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Technical Report

Choosing the Right Instrumentation for Cannabinoid Analysis

Bob Clifford[1], Craig Young[1], Alan Owens[1], Jim Mott[1], and Rachel Lieberman[1]
[1]Shimadzu Scientific Instruments, Columbia, MD, USA

Abstract:

The "Green Rush" of cannabis and hemp continues to increase because of the potential medicinal and health benefits of compounds derived from these two plants. The two major beneficial compound classes are the cannabinoids and terpenes. This article will explore the various techniques for conducting analysis of these compounds.

Keywords: Cannabis, Cannabinoid Analysis, Terpenes

■ Modular vs Integrated HPLC

The most widely used technique for cannabinoid analysis is high-performance liquid chromatography (HPLC). Examples of systems include Shimadzu's "Cannabis Analyzer for Potency" and "Hemp Analyzer". HPLC systems vary by many factors, such as capacity, versatility, and price. A lower-priced HPLC would be the turnkey Cannabis Analyzer (Figure 1A) with the autosampler, pumps, column(s), and detector(s) all built into an integrated housing. This truly turnkey package provides all the tools necessary to analyze samples on day one, including the instrumentation, methods, standards, solvents, guard columns, analytical columns, and dedicated, user-friendly software with custom reporting templates. Figure 1B is an example of a modular HPLC, a system that provides greater versatility as components can be interchanged as needed.

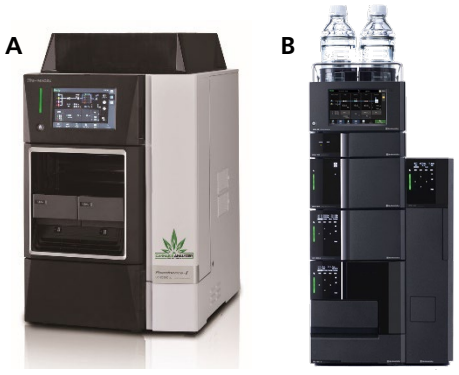

Figure 1: A) Integrated HPLC "Cannabis Analyzer" and B) Modular HPLC

■ HPLC vs UHPLC

Once the decision has been made to purchase an integrated or modular HPLC system, the next decision is to choose between conventional HPLC and ultrahigh-performance liquid chromatography (UHPLC); each has its own advantages and disadvantages. It should be noted that HPLC and UHPLC can both be obtained in integrated or modular formats. Shown in Figure 2 is a chromatogram of 11 cannabinoids by HPLC in 10 minutes, while Figure 3 displays the UHPLC chromatogram of 16 cannabinoids in 5 minutes. Table 1 shows examples of the cannabinoids analyzed by HPLC and UHPLC. Armed with this information the consumer may prefer the greater efficiency of separation from the UHPLC system. However, there are other factors which must be discussed.

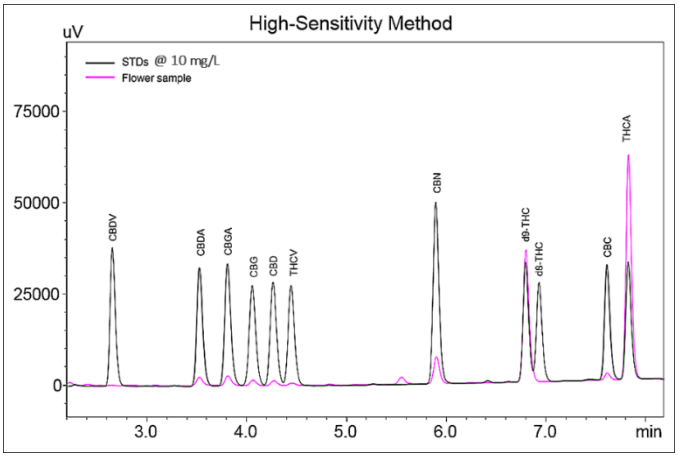

Figure 2: Chromatogram of 11 cannabinoids by HPLC in 10 minutes

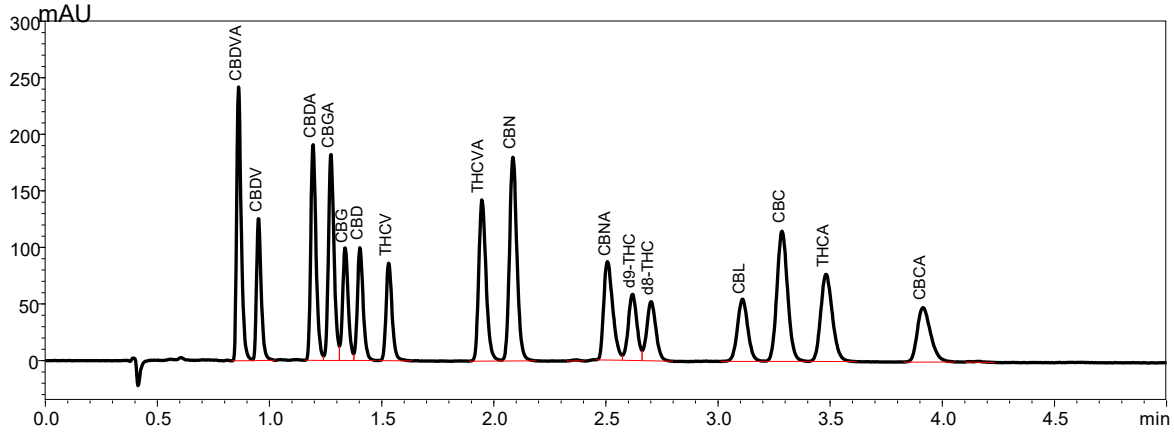

Figure 3: Chromatogram of 16 cannabinoids by UHPLC in 5 minutes-

Table 1: Cannabinoids measured by HPLC and UHPLC

HPLC Analysis	Compound Names	UHPLC Analys
THCV	Tetrahydrocannabivarin	THCV
Δ8-THC	Δ8-Tetrahydrocannabinol	Δ8-THC
Δ9-THC	Δ9-Tetrahydrocannabinol	Δ9-THC
THCA	Δ9-Tetrahydrocannabinolic acid	THCA
CBD	Cannabidiol	CBD
CBDA	Cannabidiolic acid	CBDA
CBDV	Cannabidivarin	CBDV
CBN	Cannabinol	CBN
CBG	Cannabigerol	CBG
CBGA	Cannabigerolic acid	CBGA
CBC	Cannabichromene	CBC
	Cannabidivarinic acid	CBDVA
	Tetrahydrocannabivarinic acid	THCVA
	Cannabinolic acid	CBNA
	Cannabichromenic acid	CBCA
	Cannabicyclol	CBL
	Exo-tetrahydrocannabinol	exo-THC

HPLC is a more robust method. "The robustness/ruggedness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage" [1]. Due to the robustness of HPLC, a lower-salaried technician could be utilized in place of a higher-salaried chemist for operation, sample preparation, and maintenance. Conversely, UHPLC demands the use of the highest quality solvents (UHPLC or LCMS grade) and that samples be filtered free of particulates.

In addition to robustness, the capital and operating costs for HPLC are lower. Instrument costs for HPLCs are approximately 20% lower than for UHPLCs. This takes into account the allowable use of HPLC-grade solvents instead of higher-cost UHPLC or LCMS-grade solvents. There is a lesser need to filter the mobile phase when using HPLC. A lower frequency of maintenance is another HPLC benefit, as a 30 - 50% reduction in the replacement of consumables, such as seals, plungers, rotors and stators, can be realized.

■ (U)HPLC-UV vs (U)HPLC-PDA Detectors

The acronym (U)HPLC implies the system can be utilized either as a HPLC or UHPLC system. There are multiple types of detectors that may be employed for cannabinoid analysis. This section will focus on systems based on absorbance detection in the ultraviolent/visible region of the electromagnetic spectrum (190 - 900 nanometer wavelength range), often abbreviated UV-Vis or UV/Vis.

For cannabinoid analysis the most important part of the spectrum is in the UV region of 190 - 350 nanometers (nm). The HPLC/UV is typically used to only measure a couple of specific wavelengths, such as 220 nm or 228 nm. With UV detection, confirmation of the specific cannabinoid is based on the retention time, as shown in Figures 2 and 3. The photodiode array detector (PDA), also known as a diode array detector (DAD), can measure the entire wavelength range simultaneously, which may provide other advantages. Figure 4 shows an example of the cannabinoid spectral absorbance profiles, which can provide a second form of identity confirmation. Notice the neutral cannabinoids (delta-9-THC, delta-8-THC, THCV, CBD, CBDV, and CBG) in the blue traces have similar spectral profiles that differ from the acidic forms (THCA, CBDA, and CGBA) shown in the red traces.

The carboxyl group (-COOH) of the acidic cannabinoids provides additional conjugation of the electron structure of the molecule. This results in longer wavelengths of the peak maximums. The PDA could be used to distinguish the neutral cannabinoids from the acidic forms but may not be sufficiently reliable to confirm cannabinoids within the same class. Also, other cannabinoids (CBN and CBC, shown in the green traces) have substantially different spectral profiles based on their structures.

PDA detection has other advantages in that the spectral profile may assist in determining an unknown peak in the chromatograms, such as another cannabinoid or terpene. Full confirmation analysis should be performed by a mass detector-based system, as discussed later in this article. In the pharmaceutical industry, a PDA detector is often used to determine peak purity of the target compound.

The absorbance spectra are compared at multiple points across the peak, as shown in Figure 5. Differences in the spectra can be indicative of co-elution in the chromatographic peak. A peak purity index of 1.000000 indicates a pure compound is eluting. As the peak purity index becomes lower, it can be concluded that co-elution of multiple compounds exists.

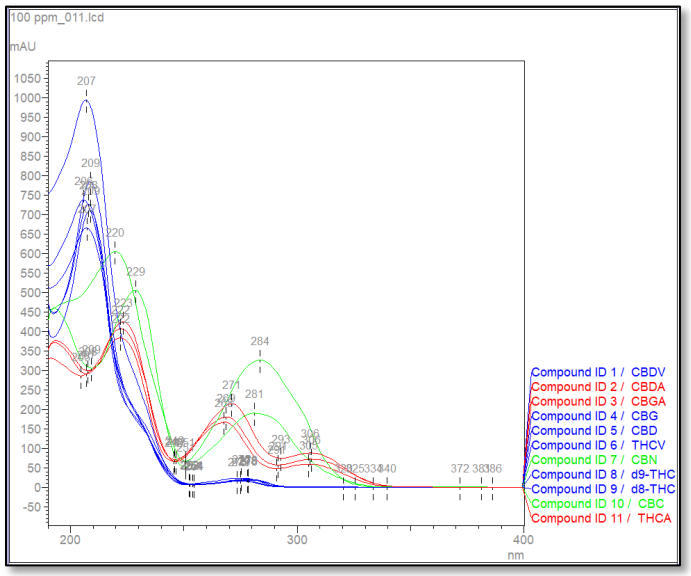

Figure 4: Simplified schematic of Peak Purity measurement across the peak and comparing with the spectral absorbance

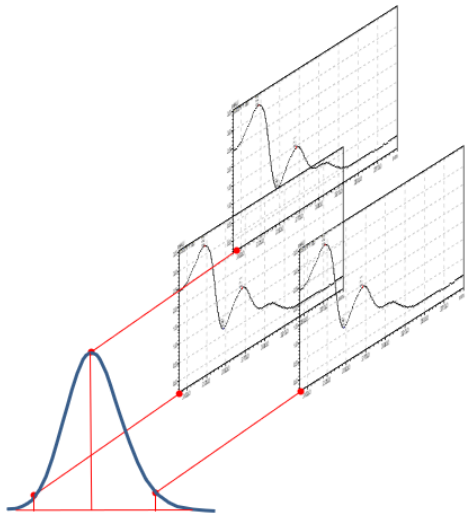

Figure 5: Deconvoluting co-eluting peaks utilizing time information (the chromatogram) as well as spectral information (UV spectrum)

Peak deconvolution is another possibility with PDA detection systems. A PDA detector collects time information (the chromatogram) and spectral information (UV spectrum), shown in Figure 6. By using both sets of information, it is possible to deconvolute the data and determine the quantity of each analyte in a co-eluting peak. Deconvolution relies on sound scientific principles, not estimation based on gaussian modeling, which has been used in the past.

Figure 7 shows an example of 5 cannabinoid peaks being deconvoluted using Shimadzu's Intelligent Peak Deconvolution Analysis (i-PDeA) software. Two forms of peak identification, including retention time and absorbance spectral ID, are now easily obtained. A third confirmation of mass spectral ID can be obtained by adding a mass spectrometer as described in the next section.

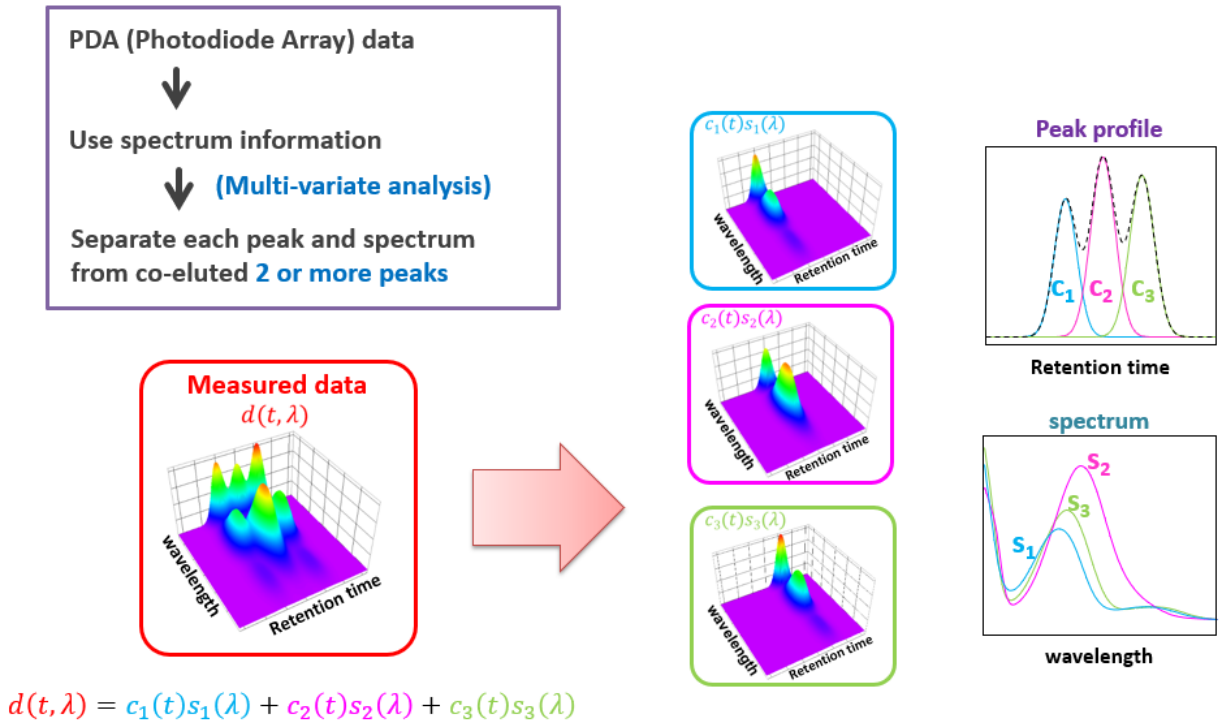

Figure 6: Example of 5 cannabinoid co-elution peaks being deconvoluted using Shimadzu's i-PDeA software

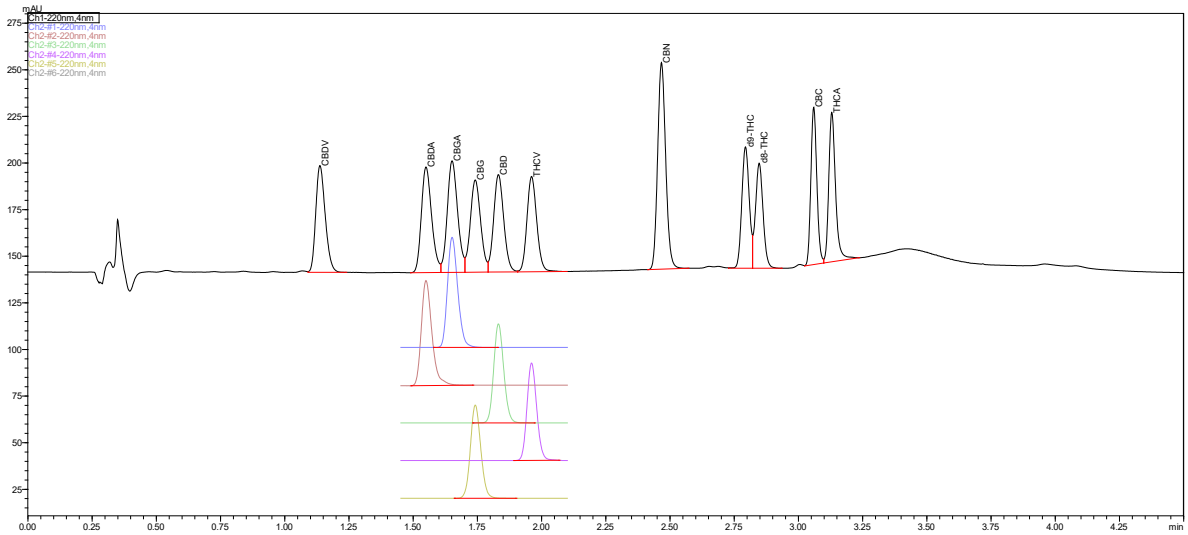

Figure 7: Example of 5 cannabinoid co-elution peaks being deconvoluted using Shimadzu's i-PDeA software.

■ **HPLC/UV vs LCMS vs LC-MS/MS**

Instead of an absorbance spectral detection of an HPLC/UV or HPLC/PDA, the analyst may prefer a mass spectrometry-based system, such as a single quadrupole liquid chromatograph mass spectrometer (LCMS) or a triple quadrupole mass spectrometer (LC-MS/MS), also known as tandem mass spectrometry. These types of mass spectrometers are referred to as high-speed mass spectrometers. They provide a fingerprint of the eluting chromatographic peak, especially with LC-MS/MS. A simplistic difference between LCMS and LC-MS/MS is the latter can assist in removing compound interferences and provide more confidence in compound identification.

Mass spectrometers can provide the analyst valuable insight with information about molecular weight and structures. They may also offer libraries for compound matching. For scientists in the research and development (R&D) arena, a mass spectrometer is often considered vital in the pursuit of critical molecular knowledge. LCMS and LC-MS/MS can also be combined with a UV/Vis or PDA detector to provide three forms of compound identification: 1) retention time, 2) absorbance spectra, and 3) mass spectra.

Each of these techniques have a certain cost. For example, a HPLC/UV costs in the range of $40 - $50K while an HPLC/PDA system is in the $50 - $60K range. UHPLC-based systems usually cost about 20% more. Most QA/QC labs believe these instruments are sufficient. High-speed LCMS systems are in the $100-$120K range, while LC-MS/MS are in the $300-$400K range and often are reserved for researchers. Many states permit using an HPLC/UV for cannabinoid analysis. An exception is the State of Tennessee, where the Department of Agriculture requires LC-MS/MS. In addition to a clear instrument cost advantage, conventional HPLC holds numerous other advantages, including a better linear dynamic range, better detector stability, reduced frequency of calibration, and a lower operator salary level (i.e. technician vs chemist).

Generally, QC labs using HPLC/UV or PDA do not use internal standards; however, some states, like New York require an internal standard (Norgestrel) as well as a surrogate (4-Pentylphenyl 4-Methylbenzoate). With LCMS(/MS), internal standards are usually a requirement. Also, the most popular LC-MS/MS sample introduction method is electrospray ionization (ESI), but for cannabinoid analysis, atmospheric pressure chemical ionization (APCI) is required.

Finally, contract labs may charge in the $50 range for cannabinoid analysis, so those incentivized by money are not likely to tie up the very expensive LC-MS/MS system, unless required to do so. LC-MS/MS would be more likely used for a higher billing pesticide analysis, typically in the $225 range per sample, and for simultaneous mycotoxins/aflatoxins analysis, which yields another $75, for a total of $300 per sample.

■ **Q-TOF MS vs MALDI-TOF MS**

Quadrupole Time-of-Flight Mass Spectrometers (Q-TOF MS) and Matrix Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometers (MALDI-TOF MS) are referred to a High-Resolution Mass Spectrometers (HRMS). Mass spectrometry (MS) measures the mass (m) to charge (z) ratio (m/z) in a sample. High-speed LCMS and LC-MS/MS measures the nominal mass of a compound, while HRMS measures the exact mass to several decimal places, providing more confidence for the analysis of targeted and untargeted compounds. Q-TOF also has the advantage of chromatographic separation by UHPLC. A Q-TOF MS costs in the range of $400K.

MALDI-TOF MS is a broad term encompassing a range of instrumentation types, including Ion Trap, benchtop and floor linear models, linear reflectron, and TOF-TOF, costing in the $150-$400K range depending on the specifications. MALDI-TOF doesn't utilize the chromatographic separation power of the previously discussed techniques so high resolution is very important.

Cannabis consists of more than 500 compounds with over 140 cannabinoids and over 200 terpenes, as reported in the literature. It also contains hydrocarbons, sugars, nitrogenous compounds, fatty acids, flavonoids, amino acids, aldehydes, ketones, esters, steroids, protein, elements, pigments, and vitamins. High resolution is important for targeted and untargeted compounds.

A bonus with using MALDI-TOF MS is it can be used to detect microorganisms. Microbes are made of proteins that will fragment differently in the mass spectrometer.

Shown in Figure 8 is the fragmentation pattern of five microorganisms. It can be easily seen that the top magenta spectrum for salmonella is different than the E. Coli spectrum in the blue trace.

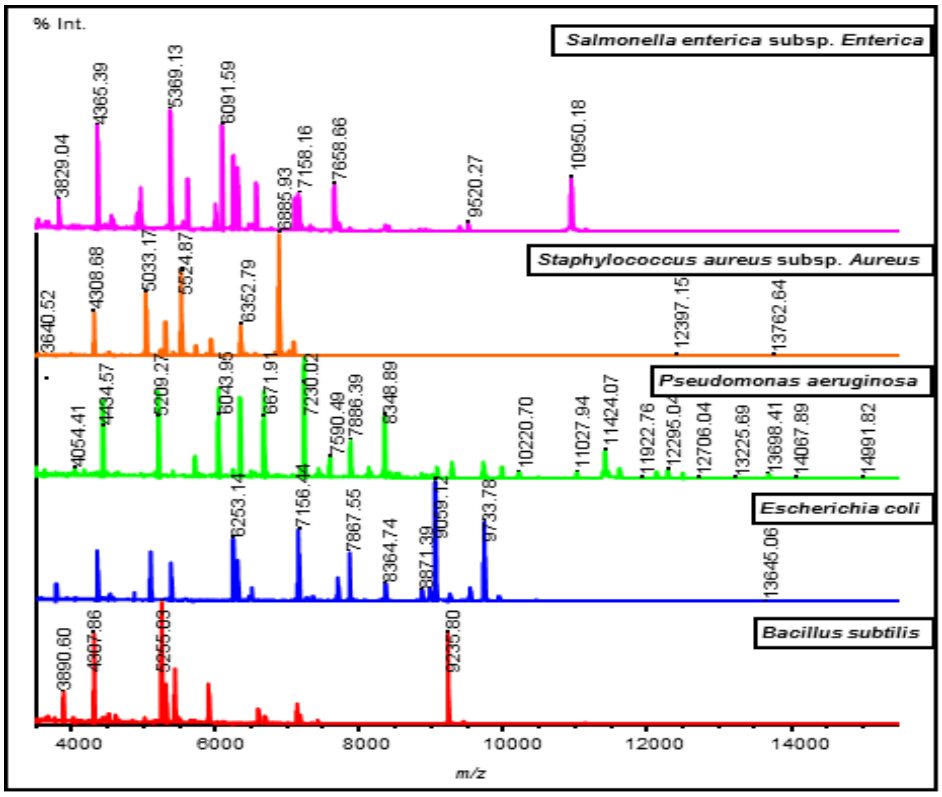

Figure 8: Detection of microorganism by MALDI-TOF MS

MALDI-TOF MS is very important in the clinical industry as a method for determining what may be causing an illness. MALDI-TOF MS is also gaining interest in the food industry. Currently, the cannabis industry uses techniques like enzyme-linked immunosorbent assay (ELISA) or quantitative polymerase chain reaction (qPCR); these techniques have capital cost advantages, but many other disadvantages, including the potential of false positives and false negatives reporting. In contrast, MALDI-TOF MS can store a library of more than 1000 microorganisms, analyze samples in two minutes for pennies a sample, and eliminate false positives and negatives found with other techniques. The disadvantage is the upfront expensive cost, but if a system is already in the lab, there is a bonus.

■ LC vs GC Based Methods

As described above, liquid chromatography (LC) may be the gold standard for cannabis analysis. However, gas chromatography (GC) systems have been reported to have better sensitivity and higher throughput then LC systems. But, due to the heated injection port and column, GC methods can only provide "Total delta-9-THC" as the delta-9-THCA is converted to the delta-9-THC. The same is true for any of the acidic cannabinoids, such as CBDA and CBGA, which will be converted to the neutral forms for CBD and CBG, respectively. It should be noted that delta-8-THC and THCV are not converted to delta-9-THC.

Figure 9 shows a chromatogram of Shimadzu's 11-cannabinoids premix standard (Part Number: 220-91239-21), which has only eight peaks because the three acidic components of THCA, CBDA, and CBGA have been converted to the neutral forms. Many analysts find this unacceptable since the cannabinoid concentrations in the original sample are missing some information. It should be noted that adding a headspace sampler to a GC-FID or GCMS allows its utilization for terpene and residual solvents analysis in the cannabis and hemp industries, and it may still be required in many instances.

Vladimiro Cardenia, et al. [2] reported on the derivatization of the acids to stabilize the structure and increase volatility for analysis by gas chromatography mass spectrometry (GCMS). The authors reported the analysis of 10 cannabinoids, including the three acidic cannabinoids. The derivatization step may be automated with the use of the proper autosampler.

Dr. Kevin Schug, Shimadzu Distinguished Professor of Analytical Chemistry, and his colleagues at the University of Texas, Arlington [3-5] have coupled a GC and GCMS to a vacuum ultraviolet (VUV) detector from VUV Analytics. The VUV region of the spectrum is below 200 nm and the working region of the authors was 120-240 nm, well below the HPLC/PDA region of 190-400 nm discussed earlier, which provided additional information. The electrons are excited from one energy level to a higher level such as σ - σ * (sigma to sigma star transition) for alkanes, n - π* (n to pi star transition) for O, N, S and halogens, and π- π* (pi to pi star transition) for unsaturated alkenes. In their publication, they showed GC-VUV and GCMS-VUV could analyze the neutral and acidic cannabinoids, but the later compounds would require derivatization.

As a bonus, the authors showed they could measure terpene isomers using the GC(/MS)-VUV system, measuring terpene alcohols, terpenes with single, double, and triple bonds, oxygenated terpenes with double bonds, and ethers, ketones, & aromatic terpenes [3-5].

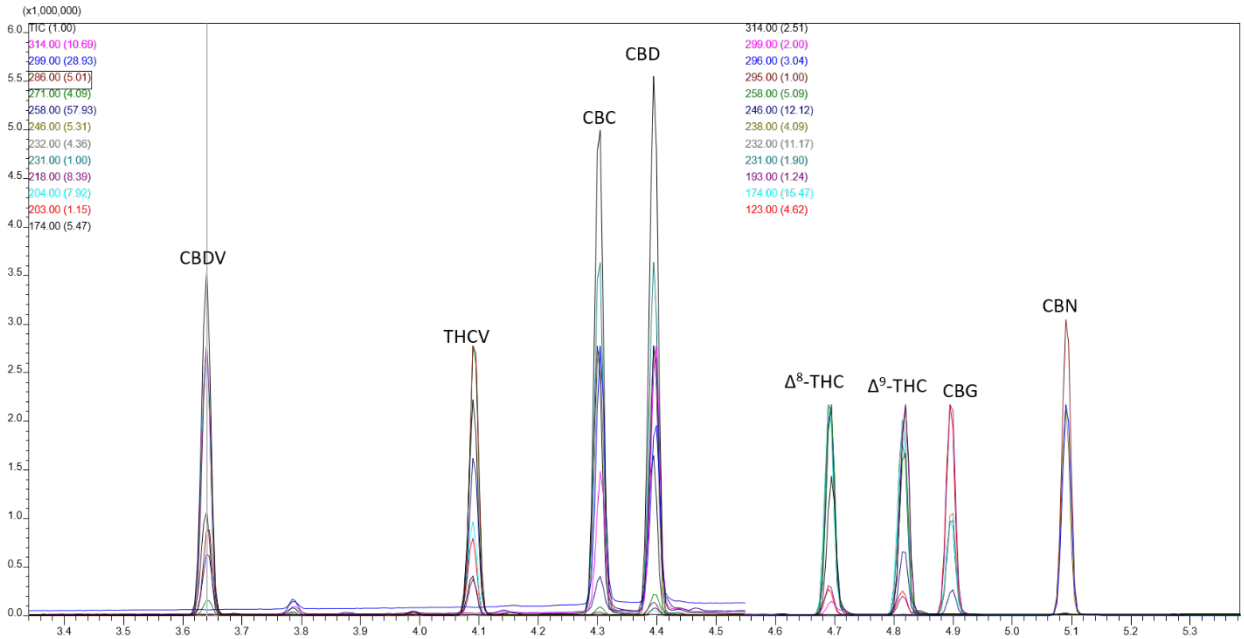

Figure 9: The GCMS analysis of the Shimadzu 11-cannabinoid premix standard only shows 8 peaks because the acidic THCA, CBDA, and CBGA are converted to the neutral THC, CBD, and CBG, respectively.

Shown in Figure 10 is a PY-GCMS-VUV system. The center is a Shimadzu Nexis GC-2030 with GCMS-QP2020 NX, on the right in the blue outline box is a VUV detector from VUV Analytics, and on the left and top are the red outline boxes for the Pyrolyzer unit from Frontier Labs, which is used for thermal desorption.

The above section only described utilizing a gas chromatograph with a VUV detector. An analysis utilizing only PY-GCMS is described in the next section. In the conclusion, there is a discussion of combining these three pieces for comprehensive experiments utilizing PY-GCMS-VUV.

Figure 10: Photo of TD-GCMS-VUV. Center is a Shimadzu Nexis GCMS-2020, on the right in the blue outline box is a VUV detector from VUV Analytics, and on the left and top are the red outline boxes for the Thermal Desorption unit from Frontier Lab.

■ Sample Preparation for LC and GC Based Methods

As cannabis and hemp are analyzed for cannabinoids, sample preparation depends on the sample type. As described above, liquid chromatography (LC) systems have been the gold standard. Sample preparation for analysis of concentrates is quite simple, typically only requiring a dilution in solvent. For flower, the sample preparation may involve extraction with one solvent and dilution with another solvent. Homogeneous foods such as gummy bears may also involve a solvent extraction with solvent dilution, which are relatively easy procedures.

The difficulty arises when the foods are non-homogenous, such as energy bars that may contain chocolate, almonds, peanut butter, and caramel. Different foods can require different sample preparation methods depending on the components of salts, sugars, sweeteners, fats, natural products, coloring, additives, preservatives, cholesterol, fiber, etc. For liquid chromatography, sample preparation would usually require a multiple step process: grinding into a homogeneous mixture, dissolving in a solvent, and then solid phase extraction (SPE).

The SPE steps then consist of conditioning a cartridge with a 1) solvent followed by 2) conditioning with water, 3) applying the sample to collect the target compounds on the cartridge while the rest goes to waste, 4) washing the cartridge with water to remove impurities, and 5) extracting the target compounds by washing the cartridge with solvent. This is very time consuming and varies depending on the matrix.

Frontier Labs have added a Pyrolyzer (PY), used for thermal desorption, as the sample introduction device to a GCMS. See Figure 10 for the GCMS and the red outline boxes for the PY unit. Sample preparation could be as simple as weighing the non-homogeneous food sample into a sample cup and having the autosampler drop the cup into an oven between 100-300 °C, which is high enough to vaporize the cannabinoids but low enough to leave behind higher boiling interfering compounds. Depending on the matrix, the sample may require grinding and, possibly, a liquid extraction step, but sample preparation steps are minimized compared to LC(/MS) sample preparation steps.

■ Conclusion

HPLC-based systems have been the gold standard for cannabinoid analysis as they offer a variety of selection between HPLC and UHPLC, integrated or modular formats, photometric detectors such as UV-Vis and PDA or DAD, and mass spectrometry detectors, such as MS, MS/MS, and Q-TOF LCMS, with each component having advantages and disadvantages. GC and GCMS-based methods are improving by reducing sample preparation with thermal desorption sample introduction for cannabinoid and terpene analysis utilizing vacuum ultraviolet detection.

Future experiments should involve combining PY-GCMS with GCMS-VUV, for a PY-GCMS-VUV configuration as shown in Figure 10, for faster sample preparation analysis of edibles with three forms of confirmation, with retention time, mass spectral identification, absorption spectral identification for cannabinoids and terpenes, and faster chromatography.

■ References

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Shimadzu Corporation
www.shimadzu.com/an/

Shimadzu Scientific Instruments
7102 Riverwood Drive, Columbia, MD 21046, USA
Phone: 800-477-1227/410-381-1227
www.ssi.shimadzu.com