Application Note AN M157

Differentiation of THC and CBD cannabis using FTIR[5]

Cannabis - ancient herb, promising future

At the end of the 19th century, cannabis was still present in all pharmacies as a medicinal plant for various purposes. Only in the course of abuse as an intoxicant it was increasingly demonized and finally banned.

Since then, cannabis and its key ingredients (cannabinoids), especially CBD and THC, are experiencing an astounding renaissance as a potent drug. The literature meanwhile also refers to the manifold applications of cannabis.[1,2,3]

In light of these findings, many countries are reviewing their existing legislation and are planning to legalize controlled medical use of cannabis, or have already legalized it. More than 60 cannabinoids of various concentrations are contained in cannabis, but legal and illegal markets are mainly dominated by THC or CBD hemp.

However, since only THC has a psychoactive effect, it is of utmost importance to reliably distinguish between different types of hemp. Such an identity verification is mostly performed by microscopy and HPLC despite the fact, that this can be done much more elegantly by using FTIR. For this, the combination of FTIR spectroscopy and software-based spectra comparison is an ideal analysis tool, which is already being used in hundreds of pharmacies worldwide with great success.

Keywords	Instrumentation and Software
Natural substances	ALPHA II FTIR spectrometer
Pharmaceutical ingredients (API)	Platinum ATR
Cannabidiol (CBD)	Quick Compare
Tetrahydrocannabinol (THC)	Quant

The focus of this application is on the rapid and reliable analysis of drugs in accordance with current pharmacopoeia regulations.

Fig. 1: CBD (left) and THC cannabis (right) are indistinguishable to the naked eye.

Identification of cannabis petals

Visually, CBD flowers are indistinguishable from THC flowers to the naked eye (see Figure 1). Thus, to differentiate between CBD and THC containing plant one must resort to chemical analysis methods based on the corresponding lead substances.

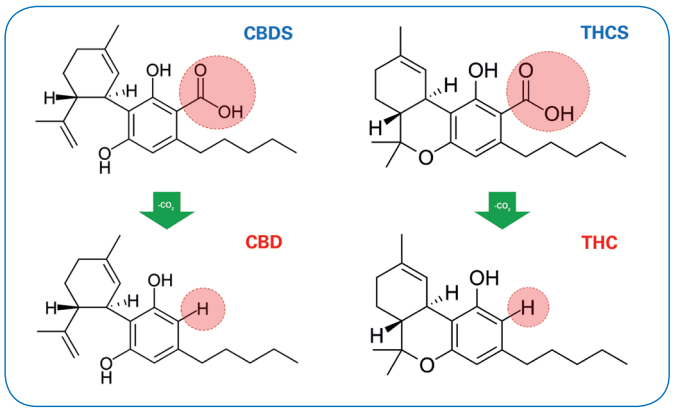

Fig. 2: Thermal decarboxylation of CBD and THCS to THC, respectively.

It should be noted that the unprocessed flowers do not directly contain CBD or THC, but the corresponding acids CBDS and THCS. By smoking or heating the flowers, the actual active ingredients CBD and THC are formed by decarboxylation.

Since both active substances naturally occur in relatively high concentrations (5 to 30%), a definite assignment to the CBD or THC type by means of FTIR is possible even by direct measurement of ground-up flowers. Additionally, these compounds can be easily extracted from the plant matrix with lipophilic solvent.

Methods and Instrumentation

In infrared spectroscopy, a sample is irradiated with infrared light and the substance-dependent absorbance used to obtain molecular information about the examined sample. The result is an IR spectrum that allows a clear identification of the substance, just like a fingerprint.

All following spectra were recorded using an ALPHA II FTIR spectrometer (Figure 3) and a platinum ATR unit. A measurement in attenuated total reflection (ATR) usually takes less than one minute, does not require any elaborate sample preparation, is uncomplicated and, above all, can be used universally for solids and liquids. The software used (OPUS or OPUS TOUCH) combines functionality with ease of use and provides extensive, automated evaluation methods.

Reference spectra of the key ingredients

In order to reproducibly compare the IR spectra of the cannabis flowers or their extracts with the corresponding identity-providing key substances, reference spectra of CBDS and THCS must be available electronically and in good quality. The substances concerned were isolated by preparative thin-layer chromatography and their spectra collected (Fig. 4; CBDS red, THCS blue).

Fig. 3: ALPHA II FTIR spectrometer equipped with Platinum ATR unit. The magnification shows the cannabis flower on the ATR diamond crystal.

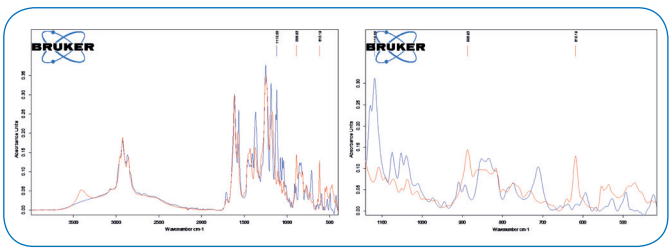

Fig. 4: On the left, the spectra comparison of THCS (blue) and CBDS (red) is shown. On the right, the spectral differences are clearly visible from 400 to 1700 cm^{-1}.

The obtained data was verified by comparison to literature KBr spectra of THCS and CBDS, respectively.[4] By conversion of CBDS to CBD and THCS to THC, the correct identification of the isolated lead substances was additionally verified (see Fig. 5). The characteristic bands of the key ingredients were determined and a method created to ensure safe and above all rapid identification of further samples.

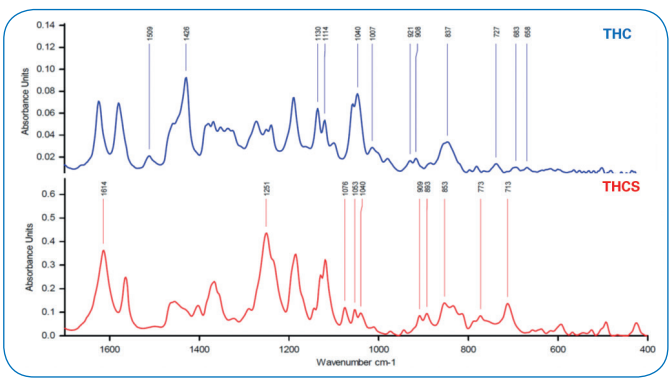

Fig. 5: Decarboxylation of THCS (red) to THC (blue).

Sample preparation

There are two approaches to minimize undesired signal contributions from the plant matrix. One possibility is the extraction of hemp flowers with petroleum ether or acetone. The resulting filtrate is concentrated by evaporation and the sticky residue applied to the measuring crystal. It is also possible to evaporate a few drops of the filtrate directly on the ATR crystal.

The second option is to analyze the flowers in their natural form. It is recommended to grind them first in a small agate mortar and then use the sticky, resinous, drug-rich plant parts for IR measurement.

As a result IR spectra of good quality with high levels of active ingredient (CBDS or THCS) and almost no undesired plant matrix can be obtained. Conveniently, the substance-specific IR signals of THCS and CBDS can also be identified by direct measurement of cannabis flowers.

Evaluation by spectra comparison

For the following measurements, the unprocessed cannabis was measured by directly pressing the flowers onto the ATR crystal. The final evaluation was done by correlating the measured sample spectrum with the entries of the previously created reference database.

For pure substances, this correlation should usually be over 95%. Depending on the specific analytical question, this value may also be higher or lower. For the present study it was shown that a correlation limit of 90% ensures a reliable discrimination and identification of THCS or CBDS hemp.

Figure 6 shows the analysis result of a THCS containing cannabis sample. Apparently, a strong agreement of the sample spectrum (black) with the THCS reference is shown.

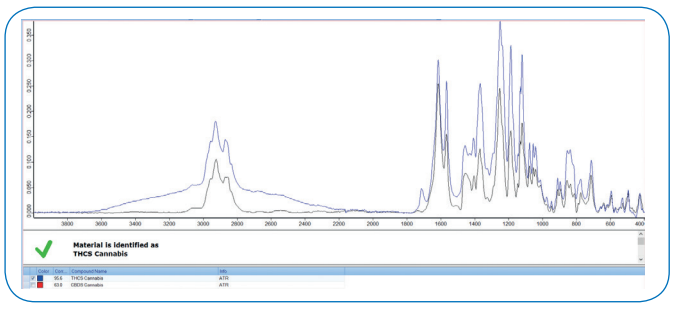

Fig. 6: Analysis of a THCS-containing cannabis sample (black), THCS Reference spectrum (blue)

According to the spectral comparison method, the correlation between the sample spectrum and the stored THCS reference is 95.6%, while the correlation with the CBDA reference is 63% and significantly below the threshold value of 90%. The sample is therefore clearly identified as THCS cannabis and would be subject to strict legislation in many countries.

Figure 7 shows the analysis of a CBDS containing cannabis sample. The correlation to the CBDS reference is very high at 92.9% and above the defined threshold of 90%. In contrast, the correlation to the THCS reference is only 49%, hence the sample was identified as CBDS hemp.

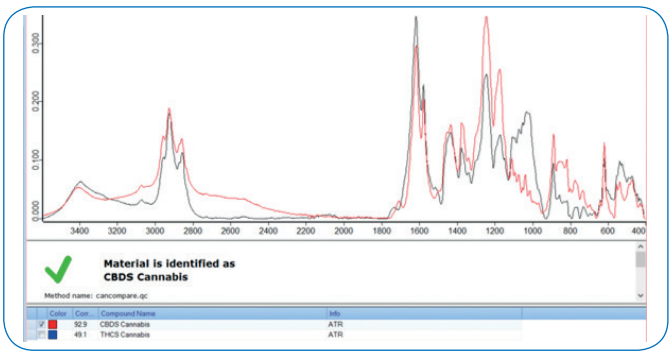

Fig. 7: Analysis of a CBDS-containing cannabis sample (black), CBDS reference spectrum (red)

Conclusion

FTIR spectroscopy allows a reliable distinction between THCS and CBDS hemp within a few seconds. However, the possibilities of FTIR analysis go far beyond that. With the help of appropriate reference samples, a calibration could be created, allowing quantitative cannabis analysis i.e. the determination of the exact content by integration of substance-specific bands.

This technique is already frequently used in pharmacies to quantify the active ingredient content of established formulations and may also be used in future for the quantitative analysis of cannabis.

[1] *Häussermann K., Grotenhermen F. und Milz E., Cannabis Arbeitshilfe für die Apotheke, Deutscher Apotheker Verlag Stuttgart, 2. Aufl. (2018);*
[2] *Grotenhermen F., Hanf als Medizin, Nachtschatten Verlag Solothurn (2015);*
[3] *Grotenhermen F. und Reckendrees B., Die Behandlung mit Cannabis und THC, Nachtschatten Verlag Solothurn, 7. Aufl. (2017);*
[4] *Hazekamp A., Peltenburg A., Verpoorte R. und Giroud Ch., J. Liquid Chromatog. & Tel. Technol. 28, 2361-2382 (2005)*
[5] *Schorno, H.J.X., Koch, H.C. Differenzierung von THC-Cannabis und CBD-Cannabis mittels FT-IR (2018)*

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