APPLICATION NOTE

FT-IR Spectroscopy

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Optimization of Cannabis Grows Using Fourier Transform Mid-Infrared Spectroscopy

Introduction

Cannabis use is becoming increasingly legal in the United States. One of the main products of this industry is dried cannabis flowers. The inflorescences, or flowers, of cannabis plants contain tetrahydrocannabinolic acid (THCA), which decarboxylates upon the addition of heat, as shown in Figure 1, to produce carbon dioxide and the psychoactive form of THCA, tetrahydrocannabinol (THC).

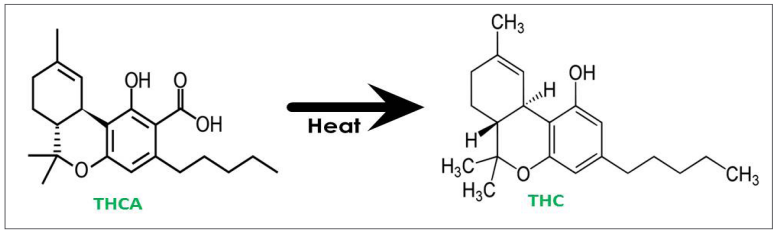

Figure 1. The conversion of acid form to neutral form via decarboxylation reaction that occurs upon addition of heat to THCA, producing the psychoactive compound THC.

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In Type II and Type III medicinal cannabis cultivars, or cannabis plants that contain cannabidiolic acid (CBDA), a similar reaction occurs wherein CBDA is converted to cannabidiol (CBD).

Since THCA and CBDA are produced by the plant in acid form, most cannabinoids are detected as their respective acids. Therefore, Type I cannabis flowers usually contain 15-25% THCA by weight and minimal amounts of THC. Similarly, CBD containing cannabis flowers have significant amounts of CBDA and small amounts of CBD. Traditionally, cannabinoid analyses have been performed by first extracting the cannabinoids with a solvent and then performing gas chromatography (GC) or high pressure liquid chromatography (HPLC). Currently, a grower or dispensary must send several grams of valuable product off to a third party lab, pay a fee for each sample to be analyzed, and often wait days or weeks for results. This delay makes it difficult to utilize the data in a meaningful time frame. Because of these problems, the cannabis industry would benefit from a real time potency spot test that is inexpensive, fast, portable, and easy to perform. To this end, the use of Fourier Transform Infrared (FT-IR) spectroscopy for determining THCA, CBDA, and other cannabinoids in cannabis flowers was investigated.

The infrared spectra of pure THCA and a cannabis flower are shown in Figure 2

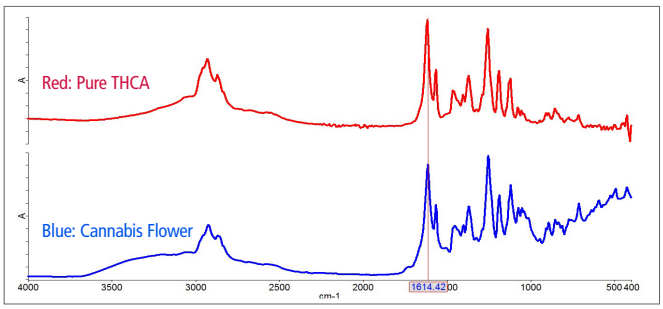

Figure 2. Top (red): The infrared spectrum of pure tetrahydrocannabinol (THCA). Bottom (blue): The infrared spectrum of a cannabis flower.

The infrared spectra of pure THCA and a cannabis flower in Figure 2 are plotted with wavenumber (related to wavelength) on the x-axis and absorbance, a measure of the light absorbed, on the y-axis. In infrared spectra the peak positions correlate to molecular structure, while the measured absorbance is proportional to concentration. Thus, FT-IR can be used to determine unknown molecular structures and measure concentrations of molecules in samples. The two spectra in Figure 2 are similar because in recreational cultivars THCA is the molecule present in highest concentration so it dominates the spectrum of cannabis flowers.

Quantitative spectroscopy is based upon Beer's Law as seen in equation 1.[1]

$$A = \varepsilon l c \quad (1)$$

A = Absorbance (peak height or area in a spectrum)

ε = Absorptivity (a constant for a given molecule and absorbance wavelength)

l = Pathlength (thickness of sample seen by light beam)

c = Concentration of absorber (analyte)

The tricky part of applying Beer's Law to the analysis of cannabis flowers is that they contain many different molecules, and it is not always possible to find an infrared peak that is solely due to a specific analyte. Chemometrics can be used to solve this problem. Chemometrics is the application of statistical algorithms to chemical data. Chemometric algorithms have the advantage of tolerating overlapped peaks, models do not need to include the concentration of every chemical species present, and multiple analytes are easily determined. For more information on chemometric algorithms please see this book on quantitative spectroscopy.[1]

Experimental

A PerkinElmer Spectrum Two™ FT-IR equipped with a deuterated tryglycine sulfate (DTGS) detector was used to measure all infrared spectra. Each spectrum was collected from 4000 cm^{-1} to 400 cm^{-1} using 4 scans and 8 cm^{-1} instrumental resolution. PerkinElmer Spectrum 10™ software was used to collect and process all spectral data. A PerkinElmer Universal Attenuated Total Reflectance (uATR) accessory was installed in the sample compartment of the Spectrum Two. The uATR was equipped with a single bounce diamond ATR crystal. The depth of penetration (pathlength) for cannabis flowers was 1.7 microns, and is essentially sample independent. Consistent force was applied to each sample using the pressure monitoring system provided. The flat pressure clamp tip was used. The limit of detection of the ATR technique for many analytes is about 0.1%.[2]

The ATR crystal was cleaned before and after each sample by squirting a few drops of methanol on a paper towel, and then wiping down the sampling surface. Some bud samples were ground for one minute in a coffee grinder and then had their spectra measured. Other bud samples were analyzed intact as seen in Figure 3. When intact flowers are analyzed the test is non-destructive and the valuable product can be used or sold.

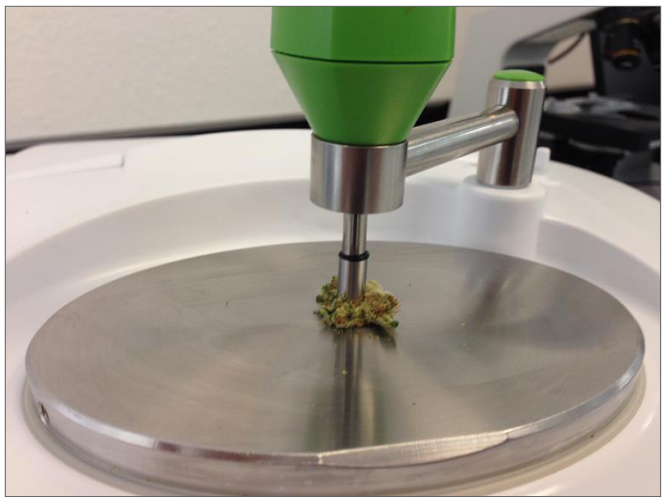

Figure 3. A dried cannabis flower clamped to the diamond ATR crystal of a PerkinElmer uATR accessory, which is installed in the sample compartment of a PerkinElmer Spectrum Two FT-IR.

It was found that compressing samples once, releasing the pressure, and then re-applying the pressure gave more reproducible results. Initially models for ground and intact flowers were developed separately. However, it was discovered that these two sets of models gave equivalent accuracy. Thus, for the final models discussed below spectra of ground and intact flowers were combined.

Quantitative FT-IR models were developed using PerkinElmer Spectrum Quant software. One model per analyte was generated to allow calibration optimization. All models were built using the Partial Least Squares One (PLS1) algorithm using second derivative spectra. Outliers were eliminated as justified. Reference cannabinoid concentrations were obtained using the peer reviewed HPLC method developed by NaPro Research in California.[3] Cannabinoid concentrations for about 100 different cannabis samples were generated representing dozens of different cultivars. Typically three spectra of each sample were measured, giving a database of about 300 spectra. A set of 27 validation samples were used to determine each model's accuracy as a standard error of prediction (SEP).[1]

Results and Discussion

Quantitation of Cannabinoids in Dried Cannabis Flowers by FT-IR

Table 1 lists the accuracy, the calibration concentration range, and correlation coefficient (R^2) for the seven cannabinoids quantitated by FT-IR. R^2 is a measure of model quality and equals 100 for a perfect calibration.

Table 1. Results for FT-IR Cannabinoid Determinations.

Cannabinoid Quantitated	Range Weight %	R^2	Accuracy Wt. %
THCA	0 - 23.8	94.6	±0.86
THC	0 - 3.7	95.1	±0.13
CBDA	0 - 20.8	93.2	±0.8
CBD	0 - 2.3	90.2	±0.08
CBGA	0 - 3.0	89.7	±0.12
CBG	0 - 0.25	78.2	±0.02
THCVA	0 - 0.14	70.3	±0.01

The weight percent ranges for each analyte were determined by the upper and lower limit of each analyte found in the standard samples. Only 7 cannabinoids were quantitated by FT-IR because these were the only ones found by HPLC in the standard samples.

In addition to the four cannabinoids discussed above, calibrations for cannabigerolic acid (CBGA), cannabigerol (CBG), and tetrahydrocannabivarinic acid (THCVA) were obtained. CBGA is the chemical precursor of THCA and CBDA, and CBG is CBGA's non-acid form. THCVA and THCA are similar, but differ in that THCVA contains a propyl chain, whereas THCA contains a pentyl chain. THCVA is found in measureable concentrations in some cultivars, and its presence can be used to distinguish cultivars from each other.

The accuracies listed in Table 1 are the absolute errors in the measurements. For example, the accuracy for THCA is ±0.86 weight %. This means the error bar on all THCA determinations is ±0.86%, regardless of whether the predicted value is 1 wt. % or 25 wt. %. Given this accuracy, the FT-IR method is capable of distinguishing flowers that are 19% and 21% THCA from each other.

It is important for any analysis of cannabis flowers to be able to distinguish between the acid and non-acid forms of the cannabinoids, for example to be able to separately quantitate THCA from THC, and CBDA from CBD. Table 1 shows that separate and accurate FT-IR models have been developed for all of these cannabinoids, proving that FT-IR can distinguish between and quantitate the acid and non-acid forms of cannabinoids.

The HPLC methodology developed by NaPro Research quantitates the concentrations of 16 cannabinoids,[3] which is considered a full cannabinoid profile. The FT-IR method determines seven cannabinoids as seen in Table 1, which comprises a partial cannabinoid profile. It should also be noted that the list above covers the main cannabinoid constituents responsible for observed pharmacology and psychoactivity most commonly contained by flowers in the marketplace. Growers and breeders can thus use FT-IR to obtain a partial cannabinoid profile in a fast, easy, and portable manner, which can be used to optimize growing conditions or design breeding programs.

Using FT-IR to Monitor and Optimize Cannabis Grows

Growing Time

Monitoring the potency of cannabis grows is useful for optimizing growing conditions, maximizing product value, and determining harvest time. It would be expected that cannabinoid concentration would increase with growing time. The FT-IR method described herein was used to determine the CBDA concentration of a type III medicinal cannabis cultivar, as determined by weight % CBDA, as seen in Table 2.

Table 2. Growing Time and Potency for Cannabis Strain CBD 11.

Growing Time	Weight % CBDA by FT-IR	Weight % CBDA by HPLC
Week 3	5.2%	6.5%
Week 4	11.6%	10.4%
Week 5	12.4%	13.1%
Week 6	15.5%	17.2%

Table 2 shows weight percent CBDA increases proportionally with growing time as expected. Both FT-IR calculations and weight percent CBDA as determined by HPLC[3] demonstrate the same trend in Table 2. The consistent measurements between the two methodologies further underscores the accuracy of FT-IR as a tool for cannabis analysis.

Lighting Conditions

In addition to growing time, another variable expected to determine bud potency is the light intensity a cannabis plant is exposed to while growing. The thought being that increased light energy will lead to increased amounts of photosynthesis and hence increased cannabinoid concentrations. This hypothesis was tested by using FT-IR to determine the THCA level in flowers grown under different lighting conditions as seen in Table 3. The FT-IR tests were performed at a grow site, confirming the portability of the system. Three samples of each cultivar were analyzed by FT-IR, and their potency values were averaged.

Table 3. Effect of Lighting Conditions on THCA Concentration as Measured by FT-IR.

Strain and Lighting Condition	Weight % THCA by FT-IR
Star, new light bulbs	18.1%
Star, 6 month old bulbs	15.6%
Prince, new bulbs	15.0%
Prince, 6 month old bulbs	13.4%
Star, LEDs	15.9%

Samples of the cannabis cultivar "Star" were grown using new and six month old light bulbs. It would be expected that the older light bulbs would be dimmer, leading to less photosynthesis and lower bud potency. This is confirmed by the THCA concentration of the flowers grown with new light bulbs being 18.1% versus 15.6% with older bulbs. The same trend was seen for the cannabis cultivar "Prince", where the potency with newer and older bulbs was 15.0% and 13.4% respectively.

Light emitting diodes (LEDs) can be used as a light source for cannabis grows. The advantage of LEDs is that they last a long time (years versus months for regular light bulbs), but the disadvantage is that they cost more. The cannabis cultivar Star was grown using LEDs and the THCA concentration of the flowers was determined by FT-IR to be 15.9%. The data in Table 3 show this is less than the same strain grown with new light bulbs, but is equivalent to six month old light bulbs. This detailed knowledge of the relationship between potency and growing conditions, combined with knowledge of how sales price varies with potency, may allow growers to optimize lighting conditions by minimizing cost and maximizing profit.

Conclusions

1. FT-IR can accurately determine THCA and CBDA in dried cannabis flowers, giving a quick, convenient, and portable potency test for recreational and medicinal cannabis cultivars.
2. Seven cannabinoids total have been accurately quantitated in dried cannabis flowers by FT-IR, giving a partial cannabinoid profile that covers the main drivers of cannabis pharmacology.
3. FT-IR has been used to monitor how potency changes with growing time and growing conditions. This knowledge should allow cannabis growing conditions to be optimized.

Acknowledgements

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