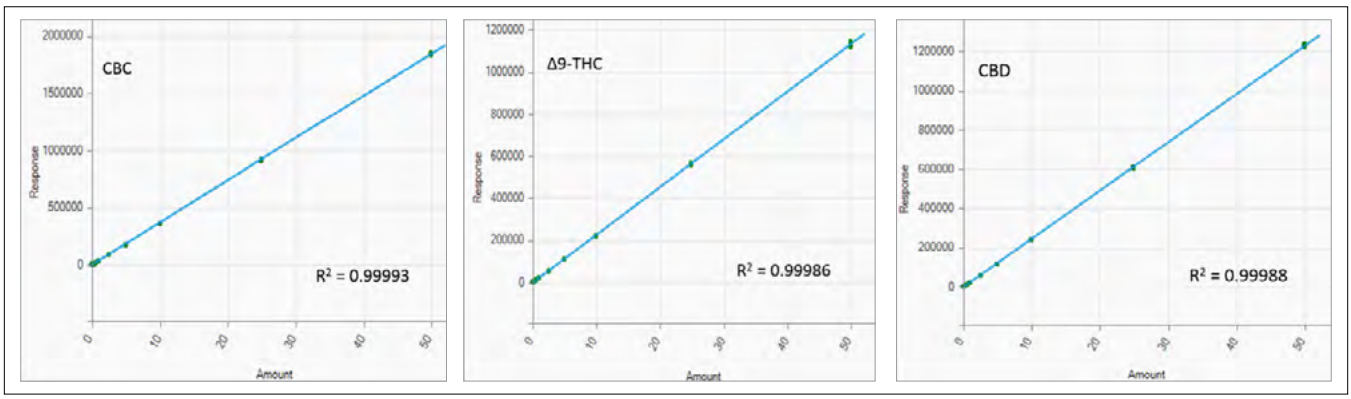

Figure 4. Linearity plots for three example cannabinoids.

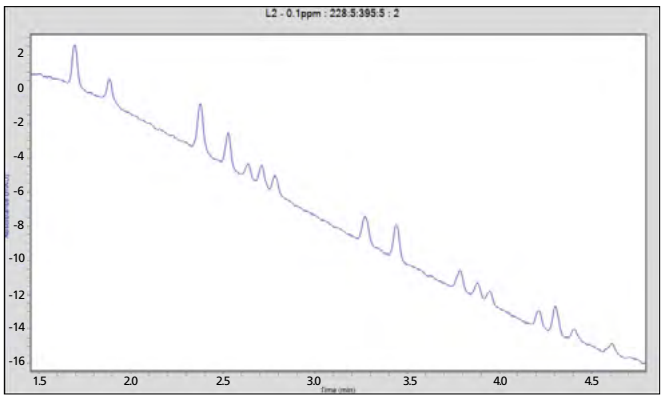

Figure 5. Chromatogram of the the 0.1-µg/mL cannabinoid standard.

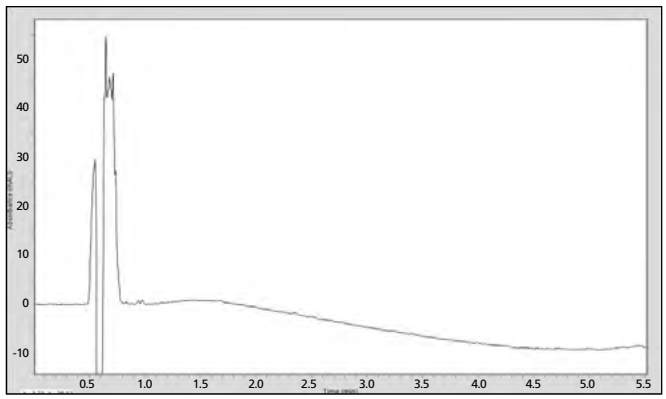

Figure 6. Chromatogram of a diluent "blank" injection.

As listed in Table 2, LOQ (limit of quantitation) levels were established for each analyte, based upon the averaged response for the 0.1 µg/mL calibration standard, run in triplicate.

Conclusions

- This work has demonstrated the fast and robust chromatographic separation and quantitation of 16 cannabinoids commonly analyzed in cannabis-centric labs, using the PerkinElmer Flexar HPLC system with a PDA detector.
- The method provides very good chromatographic repeatability and affords LOQs of ≤ 0.1 µg/mL for most analytes.
- If needed, additional sensitivity can be gained using the PDA's optional 50-mm flow cell.

Table 2. LOQs for the sixteen analytes, in order of elution.

Analyte	Calculated LOQ (µg/mL; S/N = 10)	Analyte	Calculated LOQ (µg/mL; S/N = 10)
CBDV	0.05	CBC	0.05
CBDVA	0.03	CBCA	0.11
CBG	0.10	CBL	0.08
CBGA	0.04	Δ9-THC	0.09
CBD	0.07	Δ8-THC	0.11
CBDA	0.03	THCA-A	0.12
CBN	0.04	THCV	0.08
CBNA	0.07	THCVA	0.05

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APPLICATION SUMMARY

Shining a Light on total THC and CBD With Near-infrared Spectroscopy?

The cannabis plant is biologically and chemically complex. More than 400 chemical components – over 60 of which are cannabinoids – contribute to its unique pharmacological and toxicological properties[1-2]. Delta-9-tetrahydrocannabinol (Δ^9-THC) and cannabidiol (CBD) have garnered much attention over the years and are two of the most researched and well characterized cannabinoids. THC is considered the main psychoactive ingredient of cannabis, whilst CBD, a non-psychotropic agent, is regarded as the primary therapeutic component[3]. These opposing effects make understanding the THC:CBD ratio important for researchers, healthcare professionals and patients, as cannabis and cannabis-derived products are increasingly used for medicinal purposes. Potency testing allows the quantitation of THC and CBD and other "major" cannabinoids, which is essential for precise labelling of cannabis products.

Tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA) decarboxylate to form THC and CBD, respectively. These naturally occurring precursors are often preferred for edible materials and extracted mixtures. High performance liquid chromatography (HPLC) is an analytical technique which can be used for cannabis potency testing[3]. Despite providing a full cannabinoid profile limitations such as sample destruction, complex instrumentation and longer sample preparation times have led researchers to seek faster and more user-friendly solutions[4].

Fourier transform near-infrared (FT-NIR) spectroscopy is one such technique. NIR instruments use infrared light to analyze materials by measuring the proportion of light that is reflected by the sample. Higher concentrations of compounds absorb more infrared light and therefore reflect less to the NIR instrument. In FT-NIR spectroscopy, sample analysis time is reduced using an infrared beam that contains many frequencies of light (polychromatic) at once. This enables all wavelengths to be measured simultaneously.

FT-NIR is a remarkably versatile, accurate and robust approach that requires little to no sample preparation, zero hazardous chemicals, and allows the sample to be reused in other analyses. As a result, it offers cannabis cultivators a more rapid and cost-effective method for the quantitative determination of cannabis potency, reducing the overall research and development costs[6].

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APPLICATION NOTE

FT-IR Spectroscopy

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The Determination of Total THC and CBD Content in Cannabis Flower by Fourier Transform Near Infrared Spectroscopy

Introduction

The medicinal use of cannabis and cannabis-related products has experienced astronomical growth over the past decade. Canada, Germany, New Zealand and Columbia now have legal cannabis markets. In the United States, 30 states plus the District of Columbia have passed legislation permitting the use of medicinal cannabis. Of these 30 states, nine have laws permitting the use of recreational cannabis. As the industry moves towards legitimization, understanding the cannabinoid concentrations along the cultivation and processing path is critical for assuring the quality and safety of cannabis products.

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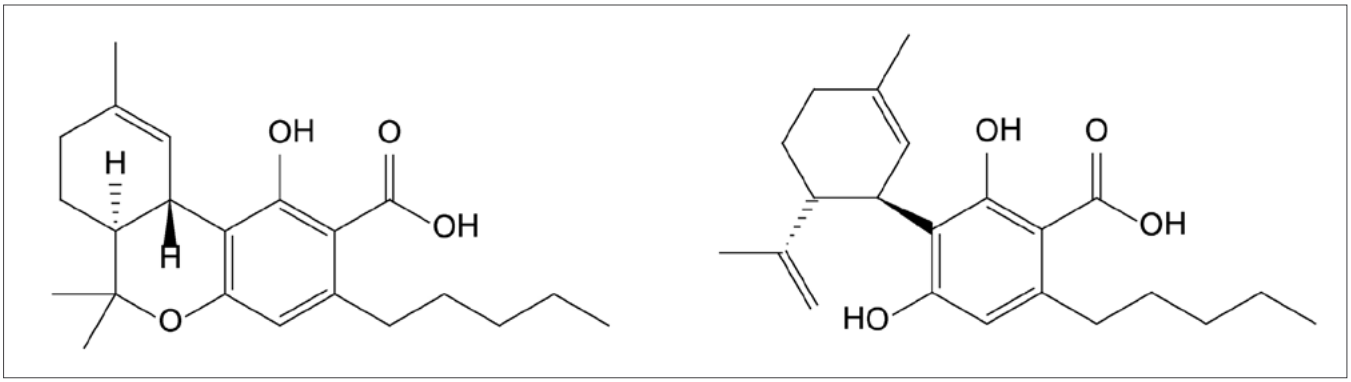

Figure 1. Chemical structures of the two predominant cannabinoids THCA (left) and CBDA (right).

The potency of cannabis flower is commonly defined as the concentration of the two predominant cannabinoids, namely tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA) (Figure 1). Valuation of the flower is influenced by this potency metric. As such, the accurate and reliable determination of potency is of great economic importance to all stakeholders in the cannabis supply chain.

Traditionally, potency has been determined by high performance liquid chromatography (HPLC). Although HPLC provides a full cannabinoid profile, several disadvantages are associated with its use. Sample destruction, complex instrumentation, hazardous chemicals and longer sample preparation times limit its deployment directly at the grow site where a rapid and non-destructive process is desirable. These limitations have prompted cultivators to seek a faster and user-friendly alternative to HPLC.

One such technique is Fourier transform near-infrared (FT-NIR) spectroscopy. FT-NIR is a remarkably versatile and robust analytical technique and has enjoyed tremendous success in a variety of applications for both the qualitative and quantitative determination of analytes on the percent level. To this end, we present FT-NIR spectroscopy as a rapid and cost-effective tool for the quantitative determination of potency in dried cannabis flower.

FT-NIR Measures the Chemical Composition of Substances

FT-NIR utilizes energy in the 14,000 – 4000 cm^{-1}, or 714 – 2500 nm, region of the electromagnetic spectrum to elucidate sample chemistry. When a sample is irradiated with near infrared light, the chemical bonds of the sample absorb light at discrete wavelengths. The resulting spectrum gives information about the chemical bonds present in the sample. This is where FT-NIR derives its power; it allows users to chemically fingerprint substances and when calibrated correctly, yields quantitative results.

Potency Determination by FT-NIR Poses a Unique Challenge

Botanicals, such as the cannabis flower, are heterogeneous by nature. This poses a unique challenge as the cannabis flower is a complex matrix consisting of a variety of plant tissue types and over 500 naturally produced chemicals. It is also widely accepted that the distribution of these chemicals can vary greatly between plants of the same cultivar, over an individual plant and even within a single flower.[1] Consequently, no two parts of the cannabis flower are the same and are likely to vary greatly in their cannabinoid content. Therefore, a technique that can overcome the inherent heterogeneity of the cannabis flower is of great analytical importance to ensure an accurate and representative measurement of flower potency.

FT-NIR is ideally suited for the analysis of heterogeneous substances due to the high penetration depth of NIR light and relative insensitivity to scattering effects. Using the Near Infrared Reflectance Module (NIRM) which employs the diffuse reflectance technique, a large sample volume is irradiated with NIR light through the use of the Sample Spinner. The light reflected by the sample is then recorded, resulting in the generation of a spectrum. Peak positions give insight to the chemical bonds present within the sample while peak intensities correlate to concentration.

Spectrum Two NIR

The PerkinElmer Spectrum Two FT-NIR featuring the Near Infrared Reflectance Module (NIRM) offers fast and easy measurements of potency in dried cannabis flower (Figure 2). The instrument optics are designed to provide tight control of the optical geometry, generating excellent spectral uniformity across the beam and a high collection efficiency. Gold-coated optics are used to maximize the energy throughput and a high sensitivity Indium Gallium Arsenide (InGaAs) detector gives excellent performance over the entire NIR spectral range. The combination of all these design optimizations results in a sampling module that facilitates easy transferability of methods from one instrument to another, a key requirement for method deployment on multiple instruments.[2]

Figure 2. Spectrum Two NIR equipped with a Near Infrared Reflectance Module and sample spinner.

Experimental

FT-NIR spectra from 302 unique flower samples were acquired. Flower samples were cured at the cultivation site prior to sample submission to the analytical laboratories (LightScale® Labs, Portland, OR; Napro Research, Sacramento, CA). Dried flower was homogenized using a manual or electric grinder and 1.0 – 1.5 grams of the homogenized flower was placed in a 60 mm glass petri dish, which was then placed atop the NIRM window. As the sample spectrum is being measured, the spinner rotates such that different parts of the sample are being scanned and the inhomogeneities averaged out. The larger sampling area results in a truly representative measurement.

Spectra were collected over the 10,000 – 4,000 cm^{-1} spectral range at 8 cm^{-1} resolution with each spectrum being the result of 64 averaged spectra. Data were acquired in interleaved mode for a total measurement time of approximately 90 seconds. This measurement configuration employs the use of an internal mirror which moves to direct the light either to an internal standard or to the sample allowing the collection of background scans periodically throughout the collection of sample spectra. The collection of background scans during each sample measurement increases the reproducibility of the analysis by eliminating the potential for errors associated with environmental fluctuations in temperature and humidity.

Following FT-NIR data acquisition, flower samples were removed from the petri dish and subjected to HPLC analysis for determination of potency. HPLC was used to determine potency values for THC, CBD, THCA and CBDA and total THC and total CBD was calculated as described in the literature.[3] The reference chromatographic data was then used in the generation of FT-NIR models for total THC and total CBD.

Chemometric Model Development

Although the FT-NIR spectra appear very similar between different flowers, small subtle differences are present and are significant enough to build quantitative models for potency. Example near infrared spectra of dried cannabis flower are shown in Figure 3. Building a robust model for cannabis flower requires the measurement of a variety of samples types for calibration. The calibration set should cover all sources of variation normally encountered for cannabis flower, such as different harvest batches, cultivars, chemical compositions, and flower morphologies. Capturing this variation is critical to ensure a stable model.

Principal component regression (PCR) was chosen for the determination of cannabis flower potency. PCR seeks to explain the observed variability in the spectral data set by reconstructing the NIR spectra into principal components. These principal components are linear combinations of the original NIR spectra and represent the most significant sources of variation within the data. A linear regression of the potency values on the principal components is then performed to obtain a calibration curve.

Spectra were randomly split 80:20 into calibration and validation datasets, respectively. Spectral pre-processing was optimized independently for each property value, total THC and total CBD. K-fold cross-validation was performed on the calibration data set by randomly segmenting the data set into equally sized subgroups (k = 10). During cross-validation a model is calculated using k-1 of the subgroups, with the remaining group then used as a prediction data set. This process repeats until each group has been left out of the model thereby generating a series of prediction results from each of the subgroups. The cross-validation process aids in model optimization by assessing how the model will perform when presented with an independent data set.

Following model generation and optimization, the cross-validated model was independently validated using the remaining 20% of spectra that were randomly selected from the original 80:20 split. The independent validation step determines how well the model predicts new data that was not included in the calibration step. This process gives insight to how the model will perform once implemented in the laboratory setting.

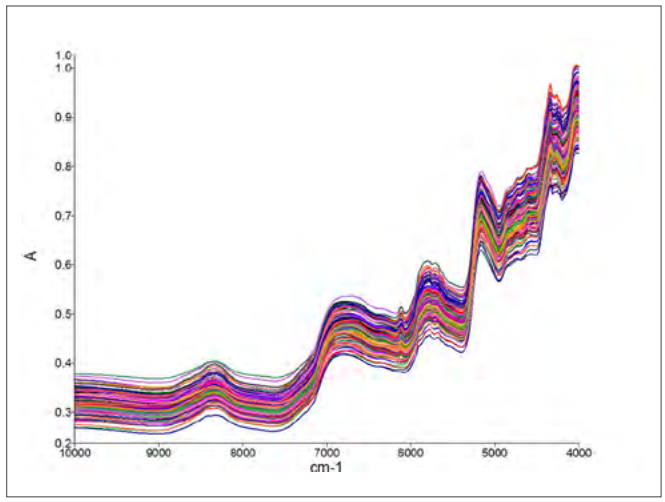

Figure 3. Raw spectra collected from the dried cannabis flower samples. Vertical offsets are due to scattering.

Quantifying Potency in Cannabis Flower

The results of the calibration curve for total THC and total CBD are shown in Figure 4 with regression details summarized in Table 1. Flower samples ranged between 0.1 – 23.4% total THC with a mean of 9.9% and 0 – 20.4 % total CBD with a mean of 8.7%. Both calibration curves show excellent correlation between the predicted and specified potency values. The coefficient of determination, R^2, is 0.98 and the cross validated standard error of prediction (CV-SEP) is 0.98% for total THC. The CBD model shows very similar performance with a coefficient of determination of 0.97 and a CV-SEP of 0.94%. The CV-SEP can be thought of as the magnitude of error expected when independent samples are predicted by the model. The low CV-SEP in conjunction with a high coefficient of determination indicates the ability of the model to make precise measurements of potency across the entire calibration range.

The independent validation results are summarized in Table 2. These results show accurate prediction across the entire calibration range indicating that there aren't any gaps in the calibration curve.

Starter calibrations are available for use with the Spectrum Two N and NIRM for determination of total THC and total CBD in dried cannabis flower. The use of starter calibrations greatly reduces method development time as a quantitative chemometric model has already been developed with consideration in mind for the large amount of variation inherent in the cannabis flower. Implementation of these starter calibrations requires on-site method validation to ensure the selected cannabis flower is representative of those used in generating the calibration model. On-site validation entails measurement of a small sample population with reference HPLC potency values. These on-site validation samples are then incorporated into the starter calibration model, further increasing the modelled sample variation.

Process Control and Optimization

FT-NIR spectroscopy has the potential to fundamentally change our understanding of the cannabis growth process by delivering time-resolved potency data that can be used to optimize growth conditions and trend cannabinoid expression in real-time. The near infrared spectrum of a cannabis flower provides a unique chemical snapshot, or immediate potency update, that can be used to establish batch trajectories. The ability to trend batches over time allows for identification of abnormal trends before harvest. Alternatively, trending data can be used to investigate conditions to maximize the cannabinoid expression behavior of the plant by manipulating external factors such as temperature, feeding schedules and light exposure. This data would provide cultivators with critical insight to early plant growth as well as future cannabinoid and potency yield.

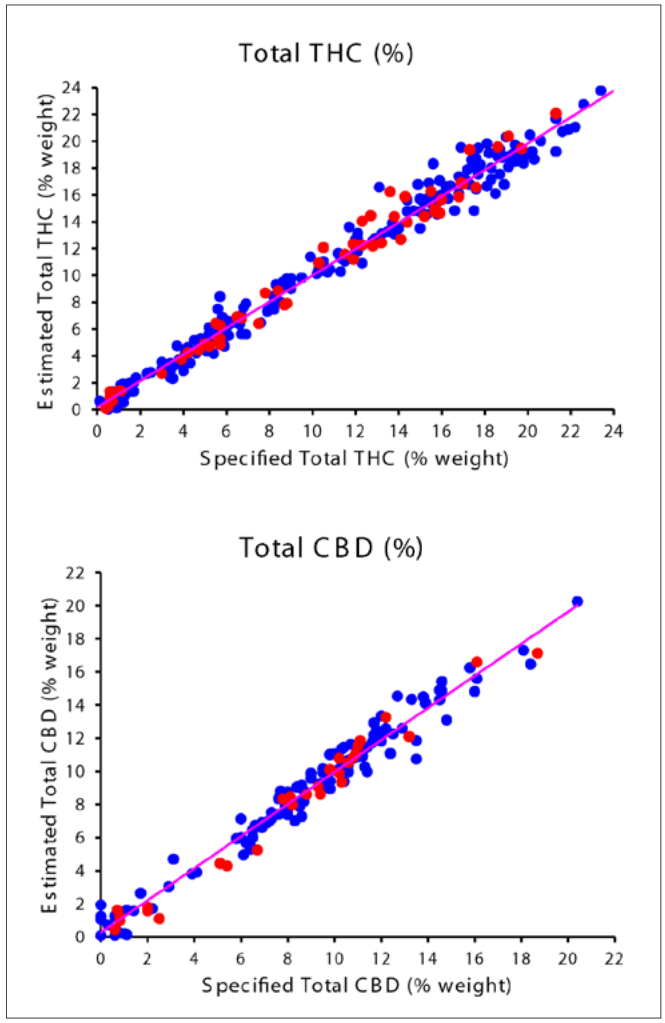

Figure 4. Correlation plots showing calibration (blue) and validation (red) data points for percent Total THC (top) and Total CBD (bottom).

Table 1. Summary of the regression details for Total THCA and Total CBDA chemometric models.

Property Value	Average	Range	R^2	Number of PCs	CV-SEP (%)	SEP (%)
Total THC (%)	9.8	0.1 – 23.4	0.98	9	0.98	0.92
Total CBD (%)	8.7	0 – 20.4	0.97	12	0.94	0.72

Table 2. Results from the independent validation procedure.

Property Value	R^2	Average Reference Value	Average Predicted Value	SEP (%)
Total THC (%)	0.989	9.9	9.9	0.92
Total CBD (%)	0.988	8.1	7.9	0.73

Conclusion

The cannabis cultivation industry would benefit from an on-site, rapid and low-cost technique for the accurate measurement of flower potency. FT-NIR spectroscopy provides rich information regarding the chemical composition of cannabis flower. When combined with chemometrics, the FT-NIR offers unparalleled speed and simplicity that cannot be matched by traditional techniques. In this application note, we have shown how the PerkinElmer Spectrum Two NIR with the Near Infrared Reflectance Module (NIRM) can be used to quantify total THC and total CBD in dried cannabis flower. The use of FT-NIR directly at the grow site would eliminate the lag time and cost associated with submitting samples to third party laboratories and reduce overall research and development costs for cultivators.

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Why the Decarboxylation Reaction is Key to a Quality Cannabis Extract

The most studied of the 480 chemical compounds found in cannabis are the chemicals that create the drug's desired effects. Of these compounds, tetrahydrocannabinol (THC) and cannabidiol (CBD) are undoubtedly the most well-known, but their progenitors, tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA) may still hold some scientific significance. CBDA, for example, has been shown to be a thousand times more powerful than CBD[1] in binding to a specific serotonin receptor linked to anti-nausea and anti-anxiety effects.

To best understand these precursor cannabis compounds, it is important to know why they are not present in the final product. The decarboxylation reaction which is typically ignited by a source of heat, such as a lighter, causes the THCA and CBDA molecules to lose their acidic carboxyl groups (they become decarboxylated) to form THC and CBD, respectively.

Despite its importance, there's remarkably little agreement on the ideal factors for the decarboxylation reaction. As most cannabis extractors do not monitor the decarboxylation process when heating the extract, the exact temperature the reaction occurs at is not properly known.

To remedy this oversight, a team from PerkinElmer and the extraction company Outco set up an experiment. Using a machine called a Fourier-transform infrared spectrometer (with a PerkinElmer universal attenuated total reflectance accessory), they measured several samples from some cannabis extract over the course of its heating. Once the data had been analyted the team had a real time-solution to decarboxylation reaction monitoring.

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APPLICATION NOTE

FT-IR Spectroscopy

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Optimization of the Decarboxylation Reaction in Cannabis Extract

Introduction

The production of cannabis extracts and oils for medicinal and recreational products has increased significantly in North America. This growth has been driven by both market demand in newly legalized states and patient demand for a greater diversity in cannabis products.[1,2,3] Most cannabis extraction processes, independent of solvent or instrument choice, undergo a decarboxylation step whereby the carboxylic acid functional group is removed from the cannabinoids. The decarboxylation reaction converts the naturally occurring acid forms of the cannabinoids, e.g. tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA), to their more potent neutral forms, e.g. tetrahydrocannabinol (THC) and cannabidiol (CBD). Because the carboxylic acid group is thermally labile, the industry typically applies a heat source, and at times a catalyst, to decarboxylate the cannabinoids.

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The heat-promoted decarboxylation reaction has been discussed at length within the industry, but an extensive literature search reveals very few papers on the process.[4, 5, 6] The data available represents a large spectrum of reaction conditions, including a range in reaction temperature, time and instrumental setup. As such, there is a lack of universal agreement surrounding the optimal reaction conditions for the decarboxylation process in cannabis extract. This reaction is further complicated by its sensitivity to water, with increased water content promoting the reaction.[7] Additionally, studies show that competing isomerization, oxidation, and decomposition reactions can occur at elevated temperatures.[7] These factors can lead to inconsistent cannabis extract products and an overall lack of quality control in the laboratory.

Cannabis quality control laboratory technicians and extraction manufacturing staff have difficulty predicting the optimal reaction time and temperature to attain maximum decarboxylation. Current manufacturing practices typically involve placing the cannabis extract on aluminum sheet trays in a vacuum oven or in a glass beaker on a stirred hot plate. The conditions required for these techniques to reach reaction completion is largely undefined and decarboxylation is rarely monitored during the heating process. When decarboxylation is monitored, extractors rely on physical observations such as a reduction in carbon dioxide off-gassing.

The lack of chemical information during this critical processing step leads to a highly subjective determination of reaction completeness. This results in extraction processes that lack scientific robustness and are far less efficient at achieving decarboxylation. Furthermore, the lengthy reaction times coupled with the uncertainty in optimum temperature can result in an inefficient use of laboratory resources and overall lack of process control. To this end, we investigated the use of Fourier transform infrared spectroscopy (FT-IR) with Attenuated Total Reflectance (ATR) to provide a quantitative estimation of the decarboxylation reaction progress in cannabis extract.

Experimental

Cannabis plant matter was milled to a 2 mm particle size (Fritsch P19, Germany). Cannabis extracts were produced by carbon dioxide supercritical extraction in a 20L, 2000 psi system (Apeks Supercritical®, USA). The cannabis extracts differed in THCA and THC concentrations. Also, different cannabis oil amounts were tested.

Table 1. Details for the different cannabis extracts tested by FT-IR-ATR.

Reaction	Reaction Temperature (˚C)	Extract (g)	Initial THC (%)	Initial THCA (%)
A	150	100	19.1	29.1
B	140	100	59.0	20.9
C	140	370	18.7	21.8

Decarboxylation Reaction

Decarboxylation was achieved by heating the cannabis extract in a oil bath with a programmable hot plate. An overhead stirrer was utilized to promote even heat distribution throughout the experiment. Oil bath and extract temperatures were recorded every five minutes throughout the 80-minute heating process. Mid-infrared spectra were collected every five minutes by pipetting a small amount of the heated extract onto the crystal of a PerkinElmer Universal Attenuated Total Reflectance (UATR) accessory. Aliquots at varying time points throughout the experiment were collected and submitted for cannabinoid concentration determination using high performance liquid chromatography (HPLC).

Spectral Data Collection

Infrared spectra were acquired using the PerkinElmer Spectrum Two™ equipped with a UATR accessory. Spectra were collected over the 4,000 – 450 cm^{-1} spectral range having 4 cm^{-1} resolution with each spectrum being the result of four averaged spectra.

Sample spectra and corresponding reference HPLC cannabinoid concentrations were imported into PerkinElmer Spectrum Quant™ software for the development of quantitative chemometric models. Spectral pre-processing was performed to include the 3,665 – 2,775 cm^{-1} and 1,755 – 450 cm^{-1} regions with absorbance threshold blanking at values greater than 1.5 absorbance units. The regions excluded from the model development are of very little chemical significance. Principal Component Regression (PCR) was used to generate quantitative models for THCA and THC. Leave-one-out cross-validation was performed during the calibration step. A total of 29 calibration spectra were used to build models for predicting concentrations of THCA and THC in the cannabis extract.

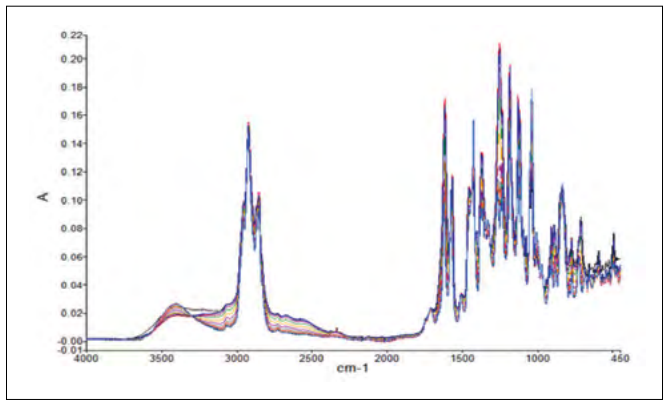

Figure 1. Example spectra of cannabis extract throughout the course of decarboxylation by the application of heat.

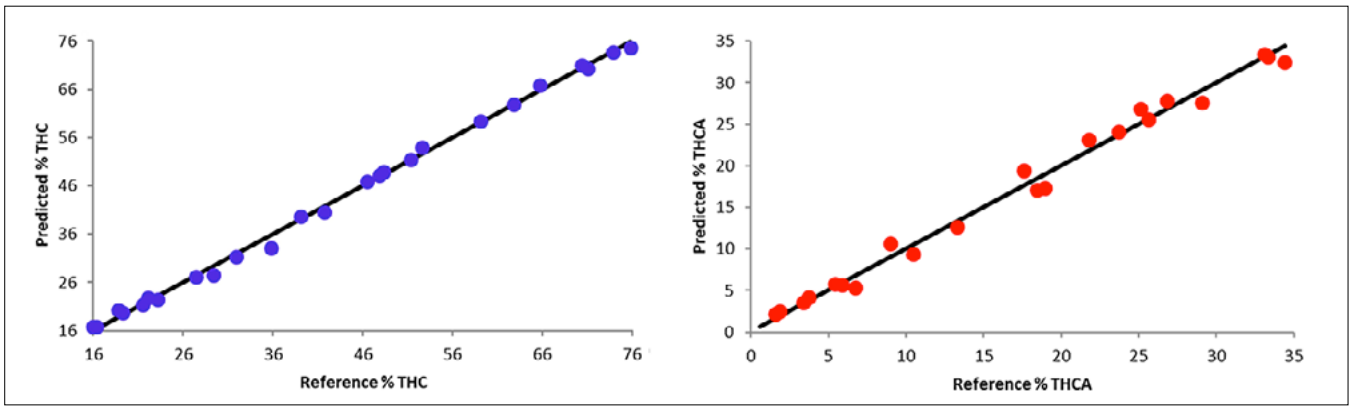

Figure 2. Correlation plots showing the relationship between reference HPLC cannabinoid content and those predicted using the PCR models for THC (left) and THCA (right).

Results and Discussion

Infrared spectra of the in-process extracts are shown in Figure 1. Changes in the infrared spectra indicate a loss of water and the conversion of THCA to THC as the reaction progresses.

The PCR models were individually optimized for each cannabinoid, THCA and THC. Table 2 highlights the regression details for the final regression models. The coefficient of determination, R^2, values of 0.990 and 0.998 for THCA and THC, respectively, indicate excellent correlation between reference HPLC cannabinoid concentrations and those predicted by FT-IR. Correlation plots for calibration data points are shown for both THCA and THC models in Figure 2. As shown in the correlation plots, calibration data points are evenly distributed throughout the entire calibration range for each cannabinoid.

Table 2. Regression summary for THC and THCA PCR models (where SEP is the standard error of prediction and CVSEP is the cross validation standard error of prediction).

Property Value	Average Property Value	Number of PCs	SEP	CV-SEP	R^2
THC (%)	42.61 (75.69-15.9)	4	1.24	1.62	0.998
THCA (%)	16.08 (34.42-0.6)	5	1.48	1.73	0.990

Decarboxylation Reaction Monitoring

Three separate decarboxylation reactions were performed with oil bath temperatures ranging from 140 – 150˚C. Starting THCA and THC concentrations ranged from 20.9 – 29.0% and 18.7 – 59.0%, respectively.

As the decarboxylation reaction progresses, THCA is converted to THC. This trend is illustrated in Figure 3 which plots the cannabinoid concentrations throughout the reaction. Cannabinoid concentrations predicted by FT-IR-ATR are overlaid with the HPLC reference values. The excellent agreement between the two techniques highlights the ability of FT-IR-ATR to accurately monitor the decarboxylation reaction in real time.

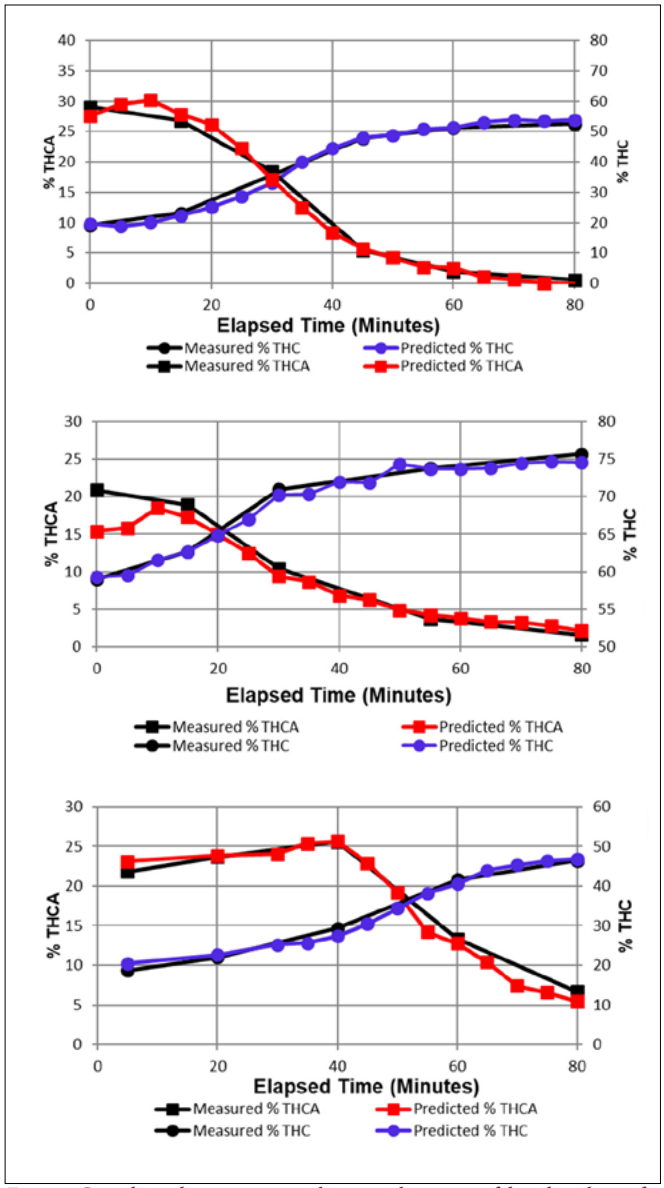

Figure 3. Cannabinoid concentration plots over the course of decarboxylation for three separate decarboxylation reactions.

Conclusion

The PerkinElmer Spectrum Two with the UATR accessory allows for the simple and rapid determination of cannabinoid concentrations in cannabis extract. The FT-IR-ATR technique offers a real-time solution to decarboxylation reaction monitoring. The ability to monitor this reaction over time would enable manufacturers of cannabis extract to optimize extraction conditions and identify process deviations. The FT-IR-ATR technique requires small sample quantities, zero sample preparation and minimal operator training.

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What Are Terpenes and Why Should You Test for Them?

Terpenes are a class of compounds found in the oils of all plants and botanicals and are responsible for their unique flavors and aromas. In total, at least 20,000 different terpenes exist, with over 100 of these found within the cannabis plant. For most consumers, the presence of these volatile terpenes will influence their preferences, as each terpene possesses unique pharmacological properties. For example, myrcene – the primary terpene found in cannabis plants – is known for its relaxing and anti-inflammatory properties. By contrast, the second most common terpene, limonene, has powerful antimicrobial and antidepressant properties and has been reported to reduce anxiety and elevate mood. Due to its citrus aroma, limonene is used as a fragrance ingredient in many household cleaning products and cosmetics[1].

In recent years, research efforts have been directed towards exploring the ability of terpenes to interact synergistically with cannabinoids – known as the "entourage effect." Some terpenes – beta-caryophyllene, for example – interact with cannabinoid receptors, and may therefore influence the activity of cannabinoids. This idea has led some to argue that terpenes can significantly increase the medicinal value of cannabis, and that terpene analysis can help characterize different chemotypes (strains of cannabis with different chemical properties).[1-3]

However, terpene profiles are diverse and can vary significantly depending on factors such as temperature, growth medium, nutrients, and sunlight.[4,5] This complexity makes accurate and reliable methods for testing and analysis important for consumers and healthcare professionals, as well as growers – and sellers – to ensure the quality and consistency of the cannabis is documented and labelled accurately.[6]

Gas chromatography–mass spectrometry (GC-MS) is an analytical technique that combines the features of gas chromatography (GC) and mass spectrometry (MS) to separate chemical mixtures (GC) and identify their components at a molecular level.[7] Within GC, headspace sampling can be used to extract volatile material from a heavier sample matrix and inject it into a gas chromatograph for analysis. Volatile compounds – such as terpenes – move into the gas phase (or headspace), whilst less volatile components remain in the liquid phase therefore, the more volatile a compound, the more concentrated it is in the headspace. The headspace sample can then be extracted into a gas chromatograph resulting in a much cleaner, faster and easier process.[8] In some systems, a pressure-balanced sampling technique is used whereby carrier gas is added to the sample vial to elevate the pressure before sampling. This technique generates results with a high degree of repeatability and decreases the probability of compound adsorption and loss via leaks.[9]

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APPLICATION NOTE

Gas Chromatography/
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To further validate the performance of this method for the industry, The Emerald Test ProficiencyTest (PT) for Terpenes was conducted. The Emerald Test™ is an Inter-Laboratory Comparison and Proficiency Test (ILC/PT) program for cannabis testing labs. The results from the PT inter-laboratory samples passed; therefore, the method meets inter-laboratory reproducibility and accuracy. The method was awarded the Emerald Test badge of approval seen on the right.
https://pt.emeraldscientific.com/

Fast, Accurate, and Precise Terpene Testing of Cannabis Samples

Introduction

Like all botanicals and plants found in nature, cannabis also contains terpenes, which are the aromatic oils that give rise to the distinctive flavors and aromas found in cannabis varieties. There have been up to 140 different types of terpenes reported in cannabis, but multiple studies suggest that approximately 17 are the most common and can be used for examining their chemotype (chemotype: those strains that have chemical properties that differ from each other's).[1] Among them are monoterpenes, diterpenes, and sesquiterpenes, which are characterized by the number of repeating units of a five-carbon molecule, called isoprene, the structural hallmark of all terpenoid compounds.

The diverse palate of cannabis terpenes is impressive enough, but arguably their most fascinating characteristic is their ability to interact synergistically with other compounds in the plant, like cannabinoids. In the past few decades, a significant amount of work has been performed to understand the 'entourage effect', which scientists refer to as synergistic interaction between terpenes and cannabinoids in the human body. This effect is believed to magnify the therapeutic benefits of the plant's individual components — so that the medicinal impact of the whole plant is greater than the sum of its parts quantifying which terpenes are present is an important aspect of understanding the unique effects of cannabis for both medicinal and recreational users.

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This paper will demonstrate a turnkey solution for the analysis of terpenes in cannabis samples by pressure-balanced headspace (HS) sample introduction and gas chromatography-mass spectrometry (GC/MS). In addition to a discussion of the instrumental parameters, optimization of the method to allow the highest sample throughput will be presented.

Headspace Sample Introduction Technique

One of the many benefits of this approach is that headspace using the pressure-balanced injection, is a fast, simple, accurate and precise solution, which allows the components of interest (e.g. terpenes and residual solvents) to be introduced into the analytical system. The non-volatile matrix components remain in the sample vial and do not enter the GC, which results in a mostly maintenance-free system, and faster analysis time.[2] In addition, the technique is mature and has already been accepted for quantitation in several regulatory industries including pharmaceutical (FDA), forensics, and environmental.[3-5] It is routinely used for the characterization of flavors and fragrances in several matrices.[6]

Instrumentation

The TurboMatrix™ HS sampler and a Clarus® SQ 8 GC/MS (PerkinElmer Inc., Shelton, CT) was used for this study. It's worth emphasizing that MS detection was chosen so that the targeted compounds may be identified by their spectra, which ensures that any unknown components found in the sample that are not included in the standard, can be identified if present in the mass spectral library being used. In addition, the human taste threshold is very sensitive; therefore, a flavor can be present at very low levels, but still have high potency, which requires the high sensitivity of MS detection (compared to the flame ionization detector). Identifying and quantifying all the terpene and other flavor components using this turnkey solution, results in a faster analysis, enhanced productivity, quicker release of product, and maximized system uptime for high throughput cannabis testing labs.

Experimental

Fast chromatography was achieved using a commercially-available standard containing 42 terpenes (CAN-TERP-MIX1H and CAN-TERP-MIX2H, SPEX CertiPrep®, Metuchen, NJ) at 1000 µg/mL stock solution. Figure 1 represents the fast chromatogram at 20 ppm of both MIX 1 and MIX 2, in less than 12.5 minutes.

Alternatively, a faster time of under 8.5 minutes may be achieved which is seen in Figure 2. The difference between these two chromatograms is that there is one co-elution with an uncommon terpene.

For samples whose terpene concentrations exceeded the 500 ppm calibration using the SPEX CertiPrep® stock standard solution, a higher concentration commercially available standard (Restek® Corporation, Bellefonte, PA - catalog number 34095), was analyzed.

Figure 3 displays the chromatography of the Restek® standard using the fast 8.5 minute method.

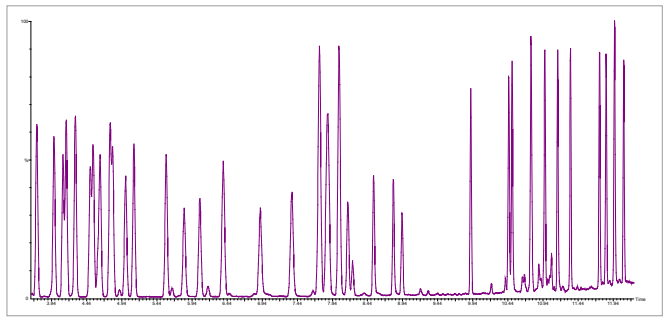

Figure 1. Fast total ion chromatogram (TIC) at standard concentration of 20 ppm of 42 terpenes in less than 12.5 minutes.

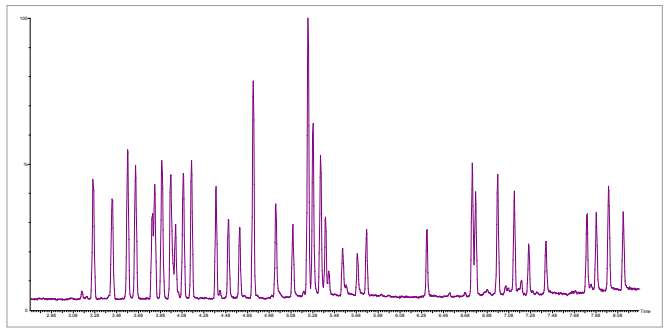

Figure 2. Fast total ion chromatogram (TIC) of a standard mixture of 42 terpenes from SPEX CertiPrep® in less than 8.5 minutes at a concentration of 20 ppm.

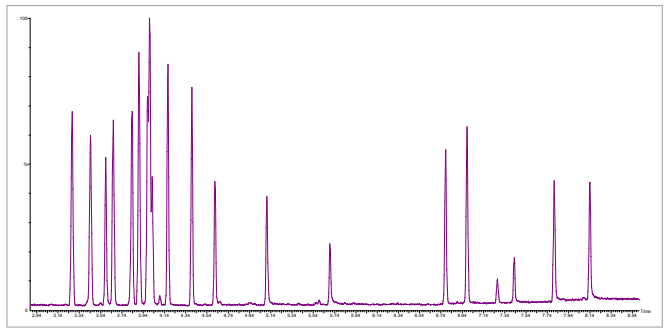

Figure 3. Fast total ion chromatogram (TIC) at standard concentration of 20 ppm of 42 terpenes in less than 8.5 minutes.

Validation

The experiments performed for validation of the method and analytical performance included dynamic range (linearity) and precision (repeatability). In addition, a cannabidiol (CBD) oil was spiked with a known standard concentration to determine compound matrix recoveries from a real sample, and proficiency test (PT)[7] samples were analyzed for inter-laboratory reproducibility. Both the matrix spike and the PT results will demonstrate method accuracy.

Calibration

A seven (7) point calibration standard of 1.9 to 500 ppm, using the SPEX CertiPrep® standard was generated for all the terpenes under investigation. An example of the 1st order calibration plot of Linalool, a naturally-occurring terpene, is found in Figure 4.

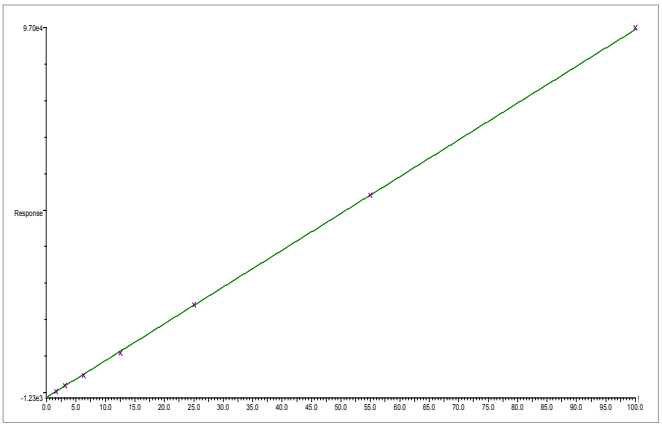

Figure 4. Graphical representation of the calibration for linalool from 1.9 to 500 ppm.

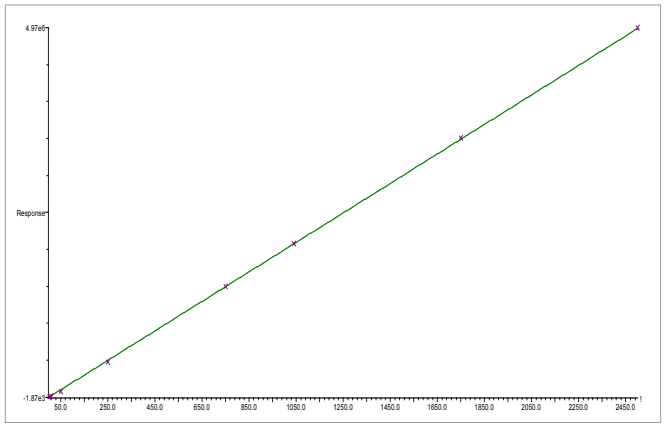

Figure 5. Calibration plot for β-myrcene from 2.5 to 2500 ppm.

In addition, a seven-point calibration range of 2.5 to 2500 ppm was generated using the Restek® standard. An example of the 1st order calibration plot of ß-myrcene is found in Figure 5.

Note: It's also important to emphasize that lower detection limits of terpenes can be achieved by this method if required.

Repeatability

Repeatability (precision) was determined using a 20 ppm SPEX CertiPrep® standard and a 50 ppm Restek® standard, inserting 20 µL into eight separate headspace vials and analyzing them consecutively.

Spike Recovery

A cannabidiol (CBD) oil was spiked at a concentration of 125 ppm using the SPEX CertiPrep® standard to test for recoveries, and 20 µL was inserted into a headspace vial and analyzed.

Results

The results for calibration were demonstrated by calculating the correlation coefficients (r^2) for both standards. These data are exemplified in Table 1, which shows the results for the dynamic range, precision testing protocol and matrix spike recoveries, thus validating the terpene turnkey solution using the 12.5 minute methodology.

Table 1. The results for dynamic range (linearity), precision and recovery testing protocol.

Standard	SPEX CertiPrep Standard		Restek Standard		Matrix Spike
Compound	Calibration 1.9 to 500 ppm	Precision @ 20 ppm (n=8)	Calibration 2.5 to 2500 ppm	Precision @ 50 ppm (n=8)	% Recovery
α-Pinene	0.9997	1.99	0.9997	2.24	95.6%
Camphene	0.9998	1.60	0.9996	3.10	96.9%
β-Myrcene	0.9999	1.57	0.9998	1.87	99.8%
Sabinene	0.9999	1.57	np*	np	86.4%
ß-Pinene	0.9998	1.98	0.9995	1.19	99.9%
α-Phellandrene	0.9997	1.77	np	np	92.1%
3-Carene	0.9999	0.98	0.9998	2.78	96.0%
α-Terpinene	0.9999	1.21	0.9990	2.48	102.9%
Limonene	0.9997	1.88	0.9994	1.49	92.6%
p-Cymene	np	np	0.9992	1.98	np
Ocimene (Isomers)	0.9995	2.47	0.9992	2.96	84.4%
Eucalyptol	0.9999	0.79	np	np	99.4%
γ-Terpinene	0.9998	1.10	0.9995	2.40	96.9%
Terpinolene	0.9997	1.89	0.9978	2.11	101.0%
Sabinene Hydrate	0.9996	2.10	np	np	88.6%
Linalool	0.9999	0.98	0.9982	2.67	88.8%
Fenchone (Isomers)	0.9996	1.55	0.9997	2.41	101.0%
Fenchol	0.9995	2.40	0.9992	3.19	89.3%
Isopulegol	0.9999	1.99	0.9995	2.50	107.0%
Camphor (Isomers)	0.9997	1.50	np	np	98.7%
Isoborneol	0.9996	1.70	np	np	99.2%

Table 1. CONTINUED

Standard	SPEX CertiPrep Standard		Restek Standard		Matrix Spike
Compound	Calibration 1.9 to 500 ppm	Precision @ 20 ppm (n=8)	Calibration 2.5 to 2500 ppm	Precision @ 50 ppm (n=8)	% Recovery
Hexahydrothymol (Menthol)	0.9996	1.70	np	np	101.3%
Borneol (+) and (-)	0.9996	1.99	np	np	99.0%
α-Terpineol	0.9991	2.87	np	np	84.9%
γ-Terpineol	0.9990	2.99	np	np	92.1%
Nerol	0.9999	1.50	np	np	106.3%
Geraniol	0.9997	1.61	0.9999	1.95	103.0%
Pulegone	0.9996	1.55	np	np	98.7%
Geranyl acetate	0.9995	1.43	np	np	112.0%
α-Cedrene	0.9999	1.11	np	np	95.1%
trans-β-Caryophyllene	0.9998	1.85	0.9997	2.95	97.7%
Farnesene (Isomers)	0.9995**	3.56	np	np	101.1%
α-Humulene	0.9997	2.85	0.9990	3.22	97.7%
Valencene	0.9999	2.10	np	np	100.3%
cis-Nerolidol	0.9990	3.10	0.9990	4.50	100.4%
trans-Nerolidol	0.9991	2.99	0.9993	4.35	97.3%
Guaiol	0.9999	2.77	0.9990	3.79	99.1%
Caryophyllene Oxide	0.9991	3.60	np	np	106.5%
Cedrol	0.9990	3.89	np	np	106.1%
α-Bisabolol	0.9990	3.85	0.9976	4.06	98.3%

*Component not present in this commercial stock standard

** Data from one isomer

Table 2 displays the results of terpenes found from an extracted flower analyzed on the TurboMatrix HS sampler and a Clarus SQ 8 GC/MS by Cassandra (Cassie) Ereman, Juniper Analytics, Bend, Oregon.

Table 2. Terpene results from an extracted flower courtesy of Cassandra (Cassie) Ereman.

Terpene Profile					
Compound	µg/g	%	Compound	µg/g	%
α-Pinene	1327.33	0.133	Isopulegol	nd	0.000
Camphene	125.78	0.013	Camphor	nd	0.000
Sabinene	80.96	0.008	Isoborneol	nd	0.000
β-Myrcene	3997.07	0.400	Borneol	78.67	0.008
β-Pinene	1711.53	0.171	Terpineol	1030.94	0.103
α-Phellandrene	665.95	0.067	y-Terpineol	nd	0.000
3-Carene	481.17	0.048	Nerol	nd	0.000
α-Terpinene	252.93	0.025	Geraniol	nd	0.000
trans-β-Ocimene	nd*	0.000	(+)-Pulegone	nd	0.000
Limonene	4496.99	0.450	Geranyl acetate	nd	0.000
p-Cymene	nd	0.000	a-Cedrene	nd	0.000
cis-β-Ocimene	nd	0.000	trans-β-Caryophyllene	2882.43	0.288
Eucalyptol	nd	0.000	α-Humulene	1014.02	0.101
y-Terpinene	309.19	0.031	Valencene	nd	0.000
Terpinolene	9445.88	0.945	cis-Nerolidol	nd	0.000
Sabinene Hydrate	nd	0.000	trans-Nerolidol	nd	0.000
Linalool	733.19	0.073	Guaiol	nd	0.000
allo-Ocimene	nd	0.000	Caryophyllene oxide	nd	0.000
Fenchone	50.77	0.005	Cedrol	nd	0.000
Fenchol	474.76	0.047	α-Bisabolol	nd	0.000
Total				29159.57	2.916

*not detected compound in this sample

Figure 6 is a chart displaying the terpene profile of this extract.

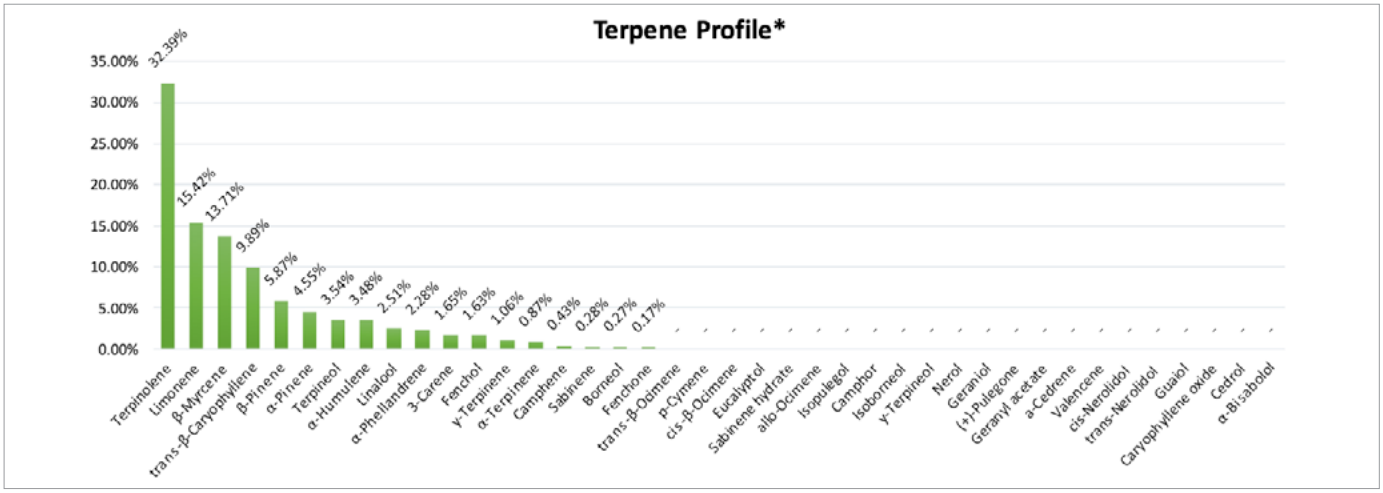

Figure 6. Terpene profile of the results from an extracted flower.

Discussion of Results

The objective of this study was to create a method to determine the concentration of terpenes in cannabis and cannabis products that is fast, linear, precise and accurate, which has clearly been achieved. In addition, since the Clarus 690 GC has rapid cooling returning to initial temperature only takes 1.6 minutes, so as a result the sample throughput is 10.5 or 14.0 minutes, depending on which of the two methods the laboratory chooses.

The correlation coefficient for all compounds is at or better than 0.999, most being greater than 0.9996, demonstrating excellent linearity. Precision and spiked recovery values are better than the criteria of regulatory requirements.

Conclusion

In conclusion, the evidence strongly suggests that the PerkinElmer HS-GC/MS solution for the determination of terpenes is fast and robust providing the laboratory with greater sample throughput, instrument uptime, essentially a maintenance free system, and enhanced profits.

Additionally, this procedure is a turnkey solution; therefore all acquisition and processing methods and standard operating procedures (SOP) will be provided upon installation of the system.

Note: For a turnkey solution of residual solvents in cannabis concentrates using headspace (HS) sampling coupled with GC/MS, please refer the following citation.[8]

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Acknowledgement

The authors would like to thank Tom Kwoka, Miles Snow and Tony Rhoden of PerkinElmer and consultant Robert Thomas, for their review and contributions to this work

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What Are Residual Solvents and How Can We Test for Them?

Cannabis concentrate products are created by separating the resin from the flowers to extract cannabinoids and terpenes (along with other active compounds) from the plant material. As a consequence, these concentrates are more potent than the flower alone. The most common cannabis concentrate products are solvent concentrates; a solvent[1] is a liquid in which other materials are dissolved to form a solution. Examples of solvent concentrates include shatter, types of hash oil, resin or wax.

Solvent extractions can include alcohol, supercritical CO_2 (meaning the form is adopts properties between a gas and a liquid), butane and propane. Solvents are used to extract the cannabinoids and terpenes from the plant material. The extraction processes can create concentrates with 65-90 percent of active cannabinoid content. However, they can also create impure by-products, which – in conjunction with excess solvent solution – may not be completely removed from the final product.

These residual solvents can be toxic to humans if they are not removed. Consumption of residual solvents can result in health issues such as eye, nose and throat irritation, headaches, dizziness and, in some cases, death. Consequently, residual solvent analysis is a critical element of cannabis testing.

One of the best ways to test for residual solvents is using headspace (HS) gas chromatography (GC) coupled with mass spectrometry[2]. Combining these two techniques reduces the chance of false positives being reported. In headspace analysis, researches use a gas-tight syringe to obtain a sample of gases in the headspace of a sealed vial, which contains the prepared sample. In addition to being fast, simple and accurate, this headspace analysis is sensitive for most volatile compounds. Since non-volatile matrix components remain the sample vial and do not clog up the gas chromatograph, this technique is predominantly a maintenance-free system, and a trusted and accepted methodology in the regulatory and legal settings.

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APPLICATION NOTE

Gas Chromatography/
Mass Spectrometry

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Fast, Quantitative Analysis of Residual Solvents in Cannabis Concentrates

To further validate the performance of this method for the industry, The Emerald Test Proficiency Test (PT) for Residual Solvents was conducted. The Emerald Test™ is an Inter-Laboratory Comparison and Proficiency Test (ILC/PT) program for cannabis testing labs. The results from the PT inter-laboratory samples passed; therefore, the method meets inter-laboratory reproducibility and accuracy. The method was awarded the Emerald Test Badge seen on the right.
https://pt.emeraldscientific.com/

Introduction

Compared to the more traditional cannabis flower, cannabis concentrate products, such as extracts, tinctures, edibles, waxes, and oils are becoming the most commonly used cannabis products that are legally manufactured for both medicinal and recreational purposes. Most concentrates are extracted using a solvent such as supercritical CO_2, butane, propane, other hydrocarbons, water, or alcohol. These solvents are used to extract out the cannabinoids and terpenes from the plant material.

In some cases, the solvent and impurities from the solvent remain in the extracted material. These are called residual solvents and are the byproducts of the extraction process. In some cases, these impurities can be toxic, which is why residual solvent analysis is a critical element of cannabis testing. The method of choice for measuring residual solvents is headspace (HS) gas chromatography (GC) coupled with mass spectrometry (MS) detection, so false positives are not reported.

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The major benefit of this approach is that headspace is a fast, simple, accurate and precise technique that allows the components of interest (e.g. residual solvents and terpenes) to be introduced into the analytical system. The non-volatile matrix components remain in the sample vial and do not enter the GC, which results in a mostly maintenance free system, and faster analysis time. In addition, the technique is mature and already has been accepted for quantitation in several regulatory industries including pharmaceutical forensics, environmental and food, where its results have routinely stood up to scrutiny in a court of law.[1-4]

It's also important to emphasize that because there are currently no federal regulations in the U.S., the allowable concentration limits for each residual solvent are defined by the individual state or country where the cannabis is grown. For example, Table 1 shows the list of proposed residual solvents and action levels for cannabis products in the State of California.[5]

This study will focus on the analysis of residual solvents using pressure-balanced headspace (HS) sample introduction coupled with gas chromatography/mass spectrometry (GC/MS). In addition, it will discuss the objective of unambiguous separation of all compounds while maximizing sample throughput.

Table 1. List of proposed residual solvents and action limits in cannabis products for the State of California.

Compound	CA Action Levels (ppm)
Propane	1000
Butane	1000
Methanol	600
Ethylene Oxide	1
Pentane	1000
Ethanol	1000
Ethyl Ether	1000
Acetone	1000
Isopropyl Alcohol	1000
Acetonitrile	80
Methylene Chloride	1
Hexane	60
Ethyl Acetate	1000
Chloroform	1
Benzene	1
1,2-Dichloroethane	1
Heptane	1000
Trichloroethylene	1
Toluene	180
Xylenes total	430

Instrumentation

The TurboMatrix™ HS sampler and a Clarus® SQ 8 GC/MS (PerkinElmer Inc., Shelton, CT) was the system used for this analysis. The benefits of headspace sampling are well-recognized in the public domain, but it is essentially a separation technique in which volatile material such as residual solvents and terpenes, is extracted from a heavier sample matrix and injected directly into a GC for analysis.[6] The major reason for using MS detection is that many of the organic compounds associated with residual solvents elute at the same time (co-elute), so the unique mass spectrum of each compound means they can be optimally separated and detected without using additional detectors. Identifying and quantifying all the residual solvents using this solution, results in a faster analysis, enhanced productivity, quicker release of product, and maximized return on investment.

Experimental

Sample Preparation After Extraction

Many states require taking multiple sampling points from non-homogenous samples (such as waxes and edibles) to ensure a representative sample for analysis. If this is the requirement, five sampling points from one sample are recommended. For example, if 500 mg is the regulatory requirement for testing, then five - 100 mg portions should be placed in a vial and brought to a final volume of 10 mL with Dimethylacetamide (DMA). Twenty µL of the diluent is then inserted into the HS vial, which is capped and placed onto the HS autosampler for analysis.

However, if an average sampling is not required, a 40 mg aliquot of the extract can be directly weighed into the HS vial; capped; and placed onto the autosampler tray which is the preferred and easiest approach.

A Turnkey Solution

A fast, accurate, robust GC/MS-HS solution, and SOP, was developed to separate the required analyte compounds in each of the concentrates being tested using the mass and/or time domains for identifying the target compound and then quantifying the specific compounds using the following commercially-available standards (Emerald Scientific, San Luis Obispo, CA).

- California Residual Solvent Mix #1 (Inhalation) reference number STRS01102
- California Residual Solvent Mix #2 (Inhalation) reference number STRS01103

Figure 1 demonstrates the chromatographic separation of all the residual solvents previously listed. To show the benefits of mass spectrometric detection, the chromatographic peaks of two pairs of compounds that co-elute (Pentane/Ethanol at about 3.0 minutes and Benzene/1,2 -Dichloroethane at about 4.8 minutes) exemplifies how they have been further separated by mass. Note, all compounds have been eluted and identified in about seven and a half minutes.

A multi-level concentration suite of standards was prepared which represented the required ranges for quantitation of the sample, and met the required action levels. Repeatability was performed preparing eight vials with 20 µL of the same concentration standard. These data are shown in Table 2, which demonstrates the linearity and precision achieved using this method, together with the method reporting limits and California action levels.

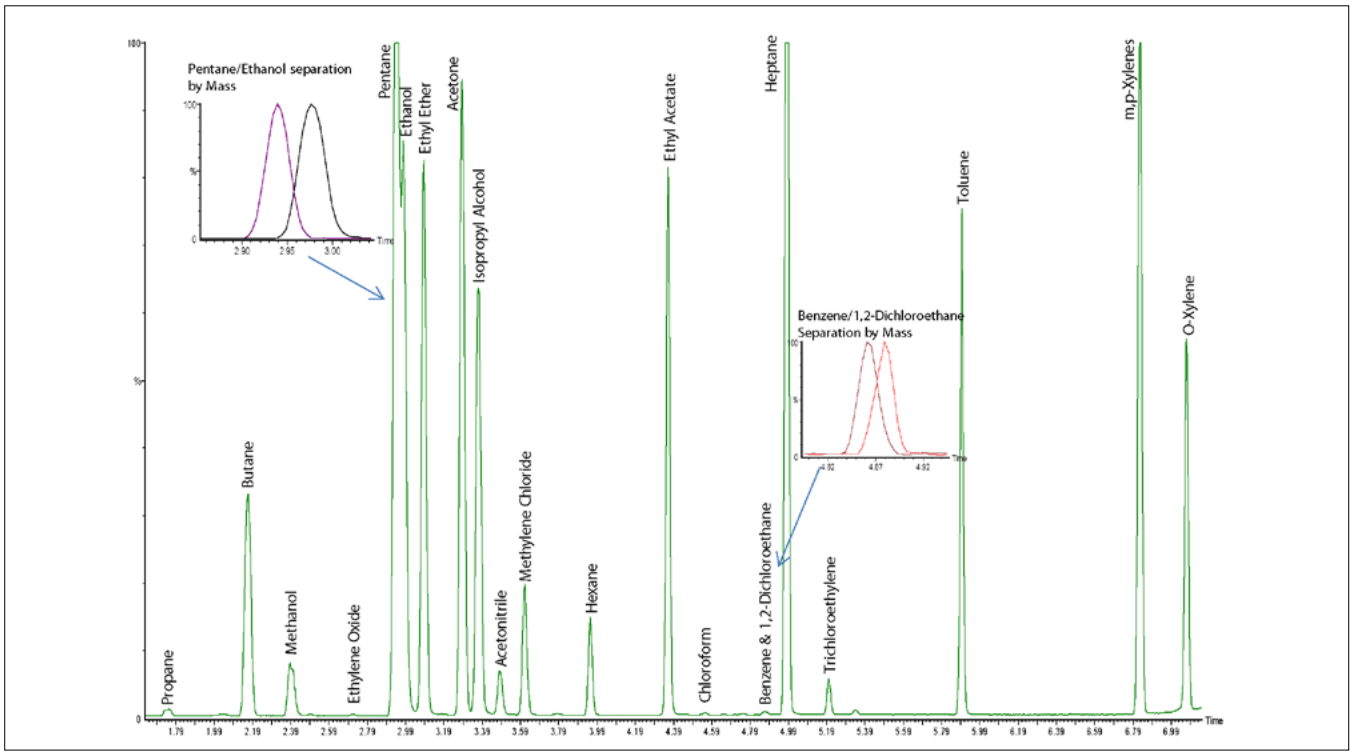


Figure 1. Chromatographic separation of all the compounds listed in Table 1.

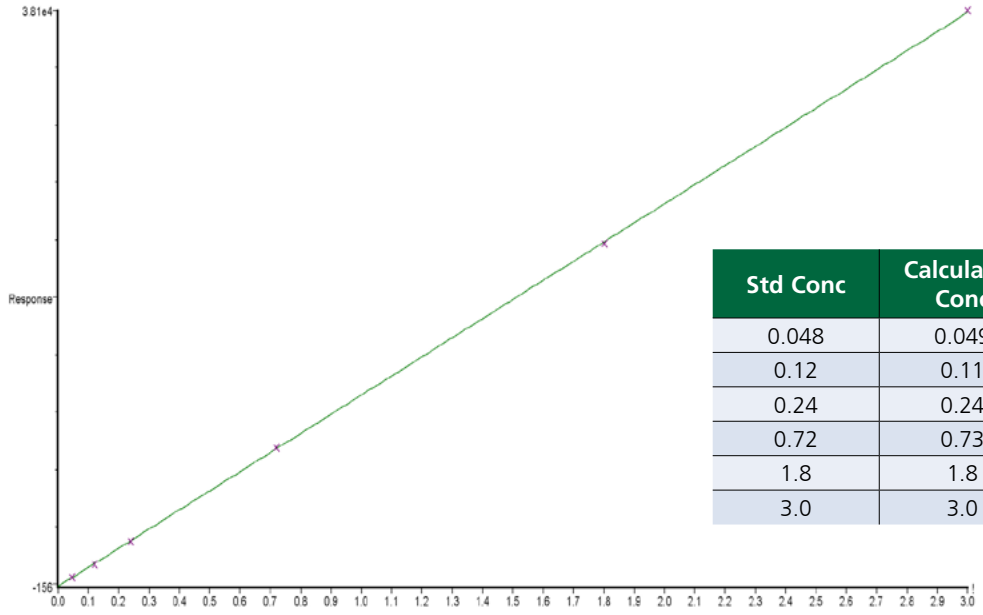
Table 2. Linearity and precision achieved using this method, together with the reporting and California action limits for cannabis products. Note: Reporting limits are based on a 1 to 20 sample dilution; therefore if no dilution is carried out, reporting limits are 20x lower.

Compound	Corelation Coefficient	Precision (n=8)	CA Action Levels (ppm)	PerkinElmer Reporting Levels (ppm)*
Propane	0.9996	2.30	1000	3.20
Butane	0.9991	1.08	1000	57.60
Methanol	0.9996	1.29	600	19.20
Ethylene Oxide	0.9994	2.29	1	1.00
Pentane	0.9997	1.95	1000	14.40
Ethanol	0.9997	1.41	1000	19.20
Ethyl Ether	0.9998	0.59	1000	9.60
Acetone	1.0000	0.94	1000	14.40
Isopropyl Alcohol	0.9996	1.33	1000	9.60
Acetonitrile	0.9998	0.45	80	1.16
Methylene Chloride	0.9999	1.08	1	1.00
Hexane	0.9996	1.08	60	0.48
Ethyl Acetate	0.9999	1.02	1000	6.80
Chloroform	0.9996	1.68	1	0.60
Benzene	1.0000	1.02	1	0.96
1,2-Dichloroethane	0.9993	2.15	1	0.96
Heptane	0.9997	1.08	1000	9.60
Trichloroethylene	0.9998	2.12	1	0.48
Toluene	0.9998	1.46	180	2.88
Xylenes total	0.9999	0.86	430	2.88

Discussion of Results

As seen in Figure 1, the chromatographic peaks are well separated with a runtime of about seven and a half minutes and a sample to sample cycle time of less than 11 minutes. Using mass spectrometry, it allows for the identification of components without concern for false positives, while still maintaining extremely fast run times. The two pairs that co-elute in time, ethanol/pentane and benzene/1,2-dichloroethane have very unique spectra and quantitation ions as seen in greater detail in Figure 3. Since quantitation is performed on the mass chromatogram (also referred to as the quantitation ion) of the unique mass, it offers the advantage of interference free integration and quantitation, which would not be possible if the analysis was carried out by flame ionization detection.

Figure 2 displays an example of the calibration curve for the target compound benzene. A table is inserted in this graphic documenting the quantitation of each point using this curve. The % deviation calculated for each point clearly shows excellent correlation with the calibration standards.



Std Conc	Calculated Conc	% Dev
0.048	0.049	7
0.12	0.11	-6
0.24	0.24	-1
0.72	0.73	1
1.8	1.8	-1
3.0	3.0	0

Figure 2. Calibration curve for benzene, showing the % deviation for each standard.

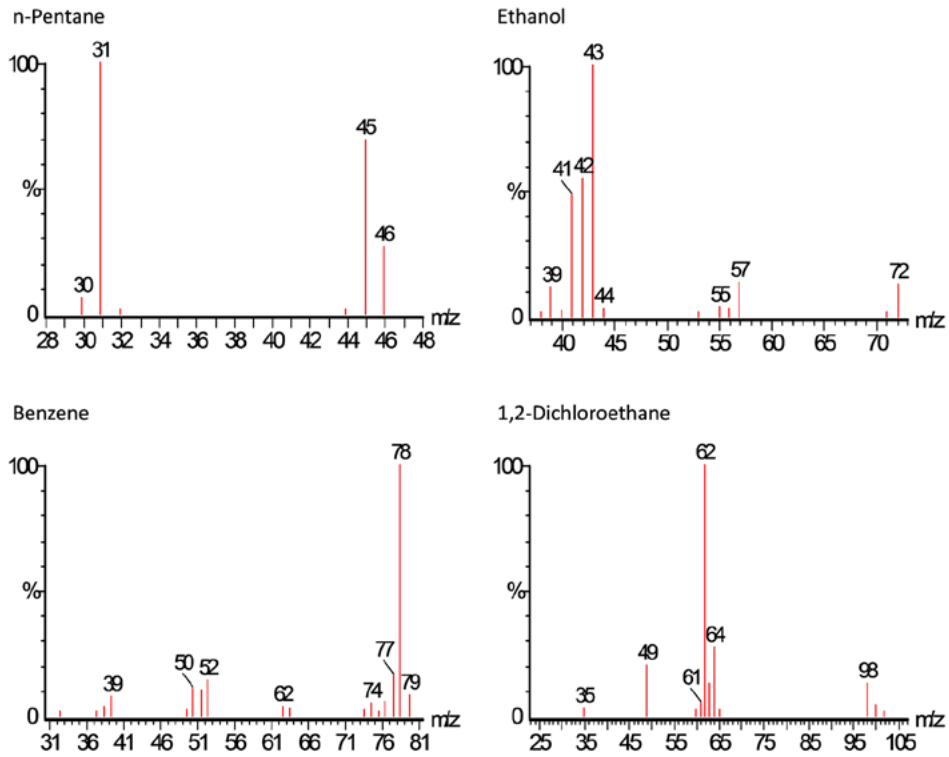


Figure 3. The mass chromatogram of two pairs of compounds that co-elute in time, ethanol/pentane and benzene/1,2- dichloroethane, show they have very unique spectra and quantitation ions.

As demonstrated in Table 2, linearity across the compound range is excellent using a multi-level calibration with all targets having a correlation coefficient value greater than 0.9993. The turnkey solution is also precise with the relative standard deviation of less than 2.3 % for all compounds. It's also important to emphasize that the reporting limits are all based upon a 1:20 sample dilution factor; therefore the reporting limit could be improved even further if smaller dilutions or no dilutions were used.

Conclusion

This study has clearly shown that headspace technology coupled with GC/MS is the perfect solution for identifying and measuring residual solvents in cannabis concentrates and its many products. The benefit of headspace sample introduction over other approaches is that it requires minimum sample preparation, with very little interaction required by the operator. When headspace sampling is combined with GC/MS, it allows for rapid, unambiguous and interference free integrations, with very little likelihood of false positives. In addition, MS provides the ability to identify unknown components that may be present in the sample that are not target compounds. This capability offers significant benefits over a single detector such as flame ionization detection (FID) where a non-targeted compound eluting at the same time as a targeted compound would produce a result which was over the action limit, resulting in a failed batch and a cannabis product not viable for market.

It's also important to emphasize that this technique is fast and capable of quantifying residual solvents in all concentrate samples and other required matrices. Combined with essentially maintenance-free operation, a GC/MS-HS method will enhance productivity and strengthen the lab's business operations.

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The Importance of Pesticide Residue Analysis

In any agricultural setting, there will always be negative factors that affect the optimal growth of a plant. Threats can come in various forms; from insect pests that consume the plants to pathogenic fungi that reduce the yield by infecting and killing the crop. To combat this, growers typically apply pesticides. The term "pesticides" can cover a wide range of compounds, including insecticides, fungicides, herbicides, rodenticides, nematicides and plant growth regulators[1]. These chemical compounds improve productivity and protect the crop by providing a vector disease control method and killing the pests.

Use of synthetic pesticides accelerated in the 1940s. Whilst organochlorine insecticides effectively controlled human diseases such as malaria and typhus through their ability to kill insect vectors, they were banned in the 1960s as they were found to accumulate in the food chain. Since the ban, more synthetic pesticides have been produced. However, resistance is starting to occur in some pest species. Whilst pesticides enable growers to optimize the yield of their cannabis crops, as demonstrated above, some of the chemicals may be harmful to humans, other organisms and the wider environment.

In recreational and medicinal use, cannabis can be delivered by smoke inhalation of the dried cannabis flowers. Remaining pesticide residues can be transferred into the smoke produced by cannabis and can cause toxicity. One example of the toxic effects of pesticides comes from myclobutanil, which is a persistent fungicide used by growers. Myclobutanil is stable at room temperature, but its boiling point is 205°C. Cannabis that is heated with a butane lighter, can produce temperatures above 450°C, and cause the release of hydrogen cyanide[2,3]. Hydrogen cyanide is very toxic to humans, and large doses of cyanide can prevent cells from using oxygen, causing these cells to die. Long term inhalation primarily affects cells in the central nervous system (CNS), which can cause a loss of consciousness and potentially death. Other effects to humans include cardiovascular and respiratory effects, irritation to the eyes and skin, and an enlarged thyroid gland[4].

To protect public health, the U.S. Environmental Protection Agency (EPA) regulates pesticides in agricultural use. The federal government considers cannabis an illegal drug, therefore there are no federal regulations or agencies (such as EPA) for pesticide residues related to cannabis products. It is up to individual states to devise regulations and these regulations can vary widely.

California's pesticide regulations for cannabis are considered the strictest in the country. California has the Department of Pesticide Regulation (DPR) which has significant expertise in toxicology and human health risk assessment. A pesticide can only be used on the plant if the active ingredient meets a specific criterion. Of the DPR list of 66 pesticides, twenty-one is classed as category I pesticides, which means they are banned as they are not registered for food crop or pose a risk to groundwater. The other 45 chemicals are Category II pesticides, which are permitted to be in the product as long as they fall within defined safety limits. These action limits can range 0- 10 µg/g, for pesticides on inhalable cannabis in California. The state of Oregon is "more relaxed" and has issued a regulatory list for 59 pesticides in the flower with action limits ranging from 0.1-2 µg/g[5].

In the past gas chromatography–mass spectrometry (GC-MS/MS) has been used for pesticide analysis in cannabis samples. GC-MS/MS is capable of isolating and fragmenting a desired molecular weight, allowing fragments to be analyzed by mass spectrometry. However, in some cases, GC-MS/MS is not suitable for ionic and polar compounds, such as abamectin and daminozide.

To overcome this limitation of GC-MS/MS, liquid chromatography–mass spectrometry (LC-MS/MS) may be considered. Liquid chromatography separates the compounds in a liquid phase and is better-suited for analyzing more polar and larger molecules that the GC may not be able to handle. PerkinElmer's QSight® 400 Series Triple Quad Mass Spectrometer, is a highly sensitive analytical instrument that combines LC-MS/MS with electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) for low level analysis of of pesticides and mycotoxins. This dual source (ESI/APCI) technology enables cannabis testing labs to screen and quantitate residues, using a single instrument, with significantly greater detection capabilities than other traditional methods.

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APPLICATION NOTE

Liquid Chromatography/ Mass Spectrometry

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A Novel ESI and APCI LC/MS/MS Analytical Method for Meeting the Canadian Cannabis Pesticide Residues Regulatory Requirements

Introduction

Canada legalized recreational use of cannabis nationally in the fall of 2018. Within this new market, cannabis safety and quality are a prerequisite to meeting domestic and international quality control regulations. Health Canada recently unveiled a new regulatory regime that will impose mandatory pesticide testing requirements on all cannabis products. Canadian licensed producers (LPs) are required to send representative cannabis product samples to independent laboratories, where they will be screened and quantified for pesticide content before they can be sold.

To ensure the credibility and accuracy of data, high-performance liquid chromatography-tandem mass spectrometry (LC/MS/MS) has emerged as the method of choice for pesticide analysis, as it offers superior selectivity, sensitivity and ruggedness, without extensive pre-analysis sample preparation. Although gas chromatography-mass spectrometry (GC/MS/MS) methods have previously been developed for pesticide analysis in cannabis samples, they are only applicable to a relatively small subset of analytes. Compounds such as abamectin, a high molecular weight compound, are not amenable to analysis by GC/MS/MS because they are heat labile and degrade either in the GC injection port or in the GC column at high temperature. GC/MS/MS methods are not as robust as LC/MS/MS methods for pesticide analysis in complex matrices as they require extensive sample preparation to prevent GC injection port contamination from complex matrices.[1,2]

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the analysis of pesticide analytes in cannabis samples, Canada has developed comprehensive guidelines for pesticide residue analysis in cannabis, and has set regulatory limits for 96 pesticides. However, in both countries, there are no Federal method guidelines in place, so laboratories remain responsible for development and validation of their pesticide testing best practices. Although numerous reports for pesticide analysis in cannabis have been published; these studies have certain deficiencies.[3-5] Most of these studies either do not achieve detection limits to meet the Canadian action limits, or they use time-consuming sample preparation methods (e.g. QuEChERS with dSPE) which exhibit poor recoveries for some of the pesticides, and require the use of both LC/MS/MS and GC/MS/MS techniques for the analysis of all regulated pesticides. These processes substantially increase the cost, uncertainty, complexity, and turnaround time of the analysis. In this work, all 96 pesticides were analyzed (including very hydrophobic and chlorinated pesticides typically analyzed by GC/MS/MS) in cannabis flower extracts. An LC/MS/MS instrument with dual source ESI and APCI modes was used to achieve the low regulatory limits set by Health Canada for all 96 pesticides, and a simple solvent extraction method was utilized, resulting in excellent recoveries for all analytes in the acceptable range of 70-120%.

Experimental

Hardware/Software

Chromatographic separation was conducted on a PerkinElmer QSight® LX50 UHPLC system, with detection achieved using a PerkinElmer QSight 420 MS/MS detector with a dual ionization ESI and APCI source, which operate independently with two separate inlets. All instrument control, data acquisition and data processing were performed using the Simplicity™ 3Q software platform.

Sample Preparation Method

Below is the step by step sample preparation procedure with 20-fold dilution:

- Take approximately 5 g of cannabis flower as a representative sample of each batch, and grind it finely using a grinder.
- Weigh accurately 1 g of sample, and place it into a 50 mL centrifuge tube.
- Spike the sample with 10 µL of internal standard solution.
- Add three steel balls (10 mm in diameter) to the tube for efficient extraction during vortex mixing.
- Add 10 mL of LC/MS grade acetonitrile to the tube and cap it.
- Place the tube on a multi-tube vortex mixer, and allow it to vortex for 10 minutes.
- Centrifuge the extract in the tube for 10 minutes at 3000 rpm.
- Filter the solvent into a 5 mL glass amber vial using a 0.22 micron nylon syringe-filter, and then cap it.
- Label the bottle with the sample ID.
- Transfer 0.5 mL of extracted sample into a 2 mL HPLC vial and dilute it with 0.5 mL of LC/MS grade acetonitrile and mix it.

The LC method and MS source parameters are shown in Table 1.

Results and Discussion

Detectability and Reproducibility

Currently, most laboratories deploy multiple analytical instruments and tedious sample preparation methods to meet the low pesticide limits imposed by Health Canada. Herein, an LC/MS/MS method amenable to in-lab validation is presented, utilizing a PerkinElmer liquid chromatograph coupled with the tandem mass spectrometer (QSight LX50 and mass spectrometer) for complete analysis of all 96 pesticides outlined in the Health Canada regulations. All 96 pesticides were analyzed with the QSight 420 dual source mass spectrometer equipped with both APCI and ESI ionization probes. Pesticides such as kinoprene, methoprene, etridiazole, endosulfans, methyl parathion, cypermethrin, cyfluthrin, and quintozene among others, which are conventionally analyzed by gas chromatography, were all detected on the single platform PerkinElmer LC/MS/MS system.

Table 1. LC Method and MS Source Conditions.

LC Conditions	
LC Column	PerkinElmer Quasar™ SPP Pesticides (4.6 × 100 mm, 2.7 µm)
Mobile Phase A (ESI Method)	2 mM ammonium formate + 0.1% formic acid (in water)
Mobile Phase B (ESI Method)	2 mM ammonium formate + 0.1% formic acid (in methanol)
Mobile Phase A (APCI Method)	LC/MS grade water
Mobile Phase B (APCI Method)	LC/MS grade methanol
Mobile Phase Gradient	The run time for the optimized gradient elution method, including analytical column re-conditioning, was 18.5 minutes for the ESI method, and 6 minutes. for APCI. The final method ensured separation of the bulk cannabis matrix from the analytes for improved quantitation.
Column Oven Temperature	30 °C
Auto sampler Temperature	20 °C
Injection Volume	3.0 µL for LC/MS/MS method with ESI source. 10 µL for LC/MS/MS method with APCI source.
MS Source Conditions for ESI Source and APCI Source	
ESI Voltage (Positive)	+5100 V
ESI Voltage (Negative)	-4200V
APCI Corona Discharge	-3 µA
Drying Gas	150 arbitrary units
Nebulizer Gas	350 arbitrary units
Source Temperature (ESI Method)	315 °C
Source Temperature (APCI Method)	275 °C
HSID Temperature (ESI Method)	200 °C
HSID Temperature (APCI Method)	200 °C
Detection Mode	Time-managed MRM™

The limits of quantification (LOQs) and response reproducibility at the LOQ level for each of the pesticides in the cannabis extract are summarized in Table 2. The LOQs were determined by considering both the signals of the quantifier and qualifier ions (S/N > 10 for both), while ensuring that the product ion ratios were within the 30% tolerance windows of the expected ratio. The response relative standard deviation (RSD) for each pesticide at its LOQ level was less than 30%. The retention time for each analyte was reproducible within ± 0.1 minute over a 24 hour period. This demonstrates that the method is more than adequately sensitive and reproducible for pesticides analysis in cannabis.

Table 2. LOQs for Health Canada Regulated Pesticides with LC/MS/MS in Cannabis.

S. No.	Pesticide	LOQ		Action Level (µg/g)	Action Level/LOQ
		LC/MS/MS (µg/g)	%CV (n=3)		
1	Abamectin	0.1	8.2	0.1	1
2	Acephate	0.01	4.8	0.02	2
3	Acetamiprid	0.02	2.8	0.1	5
4	Acequinocyl	0.01	6.5	0.03	3
5	Aldicarb	0.01	8.6	1	100
6	Allethrin	0.05	3.5	0.2	4
7	Azadirachtin	0.1	18.6	1	10
8	Azoxystrobin	0.0025	12.2	0.02	8
9	Benzovindiflupyr	0.01	14.5	0.02	2
10	Bifenazate	0.0025	4.8	0.02	8
11	Bifenthrin	0.005	18.6	1	200
12	Boscalid	0.01	8.0	0.02	2
13	Buprofezin	0.0025	3.1	0.02	8
14	Carbaryl	0.01	9.1	0.05	5
15	Carbofuran	0.01	5.1	0.02	2
16	Chlorantraniliprole	0.01	6.2	0.02	2
17	Chlorphenapyr	0.02	3.3	0.05	2.5
18	Chlorpyrifos	0.01	8.5	0.04	4
19	Clofentezine	0.01	6.9	0.02	2
20	Clothianidin	0.02	10.6	0.05	2.5
21	Coumaphos	0.01	9.9	0.02	2
22	Cyantranilipole	0.01	4.8	0.02	2
23	Cyfluthrin	0.2	15.8	0.2	1
24	Cypermethrin	0.2	11.3	0.3	1.5
25	Cyprodinil	0.01	14.0	0.25	25
26	Daminozide	0.02	1.7	0.1	5
27	Deltamethrin	0.1	11.1	0.5	5
28	Diazinon	0.01	8.5	0.02	2
29	Dichlorvos	0.01	10.2	0.1	10
30	Dimethoate	0.0025	5.9	0.02	8
31	Dimethomorph	0.01	5.6	0.05	5
32	Dinotefuran	0.0025	7.9	0.1	40
33	Dodemorph	0.01	13.0	0.05	5
34	Endosulfan-alpha	0.1	16.2	0.2	2
35	Endosulfan-beta	0.05	10.2	0.05	1
36	Endosulfan sulfate	0.01	12.3	0.05	5
37	Ethoprophos	0.01	12.4	0.02	2
38	Etofenprox	0.0025	9.8	0.05	20
39	Etoxazole	0.01	13.0	0.02	2
40	Etridiazole	0.02	18.4	0.03	1.5
41	Fenoxycarb	0.01	1.8	0.02	2
42	Fenpyroximate	0.0025	12.4	0.02	8
43	Fensulfothion	0.0025	5.1	0.02	8
44	Fenthion	0.01	3.2	0.02	2
45	Fenvalerate	0.02	14.7	0.1	5
46	Fipronil	0.01	4.6	0.06	6

Table 2. continued.

S. No.	Pesticide	LOQ		Action Level (µg/g)	Action Level/LOQ
		LC/MS/MS (µg/g)	%CV (n=3)		
47	Flonicamid	0.01	7.0	0.05	5
48	Fludioxonil	0.01	7.4	0.02	2
49	Fluopyram	0.0025	9.3	0.02	8
50	Hexythiazox	0.0025	7.4	0.01	4
51	Imazalil	0.02	6.0	0.05	2.5
52	Imidacloprid	0.01	4.8	0.02	2
53	Iprodione	0.02	4.5	1	50
54	Kinoprene	0.2	8.8	0.5	2.5
55	Kresoxim-methyl	0.01	6.5	0.02	2
56	Malathion	0.0025	11.0	0.02	8
57	Metalaxyl	0.0025	5.8	0.02	8
58	Methiocarb	0.0025	4.5	0.02	8
59	Methomyl	0.01	5.7	0.05	5
60	Methoprene	0.5	9.0	2	4
61	Methyl parathion	0.02	9.4	0.05	2.5
62	Mevinphos	0.01	3.7	0.05	5
63	MGK-264	0.01	2.7	0.05	5
64	Myclobutanil	0.01	6.0	0.02	2
65	Naled	0.01	8.2	0.1	10
66	Novaluron	0.01	4.1	0.05	5
67	Oxamyl	0.01	2.5	3	300
68	Paclobutrazol	0.01	9.4	0.02	2
69	Permethrin	0.01	10.9	0.5	50
70	Phenothrin	0.05	5.1	0.05	1
71	Phosmet	0.01	8.3	0.02	2
72	Piperonyl butoxide	0.01	6.2	0.2	20
73	Pirimicarb	0.01	4.3	0.02	2
74	Prallethrin	0.01	9.1	0.05	5
75	Propiconazole	0.04	5.7	0.1	2.5
76	Propoxur	0.01	11.1	0.02	2
77	Pyraclostrobin	0.01	6.2	0.02	2
78	Pyrethrins	0.025	5.7	0.05	2
79	Pyridaben	0.0025	1.7	0.05	20
80	Quintozene	0.01	26.8	0.02	2
81	Resmethrin	0.1	7.2	0.1	1
82	Spinetoram	0.01	8.5	0.02	2
83	Spinosad	0.01	12.6	0.1	10
84	Spirodiclofen	0.02	12.7	0.25	12.5
85	Spiromesifen	0.01	5.7	3	300
86	Spirotetramat	0.01	9.8	0.02	2
87	Spiroxamine	0.01	8.3	0.1	10
88	Tebuconazole	0.01	10.9	0.05	5
89	Tebufenozide	0.01	6.6	0.02	2
90	Teflubenzuron	0.01	11.5	0.05	5
91	Tetrachlorvinphos	0.01	6.9	0.02	2
92	Tetramethrin	0.05	7.3	0.1	2
93	Thiacloprid	0.01	4.7	0.02	2
94	Thiamethoxam	0.0025	3.0	0.02	8
95	Thiophanate-methyl	0.01	7.3	0.05	5
96	Trifloxystrobin	0.0025	6.4	0.02	8

Note: Pesticides typically analyzed by GC/MS/MS are highlighted in red/green. Pesticides analyzed by ESI are highlighted in black or red. Pesticides Analyzed by APCI are highlighted in green.

Recovery Studies with Solvent Extraction

Sample preparation is a crucial step for the analysis of pesticides in cannabis matrices. Solvent extraction is a quick and easy way to achieve high extraction recovery when compared to other time consuming sample preparation techniques such as solid phase extraction (SPE) and QUECHERS, which require multiple steps and large sample volumes. Therefore, a solvent extraction method was selected for extraction of pesticides in cannabis. To confirm this method, fortified cannabis flower samples were used to determine pesticide recovery. The cannabis flower samples were tested to confirm the absence of pesticides before they were spiked. Three cannabis flower samples were spiked at two levels (0.02 and 0.2 µg/g) with all pesticide compounds in the standard. Table 3 shows that absolute recoveries of all 96 pesticides at both spiked levels were within acceptable range of 70-120%, with RSD less than 20% for the three cannabis flower samples. For pesticides marked with an asterisk (*) in Table 3, recovery data was collected at low levels of 0.1 µg/g and high levels of 1 ug/g, since recovery data at a low level of 0.02 µg/g cannot be obtained for these pesticides, given that their LOQs are higher than 0.02 µg/g. Also, no recovery data could be obtained for cyfluthrin, cypermethrin, kinoprene, and methoprene at the lower level of 0.1 µg/g since their LOQs are higher than 0.1 µg/g.

Table 3. Recovery of all pesticides at two different levels with recommended sample preparation.

S. No.	Canada Pesticide	Low Level 0.02 µg/g		High Level 0.2 µg/g	
		Recovery/%	RSD/% (n=3)	Recovery/%	RSD/% (n=3)
1	Abamectin*	100	10	100	8
2	Acephate	103	2	102	9
3	Acetamiprid	94	2	96	2
4	Acequinocyl*	88	3	78	3
5	Aldicarb	86	4	90	2
6	Allethrin*	74	10	99	3
7	Azadirachtin*	108	17	90	5
8	Azoxystrobin	88	17	89	7
9	Benzovindiflupyr	119	9	87	3
10	Bifenazate	91	10	90	4
11	Bifenthrin	95	3	85	3
12	Boscalid	88	10	83	5
13	Buprofezin	101	3	76	7
14	Carbaryl	93	2	95	4
15	Carbofuran	94	6	94	3
16	Chlorantraniliprole	94	7	98	12
17	Chlorphenapyr	92	6	85	6
18	Chlorpyrifos	101	13	88	2
19	Clofentezine	89	19	79	11
20	Clothianidin	92	4	92	5
21	Coumaphos	109	8	80	8
22	Cyantranilipole	93	3	79	5
23	Cyfluthrin*	-	-	100	12
24	Cypermethrin*	-	-	103	8
25	Cyprodinil	77	15	105	17
26	Daminozide*	107	3	94	1
27	Deltamethrin	117	-20	81	7
28	Diazinon	93	9	79	5
29	Dichlorvos	73	4	72	1
30	Dimethoate	99	5	94	1
31	Dimethomorph	98	10	94	1
32	Dinotefuran	97	3	94	2
33	Dodemorph	82	7	77	4
34	Endosulfan-alpha*	83	6	84	2
35	Endosulfan-beta*	91	7	94	3
36	Endosulfan sulfate	92	4	94	6
37	Ethoprophos	108	6	87	6
38	Etofenprox	91	5	88	5
39	Etoxazole	91	13	92	5
40	Etridiazol*	75	9	75	7

Table 3. continued.

S. No.	Canada Pesticide	Low Level 0.02 μg/g		High Level 0.2 μg/g	
		Recovery/%	RSD/% (n=3)	Recovery/%	RSD/% (n=3)
41	Fenoxycarb	85	14	81	7
42	Fenpyroximate	117	9	100	4
43	Fensulfothion	99	7	84	5
44	Fenthion	89	19	88	7
45	Fenvalerate*	92	12	98	9
46	Fipronil	118	12	103	2
47	Flonicamid	102	4	103	4
48	Fludioxonil	104	3	103	8
49	Fluopyram	90	7	80	3
50	Hexythiazox	86	9	102	3
51	Imazalil	104	11	87	3
52	Imidacloprid	95	5	96	5
53	Iprodione*	80	6	74	3
54	Kinoprene*	-	-	106	11
55	Kresoxim-methyl	93	10	93	7
56	Malathion	88	5	96	5
57	Metalaxyl	100	9	86	7
58	Methiocarb	101	3	86	3
59	Methomyl	93	12	93	5
60	Methoprene*	-	-	110	8
61	Methyl parathion	91	10	82	13
62	Mevinphos	94	2	98	4
63	MGK-264	73	9	80	20
64	Myclobutanil	98	2	81	14
65	Naled	117	9	104	2
66	Novaluron	101	2	74	11
67	Oxamyl	102	10	94	3
68	Paclobutrazol	97	4	89	6
69	Permethrin	98	15	99	4
70	Phenothrin*	84	11	86	3
71	Phosmet	92	5	90	2
72	Piperonyl butoxide	87	8	82	18
73	Pirimicarb	93	3	97	6
74	Prallethrin	82	20	93	11
75	Propiconazole*	84	8	88	7
76	Propoxur	93	2	91	2
77	Pyraclostrobin	113	8	85	5
78	Pyrethrins*	106	12	90	6
79	Pyridaben	81	5	73	14
80	Quintozene	99	12	90	5
81	Resmethrin*	80	5	83	5
82	Spinetoram	110	10	108	10
83	Spinosad	85	13	94	7
84	Spirodiclofen	110	5	91	2
85	Spiromesifen	110	8	106	3
86	Spirotetramat	104	20	86	17
87	Spiroxamine	100	17	95	18
88	Tebuconazole	102	7	82	6
89	Tebufenozide	92	13	91	4
90	Teflubenzuron	109	18	80	4
91	Tetrachlorvinphos	87	17	85	7
92	Tetramethrin*	77	8	101	6
93	Thiacloprid	92	3	98	7
94	Thiamethoxam	91	2	93	3
95	Thiophanate-methyl	120	7	95	2
96	Trifloxystrobin	108	3	85	11

Sample Matrix Effect (Ion Suppression)

Sample matrix effects remain the primary concern for LC/MS/MS analyses, especially for cannabis analysis, owing to the diversity and complexity of the cannabis samples. To overcome sample matrix effects, numerous strategies have been widely applied to LC/MS/MS method development, including sample dilution, the use of stable isotope internal standards, sample matrix-matched standard calibration, a standard addition method, sample clean-up, the use of high efficiency UHPLC columns for better separation, and the use of alternative ionization sources.[1-7] The most common sample matrix effect from co-extracted matrix compounds is discussed in detail in this section.

Sample matrix effect (ion suppression) was evaluated by spiking the same amount of pesticide standard and internal standard mix into the extract solutions that were diluted to different levels with acetonitrile. As shown in Figure 1, acequinocyl showed significant ion suppression (~80%) in the cannabis flower extract diluted 20-fold with acetonitrile. The extracts with a 100-fold dilution showed lower ion suppression at around 40% for acequinocyl, in comparison to acequinocyl spiked in pure acetonitrile, suggesting that the dilution helps minimize the effects of ion suppression, as shown in Figure 1. In this application, a 20-fold dilution of the cannabis extract was used to minimize ion suppression of late eluting analytes from the sample matrix without compromising the detection limits of earlier eluting analytes that do not experience significant ion suppression. Thirty-three internal standards were used for further compensation of ion suppression of the analytes eluting in different regions of the chromatogram.

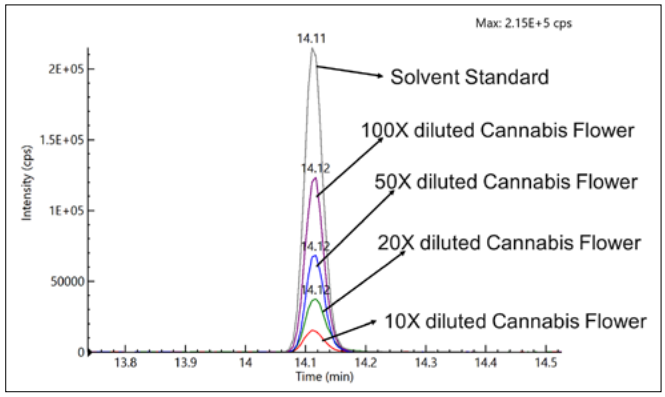


Figure 1. Chromatographic results for ion suppression at different dilution levels of cannabis flower spiked with 100 ppb of acequinocyl, and a solvent standard with 100 ppb acequinocyl.

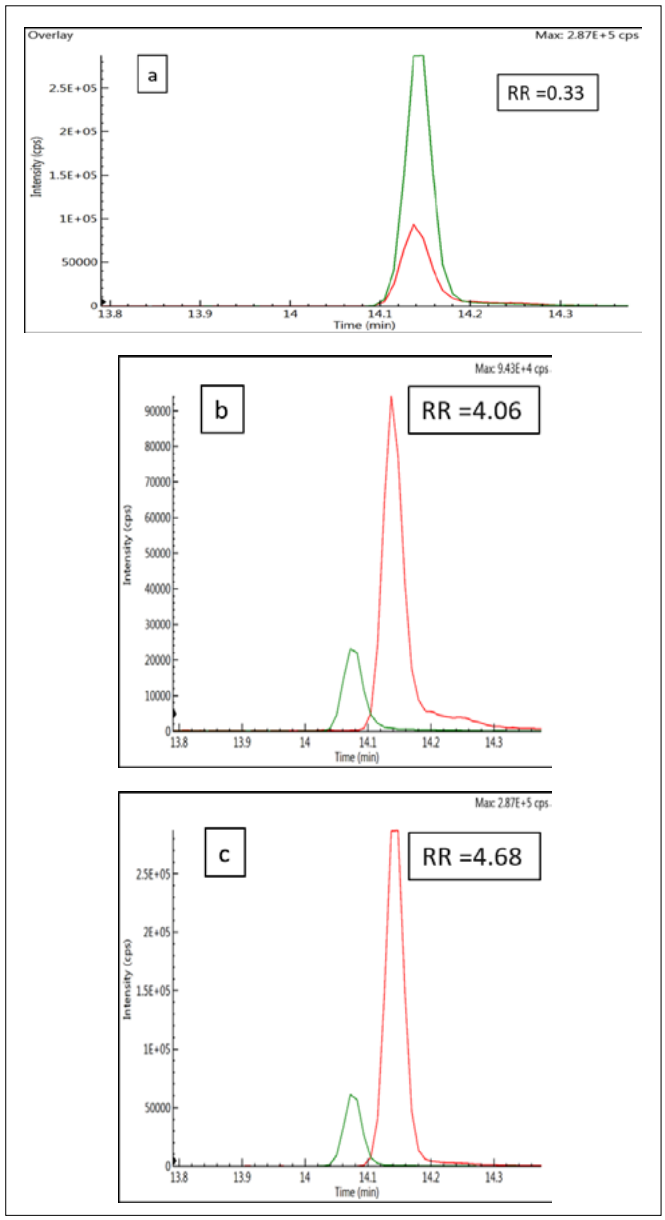


Figure 2. (a) Overlay of the responses of acequinocyl in solvent (green), and the pre-spiked cannabis flower matrix without internal standard (red). The response ratios of acequinocyl in cannabis extract to solvent standard was 0.33. (b) Overlay of the responses of acequinocyl (red) and the acequinocyl internal standard (green) in a pre-spiked cannabis flower matrix with response ratio (RR) of 4.06 for the analyte to the internal standard. (c) Overlay of the responses of acequinocyl (red) and acequinocyl internal standard (green) in solvent with response ratio (RR) of 4.68 for the analyte to the internal standard.

Internal Standards

Since complex cannabis samples exhibit a severe matrix effect, 33 internal standards were selected to improve the quantitative analysis and overall recovery, in addition to correcting for any analyte loss during sample preparation. According to the experimental results presented in Figure 2, the use of internal standards significantly increased the overall recovery in the analysis. Calculated as the extracted concentration of pre-spiked analyte versus the neat solution (unextracted) concentration, the recovery improved from 33% to 86.5%, owing to the correction of matrix effects and analyte loss during the extraction step. Finally, overall recoveries of 70-120% were achieved for 97% of the analytes, while recoveries of 60-140% were reported for remaining 3% of analytes. To expedite validation, PerkinElmer supplies a standard operating procedure (SOP) that details the required internal standards, reagent suppliers and method parameters.

LC/MS/MS Method with Optimum MRM Transitions for Challenging Analytes

As earlier stated, cannabis is a challenging matrix to test, compounded by the low concentration level of the pesticides. To ensure the highest analytical confidence, a number of MRM transitions for a number of pesticides with minimal matrix interference in the cannabis matrix were determined for low level detection. For example, propiconazole can be ionized easily as a protonated molecular ion in a standard, but the

MRM transitions, based on monoisotopic mass ion in the cannabis matrix, showed a poor LOQ of 0.2 µg/g. Therefore, MRM transitions based on other masses were determined to reduce matrix interference, and achieve an LOQ of 0.04 µg/g for propiconazole. Figure 3 shows the signal overlay of the blank cannabis matrix and propiconazole spiked at a level of 0.1 µg/g, with MRM transitions with and without matrix interference. This figure displays that optimum propiconazole MRM transitions helped in achieving lower detection limits due to minimal matrix interference.

Analysis of Pesticides by APCI

Hydrophobic and halogenated pesticides (eg. Pentachloronitrobenzene, chlorfenapyr and others) are traditionally analyzed by GC/MS/MS as they do not ionize effectively by LC/MS/MS with an ESI source. Since Pentachloronitrobenzene (PCNB) does not contain either hydrogen atoms (for loss of protons), or functional groups with either high proton affinity or those which can form ammonia or sodium adducts, it cannot be ionized with the ESI source. Since an APCI ion source is better suited for ionization of very hydrophobic and non-polar analytes, APCI was used to determine the detection limits of chlorfenapyr, pentachloronitrobenzene, methyl parathion endosulfan-alpha, endosulfan-beta, iprodione, fenvalerate, endosulfan sulfate and Etridiazol. Figure 4 shows excellent signal to noise (S/N >=100) for pentachloronitrobenzene (PCNB) spiked at level of 0.1 µg/g using an LC/MS/MS system with an APCI source, and a fast six minute short LC gradient.

Stability Studies

As can be seen in Figure 5, method stability was investigated by monitoring 500 sample injections over a period of a week. The % RSDs for all analytes in APCI mode were less than 20%, and in ESI mode, they were less than 25%. These results demonstrated that the heated self-cleaning dual ESI/APCI ion source with laminar flow in the QSight LC/MS/MS system would reduce the need for maintenance activities that are usually prevalent with this dirty and challenging cannabis flower matrix.

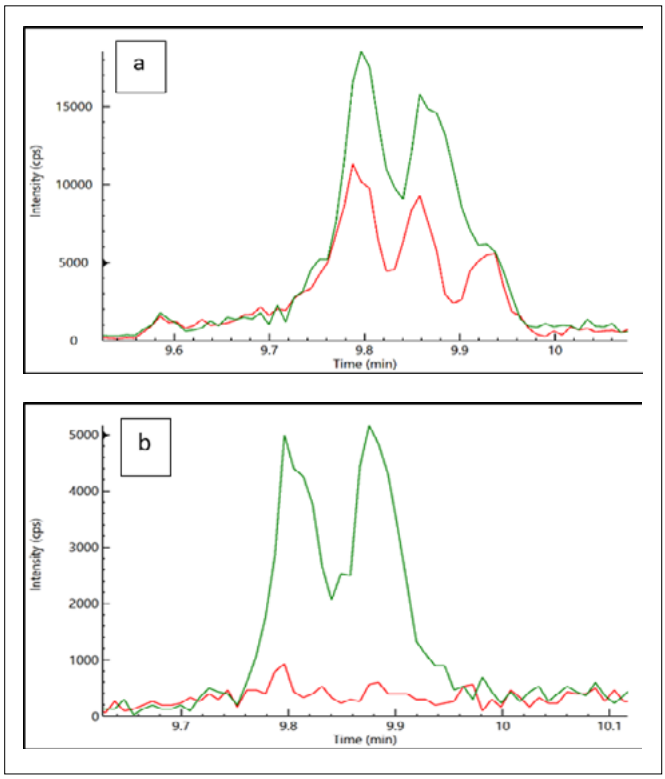


Figure 3. (a) Overlay of the response of the cannabis matrix (red) and propiconazole (green) spiked at a level of 0.1 µg/g in cannabis matrix using (a) MRM transition with matrix interference and (b) MRM transition without matrix interference.

Conclusions

This study demonstrates a unique, quantitative, rapid, and reliable LC/MS/MSmethod for the analysis of pesticide residues in cannabis samples. The proposed solvent extraction method is suitable for labs seeking to comply with Canadian regulations, as the recovery of all pesticides were in the acceptable range of 70-120%, with RSDs less than 20%. This method allowed for the identification and quantification of all 96 pesticides at low levels (0.0025 to 0.5 µg/g). The ability to screen and quantitate all 96 pesticides, including the very hydrophobic and chlorinated compounds normally analyzed utilizing GC/MS/MS, makes this method a novel way to screen and quantitate pesticides in cannabis with a single instrument.

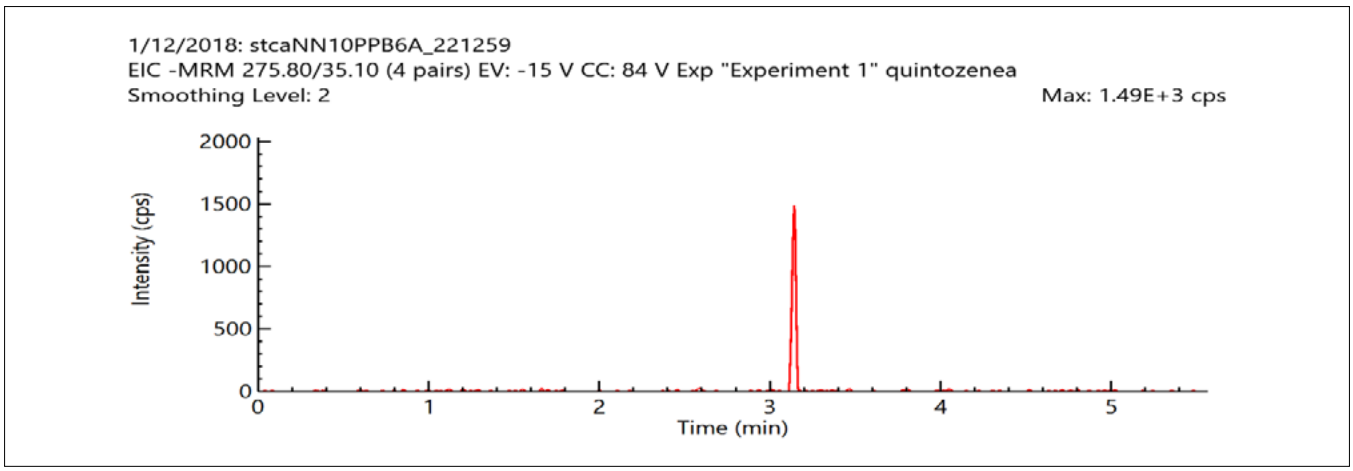
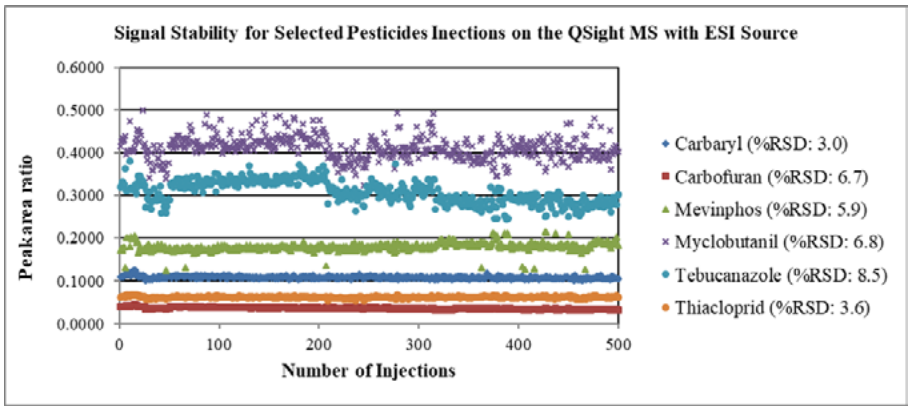


Figure 4. Sample chromatogram of pentachloronitrobenzene (PCNB) spiked at a level of 0.1 µg/g in a cannabis flower matrix using LC/MS/MS system with APCI source.



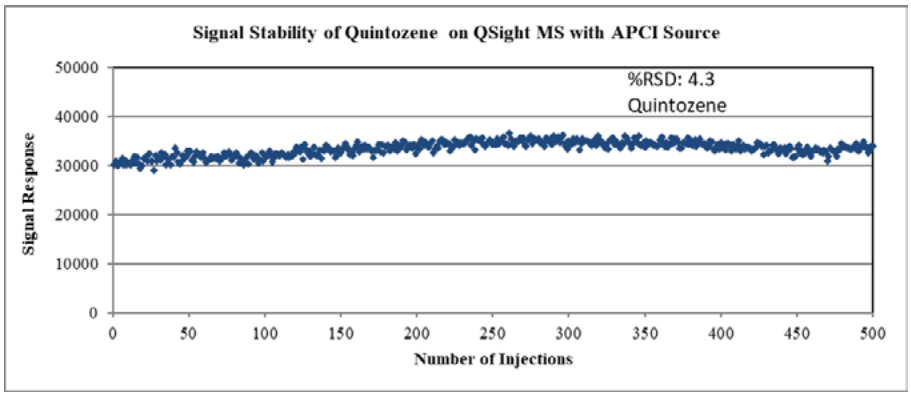


Figure 5. Long term stability data over 1 week of 500 sample injections using LC/MS/MS with ESI and APCI ion source.

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APPLICATION NOTE

Liquid Chromatography/ Mass Spectrometry

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Novel ESI and APCI LC/MS/MS Analytical Method for Testing Cannabis and Hemp Concentrate Sample Types

Introduction

As new adult-use and medicinal cannabis markets emerge in the US and Canada, the use of concentrate cannabis and CBD products (e.g. edibles, beverages, vape products, isolates, topicals, and waxes) continues to increase in popularity. According to market research, concentrates and their derivative products are expected to represent 50% of the consumer market by 2022.[1] This growth, and the diversity in sample type, presents an analytical challenge for testing laboratories. The concentrate matrix has a significant effect on the analytical method, owing to higher sample matrix effects caused by the increased concentration levels (up to 95%/wt) of cannabinoids in the sample. This effect influences the response of certain pesticide molecules, requiring laboratories to validate a pesticide method specific to the sample matrix type.

In this work, an LC/MS/MS method is presented for the analysis of 66 pesticides, including hydrophobic and chlorinated pesticides typically analyzed by GC/MS/MS, and five mycotoxins. Utilizing a cannabis concentrate matrix, the method features a simple solvent extraction, followed by analysis using an LC/MS/MS instrument with dual ESI and APCI sources. The analysis yielded excellent recoveries and detection limits, well below those specified by the State of California cannabis regulations, for all analytes.

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Experimental

Hardware/Software

Chromatographic separation was conducted utilizing a PerkinElmer QSight® LX50 UHPLC system. Subsequent detection was achieved using a PerkinElmer QSight 420 MS/MS detector with dual ESI and APCI ionization sources, which operate independently with two separate inlets. All instrument control, data acquisition and data processing were performed using the Simplicity 3Q™ software platform.

Sample Preparation

Below is the step-by-step sample preparation procedure with a 50-fold dilution for the ESI source, and a 25-fold dilution for the APCI source.

1. Measure out approximately five grams of cannabis concentrate as a representative sample for each sample batch.
2. Measure out one gram of sample, and place it into a 50 mL centrifuge tube.
3. Add 10 mL of LC/MS grade acetonitrile with 0.1 % formic acid to the tube, and cap it.
4. Place the tube in a multi-tube vortex mixer, and allow it to vortex for 10 minutes.
5. Centrifuge the extract in the tube for ten minutes at 3000 rpm.
6. Transfer the solvent into a 10 mL glass amber vial and cap it.
7. Label the bottle with the sample ID.
8. For the APCI method, transfer 400 µL of extracted sample (from Step 7) into a 2 mL HPLC vial. Spike with 10 µL of internal standard, and then dilute with 590 µL of LC/MS grade acetonitrile with 0.1 % formic acid and mix it.
9. For the ESI method, transfer 200 µL of extracted sample (from Step 7) into a 2 mL HPLC vial. Spike with 10 µL of internal standard, and then dilute with 790 µL of LC/MS grade acetonitrile with 0.1 % formic acid and mix it.
10. Inject sample for LC/MS/MS analysis, using pesticide methods.

Results and Discussion

Detectability and Reproducibility

Currently, most laboratories deploy multiple analytical instruments (LC/MS/MS and GC/MS/MS) and tedious sample preparation methods (such as QuEChERS) to meet the low pesticide regulatory limits in various food matrices. Herein, a validated LC/MS/MS analytical method with a fast solvent extraction is presented. Utilizing a PerkinElmer liquid chromatograph coupled to a tandem mass spectrometer, the complete analysis of all 66 pesticides and five mycotoxins outlined in the California regulations for cannabis concentrates is detailed. All compounds of concern were analyzed with a QSight 420 dual source mass spectrometer, equipped with both APCI and ESI ionization probes. Pesticides conventionally analyzed by gas chromatography, such as methyl parathion, cypermethrin and pentochloronitrobenzene (quintozene) among others, were all detected utilizing this single platform LC/MS/MS system.

LC Method and MS Source Conditions

The LC method and MS source parameters are shown in Table 1.

Table 1. LC Method and MS Source Conditions.

LC Conditions	
LC Column	PerkinElmer Quasar™ SPP Pesticides (4.6 × 100 mm, 2.7 µm)
Mobile Phase A (ESI method)	2 mM ammonium formate + 0.1% formic acid (in LC/MS grade water)
Mobile Phase B (ESI method)	2 mM ammonium formate + 0.1% formic acid (in LC/MS grade methanol)
Mobile Phase A (APCI method)	LC/MS grade water
Mobile Phase B (APCI method)	LC/MS grade methanol
Mobile Phase Gradient	The run time for the optimized gradient elution method, including analytical column re-conditioning, was 18 minutes for the ESI method, and 12 minutes for the APCI method. The final method ensured separation of the bulk cannabis matrix from the analytes for improved quantitation.
Column Oven Temperature	30 °C
Auto sampler Temperature	20 °C
Injection Volume	3 µL and 10 µL for LC/MS/MS method with ESI and APCI source, respectively.

MS Source Conditions for ESI Source and APCI Source	
ESI Voltage (Positive)	+5100 V
ESI Voltage (Negative)	-4200V
APCI Corona Discharge	-3 µA
Drying Gas	150 arbitrary units
Nebulizer Gas	350 arbitrary units
Source Temperature (ESI Method))	315 °C
Source Temperature (APCI Method)	250 °C
HSID Temperature (ESI Method)	200 °C
HSID Temperature (APCI Method)	180 °C
Detection Mode	Time-managed MRM™

The limit of quantification (LOQ) and response reproducibility at the LOQ for each of the pesticides (Category II and I) and mycotoxins in the cannabis concentrate sample are summarized in Tables 2, 3 and 4. The LOQs were determined by considering the signal of the quantifier ion (S/N > 10), and ensuring that the product ion ratios were within the 30 % tolerance windows of the expected ion ratio. The response RSD for each pesticide and mycotoxin at its LOQ level in the cannabis matrix were less than 20%. As demonstrated in Tables 2, 3 and 4, the LOQs determined in this study are well below the California action limit by a factor of 1.2 to 1,000 for all pesticides and mycotoxins listed. This demonstrates the sensitivity and reproducibility of the method in the analysis of pesticides and mycotoxins in cannabis concentrate samples, in support of California state regulatory program adherence.

Table 2. LOQs for California Category II Pesticides with LC/MS/MS in Cannabis Concentrate. **Red**/**Green**: Pesticides typically analyzed by GC/MS/MS. Of those, analytes highlighted in **red** were analyzed on the QSight by ESI, and those in **green** were analyzed on the QSight by APCI. Pesticides in black were analyzed on the QSight by ESI.

S. No.	Category II Residual Pesticide	LOQ		Action Level (µg/g)	Action Level/LOQ
		LC/MS/MS (µg/g)	%CV (n=7)		
1	Abamectin	0.08	14.0	0.1	1.2
2	Acephate	0.01	4.5	0.1	10
3	Acequinocyl	0.05	10.8	0.1	2
4	Acetamiprid	0.01	3.3	0.1	10
5	Azoxystrobin	0.005	11.9	0.1	20
6	Bifenazate	0.005	15.2	0.1	20
7	Bifenthrin	0.005	5.3	0.5	100
8	Boscalid	0.005	14.2	0.1	20
9	**Captan**	0.5	13.0	0.7	1.4
10	Carbaryl	0.005	7.4	0.5	100
11	Chlorantraniliprole	0.01	10.0	10.0	1000
12	Clofentezine	0.01	14.4	0.1	10
13	**Cyfluthrin**	0.9	16.0	2.0	2.2
14	**Cypermethrin**	0.15	7.8	1.0	6.66
15	Diazinon	0.01	7.5	0.2	20
16	Dimethomorph	0.005	17.4	2.0	400
17	Etoxazole	0.01	5.4	0.1	10
18	Fenhexamid	0.05	8.3	0.1	2
19	Fenpyroximate	0.01	6.9	0.1	10
20	Flonicamid	0.01	6.4	0.1	10
21	Fludioxonil	0.005	11.9	0.1	20
22	Hexythiazox	0.005	10.1	0.1	20
23	Imidacloprid	0.01	9.9	3.0	300
24	Kresoxim-methyl	0.05	4.8	0.1	2
25	Malathion	0.005	6.1	0.5	100
26	Metalaxyl	0.005	3.3	2.0	400
27	Methomyl	0.01	12.3	0.1	10
28	Myclobutanil	0.005	5.4	0.1	20
29	**Naled**	0.05	13.0	0.1	2
30	Oxamyl	0.01	4.1	0.2	20
31	**Pentachloronitrobenzene**	0.025	10.2	0.1	4
32	**Permethrin**	0.05	6.8	0.5	10
33	Phosmet	0.01	12.0	0.1	10
34	Piperonylbutoxide	0.15	4.0	3.0	20
35	**Prallethrin**	0.08	14.4	0.1	1.2
36	Propiconazole	0.005	4.5	0.1	20
37	**Pyrethrins**	0.37	5.1	0.5	1.3
38	Pyridaben	0.01	10.4	0.1	10
39	Spinetoram	0.008	9.0	0.1	12.5
40	Spinosad	0.01	10.7	0.1	10
41	Spiromesifen	0.05	5.2	0.1	2
42	Spirotetramat	0.005	12.5	0.1	20
43	Tebuconazole	0.01	13.8	0.1	10
44	Thiamethoxam	0.005	8.5	4.5	900
45	Trifloxystrobin	0.005	4.9	0.1	20

Table 3. LOQs for California Category I Pesticides with LC/MS/MS in Cannabis Concentrate. **Red**/**Green**: Pesticides typically analyzed by GC/MS/MS. Of those, analytes highlighted in **red** were analyzed on the QSight by ESI, and those in **green** were analyzed on the QSight by APCI. Pesticides in black were analyzed on the QSight by ESI.

S. No.	Category I Residual Pesticide	LOQ		Action Level (µg/g)	Action Level/LOQ
		LC/MS/MS (µg/g)	%CV (n=7)		
1	Aldicarb	0.025	9.5	0.1	4
2	Carbofuran	0.005	8.5	0.1	20
3	**Chlordane**	0.08	15.3	0.1	1.2
4	**Chlorfenpyr**	0.05	18.0	0.1	2
5	**Chlorpyrifos**	0.05	8.5	0.1	2
6	**Coumaphos**	0.01	15.7	0.1	10
7	Daminozide	0.05	11.3	0.1	2
8	DDVP (Dichlorvos)	0.025	4.2	0.1	4
9	Dimethoate	0.005	5.1	0.1	20
10	Ethoprop(hos)	0.01	12.5	0.1	10
11	Etofenprox	0.01	8.6	0.1	10
12	Fenoxycarb	0.005	5.5	0.1	20
13	Fipronil	0.005	9.8	0.1	20
14	Imazalil	0.005	19.3	0.1	20
15	Methiocarb	0.005	10.9	0.1	20
16	**Methyl Parathion**	0.05	3.0	0.1	2
17	Mevinphos	0.01	8.1	0.1	10
18	Paclobutrazol	0.01	10.2	0.1	10
19	Propoxur	0.01	11.8	0.1	10
20	Spiroxamine	0.01	6.3	0.1	10
21	Thiacloprid	0.005	6.5	0.1	20

Table 4. LOQs for Mycotoxins with LC/MS/MS in the Cannabis Concentrate.

S. No.	Category II Mycotoxin	LOQ		Action Level (µg/g)	Action Level/LOQ
		LC/MS/MS (µg/g)	%CV (n=7)		
1	Ochratoxin A	0.0125	12.6	0.020	1.6
2	Aflatoxin B1	0.003	12.4	NA	NA
3	Aflatoxin B2	0.003	13.0	NA	NA
4	Aflatoxin G1	0.004	8.2	NA	NA
5	Aflatoxin G2	0.005	10.5	NA	NA
6	Aflatoxin (B1+B2+G1+G2)	0.015	NA	0.020	1.33

Recovery Studies with Solvent Extraction

In cannabis concentrate testing, sample preparation is often identified as the main bottleneck associated with the analysis of pesticides and mycotoxins. Techniques such as solid phase multiple steps and large amounts of expensive sorbent materials compounds.[3] Solvent extraction, in comparison, offers an efficient, easy and high throughput means of achieving high extraction recovery. As such, a solvent extraction method was utilized in this study for the extraction of pesticides and mycotoxins.

To confirm the recovery performance of the method, spiked cannabis concentrate samples were utilized. The cannabis concentrate samples were analyzed to confirm the absence of pesticides and mycotoxins prior to spiking. Cannabis concentrate samples were then spiked at two levels for each contaminant of concern; 0.1 µg/g (low) and 1.0 µg/g (high) for pesticides, and 0.02 µg/g (low) and 0.2 µg/g (high) for mycotoxins. Tables 5, 6 and 7 show that the absolute recoveries at both spiking levels for all mycotoxins and pesticides were within the acceptable range of 70-120%, with RSD values less than 20%. No recovery data could be obtained for pesticides captan, cyfluthrin and cypermethrin at the lower level of 0.1 µg/g, since their LOQ is higher than 0.1 µg/g.

Table 5. Recoveries of Category II pesticides in cannabis concentrate matrix at two different levels with solvent extraction.

	Category II Residual Pesticide	Recovery/%	RSD/% (n=3)	Recovery/%	RSD/% (n=3)
1	Abamectin	81.6	4.7	83.7	15.5
2	Acephate	98.3	2.0	93.4	1.1
3	Acequinocyl	99.2	5.3	84.7	1.6
4	Acetamiprid	94.7	1.0	94.4	0.7
5	Azoxystrobin	93.0	2.2	98.5	5.3
6	Bifenazate	91.9	3.2	91.6	0.9
7	Bifenthrin	94.5	3.4	93.7	0.3
8	Boscalid	82.0	3.1	98.7	10.5
9	Captan*	-	-	96.4	18.9
10	Carbaryl	93.6	6.1	93.9	4.3
11	Chlorantraniliprole	87.8	5.1	98.1	8.8
12	Clofentezine	71.9	3.3	87.1	16.4
13	Cyfluthrin*	-	-	95.4	5.5
14	Cypermethrin*	-	-	93.4	5.5
15	Diazinon	89.1	1.1	94.5	4.1
16	Dimethomorph	83.7	2.6	93.8	4.0
17	Etoxazole	97.6	1.9	96.9	3.1
18	Fenhexamid	102.8	10.6	103.0	13.5
19	Fenpyroximate	91.1	1.7	95.7	1.2
20	Flonicamid	102.6	5.7	97.8	0.9
21	Fludioxonil	103.3	3.9	96.1	1.6
22	Hexythiazox	79.8	2.7	96.6	11.7
23	Imidacloprid	95.9	2.4	95.4	1.2
24	Kresoxim-methyl	93.4	3.0	96.1	2.5
25	Malathion	95.5	5.2	93.6	3.4
26	Metalaxyl	93.2	2.8	95.1	3.7
27	Methomyl	97.4	2.7	97.4	2.1
28	Myclobutanil	85.7	3.2	94.9	1.6
29	Naled	100.0	8.2	96.9	5.0
30	Oxamyl	98.9	1.7	95.1	0.9
31	Pentachloronitrobenzene	92.8	4.3	96.0	3.5
32	Permethrin	92.8	13.1	98.9	3.0
33	Phosmet	80.2	3.9	94.3	3.3
34	Piperonylbutoxide	90.3	2.0	95.2	2.1
35	Prallethrin	90.5	14.4	101.7	8.3
36	Propiconazole	81.3	1.8	93.9	12.0
37	Pyrethrins	109	16.9	101.0	14.4
38	Pyridaben	91.9	3.5	95.2	2.8
39	Spinetoram	92.1	1.6	93.4	1.8
40	Spinosad	95.1	8.7	97.7	3.4
41	Spiromesifen	99.8	5.0	99.0	5.6
42	Spirotetramat	95.8	2.6	94.7	1.8
43	Tebuconazole	96.4	2.7	94.9	1.7
44	Thiamethoxam	97.6	2.4	96.7	1.7
45	Trifloxystrobin	92.7	3.5	97.0	0.9

Table 6. Recoveries of Category I pesticides in cannabis concentrate matrix at two different levels with solvent extraction.

S. No.	Category I Residual Pesticide	Low Level 0.1 µg/g		High Level 1 µg/g	
		Recovery/%	RSD/% (n=3)	Recovery/%	RSD/% (n=3)
1	Aldicarb	88.9	14.2	95.5	4.1
2	Carbofuran	91.9	1.5	93.8	3.9
3	Chlordane	102.3	15.3	105.2	4.4
4	Chlorfenapyr	94.8	3.0	94.7	4.8
5	Chlorpyrifos	108.6	4.9	97.9	13.4
6	Coumaphos	73.6	5.1	93.5	13.8
7	Daminozide	95.1	6.0	95.2	1.6
8	DDVP (Dichlorvos)	92.6	3.5	95.5	1.2
9	Dimethoate	94.2	0.9	96.9	1.1
10	Ethoprop(hos)	88.0	5.7	95.7	2.7
11	Etofenprox	101.3	4.4	97.4	3.7
12	Fenoxycarb	97.1	3.5	96.9	1.5
13	Fipronil	98.1	3.6	95.8	3.7
14	Imazalil	88.5	9.8	98.0	4.5
15	Methiocarb	94.8	4.4	101.9	1.3
16	Methyl parathion	94.4	4.8	95.6	6.3
17	Mevinphos	93.4	4.0	96.4	1.7
18	Paclobutrazol	94.2	2.6	97.8	1.7
19	Propoxur	90.9	2.7	94.3	3.8
20	Spiroxamine	97.3	0.9	95.9	1.9
21	Thiacloprid	92.9	2.5	93.6	3.0

Table 7. Recoveries of mycotoxins in cannabis concentrate matrix at 2 different levels with solvent extraction.

S. No.	Category II Mycotoxin	Low Level 0.02 µg/g		High Level 0.2 µg/g	
		Recovery/%	RSD/% (n=3)	Recovery/%	RSD/% (n=3)
1	Aflatoxin B1	92	8	93	5
2	Aflatoxin B2	94	9	92	6
3	Aflatoxin G1	81	18	98	9
4	Aflatoxin G2	96	17	91	10
5	Ochratoxin A	87	12	85	3

Internal Standards

As cannabis concentrate samples exhibit a significant matrix effect, owing to the large amount of cannabinoids present (50-95%) in them, 30 internal standards were utilized to improve the quantitative analysis and overall recovery. The use of internal standards compensated for matrix ion suppression effects, and corrected for any analyte loss during sample preparation. According to experimental results shown in Figure 1, use of internals standards significantly increased the overall recovery of coumaphos, calculated based on extracted concentration of pre-spiked analyte versus neat solution (unextracted) concentration, from 56% to 86% owing to correction of matrix effects and analyte loss during extraction step. Finally, the overall recoveries of 70-130 % were achieved for all of 66 pesticides and five mycotoxins with addition of 30 internal standards to cannabis concentrate matrix.

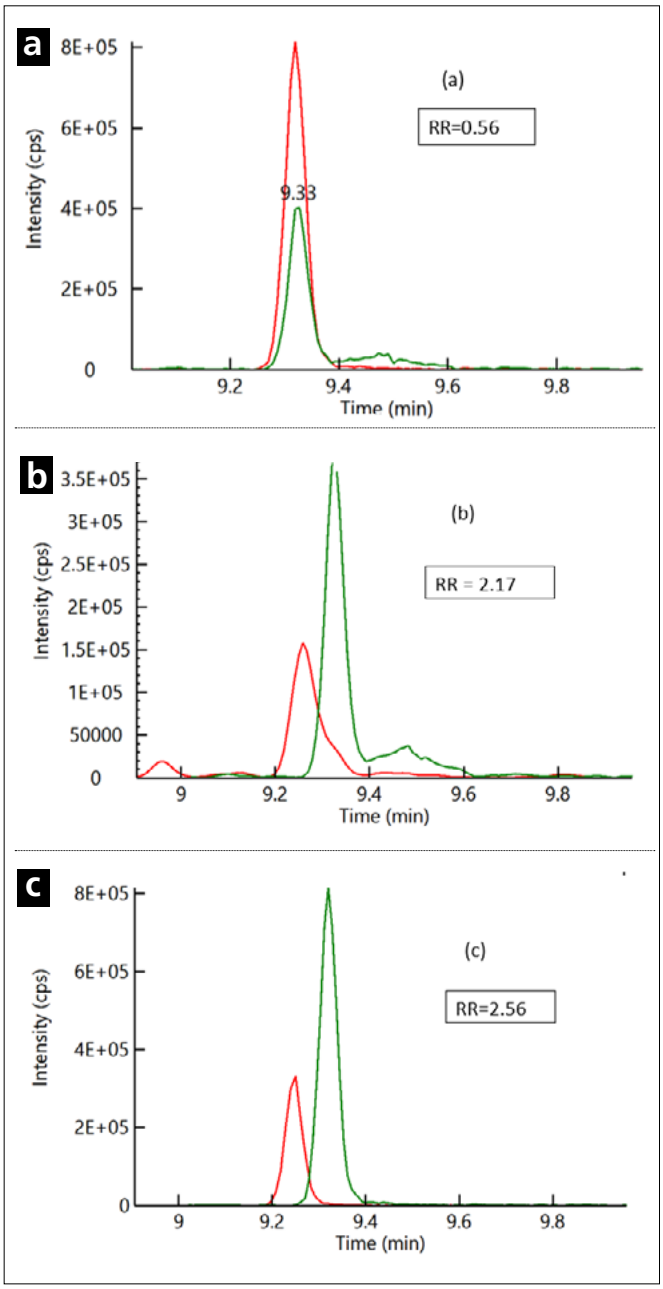

Figure 1. (a) Overlay of the response of coumaphos in solvent (**red**) and coumaphos (**green**) pre-spiked in the cannabis concentrate matrix, without an internal standard. The response ratio (RR) of coumaphos in cannabis extract to solvent standard was 0.56. (b) Overlay of the response of coumaphos (**green**) and coumaphos-D10 internal standard (**red**) in the pre-spiked cannabis concentrate matrix with a response ratio (RR) of 2.17 for the analyte to internal standard. (c) Overlay of the response of coumaphos (**green**) and coumaphos-D10 internal standard (red) in the solvent with a response ratio (RR) of 2.56 for the analyte to internal standard.

Analysis of Pesticides, Typically Analyzed by GC-MS/MS, Using LC/MS/MS With Dual ESI and APCI Ion Source

A number of pesticides, regulated in cannabis by California and other states, are traditionally analyzed using GC/MS/MS with an EI source, as these pesticides exhibit either low proton affinity (which results in low ionization efficiency with the ESI source), or they cannot be ionized by the ESI ion source used in conventional LC/MS/MS systems. Examples of such pesticides, typically analyzed by GC/MS/MS are cypermethrin, cyfluthrin, captan, naled, permethrin, pentachlornitrobenzene, chlorfenapyr, chlordane, methyl parathion, pyrethrins and others.

To achieve the required sensitivity for a number of these pesticides (cypermethrin, cyfluthrin, captan, naled, permethrin, prallethrin, chlorpyrifos, coumaphos, and pyrethrins), the selected MRMs and source conditions (temperature and flow) were optimized with a heated electrospray source to get low detection limits. The other pesticides (pentachlornitrobenzene, chlorfenapyr, chlordane and methyl parathion) were measured at low limits in the cannabis concentrate matrix using the APCI source in the LC/MS/MS instrument. The LOQs for these analytes were in the range of 0.05 to 0.9 µg/g, well below the California action limits. Figure 2 presents a sample chromatogram of cannabis concentrate spiked at a level of 0.1 µg/g with pesticides naled and chlorfenapyr, which are analyzed by LC/MS/MS with ESI and APCI source, respectively.

Method Optimization to Overcome Matrix Ion Suppression Effects from a Challenging Cannabis Concentrate Matrix

As cannabis concentrates are prepared by the extraction of cannabis flowers, they typically exhibit 3-5 times higher levels of cannabinoids (THC and CBD) than cannabis flower raw materials. The higher concentration of cannabinoids (50-95%) in cannabis concentrate matrices can result in a considerably more challenging matrix when compared to cannabis flower samples. This complexity is further compounded by the low concentration levels of the pesticides and mycotoxins in the samples. For pesticide analysis in a cannabis flower sample, the matrix ion suppression effects are minimized by using an overall dilution factor with solvent in the range of 10-20x.

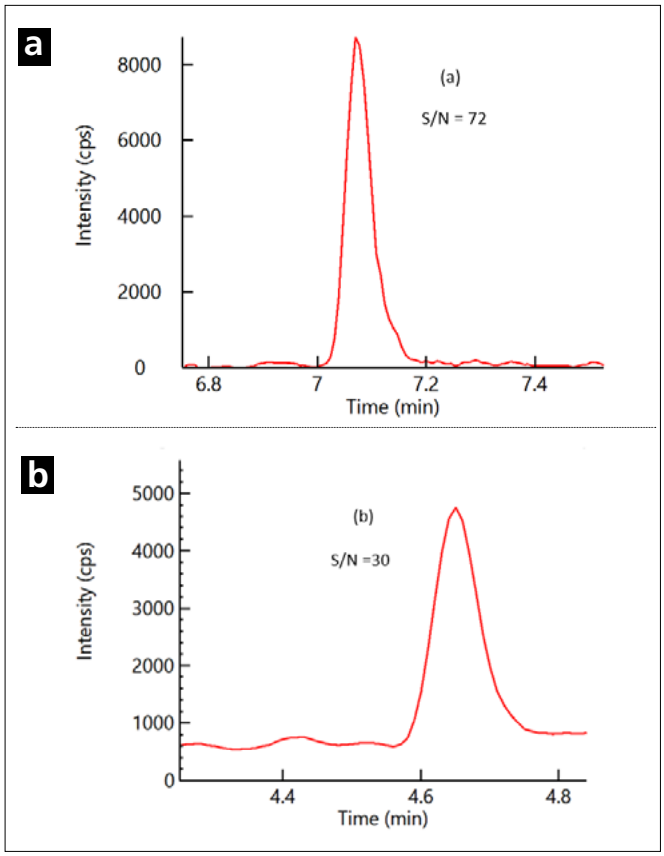

Figure 2. Sample chromatogram of (a) naled and (b) chlorfenapyr spiked at a level of 0.1 µg/g in a cannabis concentrate matrix using an LC/MS/MS system with an ESI and APCI source, respectively.

However, in the case of pesticide analysis in cannabis concentrate with our LC/MS/MS method, a considerably higher overall dilution factor of 25x for the APCI source and 50x for the ESI source was utilized to minimize matrix effects. In a previous study, a fast six-minute LC gradient with the APCI source for the analysis of four pesticides in a cannabis flower matrix was evaluated.[4] When the same six-minute LC gradient method was utilized for the analysis of the same four pesticides in a cannabis concentrate matrix, a significant ion suppression matrix effect was observed, which resulted in a much lower signal and reduced sensitivity. Apart from the higher dilution factor for the cannabis concentrate matrix, a 12-minute slower LC gradient method with an APCI source was developed to separate pesticides and cannabinoids on the LC column and reduce the ion signal suppression effects. Figure 3 illustrates that, when compared to the six-minute fast LC gradient method, the signal-to-noise for PCNB in the cannabis concentrate matrix was improved by a factor of 60 using the 12-minute optimized LC gradient method.

Selectivity of PCNB Analysis and Mechanism of PCNB Ionization With APCI Source

As PCNB does not have a hydrogen atom to lose, it cannot be ionized using an ESI source in negative ion mode. The nonpolar nature of the compound, the low proton affinity, and the inability to form adducts with ammonia and other metal ions further precludes PCNB from forming ions utilizing an ESI source in positive ion mode. Owing to these impediments, the ESI source could not be used for the detection of PCNB. Thus, the APCI source, in negative ion mode, was utilized for selective analysis of PCNB in different cannabis matrices.

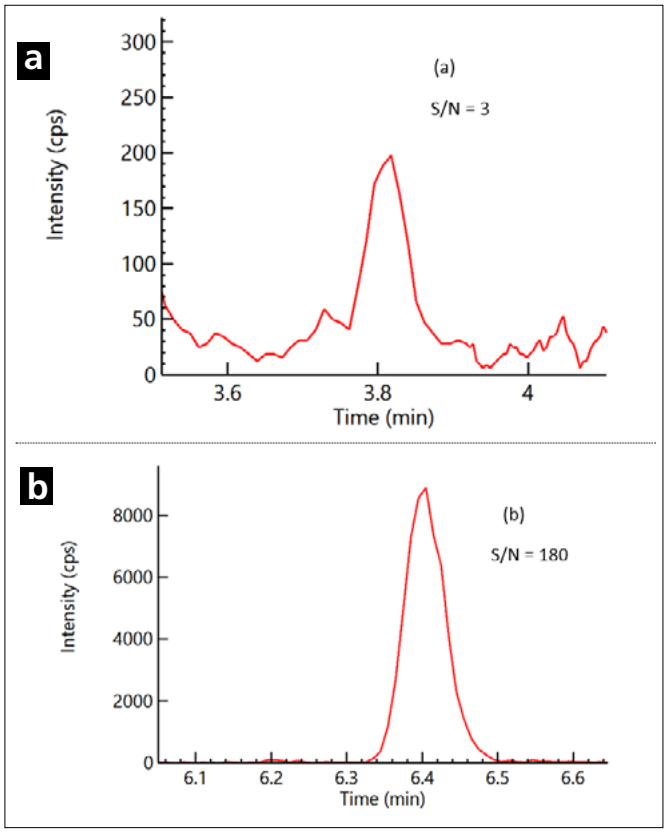

Figure 3. (a) Response for PCNB spiked at level of 1 µg/g in a cannabis concentrate matrix using a fast 6-min LC gradient method, coupled with the QSight MS/MS system with APCI source. (b) Response for PCNB spiked at a level of 1 µg/g in a cannabis concentrate matrix using an optimized 12-minute LC gradient method, coupled with a QSight MS/MS system with APCI source.

Figure 4 illustrates the response for PCNB in a blank cannabis concentrate matrix, and in a cannabis concentrate matrix spiked with 0.1 µg/g of PCNB. FDA method validation guidelines concerning the selectivity of an analysis specify that matrix blanks should be free of any matrix interference peaks at the retention time of an analyte.[5] As shown in figure 4a, the matrix response for PCNB in the blank cannabis concentrate shows low background signal with no matrix interference peak at the retention time of PCNB, thus demonstrating that the measurement of PCNB in the cannabis concentrate matrix is very selective. Further, a good signal-to-noise ratio for PCNB spiked at the California action limit of 0.1 ug/g in the cannabis concentrate matrix demonstrates that PCNB can be determined using an APCI source in LC/MS/MS systems with good selectivity and sensitivity.

Figure 5 details the excellent linearity of the PCNB response over a concentration range of 1-3000 ppb (corresponds to 25-75000 ppb in cannabis concentrate) in the 25x diluted cannabis concentrate extract, with a regression fit (R^2) of 0.9999. As the regression fit value for PCNB is greater than 0.99, the result meets the requirement outlined by the California Bureau of Cannabis Control, which stipulates that regression fits be higher than 0.99.[6] The accuracy of the calibration curve was checked by comparing back-calculated concentrations from the calibration curve with known concentrations of PCNB, ensuring that the strict criterion of a maximum deviation of 10% was met for all concentration levels. The literature claims that analysis of PCNB with an APCI LC/MS source is not selective and may require a quadratic calibration curve susceptible to a poor correlation coefficient, however, this experimental work outlines a robust APCI method that exhibits excellent sensitivity, selectivity and linearity of PCNB analysis in a cannabis sample.[7]

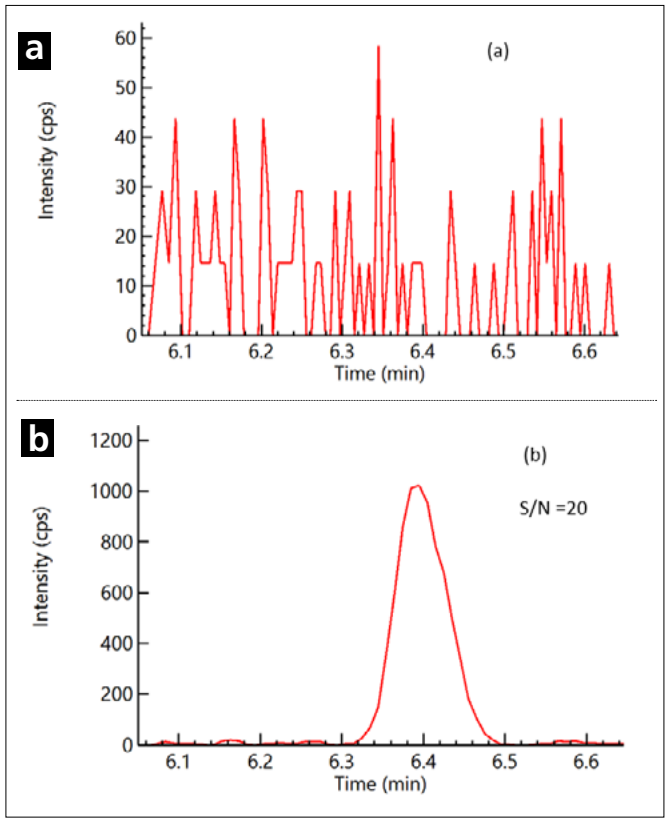

Figure 4. PCNB response in a blank cannabis concentrate matrix (a), and from spiked level of 0.1 µg/g in cannabis concentrate matrix (b).

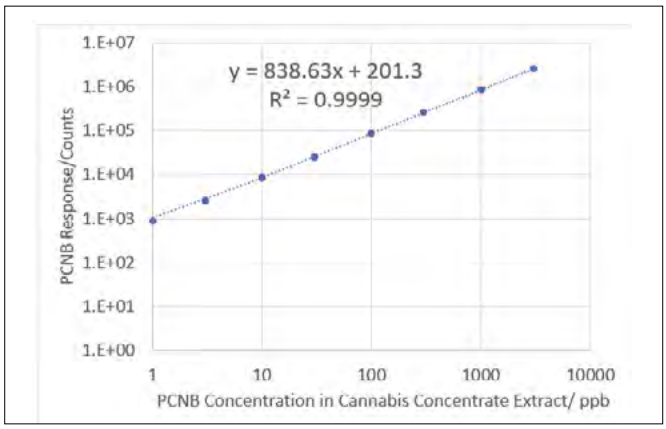

Figure 5. Linearity of PCNB response over 3.5 orders of magnitude in 25 times diluted cannabis concentrate concentrate.

In past studies, different mechanisms for negative APCI ionization, such as proton abstraction, anion adduction, electron capture and dissociative electron capture have been proposed.[8] It has been demonstrated that chlorinated nitrobenzene compounds can form phenoxide ions under negative APCI conditions.[9] Similarly, the following mechanism for ionization of PCNB was proposed in a previous publication, with the APCI source in negative ion mode (where M is PCNB):[10]

$$O_2 + e^- \rightarrow O_2^-$$

$$M + O_2^- \rightarrow [M - Cl + O]^- + ClO$$

Herein, the formation of $[M\text{-}Cl+O]^-$ can be attributed to the formation of the superoxide ion (O_2^-) by electron capture, followed by its chemical reaction with PCNB. This mechanism can be explained further by analyzing Q1 scan data for PCNB infusion into the APCI source. The Q1 scan data showed a monoisotopic base peak at a nominal mass of 274 dalton. The nominal monoisotopic mass of PCNB is 293 dalton, and therefore the mass loss of 19 dalton from an intact molecule of PCNB can be explained by the loss of chlorine (nominal monoisotopic mass of 35) and the addition of an oxygen (nominal monoisotopic mass of 16) atom to the PCNB molecule to form a negatively charged ion. Further, an experimentally observed isotope pattern or ratio of the PCNB ion matched very closely to the theoretical isotope pattern of an ion with four chlorine atoms, and this proved further that PCNB loses one chlorine atom in the APCI ion source. The low mass spectra of the APCI ion source was checked to confirm the formation of the superoxide reagent ion species, which could interact with PCNB to ionize it. It was observed that both the superoxide ion (O_2^-) and the PCNB signal increased roughly by a factor of 300 and 30, respectively, when the mobile phase was changed from 75:25 methanol:water with 0.1% formic acid and 2 mM ammonium formate to just 75:25 methanol:water. This further established that the superoxide ion plays an important role in the ionization of PCNB in the APCI source.

Conclusions

This study demonstrates a unique, quantitative, rapid, and reliable LC/MS/MS method, with dual ESI and APCI sources, for the analysis of various pesticides and mycotoxin residues in cannabis concentrates. In the proposed method, 62 pesticides and five mycotoxins were analyzed with an ESI source and run time of 18 minutes, with an additional four pesticides analyzed with an APCI source and run time of 12 minutes.

The proposed solvent extraction method with 30 internal standards is suitable for labs analyzing samples in accordance with California regulations, as the overall recovery of all pesticides and mycotoxins from the cannabis concentrate matrix was in the acceptable range of 70-130%, with an RSD less than 20%. The method allowed for the identification and quantification of all 66 pesticides and five mycotoxins in cannabis concentrate samples at levels (0.005 to 0.9 µg/g) below State of California action limits.

It was further demonstrated that the analysis of PCNB (a pesticide normally analyzed by GC/MSMS with an EI source) utilizing an APCI source is both selective and sensitive, with excellent linearity. The ability to screen and quantitate all 66 pesticides and five mycotoxins, including the hydrophobic and chlorinated compounds normally analyzed by GC/MSMS, eliminates the requirement of using both an LC/MS/MS and GC/MS/MS instrument for this analysis. This method illustrates the use of LC/MS/MS as a novel, cost effective and efficient way to screen and quantitate pesticides and mycotoxins in a cannabis concentrate matrix with a single LC/MS/MS instrument.

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APPLICATION NOTE

Liquid Chromatography/ Mass Spectrometry

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A Single Cannabis LC/MS/MS Method to Meet California Pesticide and Mycotoxin Residues Regulatory Requirements

To further validate the performance of this method for the industry, The Emerald Test ProficiencyTest (PT) for Pesticides was conducted. The Emerald Test™ is an Inter-Laboratory Comparison and Proficiency Test (ILC/PT) program for cannabis testing labs. The results from the PT inter-laboratory samples passed; therefore, the method meets inter-laboratory reproducibility and accuracy. The method was awarded the Emerald Test badge of approval seen on the right. https://pt.emeraldscientific.com/

Introduction

Over half of the U.S. has legalized the use of medical cannabis due to its therapeutic benefits for ailments such as cancer, multiple sclerosis, and ALS.[1-3] Like traditional agriculture crops, pesticides are sometimes used in cannabis cultivation to protect plants from pests and improve growth yield. Chronic exposure to pesticides can pose serious health risks; therefore, pesticide analysis in cannabis is an important consumer safety topic. Recent news has reported an alarming percentage of cannabis products to be tainted by high levels of pesticide residue, prompting recalls and public-safety alerts. Banned pesticides like myclobutanil, imidacloprid, abamectin, etoxazole and spiromesifen, have been detected as residue on cannabis flowers and concentrated further in extracts and edibles. A case in Colorado recalled 20,000 packages of cannabis flowers in October 2015 due to pesticide contamination, and in November 2016, Oregon officials issued a health alert for specific batches of cannabis. Moreover, many of today's cannabis products are inhaled after combusting them, so there is growing concern among consumers and regulators due to the unknown effects of pesticide compounds when inhaled.[4-5] In addition to pesticides, the growing conditions for cannabis are also conducive to the growth of molds and fungi which can produce carcinogenic mycotoxins including ochratoxin A and aflatoxins. As a result, testing for the levels of pesticide and mycotoxins in cannabis is important to ensure consumer safety and quality control.

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High performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) has emerged as method of choice for pesticide and mycotoxin analysis because it offers superior selectivity, sensitivity, ruggedness, and does not require extensive sample preparation before analysis. Although gas chromatography-mass spectrometry (GC-MS/MS) methods have been developed for pesticide analysis in cannabis samples, they are only applicable to a smaller subset number of analytes. Compounds such as daminozide, a highly polar compound, and abamectin, a high molecular weight compound, are not amenable to analysis by GC-MS/MS because they are heat labile and degrade in either the GC injection port or the column at high temperature. GC-MS/MS methods are not as robust as LC-MS/MS methods for pesticide analysis in complex matrices since they require extensive sample preparation to prevent GC injection port contamination from complex matrices.[6,7]

Since there is no federal guidance for the analysis of pesticides analysis in cannabis samples, different States in the U.S. have developed their own testing guidelines. Oregon was the first state in the U.S. to develop comprehensive guidelines for pesticide residues analysis in cannabis and set regulatory limits for 59 pesticides in cannabis.[8] California has however issued more stringent action limits for 66 pesticides (including all but one of those found on Oregon state list, and eight more) and five mycotoxins residues in cannabis flower and edibles.[9] Numerous reports for pesticide analysis in cannabis have been published but these studies have certain deficiencies.[10-12] Most of these studies either do not achieve detection limits to meet the state of California's action limits; or, use time-consuming sample preparation methods (e.g. QuEChERS with dSPE) with poor recoveries for some of the pesticides, which require use of both LC-MS/MS and GC-MS/MS based instruments for analysis of all the pesticides. This increases cost, complexity, and turnaround time of analysis substantially. In this work, the PerkinElmer application development team analyzed all 66 pesticides (including very hydrophobic and chlorinated pesticides typically analyzed by GC-MS/MS) and five mycotoxins spiked in cannabis flower extracts well below the action limits specified by the state of California. A LC-MS/MS instrument was used with ESI and APCI sources and a simple solvent extraction method with excellent recoveries for all analytes in acceptable range of 70-120%.

Experimental

Hardware/Software

Chromatographic separation was conducted on a PerkinElmer LC-MS/MS QSight® LX50 UHPLC system, while detection was achieved using a PerkinElmer QSight 220 MS/MS detector with a dual ionization ESI and APCI source, which operates independently with two separate inlets. All instrument control, data acquisition and data processing was performed using the Simplicity™ 3Q software platform.

Sample Preparation Method

Below is the step by step sample preparation procedure with 10-fold dilution:

- Take approximately 5 grams of cannabis flower as a representative of each sample batch and grind it finely using a grinder.
- Measure 1 gram of sample and place it into 50 mL centrifuge tube.
- Spike 10 µL of internal standard solution.
- Add 3 steel balls (10 mm in diameter) to the tube for efficient extraction during vortex mixing.
- Add 5 mL of LC/MS grade acetonitrile to the tube and cap it.
- Place the tube on multi-tube vortex mixer and allow it to vortex for 10 minutes.
- Centrifuge extract in tube for 10 minutes at 3000 rpm.
- Filter the solvent into a 5 mL glass amber vial using 0.22 micron nylon syringe-filter and cap it.
- Label the bottle with the sample ID.
- Transfer 0.5 mL of extracted sample into a 2 mL HPLC vial and dilute it with 0.5 mL of LC/MS grade acetonitrile and mix it.
- Inject 3 µL of sample for LC-MS/MS analysis, using pesticide methods.

LC Method And MS Source Conditions

The LC method and MS source parameters are shown in Table 1.

Table 1. LC Method and MS Source Conditions.

LC Conditions	
LC Column	PerkinElmer Quasar Pesticide Column (4.6 × 100 mm, 2.7 µm) Part Number: N9306880
Mobile Phase A (ESI method)	2 mM ammonium formate + 0.1% formic acid (in water)
Mobile Phase B (ESI method)	2 mM ammonium formate + 0.1% formic acid (in methanol)
Mobile Phase Gradient	A 18.5 min. (this time includes both analysis time and column equilibration time) LC-MS/MS method with optimized gradient using ESI source was used for separation and analysis of 63 out of 66 pesticides and five mycotoxins residues at low levels in cannabis matrix with minimal matrix interference. A fast 6 min. LC-MS/MS method with short gradient, optimum mobile phase composition and APCI source was used for measurement of remaining three pesticides.
Column Oven Temperature	30 °C
Auto sampler Temperature	10 °C
Injection Volume	3.0 µL for LC-MS/MS method with ESI source. 10 µL for LC-MS/MS method with APCI source.
MS Source Conditions for ESI Source and APCI Source	
ESI Voltage (Positive)	+5500 V
ESI Voltage (Negative)	-4200V
APCI Corona Discharge	-5 µA
Drying Gas	120 arbitrary units
Nebulizer Gas	350 arbitrary units
Source Temperature	315 °C
HSID Temperature	200 °C
Detection mode	Time-managed MRM™

Results and Discussion

Analytical Challenges for Testing Pesticide Residues in Cannabis Samples

Since the pesticides tested in this study include both polar and non-polar compounds, 100% acetonitrile was used to extract all the analytes from the samples. Due to the cannabis matrix's hydrophobicity, further dilution of the extract was performed with the aqueous mobile phase to make it compatible with reverse phase column. This protocol resulted in lower recoveries of some of pesticides due to precipitation. To achieve a higher performing method, cannabis extracts are diluted with acetonitrile by overall factor of 10 to achieve high recovery of pesticides and reduce matrix effects. However, the reverse phase LC method uses aqueous mobile phase at the beginning of the LC run to help better retain the polar compounds on the column. Injecting an organic solvent such as an acetonitrile sample on the LC leads to poor chromatographic peaks for early eluting polar compounds. To overcome this problem, a small sample injection volume of three microliters was used in this study.

Pesticide analysis in cannabis is very challenging since its matrix composition is very complex and contains compounds from different classes such as cannabinoids, terpenes, hydrocarbons, sugars, fatty acids, flavonoids and others. Sample matrix effect remains the main concern for LC-MS/MS, and leads to variable signal ion suppression and matrix interference. Moreover, quantification of pesticide residues in cannabis is a difficult task due to great disparity in high concentration levels of naturally occurring cannabinoids as well as high terpene content. In this work, we used a generic extraction method with dilution, selected the best MRM transitions and optimized the LC gradient to allow low level analysis of pesticides with good recovery in a complex cannabis matrix.

Normally, analysis of pesticides in cannabis and other food matrices is done by both GC-MS/MS and LC-MS/MS since some non-polar and chlorinated pesticides are difficult to ionize with an electrospray ion source.[13-14] To demonstrate the convenience of a single method, the application team developed a LC-MS/MS method using both APCI and ESI techniques to analyze all the pesticides (California regulated pesticide list) with the additional benefits of improved throughput, reduced complexity and lower cost of analysis. Typically, the dirty matrix found with cannabis samples causes build-up on the sampling interface of a GC-MS/MS and LC-MS/MS systems and this would increase the maintenance costs and downtime resulting in a loss of productivity. It showed that the LC-MS/MS method we developed would be more immune to contamination from the dirty cannabis matrix.

Detectability and Reproducibility

Figure 1 shows MRM chromatograms with excellent signal to noise for a representative set of pesticides spiked at low level of 0.01 µg/g in the cannabis flower. The limits of quantification (LOQs) and response reproducibility at LOQ level for each of the pesticides (category II and I) and mycotoxins in cannabis extract are summarized in Table 2, 3 and 4. The LOQs were determined by considering both the signals of the quantifier and qualifier ions (S/N > 10 for both) and ensuring that the product ion ratios were within the 20% tolerance windows of the expected ratio. As demonstrated in Table 2 and 3, the LOQs determined in this study are well below the California action limit by a factor of 2 to 600 for all category II pesticides and mycotoxins listed. The response RSD for each pesticide and mycotoxin at its LOQ level in the cannabis matrix was less than 20 %. The retention time for each analyte was reproducible within ± 0.1 minute over a 24-hour period. This demonstrates that the method is more than adequately sensitive and reproducible for pesticides and mycotoxins analysis in cannabis at the regulatory limit specified by the state of California.

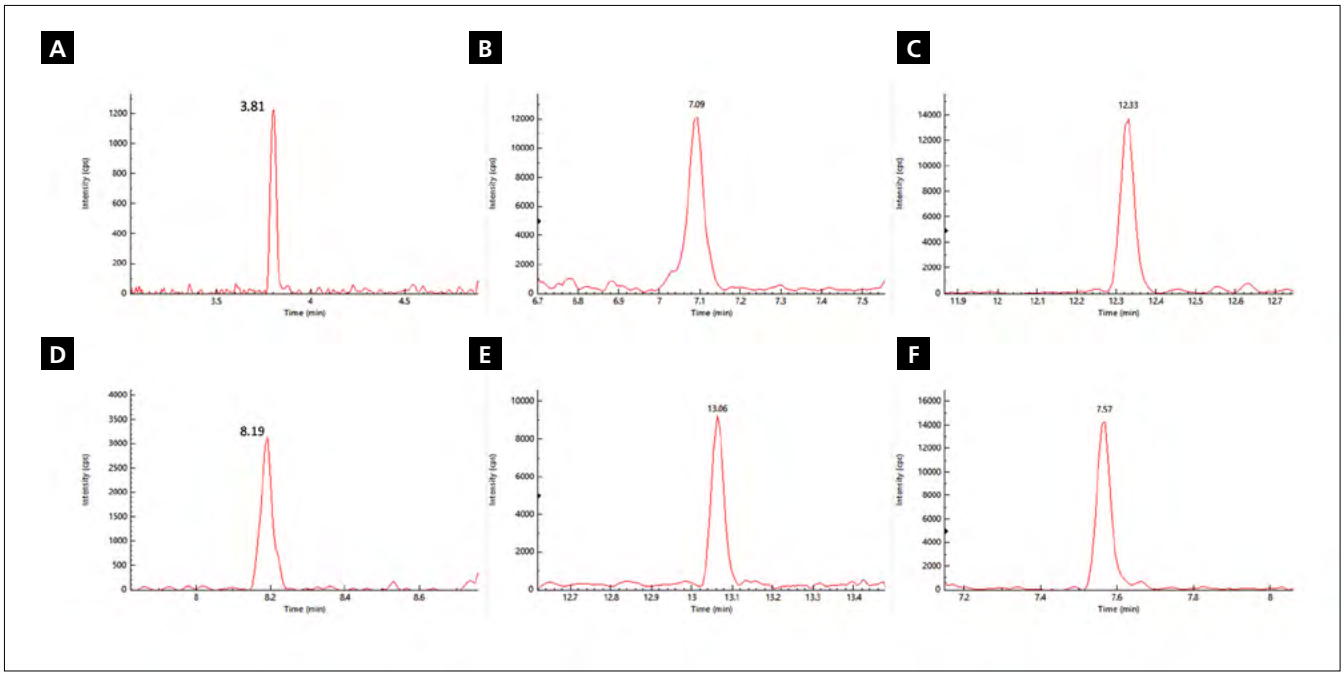

Figure 1. MRM chromatogram of representative set of pesticides-:(a) oxamyl, (b) metalaxyl, (c) fenpyroximate, (d) mycyclobutanil, (e) Etofenprox and (f) Azoxystrobin spiked at level of 0.01 µg/g in cannabis matrix.

Table 2. LOQs for California category II Pesticides with LC-MS/MS in Cannabis. Red/Green: Pesticides typically analyzed by GC-MS/MS, Red: Pesticides analyzed on LC-MS/MS by ESI, Green: Pesticides analyzed on Q-Sight by APCI

S. No.	Category II Residual Pesticide	LOQ		Action Level (µg/g)	Action Level/QSight LOQ
		QSight (µg/g)	%CV (n=7)		
1	Abamectin	0.025	10.6	0.1	4
2	Acephate	0.010	3.1	0.1	10
3	Acequinocyl	0.025	13.3	0.1	10
4	Acetamiprid	0.010	13.1	0.1	10
5	Azoxystrobin	0.005	5.0	0.1	20
6	Bifenazate	0.010	10.8	0.1	10
7	Bifenthrin	0.010	14.4	0.5	50
8	Boscalid	0.025	12.2	0.1	4
9	Captan	0.25	7.0	0.7	2.8
10	Carbaryl	0.010	9.5	0.5	50
11	Chlorantraniliprole	0.025	5.6	10.0	400
12	Clofentezine	0.010	11.3	0.1	10
13	Cyfluthrin	0.25	19.1	1.0	4
14	Cypermethrin	0.100	20.0	1.0	10
15	Diazinon	0.005	3.8	0.2	40
16	Dimethomorph	0.005	1.4	2.0	400
17	Etoxazole	0.005	13.5	0.1	20
18	Fenhexamid	0.010	12.5	0.1	10
19	Fenpyroximate	0.005	6.9	0.1	20
20	Flonicamid	0.010	10.2	0.1	10
21	Fludioxonil	0.050	9.5	0.1	2
22	Hexythiazox	0.005	8.4	0.1	20
23	Imidacloprid	0.010	10.3	3.0	300
24	Kresoxim-methyl	0.025	8.1	0.1	4
25	Malathion	0.010	14.7	0.5	50
26	Metalaxyl	0.010	8.0	2.0	200
27	Methomyl	0.010	8.5	0.1	10
28	Myclobutanil	0.010	10.4	0.1	10
29	Naled	0.010	8.4	0.1	10
30	Oxamyl	0.010	6.7	0.2	20
31	Pentachloronitrobenzene	0.010	13.0	0.1	10
32	Permethrin	0.010	16.0	0.5	50
33	Phosmet	0.005	13.3	0.1	20
34	Piperonylbutoxide	0.005	3.5	3.0	600
35	Prallethrin	0.025	7.4	0.1	4
36	Propiconazole	0.015	8.9	0.1	6.7
37	Pyrethrins	0.1	1.4	0.5	5
38	Pyridaben	0.010	7.9	0.1	10
39	Spinetoram	0.005	13.8	0.1	20
40	Spinosad	0.005	9.3	0.1	20
41	Spiromesifen	0.010	9.4	0.1	10
42	Spirotetramat	0.010	8.4	0.1	10
43	Tebuconazole	0.005	11.0	0.1	20
44	Thiamethoxam	0.010	3.6	4.5	450
45	Trifloxystrobin	0.005	8.4	0.1	20

Table 3. LOQs for California Category II Mycotoxins with LC-MS/MS in Cannabis.

S. No.	Category II Mycotoxin	LOQ		Action Level (µg/g)	Action Level/QSight LOQ
		QSight (µg/g)	%CV (n=7)		
1	Ochratoxin A	0.010	18	0.020	2.0
2	Aflatoxin B1	0.001	18	NA	NA
3	Aflatoxin B2	0.0015	14	NA	NA
4	Aflatoxin G1	0.010	18	NA	NA
5	Aflatoxin G2	0.0015	19	NA	NA
6	Aflatoxin (B1+B2+G1+G2)	0.005	NA	0.020	4.0

Table 4. LOQs for California category I Pesticides with LC-MS/MS in cannabis. Red/Green : Pesticides typically analyzed by GC-MS/MS, Red: Pesticides Analyzed on LC-MS/MS by ESI Green: Pesticides Analyzed on LC-MS/MS by APCI

S. No.	Category I Residual Pesticide	LC-MS/MS LOQ		Action Level (µg/g)	Action Level/LOQ
		(µg/g)	%CV (n=7)		
1	Aldicarb	0.010	10.6	0.1	10
2	Carbofuran	0.010	3.1	0.1	10
3	Chlordane	0.05	13.3	0.1	2
4	Chlorfenpyr	0.05	6.0	0.1	2
5	Chlorpyrifos	0.010	5.0	0.1	10
6	Coumaphos	0.010	10.8	0.1	10
7	Daminozide	0.015	14.4	0.1	6.67
8	DDVP (Dichlorvos)	0.025	12.2	0.1	4
9	Dimethoate	0.010	3.8	0.1	10
10	Ethoprophos	0.010	9.5	0.1	10
11	Etofenprox	0.010	5.6	0.1	10
12	Fenoxycarb	0.010	11.3	0.1	10
13	Fipronil	0.010	19.1	0.1	10
14	Imazalil	0.010	23.1	0.1	10
15	Methiocarb	0.010	3.8	0.1	10
16	Methyl parathion	0.040	1.4	0.1	2.5
17	Mevinphos	0.025	13.5	0.1	4
18	Paclobutrazol	0.010	12.5	0.1	10
19	Propoxur	0.010	6.9	0.1	10
20	Spiroxamine	0.010	10.2	0.1	10
21	Thiacloprid	0.010	9.5	0.1	10

Sample Matrix-Matched Calibration Standards

Matrix matched calibration is the preferred analytical procedure for quantitation because it compensates for matrix effects that are prevalent in cannabis samples. The decrease or increase in response is attributed to ion suppression of the analytes during ionization by the presence of co-eluted matrix compounds. Due to sample matrix effects, a matrix matched calibration curve was used for quantitation and generated by injecting blank cannabis flower extracts and blank cannabis flower extract samples spiked with varying concentrations of pesticides and mycotoxins over a range of 0.1-1000 ng/mL. The calibration curves for all pesticides and mycotoxins were linear with calibration fit of R^2 greater than 0.99 for all the analytes.

Recovery Studies with Solvent Extraction

Utilizing the QuEChERS extraction technique is a common method for extraction of low levels of contaminants such as pesticides from fruit and vegetable matrices with higher water content.[15] The method includes extraction of a broad range of pesticides and removal of sugars, organic acids and other compounds commonly found in fruits and vegetables.[16-20] It is not a suitable method for very polar pesticides, such as Daminozide, which are included in both the California and other states regulatory framework. Since Daminozide is too polar to be extracted efficiently with QuEChERS, it remains in the aqueous phase and does not partition into the organic solvent during salting out step. The recovery of Daminozide from a cannabis matrix with QuEChERS extraction has been reported to be less than 10%.[10] Moreover, a typical cannabis matrix contains mostly hydrophobic compounds such as cannabinoids and terpenes, and therefore the QuEChERS extraction method does not remove the matrix interfering compounds during the salting out step. Different groups have tried to develop an advanced QuEChERS method with d-SPE

step which utilizes PSA and other adsorbents to remove matrix from cannabis extract. However, the addition of the d-SPE step to the QuEChERS method not only makes this method more laborious and expensive, but also leads to low recoveries of compounds such as spinosad, spirotetramat, spioroxamine, ochratoxin A and a few others.[11-12] This is a result of these compounds binding to the PSA adsorbent in the d-SPE step, and resulting in poor recoveries. Due to above shortcomings of the QuEChERS method for extraction of pesticides from a cannabis matrix, the application team used a simple acetonitrile based solvent extraction method for extraction. To confirm this method, fortified cannabis flower samples were used to determine pesticides and mycotoxin recovery. The cannabis flower samples were tested to confirm the absence of pesticides before they were spiked. Five cannabis flower samples were spiked at two levels (low and high) of all pesticides (0.1 and 1 µg/g) and mycotoxins (0.02 and 0.1 µg/g) standard. These two levels were chosen based on regulatory limits, for pesticides and mycotoxins in cannabis, from California and other states. Tables 5-7 show that absolute recoveries of all 66 pesticides and five mycotoxins at two different levels was within acceptable range of 70-120 % with RSD less than 20% for five cannabis flower samples. For two pesticides, the recovery values were not reported at low spiked value since it was below their LOQ value.

LC-MS/MS Method with Optimum MRM Transitions for Challenging Analytes in Cannabis Matrices

As stated, cannabis is a challenging matrix to test, and this is compounded by the low concentration level of the pesticides. To ensure the highest analytical confidence, multiple MRM transitions for a number of pesticides with minimal matrix interference in the cannabis matrix were determined for low level detection. For example, acequinocyl is an insecticide and can be ionized easily as a protonated molecular ion in a standard, but the MRM transitions, based on protonated molecular ion in the cannabis matrix, showed poor LOQ of 0.5 to 1 µg/g about five to 10 times higher than its action limit for the state of California. Therefore, MRM transitions based on alternative modes of ionization, such as adduct formation, were determined to reduce matrix interference and achieve LOQ of 0.025 µg/g (fourtimes below action limits) for acequinocyl in the cannabis matrix. Figure 2 shows the signal overlay of blank cannabis matrix and acequinocyl spiked at level of 0.1 µg/g in cannabis with MRM transitions based on protonated molecular ion and adduct ion of acequinocyl. This figure displays that optimum acequinocyl MRM transitions helped in achieving lower detection limits due to minimal matrix interference.

High molecular weight compounds such as abamectin, and some early eluting polar compounds, such as daminozide, are difficult to measure at low levels using GC-MS/MS since they decompose either in a high temperature GC injector or a GC oven. Although, high molecular weight compounds such as abamectin, and polar compounds such as daminozide, can be ionized with the ESI source, they are also prone to decomposition at high temperatures. Figure 3 shows abamectin response as a function of HSID and source temperature. Based on these results, the optimum temperature values for the ESI source and HSID temperature were set to maximize signals for high molecular weight and polar pesticides. Abamectin is also prone to sodium and potassium adduct formation from the sodium and potassium ions leached into mobile phase from glassware. Since it is difficult to control amount of sodium and potassium ions leached from glassware, the use of the sodium adduct for abamectin as Q1 (parent ion) mass for analysis would lead to response variation. To reduce sodium or potassium adduct formation, a controlled amount of ammonium salt was added to the mobile phase. The combination of ammonium salt in mobile phase and optimum temperature conditions resulted in good and reproducible signals for abamectin.

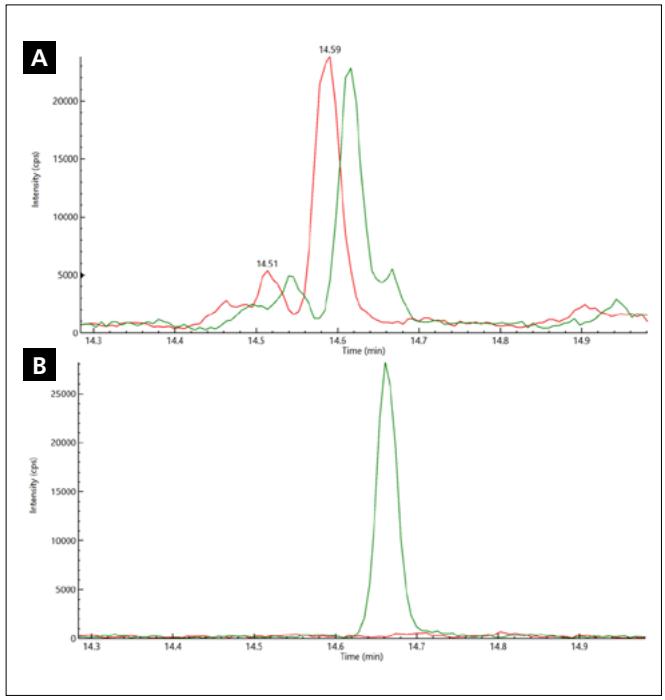

Figure 2. (a) Overlay of response of cannabis matrix (Red) and acequinocyl (Green) spiked at level of 0.1 µg/g in cannabis matrix with MRM transition based on protonated molecular ion and (b)Overlay of response of cannabis matrix (Red) and acequinocyl (Green) spiked at level of 0.1 µg/g in cannabis matrix with MRM transition based on adduct ion.

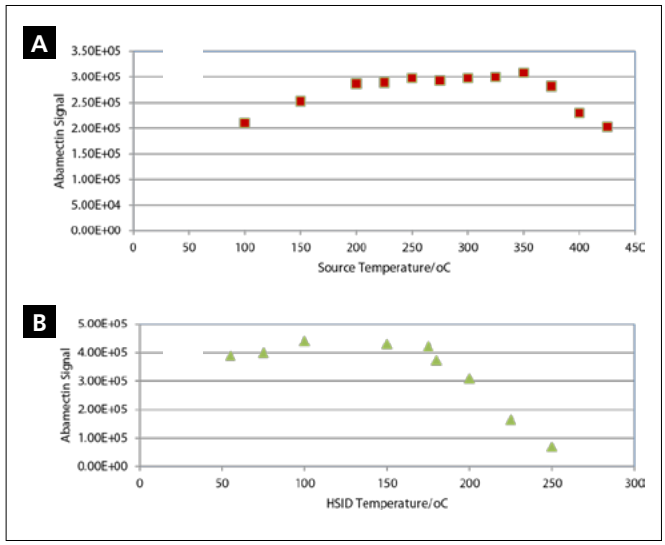

Figure 3. Abamectin signal as a function of ESI source (a) and HSID temperature (b).

Table 5. Recovery of Category II pesticides at two different levels from cannabis with acetonitrile solvent extraction method.

S.No.	Category II Residual Pesticide	Low Level 0.1 µg/g		High Level 1 µg/g	
		Recovery%	RSD % (n=5)	Recovery %	RS% (n=5)
1	Abamectin	85	10	89	9
2	Acephate	93	16	91	9
3	Acequinocyl	90	11	86	6
4	Acetamiprid	87	13	95	9
5	Azoxystrobin	87	12	92	8
6	Bifenazate	88	8	88	7
7	Bifenthrin	84	13	94	7
8	Boscalid	87	10	89	5
9	Captan	NA	NA	70	15
10	Carbaryl	84	12	92	10
11	Chlorantraniliprole	88	13	90	8
12	Clofentezine	87	13	91	12
13	Cyfluthrin	NA	NA	97	17
14	Cypermethrin	98	18	85	13
15	Diazinon	96	10	95	10
16	Dimethomorph	87	15	90	7
17	Etoxazole	89	10	92	10
18	Fenhexamid	87	12	87	7
19	Fenpyroximate	87	9	93	8
20	Flonicamid	93	15	92	12
21	Fludioxonil	94	13	93	8
22	Hexythiazox	86	11	93	7
23	Imidacloprid	89	11	91	9
24	Kresoxim-methyl	91	10	95	8
25	Malathion	90	12	91	7
26	Metalaxyl	86	10	92	8
27	Methomyl	89	10	90	9
28	Myclobutanil	84	10	93	7
29	Naled	87	10	91	7
30	Oxamyl	93	16	94	9
31	Pentachloronitrobenzene	80	16	88	8
32	Permethrin	87	17	92	9
33	Phosmet	86	11	91	7
34	Piperonylbutoxide	91	8	94	8
35	Prallethrin	88	15	94	8
36	Propiconazole	90	14	95	11
37	Pyrethrins	89	12	93	9
38	Pyridaben	84	13	92	9
39	Spinetoram	93	13	94	9
40	Spinosad	88	14	90	10
41	Spiromesifen	90	8	92	5
42	Spirotetramat	97	10	90	7
43	Tebuconazole	94	12	91	7
44	Thiamethoxam	90	10	95	10
45	Trifloxystrobin	86	12	93	9

Table 6. Recovery of Category II mycotoxins at two different levels from cannabis with acetonitrile solvent extraction method.

S.No.	Category II Mycotoxin	Low Level 0.1 µg/g		High Level 1 µg/g	
		Recovery%	RSD % (n=5)	Recovery %	RS%(n=5)
1	Aflatoxin 81	75	15	84	9
2	Aflatoxin 82	78	14	82	9
3	Aflatoxin G1	76	12	85	7
4	Aflatoxin G2	79	12	84	6
5	Ochratoxin A	78	20	83	7

Table 7. Recovery of Category I pesticides at two different levels from cannabis with acetonitrile solvent extraction method.

S.No.	Category I Residual Pesticide	Low Level 0.1 µg/g		High Level 1 µg/g	
		Recovery%	RSD % (n=5)	Recovery %	RS% (n=5)
1	Aldicarb	87	11	94	11
2	Carbofuran	86	11	91	9
3	Chlordane	87	19	92	10
4	Chlorfenapyr	95	15	99	10
5	Chlorpyrifos	94	8	92	8
6	Coumaphos	90	12	95	10
7	Daminozide	82	15	80	14
8	DDVP (Dichlorvos)	94	14	91	11
9	Dimethoate	89	11	96	9
10	Ethoprop(hos)	92	9	94	7
11	Etofenprox	88	13	93	8
12	F enoxycarb	91	11	93	7
13	Fipronil	89	9	95	8
14	Imazalil	86	10	89	10
15	Methiocarb	81	9	93	6
16	Methyl parathion	89	14	96	11
17	Mevinphos	86	10	95	10
18	Paclobutrazol	79	13	90	6
19	Propoxur	91	13	93	9
20	Spiroxamine	88	9	89	9
21	Thiacloprid	89	13	95	10

Analysis of Pesticides, Typically Analyzed by GC-MS/MS, by LC-MS/MS

A number of pesticides in cannabis, regulated by California and other states, are analyzed traditionally using GC-MS/MS with an EI source since these pesticides have low proton affinity, which results in low ionization efficiency with the ESI source. Some examples of these pesticides analyzed normally with GC/MS are cypermethrin, cyfluthrin, captan, naled, permethrin and pyrethrins. To achieve the required sensitivity, the selected MRM's were optimized with a heated electrospray source. LOQ for these analytes were in the range of 0.01 to 0.25 µg/g, well below the California action limits.

Analysis of Pyrethrin Isomers in Cannabis

The pyrethrins are a class of organic compounds normally derived from chrysanthemum cinerariifolium that have potent insecticidal activity by targeting the nervous systems of insects. Pyrethrins are a group of six isomers and their structures are displayed in Figure 4. The naturally-occurring pyrethrins, extracted from chrysanthemum flowers, are esters of chrysanthemic acid (pyrethrin I, cinerin I, and jasmolin I) and esters of pyrethric acid (pyrethrin II, cinerin II, and jasmolin II). In the U.S., the pyrethrum extract is standardized as 45–55% w/w total pyrethrins and in a commercially available pyrethrin standard, the percentage of pyrethrin I, pyrethrin II, cinerin I, cinerin II, jasmolin I and jasmolin II is about 56.1, 27.8, 5.7, 3.8, 4 and 2.6%, respectively. A number of compounds in cannabis mimic the structure of pyrethrins, and therefore the analysis of pyrethrins in cannabis is very difficult due to matrix interference. The optimum MRM transitions and LC gradient were developed to analyze the six pyrethrins at low levels in the cannabis matrix with minimal matrix interference. The LOQs, with LC-MS/MS method utilizing optimum MRM transitions and LC gradient, for six pyrethrins -: pyrethrin I, pyrethrin II, cinerin I, cinerin II, jasmolin 1 and jasmolin II were 0.1, 0.1, 0.01, 0.03, 0.025 and 0.01 µg/g, respectively in cannabis flowers.

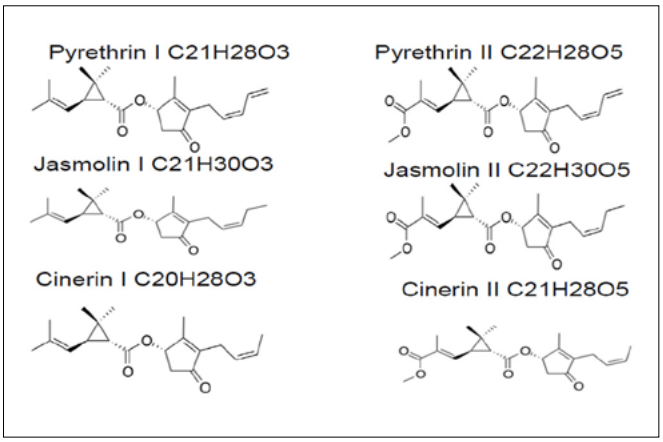

Figure 4. Structure of 6 isomers of pyrethrins.

Pesticides, Which Do Not Ionize Effectively with ESI, Analyzed with APCI

Hydrophobic and halogenated pesticides (eg. pentachloronitrobenzene and chlordane) are traditionally analyzed by GC-MS/MS since they do not ionize effectively by LC-MS/MS with an ESI source. For reference, the structure of the chlorinated pesticides is shown in Figure 5. Since Pentachloronitrobenzene (PCNB) does not contain either hydrogen atoms, for loss of protons, or functional groups with either high proton affinity or which can form ammonia or sodium adducts, it cannot be ionized with the ESI source. Similarly, chlordane is highly chlorinated and has very low proton affinity and therefore difficult to ionize efficiently with an ESI source. Since an APCI ion source is better suited for ionization of very hydrophobic and non-polar analytes, APCI was used to determine the detection limits of pentachloronitrobenzene and chlordane in cannabis. Also, the APCI ion source was used for low level analysis of chlorfenapyr in cannabis, since limits of detection for chlorfenapyr were improved by a factor of two with APCI source in comparison to ESI source due to less ion suppression. Figure 6 shows excellent signal to noise (S/N >=100) for pentachloronitrobenzene (PCNB) spiked at level of 0.1 µg/g in the cannabis matrix using a LC-MS/MS system with an APCI source. Using a fast six minute LC-MS/MS method with short LC gradient and APCI source, LOQ of pentachloronitrobenzene, chlordane and chlorfenapyr in cannabis was 0.01, 0.05 and 0.05 µg/g, respectively.

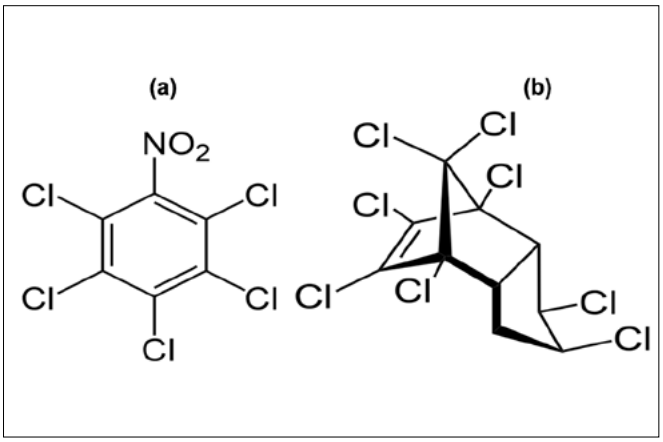

Figure 5. Structure of pentachloronitrobenzene (a) and chlordane (b).

Long Term Stability Data with StayClean™ Source in LC-MS/MS

Long term stability data for pesticide and mycotoxin analysis in cannabis samples was collected using a LC-MS/MS system, fitted with dual ESI and APCI sources, and combined with a heated and self-cleaning StayClean source with a laminar flow interface. Figure 7 shows long term response and stability of the method for 100 ng/ml of Diazinon spiked in cannabis extract over one week. Long term stability data for pesticide analysis in cannabis showed that response RSD over one week for most of pesticides and mycotoxins was between 1.5 to 20%. These results demonstrated that the heated self-cleaning source in the LC-MS/MS system would reduce maintenance needs that are usually prevalent with this matrix. Most published LC-MS/MS methods do not show long term stability data or state that they have to clean the electrospray source frequently to maintain the sensitivity of mass spectrometer.[21] Also, they divert the LC flow to waste for the first few minutes, and after the last peak elutes out to reduce contamination from unretained and late eluting matrix compounds. In this study, excellent long-term stability data was obtained without diverting the LC flow from the MS in the first few minutes, at the end of run, and without periodical cleaning of ion sources.

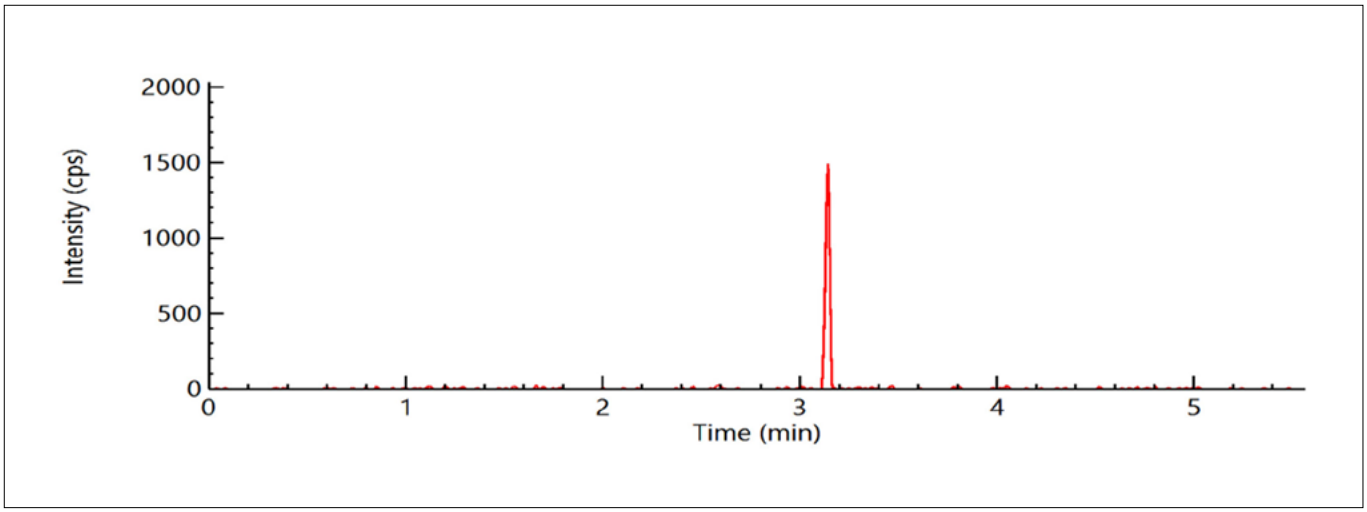

Figure 6. Sample chromatogram of pentachloronitrobenzene (PCNB) spiked at level of 0.1 µg/g in a cannabis matrix using LC-MS/MS system with APCI source.

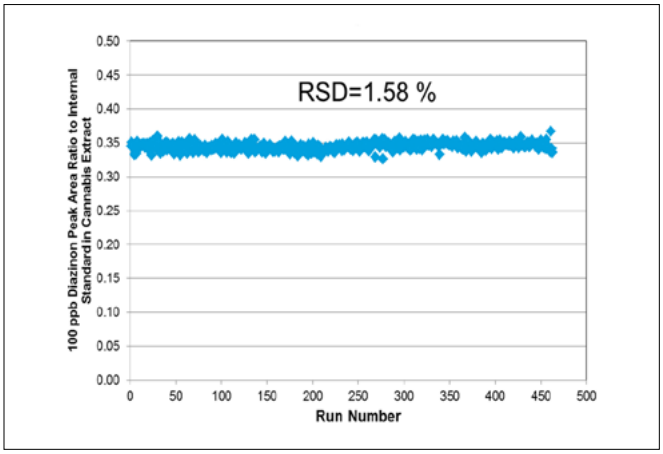

Figure 7. Long term stability data over one week of injections of diazinon at a level of 100 ng/mL spiked in cannabis flower matrix extract.

Conclusions

This study demonstrates a unique, quantitative, rapid, and reliable LC-MS/MS method for analysis of different cannabis pesticides and mycotoxins residues in cannabis samples. The proposed solvent extraction method is suitable for labs wanting to comply with the state of California regulations, as the recovery of all pesticides and mycotoxins from a cannabis matrix was in the acceptable range of 70-120% with RSD less than 20%. This method allowed identification and quantification of all 66 pesticides and five mycotoxins at low levels (0.005 to 0.25 µg/g), which is well below the actions limits set by the state of California with good precision. The ability to screen and quantitate all 66 pesticides, including the very hydrophobic and chlorinated compounds normally analyzed on a GC-MS/MS amenable, and the five mycotoxins, makes this method a novel way to screen and quantitate pesticides and mycotoxins in cannabis with a single instrument.

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APPLICATION SUMMARY

Weighing Up the Risks of Heavy Metals

Despite the name, heavy metals are hardly ever heavy – especially in trace levels in soil. But that does not mean that cannabis lab analysts should take them lightly. As natural hyper-accumulators, cannabis plants can absorb these minute metals in alarming quantities, which can cause big problems for cannabis consumers. Lead exposure alone has been linked to high blood pressure, kidney damage, and abdominal pain[1] – and that's just one of four heavy metals that most US states recommend testing for in cannabis samples.

While most states agree on which heavy metals they should be looking for in cannabis samples, they do not always agree on what amounts are permissible. California, for example, accepts up to 0.2 micrograms (µg) of arsenic per gram of inhaled cannabis goods, whilst Washington allows up to 2 µg per gram of products. And as arsenic exposure can cause immediate vomiting and abdominal pain[2], these conflicting regulations aren't exactly an encouraging sign for consumers.

But these differing directives are not the only reason why heavy metal testing can give cannabis analysts headaches. As the quantities of heavy metals in cannabis samples can be so small, stringent equipment is necessary to ensure every impurity is detected. Fortunately, the inductively coupled plasma mass spectrometry (ICP-MS) technique is well equipped for the job. Thanks to its strength in detecting low levels in complex matrices, the technique is ideal for trace metal analysis in cannabis samples.

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APPLICATION NOTE

ICP - Mass Spectrometry

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Digestion, Testing, and Validation of Heavy Metals in Cannabis

To further validate the performance of this method for the industry, The Emerald Proficiency Test (PT) for Heavy Metals was conducted. The Emerald Test™ is an Inter-Laboratory Comparison and Proficiency Test (ILC/PT) program for cannabis testing labs. The results from the PT inter-laboratory samples passed; therefore, the method meets inter-laboratory reproducibility and accuracy. The method was awarded the Emerald Test Badge seen on the right.
https://pt.emeraldscientific.com/

Introduction

Owing to the toxicity of heavy metals, it is increasingly important to test cannabis flowers and other cannabis derivatives so that patient and consumer safety is maintained as the use of cannabis becomes more common. This need has translated into an increasing demand for testing cannabis flowers and other cannabis derivatives for toxins such as the heavy metals cadmium (Cd), lead (Pb), arsenic (As), and mercury (Hg). Similar to federal pharmaceutical and nutraceutical requirements in the US[1-5], states like California[6], Oregon, and Colorado have published action limits for heavy metals.

Each jurisdiction where cannabis is permitted has published required maximum allowable heavy metals in cannabis and related products. Many of these limits are based on USP <232>/ICH Q3D recommendations. The limits differ based on the route of administration, similarly to what is set out in the ICH Q3D recommendations. Currently, Canada has not set regulations around metals in cannabis products, but is referring to USP <232> and <233> for guidance. Some of the currently known limits for heavy metals are provided in Table 1. For the purpose of this study, the California limits on "all inhaled cannabis goods" were used as they are the most stringent and most applicable to cannabis flower.

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Table 1. A list of the heavy metals and their limits based on jurisdiction and route of administration.

	Canada (Based on USP <232>)	California[6]		Colorado	Connecticut, Maryland, Nevada, New Mexico	Massachusetts		Minnesota	Washington
Heavy Metal	Inhaled Cannabis Goods (µg/g)	All Inhaled Cannabis Goods (µg/g)	Other Cannabis Goods (µg/g)	Flower, Concentrates and Infusions (ppm)	"µg/kg of body weight per day"	All Uses (µg/kg)	Ingestion Only (µg/kg)	PPM in Final Product	µg/Daily Dose (5 grams)
Cadmium (Cd)	0.2	0.2	0.5	0.4	0.09	200	500	0.3	4.1
Lead (Pb)	0.5	0.5	0.5	1	0.29	500	1000	1.0	6.0
Arsenic (As)	0.2	0.2	1.5	0.4	0.14	200	1500	1.5	10.0
Mercury (Hg)	0.1	0.1	3	0.2	0.29	100	1500	0.5	2.0

Several challenges arise in the elemental analysis of cannabis. Of primary consideration is the required sample preparation and digestion. To account for the wide variety of cannabis sample types (flower, concentrates, edibles, extracts, tinctures, waxes, and oils etc.), a robust sample preparation scheme must be employed. Typically, preparation consists of homogenization followed by microwave digestion to break down the complex matrix and extract the heavy metals. Therefore, specific sample prep protocols, microwave digestion conditions, and ICP Mass Spectrometry (ICP-MS) methodology were developed and employed to offer a robust method for all cannabis sample types.

ICP-MS is a very effective technique for trace metal analysis. Due to its ability to see low levels in complex matrices, it is the ideal tool for the determination of trace metals in cannabis samples, especially since normal levels for some analytes are extremely low (sub-ppb).

In this application note, we present data to illustrate the successful validation of the Titan MPS™ Microwave Sample Preparation System and the NexION® ICP-MS for the determination of heavy metals in cannabis flower according to the validation protocols set in USP General Chapter <233>, which are commonly used for evaluation of the levels of elemental impurities in samples.

Experimental

Sample Preparation Procedure

In this work, all samples were digested using microwave digestion (Titan MPS System: PerkinElmer Inc., Shelton, Connecticut, USA) with standard 75 mL TFM vessels. Approximately 3-5 grams of cannabis flower was ground and homogenized. The California-proposed regulations require that "the laboratory shall analyze at minimum 0.5 grams of the representative sample of cannabis goods or cannabis product to determine whether heavy metals are present".[6] Therefore, 0.50 ± 0.05 g of each sample was weighed on a weight boat and then transferred into a digestion vessel, followed by 7 mL of nitric acid (70%), and 3 mL of hydrogen peroxide (30%). The vessels were left uncapped for ten minutes to allow for any pre-reactions to occur safely before being capped and digested following the program in Table 2. To evaluate the effect of the sample preparation on analyte recovery, spikes were added to the microwave vessel prior to the addition of the reagents. To stabilize mercury, 200 ppb gold (Au) was added to each sample.

Upon completion of the digestion, all samples were diluted with deionized water to a final volume of 50 mL. This resulted in a total dilution factor of 100x with a reagent matrix of 14% HNO_3. Calibration standards were prepared in this same matrix. Figure 1 shows the cannabis flower and the resulting clear solution after digestion and preparation for analysis.

Table 2. Titan MPS System microwave digestion program for dissolution of cannabis samples.

Step	Target Temp (°C)	Pmax (bar)	Ramp (min)	Hold (min)	Power (%)
1	160	30	5	5	90
2	200	30	5	20	100
3	50	30	1	30	0

Figure 1. Cannabis flower before and after digestion.

Instrumentation

A PerkinElmer NexION ICP-MS, which includes the proprietary Universal Cell Technology™ (UCT) as well as the All Matrix Solution (AMS) system, was used for the analysis. The NexION ICP-MS was configured with the standard SMARTintro™ sample introduction module consisting of a MEINHARD® glass concentric nebulizer, glass cyclonic spray chamber, and a quartz torch with 2 mm id injector.

The instrument operating parameters are shown in Table 3. To reduce the matrix loading in the plasma and provide robust plasma conditions for the high sample matrix, an AMS dilution factor was set to approximately 3x. All analytes were acquired in Collision mode using helium. Using this simple methodology, the UCT reduces or eliminates all common polyatomic interferences using kinetic energy discrimination (KED).

Calibration

To cover the wide range of concentrations for all cannabis sample types, including concentrates and extracts, a calibration was developed using a blank and four calibration standards. The elements, masses, and standard concentrations are shown in Table 4. As stated in the previous section, the calibration blank and standard were prepared in 14% nitric acid to matrix match with the samples. To stabilize mercury, 200 ppb gold (Au) was added to the calibration blank and each standard.

To monitor the instrument response from sample to sample, internal standards (Ge, In, and Tb) were added on-line.

Results and Discussion

Method Validation

USP General Chapter <233> defines the following requirements for method validation:

Accuracy: The matrix and materials under investigation must be spiked with target elements at concentrations that are 50%, 100%, and 150% of the maximum permitted daily exposure (PDE). Mean spike recoveries for each target element must be within 70%-150% of the actual concentrations.

To calculate the appropriate spike levels, we used the California inhalational limits for all inhaled cannabis goods. The 50%, 100%, and 150% spike levels were calculated based on a nominal preparation factor of 100. The limits and spike levels used for this study are shown in Table 5.

Repeatability: Six independent samples of the material under investigation must be spiked at 100% of the target limits defined and analyzed. The measured percent relative standard deviation (%RSD) must not exceed 20% for each target element.

Ruggedness: Carrying out the repeatability measurement testing procedure by analyzing the six repeatability test solutions either on different days, either with a different instrument or by a different analyst. The %RSD of the 12 replicates must be less than 25% for each target element.

Sample Analysis

All quantitative sample data were less than the lowest calibration standard and, as a result, were less than the target limits for the heavy metals in inhalable cannabis products.

Table 3. NexION ICP-MS Operating Conditions.

Parameter	Value
RF Power (W)	1600
Nebulizer Flow (L/min)	0.88
Dilution Gas Flow (L/min)	0.11
Sample Uptake Rate (mL/min)	0.20
Collision (He) Gas Flow (mL/min)	4

Table 4. Elements and standard concentrations.

Analyte	Mass	Standard 1 (µg/L)	Standard 2 (µg/L)	Standard 3 (µg/L)	Standard 4 (µg/L)
Cadmium (Cd)	110.90	0.5	1	5	10
Lead (Pb)	207.98	1.25	2.5	12.5	25
Arsenic (As)	74.92	0.5	1	5	10
Mercury (Hg)	201.97	0.1	0.2	1	2

Table 5. PDEs and Spike Levels.

Analyte	PDE for Inhaled Products	Spike Level (µg/L)		
		50% PDE	100% PDE	150% PDE
Cadmium (Cd)	0.2	1.00	2.00	3.00
Lead (Pb)	0.5	2.50	5.00	7.50
Arsenic (As)	0.2	1.00	2.00	3.00
Mercury (Hg)	0.1	0.50	1.00	1.50

Table 6. Sample Results.

Element	Sample Results			Units (µg/g)			Pass/Fail
	1	2	3	Mean	SD	Limit	
Cadmium (Cd)	0.029	0.037	0.042	0.036	0.006	0.2	Pass
Lead (Pb)	0.009	0.021	0.010	0.013	0.007	0.5	Pass
Arsenic (As)	0.027	0.030	0.045	0.034	0.010	0.2	Pass
Mercury (Hg)	0.056	0.044	0.044	0.048	0.007	0.1	Pass

Meeting the Validation Criteria

All quantitative sample data were less than the lowest calibration standard and, as a result, were less than the target limits for the heavy metals in inhalable cannabis products.

Accuracy

The accuracy data of the methodology is exemplified in Table 7, which shows that the pre-digestion spike recovery test in the sample matrix passes at all three spike levels (50%, 100%, and 150% of the target limits) with the mean spike recoveries for each target element well within the 70-150% acceptance criteria.

Repeatability

Six independently prepared samples of a cannabis flower were digested and then spiked at 100% of the target limit and analyzed. As shown in Table 8, the %RSDs for all target elements were within 3%, which is well under the 20% acceptance limit.

Ruggedness

The six samples used for the repeatability study shown in Table 7 were prepared by two different analysts. The RSDs for these twelve measurements are all < 2.5% (as shown in Table 9), well below the method requirement of 25%.

Table 7. Accuracy Test Results.

Element	Mean Unspiked Sample (µg/g)	Mean Recovery (%)			Pass/Fail
		50%	100%	150%	
Cadmium (Cd)	0.036	87	94 %	91	Pass
Lead (Pb)	0.013	81	85 %	84	Pass
Arsenic (As)	0.034	94	96 %	98	Pass
Mercury (Hg)	0.005	97	95 %	107	Pass

Table 8. Repeatability Test Results.

Element	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Mean	%RSD	Pass/Fail
	(µg/g)	(µg/g)	(µg/g)	(µg/g)	(µg/g)	(µg/g)	(µg/g)		
Cadmium (Cd)	0.22	0.21	0.23	0.22	0.23	0.23	0.23	2.90	Pass
Lead (Pb)	0.22	0.21	0.23	0.22	0.23	0.23	0.23	2.90	Pass
Arsenic (As)	0.43	0.43	0.44	0.43	0.45	0.47	0.44	1.10	Pass
Mercury (Hg)	0.23	0.22	0.22	0.23	0.24	0.24	0.23	1.10	Pass

Table 9. Ruggedness Test Results.

Element	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	Sample 12	Mean	% RSD
	(µg/g)	(µg/g)	(µg/g)	(µg/g)	(µg/g)	(µg/g)	(µg/g)	(µg/g)	(µg/g)	(µg/g)	(µg/g)	(µg/g)	(µg/g)	
Cadmium (Cd)	0.22	0.21	0.23	0.22	0.23	0.23	0.20	0.21	0.19	0.21	0.19	0.21	0.21	7.07%
Lead (Pb)	0.43	0.43	0.44	0.43	0.45	0.47	0.38	0.43	0.38	0.42	0.38	0.43	0.42	6.85%
Arsenic (As)	0.23	0.22	0.22	0.23	0.24	0.24	0.20	0.22	0.21	0.22	0.21	0.22	0.22	5.26%
Mercury (Hg)	0.10	0.10	0.10	0.10	0.10	0.11	0.09	0.10	0.09	0.11	0.09	0.10	0.10	4.96%

Conclusion

This work has demonstrated the ability of PerkinElmer's NexION ICP-MS coupled with the Titan MPS Sample Preparation System to perform accurate and reproducible analyses of cannabis flower samples. Using PerkinElmer's AMS and Universal Cell Technology, a robust method was developed. All quantitative sample data were less than the target limits for heavy metals in "Inhaled Cannabis Goods". This work easily passed the acceptance criteria for the testing protocols described in USP General Chapter <233>.

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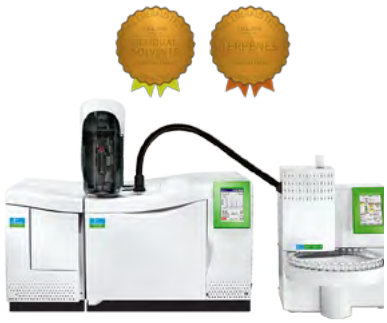

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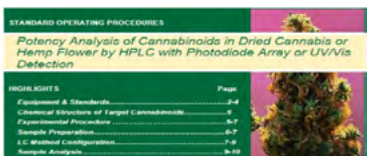

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