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miniRaman spectrometer for cannabis: Part 1. Simple Raman spectra interpretation

KEYWORDS

Cannabis; Raman spectroscopy; phytocannabinoid (pCB); delta-9 tetrahydrocannabinol (THC); delta-9 tetrahydrocannabinolic acid (THCA); cannabidiol (CBD); cannabidiolic acid (CBDA); cannabigerol (CBG); cannabigerolic acid (CBGA).

PREFACE

The measurements described in this Application Note were carried out in collaboration with Prof. Rime Bahij and Prof. Martin Aage Barsøe Hedegaard from the Department of Green Technology of the University of Southern Denmark (SDU), Odense, Denmark.

SDU has a license to cultivate, manufacture, and distribute cannabis, issued by the Danish Medicines Agency in accordance with applicable legislation.

INTRODUCTION

Cannabis is the general name of annual dioecious plants from the Cannabis genus of the Cannabaceae family. Since ancient times, mankind has widely used these plants for the production of foodstuff (flour, seeds, oil), fodders, fibers (for fabrics, ropes, paper), building materials, fuel, as well as for medical and recreational purposes due to its anesthetic and relaxing effects [1]. However, in the 20th century, the cultivation and use of cannabis were significantly restricted worldwide at the legislative level, mainly due to its psychotropic action on humans.

In last decades, the situation is changing owing to a better understanding of the medicinal potential of cannabis, social movement to legalize non-medical cannabis, fight against drug trafficking, etc. As a result, some countries/regions already permit the cannabis cultivation for medical/recreational purposes, while legal regulation is shifting rather towards controlling the chemical composition of the plant. In turn, this gives an impetus for large-scale investigations on cannabis, as well as stimulates efforts on the selection of its new varieties with designated characteristics and, accordingly, the development of effective analytical instruments for research and process control [1, 2].

This Application Note is intended to demonstrate the potential of the miniRaman spectrometers from Lightnovo ApS [3] to identify key bioactive components in cannabis plants, as well as products derived from them.

Details on YouTube

BACKGROUND

The physiological effects of cannabis are primarily due to specific complex bioactive chemicals known as phytocannabinoids (pCBs), which are also present (but in much smaller amount) in some other plants [2, 4]. The organisms of all vertebrates, including humans, produce "intrinsic" compounds that provide similar physiological effects, and accordingly they are called endocannabinoids. Together with their synthetic analogues, pCBs and endocannabinoids are simply called cannabinoids.

Human endocannabinoid system

In 1988, it has been proved the existence of the endocannabinoid system (ECS) in humans and other vertebrates [5]. This is a complex cell signaling network that plays a crucial role in homeostasis – the ability of the body or a certain of its part to maintain a stable internal state, despite external changes. The system is mainly concentrated in the central and peripheral nervous systems, but in varying degree is also present in all organs/tissues of the body. ECS has three main components:

- Endocannabinoids – a special type of neurotransmitter molecules produced within the body. They, unlike other types of neurotransmitters, are synthesized only when needed and are quickly degraded after performing their functions, without accumulating in the organism.
- Cannabinoid receptors – specific cell membrane receptors in certain types of cells that are activated by binding with endocannabinoids, triggering a corresponding cell response.
- Enzymes that are responsible for the synthesis and degradation of endocannabinoids.

In a very simplified way, the ECS work can be illustrated as follows. Consider two cells in some organ, C1 and C2, that interact through certain cell signaling process. When for any reason (e.g., due to external impact) C1 begins to affect C2 abnormally, the latter produces (using enzymes) endocannabinoids, which move towards the former and activate its cannabinoid receptors. The resulting cell response tends to alter the C1 activity so that cell interactions return to normal. After this, the endocannabinoids are degraded by enzymes, which prevents over-regulation. As a result, the organ remains in a stable state. Of course, the actual ECS mechanisms are much more complex and involve many cells and/or cell types as well as the different chemical compounds they produce. Moreover, these mechanisms affect not only individual organs, but also interactions between different ones.

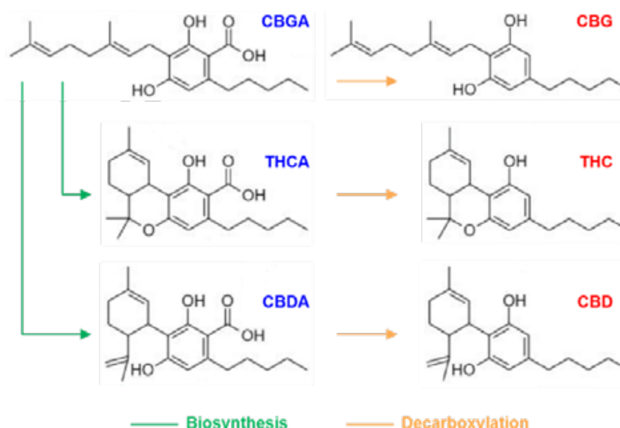
Although research into ECS is still far from complete, it is already proven that it regulates many physiological/cognitive processes in the human body: metabolism, blood pressure, immune response, reproductive function, appetite, movement and motor coordination, sensation of pain, memory, learning, emotion, etc. Accordingly, the ECS modulation is considered as a powerful therapeutic strategy for the treatment of many diseases: neurodegenerative (multiple sclerosis, Parkinson's, Huntington's and Alzheimer's diseases etc.), cardiovascular, reproductive, inflammatory, autoimmune, metabolic, oncological, as well as psychiatric disorders, addictions etc. [5].

Phytocannabinoids in cannabis

One way to modulate ECS is by targeting cannabinoid receptors with the medicines based on pCBs. In this regard, cannabis is a unique raw material, because it can produce a large number of such compounds: > 140 have been identified in the plant to date [2]. They are contained mainly in the inflorescences, oleoresin and leaves, and can be extracted by different known methods [6]. Note, that due to enormous variability of cannabis the set and/or content of pCBs in a plant can significantly depend on its variety/hybrid and agroclimatic conditions [1, 2].

In young fresh cannabis, pCBs are presented mainly in the form of carboxylic acids, which themselves have relatively weak physiological effects. However, when the plant ages or is processed (first of all, by drying), these acids are decarboxylated, producing more active neutral forms of pCBs [1]. Three of them have a strong action on humans [1, 7]:

- Delta-9-tetrahydrocannabinol (THC). Its acid precursor is delta-9-tetrahydrocannabinolic acid (THCA). THC is the most active pCB, which is responsible for the psychoactive effect of cannabis, and so, in itself, is an illegal drug. At the same time, THC has a wide range of valuable pharmacological effects: anti-inflammatory, analgesic, antispastic, antineoplastic, antiemetic, antioxidant, antipruritic and many others.
- Cannabidiol (CBD). Its acid precursor is cannabidiolic acid (CBDA). CBD is capable of reducing chronic pain, inflammation, anxiety and depression, lowering blood pressure, alleviating side effects related to cancer treatment (nausea, vomiting), and mitigating symptoms of neurological disorders etc.
- Cannabigerol (CBG). Its acid precursor is cannabigerolic acid (CBGA), which also is the common biosynthetic precursor to THCA, CBDA and most other acidic forms of pCBs. CBG is able to inhibit tumor cell growth, reduce inflammation and decrease intraocular pressure that has positive effect in glaucoma treatment. Also, CBG is known to decelerate bacterial growth.



The molecules of phyto- and endocannabinoid differ significantly [4]. So, despite similar action on the receptors, their metabolism in the body is not the same. E.g., pCBs aren't degraded by ECS's enzymes, and are excreted from the organism through other ways, in passing affecting many organs/systems. Accordingly, their uncontrolled use may cause side effects. For example, THC, in addition to psychotropic effect, can lead to anxiety, memory loss, immunosuppression etc. [1].

Due to the THC peculiarities, since 1976, most countries have introduced strict regulation or total ban for the cultivation/use of cannabis containing > 0.3 % (> 0.2 % in EU) THC by dry weight, called «marijuana» [1, 7]. Cannabis with ≤ 0.3 % (≤ 0.2 % in EU) THC content, called "hemp", has generally been subject to weaker restrictions, but in some regions has also been prohibited. This situation has reduced the industrial use of cannabis and has hampered its pharmaceutic applications.

In recent decades, attitude towards cannabis have gradually softened, and its legalization is taking place, first of all for medical use. This is partly due to the discovery of therapeutic effects in non-psychotropic pCBs, like CBD and CBG (see above). On the other hand, it was shown that CBD antagonizes the psychoactive and some other side effects of THC [1, 2]. This allows for development of safe and effective THC-based medicines. An example is "Nabiximols" ("Sativex") from GW Pharmaceuticals, that alleviates the symptoms of multiple sclerosis. It contains a standardized cannabis extract with strictly defined contents of THC and CBD in a ratio of ~1:1.

Advances in cannabinoid research and applications have also impacted the classification of cannabis varieties, which is now more practically oriented and relied on the content of main pCBs. There are five (chemo)types of varieties: THC-rich (type I); THC/CBD-balanced (type II); CDB-rich (type III); CBG-rich (type IV); and cannabinoid-free (type V) [1].

Main methods for analysis of phytocannabinoids in cannabis and its products

Effective and legal cultivation/processing of cannabis is impossible without analysis of the main pCBs composition/content. Such analysis provides general quality control, as well as being able to help solve many important technological issues, such as:

- Timely detect the undesirable changes in the plants' chemical composition that can occur due to crossing with other varieties (including wild hemp), disturbances in soil characteristics etc.
- Monitor the dynamics of changes in pCBs proportions during plant maturation in order to determine the optimal harvest time.
- Modify cannabis processing modes to achieve optimal characteristics of end products.
- Detect additionally the presence of contaminants in cannabis (pathogen, pesticides, mycotoxins etc.) that can be introduced via soil, water, air etc.

For the chemical analysis of cannabis, it is widely used various methods based on chromatography [2]. In its simplest form, this approach assumes to use a special long tube or capillary (called "column"), whose inner surface is coated (or volume is filled) uniformly with a certain fixed solid or liquid substance ("stationary phase"). The analyzed sample is dissolved in another liquid or gaseous substance ("mobile phase"), which is then passed through the column. Usually, the sample's molecules-components have different affinities for the mobile and stationary phases, and so, are held on their interface with various strengths. As a result, the components move along the column at different speeds, that provide their spatial separation. Each component reaches the column output in due time, where a detector is installed. It measures a time dependence of the analytic signal ("chromatogram"), which is a set of peaks corresponding to individual components. The comparison with calibration chromatograms, as well as analysis of peaks' parameters, gains information about the types and quantities of components.

Although chromatography-based methods are the "gold standard" in analytical chemistry and can provide excellent results, they have disadvantages when used for cannabis-cultivation tasks. First, chromatographic equipment is complex, expensive, non-portable, requires laboratory infrastructure and qualified personnel, which limits its use "in the field". Second, these methods are destructive and involve transforming the analyzed plant parts into liquid/gaseous form. This requires using reactants/solvents, filtration and heat treatment, which poses the risk to contaminate the sample as well as to lose some pCBs due to decarboxylation and/or other reactions. Third, as can be seen from the description above, the separation process, in itself, is time-consuming (sometimes ≥ 30 min per sample), so the overall analysis duration, including sample preparation and data processing, can be unreasonably long.

An alternative approach, free from the most of above problems, is to use the methods of molecular spectroscopy. It is based on the fact that in any complex molecule there are vibrations and rotations of atoms (and/or groups of atoms) relative to each other, as well as rotations of the molecule as a whole. These processes are quantized, which is reflected in the unique structure of quantum energy levels of the molecule. Accordingly, studying its electromagnetic emission, absorption or scattering spectra allows to identify the molecular type. For a complex sample, such a spectrum is a superposition of the weighted contributions from the different types of molecules the sample is composed of. Decomposition of the spectrum using chemometric techniques, and comparison of results with reference spectra allows to identify and quantify different molecules.

To date, the most popular molecular-spectroscopic method for cannabis analysis is near-infrared spectroscopy (NIRS) [2, 8]. It involves measuring the absorption spectrum in transmission or reflection mode. The sample is irradiated with polychromatic light having a continuous intensity distribution within the wavelength range λ = (780 … 2500) nm. In the simplest implementation, transmitted/reflected light passes through the spectrometer's entrance diaphragm, is dispersed by a diffraction grating, and then the spectrum is captured by a linear image sensor.

NIRS is a relatively cheap, simple and fast technique. However, it has a disadvantage, which is that water strongly absorbs near-infrared light. Consequently, when analyzing fresh cannabis, large inaccuracies may occur due to the presence of a strong background signal from water.

A good alternative/addition to NIRS may be Raman spectroscopy, which also shows high efficiency in the analysis of cannabis [7, 9]. It involves irradiation of the sample by monochromatic (laser) light with wavelength λ_0, and measuring of the inelastically-scattered (Raman) spectrum, i.e., light with wavelengths $\lambda \neq \lambda_0$. Compared to NIRS, the key advantage of this method is that in the Raman spectrum the water-

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associated peaks are located far away from the peaks corresponding to pCBs. And thus, the background from water does not affect the measurement results.

The Raman spectroscopy scheme, in general, is similar to that for NIRS, but it includes an additional edge filter that rejects elastically scattered light with $\lambda = \lambda_0$. The measurement can also be carried out in transmission or reflection mode. Raman spectrum is usually stored as the $I(\nu)$ dependence, where I is light intensity, and $\nu = (1/\lambda_0 - 1/\lambda)\cdot 10^7$ is Raman shift (wavenumber), expressed in cm^{-1} units, while λ_0 and λ are taken in nm.

For Raman measurement, one of the main issues is fluorescence background. Optimization of the optical scheme makes it possible to mitigate its manifestations. However, complete removal usually requires post-processing of the spectrum. This is helped by the fact that the fluorescence peaks are much broader than Raman ones, and so effective correction algorithms can be easily developed.

Lightnovo miniRaman spectrometer

miniRaman is a compact dispersive spectrometer with a fixed transmission diffraction grating, that operates in reflection mode (180°-backscattering) [3], Figure 1. The instrument uses diode laser to generate excitation light, which is delivered to the sample through a special optical probe (Raman probe). Backscattered light is collected by the same optical components. A CMOS image sensor is used to capture Raman spectra.

Figure 1. Two miniRaman spectrometers and a cannabis leaf.

A key peculiarity of the Lightnovo miniRaman spectrometer is the presence of a dedicated in-built reference channel, which allows to carry out the "on the fly" correction of the measuring Raman spectrum [10]. At the same time, neither moving components, nor additional light sources or image sensors are used. In combination with some other technical solutions, the reference channel allows to abandon the use of expensive high-end lasers and image sensors, and forced cooling of these components, as well as allows to reduce the background fluorescence signal. As a result, the instrument has excellent measuring characteristics with ultimate compactness.

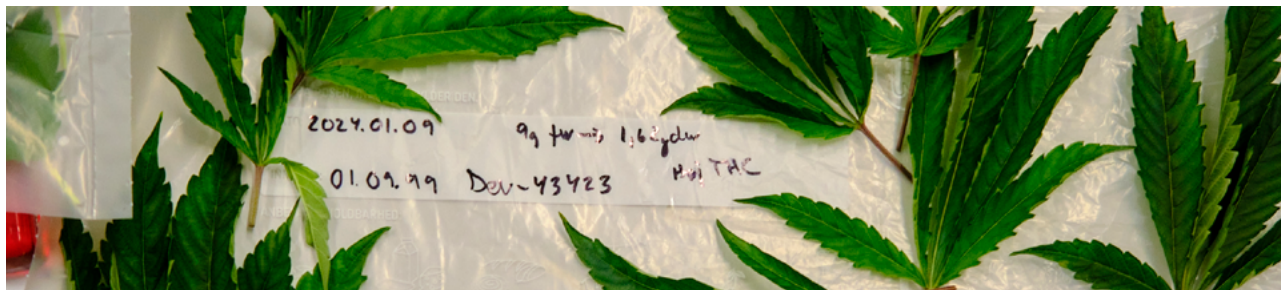
The following features make miniRaman an attractive tool for chemical analysis of cannabis and cannabis products:

- **Wide spectral range.** In the basic configuration it is ν = (400 ... 2700) cm^{-1}, while for main pCBs, Raman peaks are in the range ν = (700 ... 1700) cm^{-1} [7, 9].
- **Record low size-mass characteristics.** With sizes of 112 mm × 39 mm × 34 mm and mass of 400 g (200 g in aluminum housing), the instrument is ideal for *in vivo* analysis of cannabis "in the field", for rapid testing of cannabis products on production lines, for the activities of various regulatory authorities, custom, police etc.
- **Flexibility of use.** The spectrometer is equipped with a battery and standard interfaces: USB, Bluetooth and Wi-Fi. This allows it to be used as:
  (i) a portable instrument for *in vivo* analysis of cannabis at indoor and outdoor plantations;
  (ii) benchtop instrument in a laboratory;
  (iii) a remote instrument, e.g., as part of an automatic system.
  At the same time, a reliable connection to a workstation can be provided to collect and process large volumes of data.
- **Large number of Raman probes.** They allow to analyze plant parts from different distances, including direct contact. There is also a special accessory for vials, which makes working with cannabis extracts and other liquid samples more reliable and comfortable.
- **Microscope compatibility.** Special precise focusing stages are available that allow the spectrometer to be used as part of a confocal miniRaman microscope. This makes it possible to analyze the distributions of pCBs and other chemicals in the cannabis.

Details for miniRaman patent

MATERIALS, SAMPLE PREPARATION AND MEASUREMENTS

The data shown in this application note comes from two different kind of cannabis leaves picked from two different plant as well as a THC distillate.



The measurements were carried out with two models of miniRaman spectrometer having the excitation wavelengths and power λ_0 = 785 nm, P = 46 mW and λ_0 = 815 nm, P = 76 mW, respectively. Spectrometers were equipped with contact probes, leaving no distance between spectrometer and the leaves.

Spectra were recorded in the spectral range ν = (500 ... 2000) cm^{-1}. Image sensor gain 0. Exposure time varied between samples and was 200 ms; number of repetitions 10. The distillate was measured in a vial.

RESULTS

Figure 2 demonstrates Raman spectra I(λ) for the CBD-rich leaf, THC-rich leaf and THC distillate, measured using two miniRaman models with λ_0 = 785 nm (blue curves) and λ_0 = 815 nm (red curves). The left column of figures (a), (c) and (e) consists of raw spectra; here the intensity values I are expressed in a percentage of the saturation signal's level for the CMOS image sensor used in the spectrometer. The right column of figures (b), (d) and (f) consists of the spectra obtained from raw ones after background correction and normalization carried out using rolling circle filter in Miraspec software; here the intensity values I are expressed in arbitrary units.

For λ_0 = 785 nm, a high fluorescence background presents in all raw spectra. At the same time, the spectrum baselines have a different convexity: downward for both leaves, and upward for the THC distillate. Obviously, this is due to different fluorescence sources. It is believed, that for leaves the background is mainly due to the presence of chlorophyll B and carotenoids [9]. However, the THC distillate should not contain these components, at least not in large quantities. Most possible cause here could be the residues of chemicals used in the distillate's production. On the other hand, it must be taken into account that pCBs themselves are capable of fluorescence [11], although this effect has not yet been sufficiently studied at such a high value of λ_0.

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It is worth to noting that, for most industrial spectrometers operating at $\lambda_0 \leq 785$ nm, raw spectra of cannabis have such a high background that the Raman spectrum's details become virtually indistinguishable (e.g., see Figure 1 in [9]). In contrast, for miniRaman, many peaks are quite well defined. This peculiarity is connected with above-mentioned reference channel, which makes it possible to suppress fluorescence to some extent immediately during the measurement process.

Nevertheless, it seems that in the case of cannabis, even such advanced "hardware fluorescence suppression" isn't effective enough at $\lambda_0 = 785$ nm, since the background is still noticeable, see blue curves in Figure 2(a), (c) and (e). This is also evident from the spectra obtained after software background correction, which have distinct artifacts in the wavenumber range $\nu \leq 700$ cm^{-1}, see blue curves in Figure 2(b), (d) and (f).

A common way to eliminate the fluorescence background is to use a higher excitation wavelength [9]. But it must be remembered that, in theory, the Raman intensity depends on it as $\sim (1/\lambda_0)^4$. Thus, the increase in λ_0 should be such that (i) it does not significantly attenuate the useful Raman signal, (ii) but reduces the spurious background signal to such a level that software correction is effective.

$\lambda_0 = 815$ nm is a good option for the miniRaman spectrometer. At this wavelength, the fluorescence amplitude decreases severalfold, while the Raman peaks remain clearly visible in the raw spectra, see red lines in Figure 2(a), (c) and (e). At the same time, the background-corrected spectra don't contain evident artifacts, see red lines in Figure 2(b), (d) and (e).

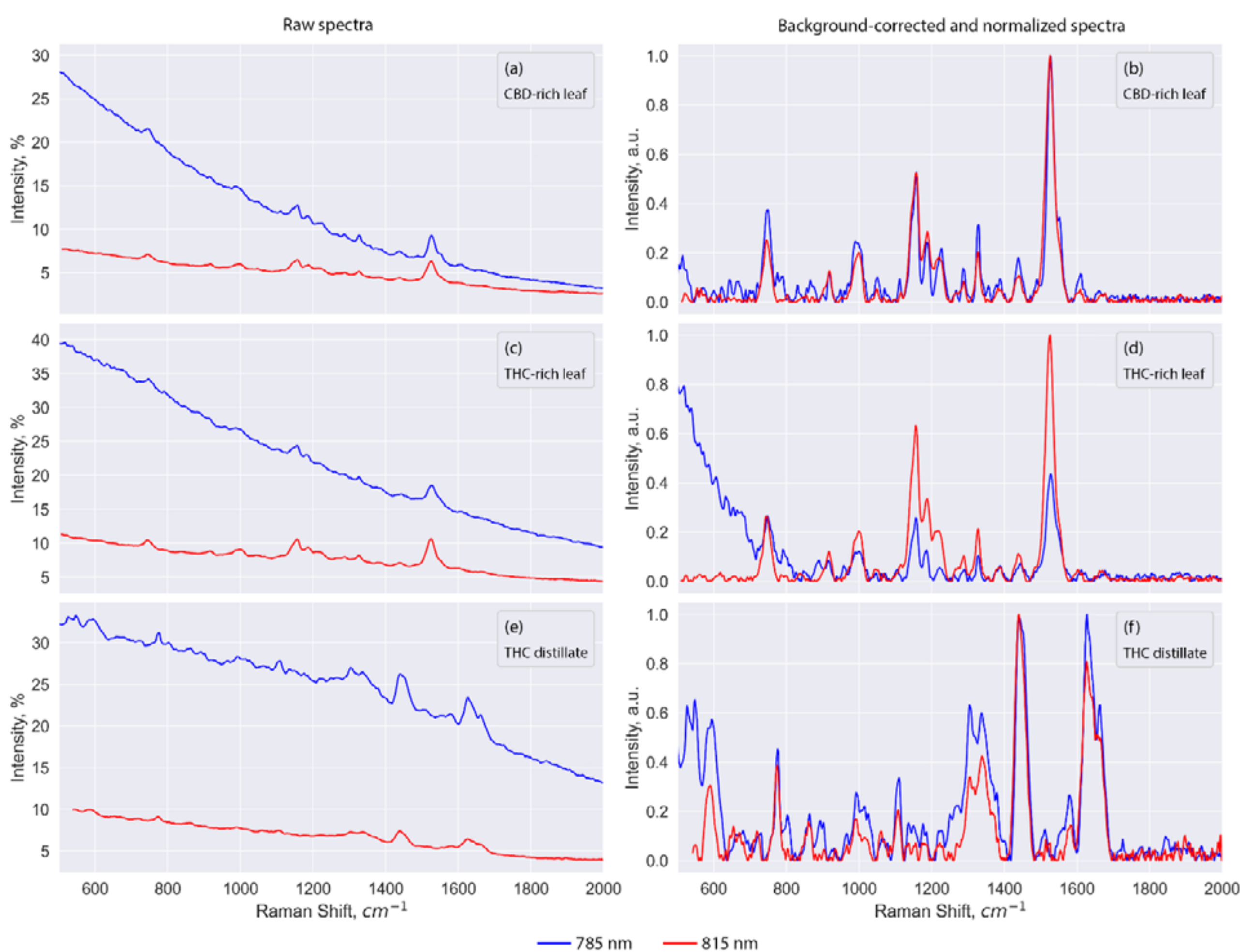

Figure 2. Raman spectra I(ν) for: (a)–(b) CBD–rich leaf, (c)–(d) THC–rich leaf, and (e)–(f) THC distillate. Data were obtained at $\lambda_0 = 785$ nm (blue curves) and $\lambda_0 = 815$ nm (red curves).
Raw spectra are shown in the left column (a), (c) and (e).
Background–corrected and normalized spectra are shown in the right column (b), (d) and (f).

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Figure 3 combines the corrected and normalized spectra I(λ), measured at λ_0 = 815 nm. The spectrums, were taken from Figure 2(b), (d) and (e) and vertically shifted to facilitate observation/comparison of different peaks. As it seen, the spectra for the leaves are very similar, and the positions of most of their peaks coincide. The THC distillate spectrum is expectedly different, but many of its peaks have the same positions as those in the leaf spectra.

A computer program was used to automatically select prominent peaks and determine the wavenumbers ν_{exp} corresponding to the centers of the peaks displayed in Figure 3. In doing so, it was taking into account the error ± 2.5 cm^{-1} in the wavenumber determining typical for the miniRaman spectrometer [3].

To identify the peaks, their wavenumbers ν_{exp} were compared with reference values ν_{ref}. The last ones were taken from [7], where it was studied in detail the spectra for cannabis, THC, THCA, CBD, CDBA, CBG, CBGA measured at λ_0 = 830 nm. Note, that this excitation wavelength is 15 nm greater than one used in the miniRaman spectrometer, but it is assumed that this difference is insignificant for a rough estimation of peak positions.

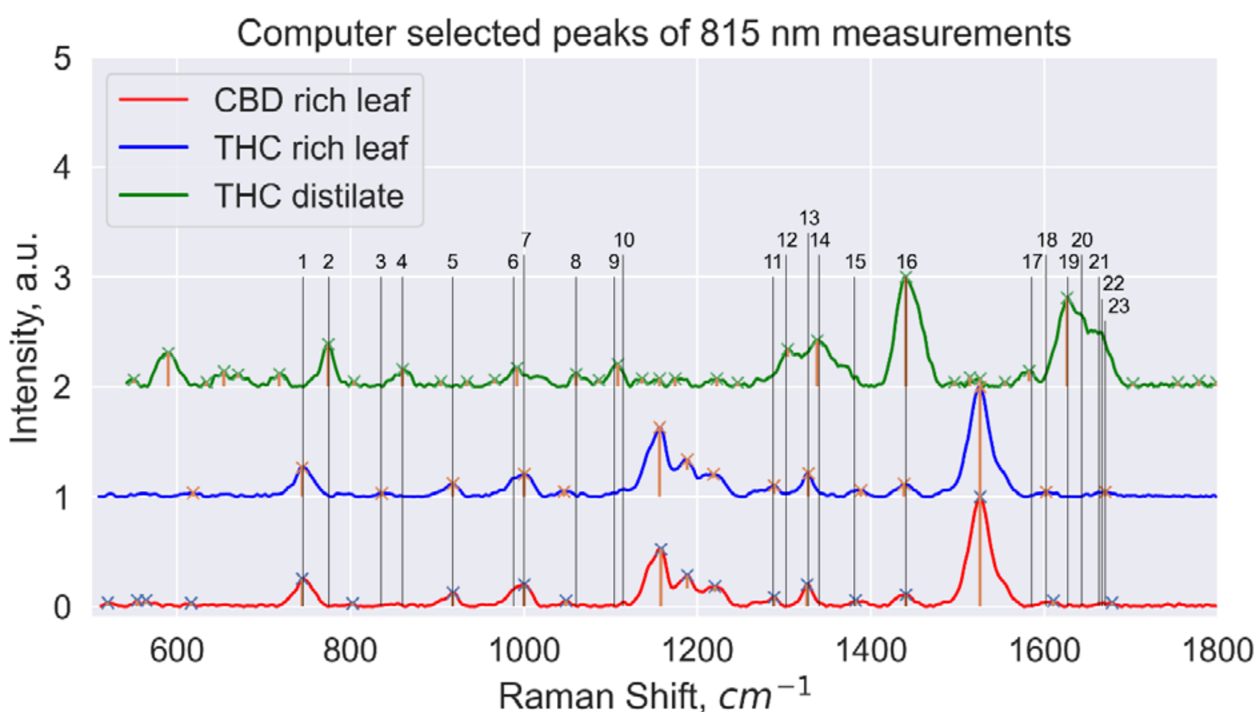

Figure 3. Background-corrected and normalized Raman spectra I(ν) for CBD-rich and THC-rich leaves, and THC distillate at λ_0 = 815 nm. Peaks marked by crosses are selected by a computer program, and relevant peaks marked with numbers 1–23.

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In Figure 3, the numbered lines indicate peaks that can be attributed to THC, THCA, CBD, CDBA, CBG and CBGA based on comparison with [7]. Some peaks are intentionally not marked, since they either cannot be identified, or simply have too low an amplitude, or not associated with these pCBs. E.g., in the leaf spectra, the peaks within the range from ν_{exp} = (1156 ± 2.5) cm^{-1} to (1269 ± 2.5) cm^{-1} correspond to cellulose, carbohydrates, xylan, lignin and aliphatic compounds; whereas the intensive peak at ν_{exp} = (1526 ± 2.5) cm^{-1} is caused by carotenoids [7]. All these peaks are absent in the THC distillate spectrum, as expected.

Table 1 contains data for the peaks marked in Figure 3 (left side of the table), and data for the corresponding reference peaks from [7] (right side).

Table 1. Identification of peaks in Raman spectra of the CBD-rich leaf, THC-rich leaf, and THC distillate, which are marked in Figure 3.

Data obtained from spectra measured using miniRaman					Reference data taken from [7]	
Peak #	Sample (*)			ν_{exp} [cm^{-1}]	ν_{ref} [cm^{-1}]	Compounds
	CBD rich leaf	THC rich leaf	THC distillate			
1	●	●		744 ± 2.5	745	CBG
2			●	774 ± 2.5	775	THC, THCA
3		●		836 ± 2.5	835	THC, THCA, CBG
4			●	860 ± 2.5	860	CBG, CBGA
5	●	●		918 ± 2.5	916	Cellulose, lignin
					918	CBG
6	○	○	●	990 ± 2.5	988	CBG
7	●	●	○	1000 ± 2.5	998	CBG, CBGA
					1000	Carotenoids, protein
8			●	1060 ± 2.5	1060	CBG
9			●	1106 ± 2.5	1104	CBD
10	●	●	○	1115 ± 2.5	1114	THC, THCA, CBD, CBDA
						Cellulose
11	●	●		1288 ± 2.5	1287	CBGA
12			●	1304 ± 2.5	1302	CBD
13	●	●	○	1327 ± 2.5	1328	CBG
14			●	1338 ± 2.5	1340	CBD
15	●	○	○	1382 ± 2.5	1381	CBG, CBGA
16	●	●	●	1439 ± 2.5	1437	CBD, CBDA
					1440	THC, THCA, CBD, CBDA
						Aliphatic compounds
17			●	1583 ± 2.5	1585	CBD, CBDA
18	○	●		1602 ± 2.5	1602	CBDA
19	○		●	1626 ± 2.5	1627	CBG
20	●	○	○	1644 ± 2.5	1643	CBD
21	○	●	○	1663 ± 2.5	1663	CBD, CBDA
22	○	○	○	1667 ± 2.5	1666	THC, THCA
23	○	●	○	1670 ± 2.5	1670	CBG, CBGA

(*) Keys: ● – the peak is present; ○ – the peak may be present but is overlapping with adjacent peaks; no icon – no peak.

Conventionally, all peaks in Figure 3 and Table 1 can be divided into well-identified and poorly-identified. The latter include those for which at least one of two conditions is met:

- The peak belongs to a few compounds. Certain Raman peaks of cannabis can be associated with multiple compounds at once, as is shown in the last column of Table 1. Most often these are the acidic and neutral forms of the same pCB and/or types of pCBs. Obviously, this is due to the fact that all phytocannabinoids, in varying degree, are close to each other in their molecular structure [7]. In this regard, it is worth noting that, when manufacturing products like distillates, the acidic forms are decarboxylated at heating. Thus, for the THC distillate, any "multiple" peaks in the table should be attributed rather to the neutral forms.
- The peak cannot be accurately resolved. In the Raman spectrum of cannabis, some peaks are very close to each other and overlap. Thus, it is not always possible to reliably determine the presence of a particular peak in the measured spectrum. In Table 1, such peaks are indicated by open circles, while filled circles correspond to well-resolved ones. In addition, even if a peak stands out from its neighbors, it is sometimes impossible to unambiguously determine which reference value ν_{ref} its position ν_{exp} is closer to, with taking into account the existence of measurement error. This is the case of peaks #5, #7, #10 and #16 in the table.

Most of the observed peaks are associated with CBGA/CBG. This is not surprising, since CBGA is the common biosynthetic precursor of all pCBs, and its content in cannabis can reach up to (25 … 35) % [9]. The leaf spectra contain peak #11, which is characteristic only of CBGA, and is absent in the distillate spectrum, as expected. Monitoring its intensity can be useful, for example, in the breeding type IV cannabis, or the manufacturing CBG-rich products.

Well-identified peaks associated with CBG are #1 and #13 (the leaves), as well as #4, #6, #8 and #19 (the distillate). They can likely be used to easily estimate the CBG content in the plant and product, respectively. It is interesting that a strong peak #1 is present in the leaf spectra and absent in the distillate one, while with #4 and #8 the situation is rather the opposite. This may be due to the different chemical environment of the CBG molecules in the two cases, and could therefore be useful, for example, for detecting unprocessed material in cannabis products.

Additionally note that, there are many poorly-identified peaks for CBGA/CBG in the spectra: #3, #5, #6, #7, #15, #19 and #23 (the leaves), as well as #7, #13 #15 and #23 (the distillate).

Peak #18 in the leaf spectra is due only to the presence of CBDA. It is weak but quite detectable, that should probably allow it to be used to assay the CBDA content in the plant. In turn, this could be valuable for cultivation of type III cannabis, as well as for manufacturing CBD-rich products.

For CBD, well-identified peaks #9, #12, #14 and #17 are present only in the distillate spectrum. The leaf spectra do not contain them. At the same time, most of the poorly-identified CBDA/CBD-associated peaks #10, #16, #20 and #21 are present for all samples.

Regarding THCA/THC, there is only one well-identified peak #2 which belongs to the THC distillate spectrum. It has a fairly high intensity and doesn't overlap with adjacent peaks, which allows it to be used to directly monitoring of the THC content in the product. Besides, the THC-rich leaf spectrum has peak #3, that is absent in the CBD-rich leaf spectrum. According to [7], this peak is conditioned by both THCA/THC and CBG. However, all other above-mentioned CBG-associated peaks are present in measured spectra for both types of leaves. This suggest that just THCA/THC is the main source of peak #3, while the CBG impact is small, if any. Accordingly, this peak could possibly be used, e.g., for the express detection of type I cannabis.

Also, three poorly-identified THCA/THC-associated peaks #10, #22 and #16 are present in all spectra. The latter is attractive for analysis because it has high intensity and is well-resolved. At the same time, this peak is difficult to interpret, since it is associated with THCA/THC, CBDA/CBD and non-cannabinoid compounds at once.

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To summarize, the ability to detect well-identified peaks potentially makes the miniRaman spectrometer an effective instrument for qualitative and semi-quantitative analysis of cannabis and its products without the need for any sophisticated processing of the measured spectra. A notable example is the THC distillate which spectrum has many such peaks associated with all three main pCBs. This allows to monitor the distillate purity just by detecting peaks for CBD and CBG, as well as evaluate its performances by simple measuring the intensities of peaks for THC, CBD and CBG.

On the other hand, poorly identified peaks carry much more information about the molecular composition. Accordingly, for full-fledged quantitative analysis of cannabis and its products, the spectra measured using the miniRaman spectrometer must be further processed and examined using modern powerful techniques, e.g., similar to those developed in chemometrics.

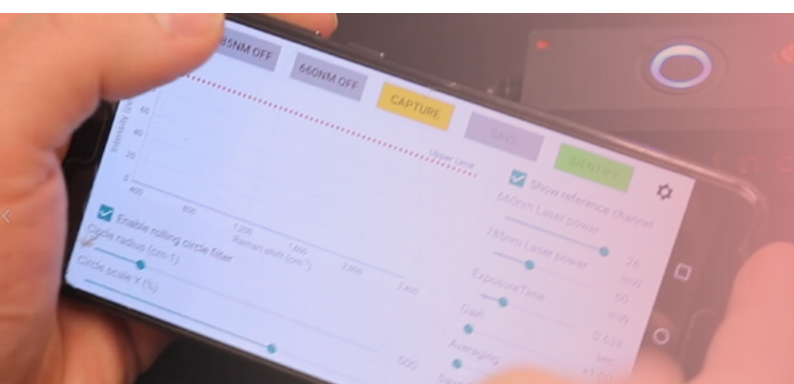
CONCLUSION

When apply the miniRaman spectrometer to analyze cannabis and its product, the use of 815-nm excitation light in combination with the bundled software correction allows to completely eliminate the spurious fluorescent background. Resulting Raman spectra are of sufficiently high quality and reveal most of the spectral peaks typical for the three main phytocannabinoids, THC, CBD and CGB, as well as their acidic forms. Peak wavenumbers, within the measurement error, coincide with the values given in the literature. This allows to identify the above compounds, and roughly estimate their content. However, the cannabis Raman spectra themselves are quite complex, and therefore, spectral chemometrics analysis is required for full-fledged quantification of the cannabinoid composition and content in the sample.

Obtained results, together with the constructional and operational advantages such as ultra-small sizes and weight, autonomy, configuration flexibility, ease of use, rapid measurements, etc., make the miniRaman spectrometer a convenient multitask analytical instrument for application in all cannabis-related technologies and industries.

REFERENCES

1. P. Romero, A. Peris, K. Vergara and . J. T. Matus, «Comprehending and improving cannabis specialized metabolism in the systems biology era,» *Plant Science*, vol. 298, p. 110571 (48 pp.), September 2020.
2. R. M. Strongin, J. Meehan-Atrash and M. Vialpando, Eds., Recent Advances in the Science of Cannabis, Boca Raton, Florida: *CRC Press*, 2022.
3. *https://lightnovo.com/lightnovo-products/raman-spectrometer/*.
4. M. Maccarrone, «Phytocannabinoids and endocannabinoids: Different in nature,» *Rendiconti Lincei. Scienze Fisiche e Naturali*, vol. 31, pp. 931-938, 15 October 2020.
5. H. L. Lowe, N. Toyang, B. Steele, J. Bryant and W. Ngwa, «The endocannabinoid system: A potential target for the treatment of various diseases,» *International Journal of Molecular Sciences*, vol. 22, no. 17, p. 9472 (42 pp.), 31 August 2021.
6. M. P. Lazarjani, O. Young, L. Kebede and A. Seyfoddin, «Processing and extraction methods of medicinal cannabis: a narrative review,» *Journal of Cannabis Research*, vol. 3, p. 32 (15 pp.), 19 July 2021.
7. L. Sanchez, D. Baltensperger and D. Kurouski, «Raman-based differentiation of hemp, cannabidiol-rich hemp, and cannabis,» *Analytical Chemistry*, vol. 92, no. 11, pp. 7733-7737, 13 May 2020.
8. C. Jaren, P. C. Zambrana, C. Perez-Roncal, A. Lopez-Maestresalas, A. Abrego and S. Arazuri, «Potential of NIRS technology for the determination of cannabinoid content in industrial hemp (Cannabis sativa L.),» *Agronomy*, vol. 12, no. 4, p. 938 (12 pp.), 14 April 2022.
9. S. Porcu, E. Tuveri, M. Palanca, C. Melis and et al., «Rapid in situ detection of THC and CBD in Cannabis sativa L. by 1064 nm Raman spectroscopy,» *Analytical Chemistry*, vol. 94, no. 29, p. 10435–10442, 18 July 2022.
10. O. Ilchenko, T. Rindzevicius, A. Boisen and R. Slipets, «An apparatus for carrying out Raman spectroscopy». International Patent WO2019145005, 2019.
11. M. Birenboim, E. Fallik, D. Kengisbuch and J. A. Shimshoni, «Quantitative and qualitative spectroscopic parameters determination of major cannabinoids,» *Journal of Luminescence*, vol. 252, p. 119387, 1 December 2022.



Harness the power of Raman spectroscopy and make it widely accessible for the benefit of mankind.

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