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ANALYTICAL TECHNIQUES FOR CANNABINOIDS IN CANNABIS AND ITS PHARMACEUTICAL PRODUCTS: A REVIEW

Arham Wakeel[1], Talha Dilber[1], Muhammad Zia-ur-Rehman[1], Muhammad Zaheer[1*], Quratulain Syed[2], Syed Hussain Imam Abidi[3]

[1]Applied Chemistry Research Centre, Pakistan Council of Scientific and Industrial Research, Laboratories Complex, Ferozepur Road Lahore 54600, Punjab Pakistan.

[2]Food and Biotechnology Research Centre, Pakistan Council of Scientific and Industrial Research, Laboratories Complex, Ferozepur Road Lahore 54600, Punjab Pakistan.

[3]Pakistan Council of Scientific and Industrial Research, Head Office Islamabad 44000, Pakistan.

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***Corresponding Author**
Muhammad Zaheer
Applied Chemistry Research Centre, Pakistan Council of Scientific and Industrial Research, Laboratories Complex, Ferozepur Road Lahore 54600, Punjab Pakistan.

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ABSTRACT

Cannabis is a multifaceted plant with several bioactive phtyocannabinoids that have both medicinal and intoxicating qualities. Forensic analysis, medicinal research, production quality control, legal compliance, and other uses all depend on the analytical characterisation of cannabis and its derivatives. An impression of the many analytical methods used to analyze cannabis is given in this abstract. The methods covered include both qualitative and quantitative approaches. These include spectroscopic approaches like mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy, which are used for compound identification and structural elucidation, and chromatographic approaches like gas chromatography (GC) and high performance liquid chromatography (HPLC), which are frequently used for separating and quantifying cannabinoids, terpenes, and other constituents. In summary, from research and development to regulatory compliance and customer safety, the cannabis sector is greatly aided by the wide range of analytical techniques available for cannabis examination. To comprehend the makeup, strength, and safety of cannabis plants and goods associated with them, study is essential. An impression of

the methods used to analyze cannabis is given in this paper, which includes plant material as well as several derived products like oils, extracts, and edibles. Quantifying cannabinoids, terpenes, and pollutants is done by the use of mass spectrometry, spectroscopic methods, and chromatography (GC and HPLC). Cannabis analysis's regulatory issues and difficulties are also covered, with a focus on the significance of established procedures and quality control measures for accurate and trustworthy results. In the end, improvements in analytical methods help us better understand the chemistry of cannabis, supporting both its medical and recreational uses while addressing issues related to public health.

KEYWORDS: Cannabinoids, Phytocannabinoids, Analytical Techniques, Pharmaceutical products.

INTRODUCTION

For many years, investigations in toxicology and medicine have focused on cannabinoids (CNBs), which are found in plants alike Cannabis sativa L. and shown to have a variety of medicinal and psychotropic effects.[1-4] It's important to remember that cannabis has been grown for centuries in many parts of the world for textile, medical, and recreational purposes. The most highlighted ingredient, the renowned Δ9-tetrahydrocannabinol (THC), is still the utmost commonly marketed narcotic when it comes to oils, resins, and herbs.[1] Ten subclasses can be used to categorize the more than 90 CNBs that have been identified to date.[5,6] Cannabigerol (CBG), cannabichromene (CBC), THC, and cannabidiol (CBD) are the main components of CNBs. To a lesser degree, these chemicals are also present. These substances, especially in particular THC and CBD when taken separately or in combination, offer potential pharmacological benefits for the cure of a several of illnesses, including cancer, multiple sclerosis, prolonged pain, epilepsy, and anxiety disorders.[2,4,7] Moreover, unlike THC, CBD does not have any psychoactive qualities but does have certain pharmacological ones.[7,8] As a result, some nations have legalized cannabis for medical purposes. The FDA authorized Epidiolex®, a medication containing cannabidiol, in 2018, and Sativex®, a medication that combines THC and CBD, is currently prescribed in a number of nations (although not in the USA). The laws are complicated and differ from nation to nation based on the amount of THC in the drug product. Furthermore, other nations have made cannabis legal for recreational use, including Canada and Uruguay.[8,9] A thorough review of the literature found that cannabinoids in cannabis plant extracts may be quantified using a variety of analytical techniques, the most popular of which use gas chromatography

(GC) in conjunction coupled mass spectrometry (MS) detectors.[9,10] Acidic analogue quantification is limited by the GC method because of hot input and oven conditions that cause decarboxylation. Moreover, it was discovered that the decarboxylation procedure was insufficient and caused problems for quantitative analysis. Extensive derivatization processes are necessary for the identification of acidic cannabinoids in GC.[11] High performance liquid chromatography (HPLC),[12] thin layer chromatography (TLC),[13] HPLC coupled with mass spectrometry (HPLC/MS),[14] ultra-high performance supercritical fluid chromatography (UHPSFC),[15] liquid chromatography coupled with MS (LC–MS),[16] and centrifugal partition chromatographic techniques,[17] have all been reported as methods for the quantification of both acidic and neutral cannabinoids. Techniques for quantifying CBD in pure isolation were previously published,[18] The HPLC approach was found to be the most straightforward of all the techniques because it can determine both acidic and neutral forms, and the system doesn't even need pricey MS detectors. Numerous HPLC-based procedures have been described.[19,20] and the majority of these procedures make use of buffer mobile phases and gradient elution techniques. Furthermore, these techniques show how to separate a mixture of cannabinoids that contains more than just THC and CBD.[21] Isocratic elution techniques, in which the mobile phase exclusively contains organic modifiers, are employed in the most basic HPLC procedures.[22,23] To meet the ever-changing legal requirements of the hemp sector, straightforward, reliable, precise, and effective systematic techniques for the quantification of key composites like THC and CBD must be developed. Here, we show that the reverse phase HPLC (RP-HPLC) method may be used to speedily and precisely conclude the quantities of THC and CBD in hemp oil products. Because this procedure takes use of isocratic elution, it can be carried out in any conventional HPLC system. Additionally, the technique simply employs an organic modifier's mobile phase and works well in room temperature environments. The requirements for assay method validation for the quantitation of CBD and THC by liquid chromatography are outlined in the International Council for Harmonization (ICH) quality guideline Q2(R1), "Validation of Analytical Procedures: Test and Methodology." The specificity, linearity, accuracy, range, precision, and robustness of the analytical method were assessed in accordance with these requirements.[24]

Quantitative analysis of cannabinoids by Gas chromatography (GC)

Gas chromatography (GC) is one of the most popular chromatographic methods for quantitative cannabis analysis.[25] It can be completed in less than 20 minutes at temperatures as high as 300 °C,[26] and it uses low polarity stationary phases, such as 5% diphenyl- and

95% dimethyl polysiloxane. It is important to keep in mind that the total amount of neutral and acidic components in a sample equals the amount of cannabis in the sample.[27] The acidic cannabinoids in gas chromatography undergo decarboxylation as they pass through the column because of the high column temperatures needed for this procedure,[27] As a result, unless acidic cannabinoids are removed prior to analysis, their identity cannot be determined,[28] By increasing the volatility of cannabis while maintaining the cannabinoid structure, derivatization enhances peak shape,[26] Dussy and Hamberg (2005) suggested measuring the concentrations of acidic and neutral cannabinoids separately in order to accurately determine the overall cannabinoid content. Gas chromatography is used to determine cannabinoids; nevertheless, elution detection presents special challenges and solutions.[29]

GC- FID/MS

To identify and measure cannabinoids, GC is typically used in conjunction with flame ionization detection (FID) or mass spectrometry (MS). Compound libraries can be used to identify the parent analyte by the use of MS, which fragments analytes using standardized electron ionization. While comparable deuterated standards, which are expensive and unavailable for all cannabinoids, are frequently needed for mass spectrometry, FID uses comparatively inexpensive authentic standards, which leads to more accurate measurement of cannabinoids,[27,28] A disadvantage of the high injection temperature is that it does not preserve the acidic form of cannabis. Two parameters need to be identified in order to validate any quantification techniques. Limits of quantification (LOQ) and detection limit (LOD). used triple quadrupole mass spectrometry in conjunction with GC-MS for the first time to analyze cannabis using Multiple Reaction Monitoring (MRM) from a surrogate hops matrix.[30] The lowest concentration of the chemical of interest that may be accurately ascertained by an analytical approach is shown by these two characteristics. They used silylated cannabinoids to stop the decarboxylation process that was brought on by the GC injection port's high temperature. They found that this technology might be used to analyze the cannabinoids found in plant materials and cannabis products. The primary finding of their study is that, in comparison to other techniques like SPE, this extraction approach requires less sample preparation in the labs due to the decreased possibility of interferences from essential oils and waxes. Vacuum ultraviolet spectroscopy, or gas chromatography with vacuum ultraviolet spectroscopy, or GC-VUV, was employed in another study. Cannabinoids and their metabolites were quickly and easily detected, making it possible to employ them for

rapid detection even in the absence of a baseline for cannabinoids to compare against. The single drawback of this approach is its high limit of detection (LODs). Because of this restriction, analytes in biological matrices cannot be identified without pretreatments.[31]

Liquid chromatography based methods

Lately, LC-based techniques have emerged as the preferred approach for both qualitative and quantitative phytocannabinoid profiling. In addition to the efficient sample preparation, low temperatures, high pressure, and high flow rates used in TLC, HPTLC, HPLC, and UHPLC, the recently developed supercritical fluid chromatography (SFC) analysis enables the preservation of samples without decarboxylation and decomposition, the reliable separation of neutral and acidic phytocannabinoid species, and the subsequent direct identification and quantification of both neutral and acidic forms of phytocannabinoids in the extracted samples.[32,33] The utilization of LC in cannabis profiling was preferred over GC due to the streamlined sample preparation procedures and the prevention of analyte loss.[34]

- **Thin Layer Chromatography and High Performance Thin Layer Chromatography Methods**

TLC is a desirable technique designed for analyzing the ingredients of herbal drugs,[35] and it is particularly well-suited for the initial semi-quantitative screening of cannabis concentration in standard assays.[36] By paralleling the retardation factors (RFs) of analytes and standards on a TLC plate prepared with the proper mobile phase, one can identify cannabinoids using TLC. Meanwhile, visual evaluation can be achieved by submerging or dousing the TLC plate with the suitable visualization reagent in the daylight or under UV light. TLC methods are promising for cannabis fingerprinting due to their accuracy, reproducibility, and acceptable LODs and LOQs in the linear dynamic range.[37] However, these characteristics are rather low when compared to more advanced LC analytical platforms. Because it was difficult to document for peer review, the "classic" TLC was used less frequently because of issues with temperature/humidity control, handspotting systematic mistakes leading to poor resolution, and inaccurate RF measurement.[38,39] Thus, it has been demonstrated that, for the primary neutral phytocannabinoids, normal-phase HPTLC with programmed spotter separates the molecules 2022, 27, 975 25 of 42 more effectively than TLC. The technique is equivalent to a validated HPLC method within a tiny error margin (±0.5%).[40] Other planar chromatography techniques, such as optimum performance laminar chromatography (OPLC) for phytocannabinoid profiling, are rarely utilized in addition to TLC and HPTLC despite having

higher reproducibility because of total automation. Additionally, AMD provides the finest resolutionwhereas extension is permitted as a semipreparative technique for sample purification in OPLC. The only known applications of OPLC for cannabinoid profiling in AMD are hexane extracts of cannabis resin and dried and reconstituted cannabis resin in toluene. On HTSorb BSLA 011 and HT Sorb BSLA 003 columns, Δ 9 -THC, CBD, and CBN were measured using optical parametric spectroscopy (OPLC). Isooctane/diethylether (90:10, v/v) was the eluent utilized. Using semi-preparative OPLC with hexane/diethylether (80:20, v/v), CBD was extracted from cannabis resin. Acetone (100, v/v), diisopropylether (100, v/v), hexane (100, v/v), and hexane (100, v/v) comprise the elution gradient 1C. were separated by AMD on HPTLC during the course of 20 steps. The Fast Blue B salt reagent is used for visualization in both OPLC and AMD.[41]

• High performance liquid chromatography (HPLC) method

The HPLC approach is becoming more and more common as the primary method for fingerprinting studies for herbal drug quality control,[42] allowing herbal medicines to be chemically characterized.[43] Compared to GC-based approaches, for all phytocannabinoids, HPLC methods yield larger linear ranges and more dependable calibration curves.[44] Reliability, repeatability, and sensitivity of high-resolution GC/FID and HPLC-UV methods for detecting Δ 9-THC, CBD, and CBN were found to be similar.[45]

I. HPLC Mobile Phases

The composition of the mobile phases consumed in the HPLC/DAD analysis of phytocannabinoids was MeCN with 0.1% o-phosphoric acid and buffered aqueous solutions.[46] For the assay of Cannabis flowers and Cannabis extractum normatum, acidic conditions are recommended for cannabinoid acids (Δ 9 -THCA, CBDA, and CBGA). These assays focus on the five primary phytocannabinoids: CBDA, CBD, CBN, Δ 9 -THC, and Δ 9 -THCA employing an aqueous solution of 85% o-phosphoric acid and MeCN as mobile phases.[47]

II. HPLC Columns

Similar columns are used by the many LC-based techniques for phytocannabinoid profiling; the difference in the instrumental conditions results in better quantification strategies. Direct injection is only used in one method,[48] eschewing the column. A wide range of phytocannabinoids (focused on, but not limited to, CBDV, CBDA, CBGA, CBG, CBD, THCV, CBN, Δ 9 -THC, Δ 8 -THC, CBC, Δ 9 -THCA) can be reliably separated and

quantified using columns with normal phase C18 stationary phase, with or without guard column or C18 guard cartridge.[49]

III. HPLC Detectors

UV, DAD, PAD, or MS are combined to (U) HPLC analytical platforms in order to perform phytocannabinoid profiling. Due to the low molar absorptivity of phytocannabinoids, LC techniques utilizing UV and DAD have comparatively low sensitivity. This limits the use of DAD detection to low wavelengths, particularly in gradient elution investigations, where the eluent components often cause significant background absorbance.[50] Cannabinoid acids (CBDA and CBGA) show three absorption maxima (λmax): one stronger at 220–223 nm, the second at 266–270 nm, and the third one around 305 nm. Two wavelengths are selected: 210 nm for neutral phytocannabinoids and 220 nm for cananbinolic acids. Typically, the ranges 190–600 nm and 200–650 nm are used for UV collection.[51,52]

Matrix Effect

The choice methodology for extraction, extraction solvent(s), HPLC column, mobile phase composition, and detection method is significantly influenced by the sample, or matrix type, and ultimately improves the sensitivity, selectivity, and specificity of the approach. The existence of medium elements that co-extract with phytocannabinoids, resulting in sign modification (overpowering or augmentation), affects all three validation parameters. In keeping with this, matrix impact is often documented in phytocannabinoid profiling conducted using LC-MS. The complex matrix of cannabis plant material contains a high concentration of fat, pigment, and polar chemicals, with flavonoids and terpenes being the most prevalent. Products derived from cannabis exhibit greater versatility in terms of fat, sugar, and polar interference concentration, making them more likely to exhibit notable matrix suppression when subjected to instrumental examination. When phytocannabinoids are extracted from honey using ethanol (EtOH), various interfering matrix components, including flavonoids, are also extracted together with the phytocannabinoids, causing significant polar matrix interferences.[53] When commercial items such as oils, lotions, and plant material are phytocannabinoid profiled, the matrix effect is also investigated. In oil, there is no discernible matrix impact for Δ 9 -THC, CBD, CBDA, and Δ 9 -THCA. The plant material exhibited a notable matrix impact of CBDA, with signal increase occurring primarily at low doses.[54]

Fourier transforms infrared spectroscopy (FTIR)

The examination of cannabinoids is done by adding KBr to the ethanolic solution of cannabinoids and then vacuum evaporating the ethanol, as KBr does not display absorption spectra in the infrared region. Moreover, KBr shows a 100% transmission window in the wave number range during the FTIR spectroscopy. The infrared spectra were measured within the region of 500–4000 cm− 1. Compared to UV spectra, IR spectra displayed higher absorption peaks.[32] The carbonyl and ester groups in cannabis extract composite samples exhibited FTIR peaks at 1775 and 1725 cm−1, respectively.[55]

Nuclear magnetic resonance spectrometry (NMR)

An additional alternative to GC and HPLC is NMR.[3,56] Unlike GS and HPLC, NMR is accurate and reliable, and it is unaffected by contaminants like lipids or chlorophyll that are present in the sample.[57] The cannabinoid quantification method based on 1 H-NMR, which does not require chromatographic purification, requires a 5-minute final analysis time. In that study, they analyzed singlets in the δ 4.0–7.0 region of the 1 H-NMR spectrum and found that their method was suitable for quantifying THCA, CBDA, CBG, CBGA, and possibly additional cannabinoids as well.[57] One important benefit of this method is that it does not require reference standards, so it can quantify cannabinoids that do not have reference standards and cannot be analysed using other methods. Despite the encouraging results of NMR, One major limitation of the method is the expensive nature of high resolution equipment.[3]

Table 1: Cannabis and cannabis-based product analytical techniques Characterizing Phytocannabinoid.

Techniques of analysis	Advantages	Drawbacks
HPLC PDA/UV	Estimation of phytocannabinoids in individually acidic and neutral form	The intricate makeup of the cannabis material results in a significant peak duplication of the phytocannabinoids. -only target analytes, not entire spectrum, may be detected - limited application for biological sample analysis Substantial peak duplication of the phytocannabinoids is caused by the omplex makeup of the cannabis extract -Only target analytes, not whole spectrum, can be measured. - Limited application in biological sample analysis
Triple quadrupole	fingerprints complex matrices more	QQQ instrument setup calls for meticulous tuning and optimization, which takes time

	specifically and accurately	and work.
HPLC-Q-Exactive Orbitrap®	- Strong complex matrix selectivity -Analyte structure confirmation -Examining the "Un-knowns"	QQQ instrument setup calls for meticulous tuning and optimization, which takes time and work.
(HP)TLC	Quick screening of numerous samples to determine whether cannabis are present, improve determination, and produce results intended for easier casework credentials in crime labs for peer review	-Poorer efficacy in comparison to alternative separation methods -reproducibility is dependent on humidity
GC-FID	Greater precision in measuring cannabinoids compared to GCMS - solvent residues and terpenoids - Enhanced resolution	Derivatization processes for acidic cannabinoids takes a long time.
GC-MSD	Compound libraries for parent analyte identification; -higher specificity and sensitivity	Applying comparable deuterated standards (which are pricey and not available for all types of cannabis compounds)
GC-QTOF/QQQ	- Highest sensitivity simultaneous analysis of residues from pesticides, aromatic compounds, and cannabinoids - Testing of "Un-knowns"	
SFC	Green method appropriate for chemicals that are intensely unstable	SFC equipment accessibility
NMR	Resistant to the ballast chemicals lipids and chlorophylls. It's not required to use reference standards.	This analyzer's high cost
RAMAN	Rapid, adaptable, and simple qualitative and	

	quantitative characterisation of the growth stages and concentrates of the cannabis plant	
FTIR, NIR, MIR	- Chemical fingerprinting of constituents - Testing of varied compounds, such as cannabis specimen, and measuring the flower's effectiveness - Quick on-site application to track cannabis growth and curing procedures	-Must be used in conjunction with chemometrics - Less precise when analyzing potency

Techniques for testing of phtyocannabinoids from cannabis plant

Different cannabinoids in Cannabis sativa have been separated, identified, and quantified using LC, HPLC, and UHPLC techniques. Because UHPLC requires less solvent in the mobile phase and requires less time to analyze, it has gained popularity in recent years.[58] Large quantities of THC, CBD, and THCA—all of which have medicinal properties—were detected in a study conducted by Bala and colleagues using UHPLC linked to MS.[59] DAD was the most widely employed detector when using UHPLC to evaluate cannabinoids from plants3.[60] A straightforward method based on a mobile phase composition of water having 0.1% formic acid and acetonitrile containing 0.1% formic acid running with gradient elution mode (ranging between 40–100%) through a Phenomenex Luna Omega C18 column having dimensions (150 × 2.1 mm × 1.6 µm) and PDA detection monitored at 280 nm was used by colleagues and Elkins to analyze THC, CBD, CBN, CBDA, CBC, and THCA.[61] While DAD is frequently used as a detector for HPLC and UHPLC, the popularity of combination detectors like UV-PDA, ESI-MS, and MS/MS has also grown. The ability to conduct analysis in individually positive and negative ion mode is one benefit of utilizing ESI-MS or MS/MS. While acidic cannabinoids produce a stronger signal in the negative ion-mode, neutral cannabinoids produce a stronger signal in the positive ion-mode.[62] Using the three integrated detectors, Brighenti and associates devised a methodology for the testing of non-psychoactive phytocannabinoids.[62] Sensitivity of LC is lower than that of HPLC and UHPLC. Simpler and less expensive equipment is needed to employ LC.[63] Dong et al. created a real-time mass spectrometry method for thermal desorption direct analysis, and they compared the

outcomes with an LC-MS system.[64] Sample preparation is usually carried out in tandem with dynamic maceration (DM), which involves extracting target analytes from plant biomass using a solvent, a vortex, or stirring at room temperature.[62] Four different extraction techniques were compared by Brighenti et al.: ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and supercritical fluid extraction (SFE), and DM. The extraction methods known as UAE and MAE use microwave radiation and ultrasonic waves to extract marijuana' secondary metabolites more quickly and thoroughly.[62] Comparing SFE vs organic solvent-based cannabinoid extraction methods from plant material, the former is more ecologically friendly.[62] Elkins et al.[61] used a biobotanical SFE liquid CO2 extractor to remove the resin from cannabis. The most effective way to extract acidic cannabinoids, such CBDA and MAE for CBD, is using DM.[62] Solid phase extraction (SPE) with supercritical carbon dioxide (scCO2) was used by Ribeiro Grijó and associates to complete the extraction procedure while removing all traces of organic solvents from the prepared sample.[65] Just four out of the 22 studies that examined cannabinoids from plants by using isocratic; the majority of studies used gradient mode elution of the mobile phase. MeOH and ACN are often the organic solvents combined with water to make up the majority of the mobile phases. Because ACN reduces the overall run duration in comparison to MeOH, it was chosen. The mobile phase flow rate varied from 0.3 mL/min to 3 mL/min; the most often utilized values were 0.4 mL/min and 0.3 mL/min.

Techniques for testing phtocannabinoids in Oil

The use of CBD oil for various ailments has grown in popularity in recent years.[66] Standardized regulations for extraction are lacking.[66,67] Hemp seed oil, black cumin seed oil, medium chain triglyceride (MCT), and olive oil are some of the several carrier oils available on the market. Because of its high viscosity, oil cannot be injected directly into an HPLC, necessitating the employment of effective extraction techniques for the detection of cannabinoids in oil.[68] For the extraction of different cannabinoids from olive oil, Bettiol and colleagues and Deidda and associates utilized the same procedure: they vortex-mixed 40 µL of sample in olive oil with 960 µL of tetrahydrofuran (TFH). Next, 50 µL of this solution was added to 950 µL of ACN and MeOH, respectively, in the studies conducted by Bettiol and colleagues and Deidda and colleagues.[69,70] Mudge and colleagues performed a solvent extraction using MeOH, but Nemeškalová and colleagues used isopropanol/ethyl acetate (1:1, v/v). Ciolino and colleagues used ethyl alcohol, or etOH[71,72,73] Benchtop nuclear magnetic resonance (NMR) equipment was used by Araneda and associates to analyze cannabis and

compare the results with those obtained using HPLC-UV. An analysis was performed on five distinct cannabis concentrations. When using benchtop NMR to examine samples, the relative standard deviation was larger than when using HPLC-UV. The amount of THC in sample 2 and CBD in sample 1 could not be determined in the benchtop NMR studies, however both samples could be determined in the HPLC analysis.[74]

Conclusions and Prospective Courses of Action

The production, industrial, medical, and recreational applications of cannabis, together with new legal regulations, have all led to the development of numerous analytical techniques for phytocannabinoid profiling. The matrix nature greatly influences the choice of extraction procedure, sample preparation, and analytical method since considerable matrix interferences might arise and worsen the overall analysis of target phytocannabinoids. For the past 40 years, versatile kinds of accelerated have been the mainstay of sample preparation for phytocannabinoid profiling. More advanced environmentally friendly processes have lately been developed. SFE and readily-automatable HS-SPME are two examples of these methods, which increase the speed, repeatability, and reproducibility of analyses. The most often used analytical platforms are TLC and HPTLC, HPLC-DAD, GC and LC combined with mass spectrometry (MS or MS/MS); nevertheless, NMR and vibrational spectroscopy approaches, like IR, NIR, FTIR, and FT-NIR, are recently emerging techniques. TLC in addition to HPTLC, which is a good technique for sample screening and is covered by pharmacopoeias in the identification procedures. The two analytical platforms that are primarily used for cannabinoid profiling in research, industry, and quality control are GC and LC. For phytocannabinoid profiling, mass analyzers are among the most established, favored, and extensively studied analytical systems because of their dependability, accuracy, speed, and sensitivity. The development of mass spectral libraries (MSLs), compound databases (DBs), public compound repositories, computational tools, and sophisticated detection methods has led to the use of GC as the analytical platform for forensic, pharmacokinetic, and phytochemical analysis of naturally occurring phytocannabinoids. Because of this, authorities also formally use GC technologies for terpene profiling, pesticide screening, and residual solvent analysis, which may be advantageous for regulatory bodies and the cannabis sector. In TLC, HPTLC, HPLC, and UHPLC, high pressure and high flow rates are used to preserve samples without decarboxylation or decomposition. The recently developed SFC technique also makes it possible to reliably separate neutral and acidic cannabinoid species, allowing for the direct identification and quantification of both neutral and acidic forms of

phytocannabinoids in the extracted samples. Over the last few decades, LC (HPLC-DAD and LC-MS) has emerged as the preferred analytical technology for potency investigations as well as for untargeted examination of cannabis and cannabis-derived products. Although vibrational spectroscopy techniques like Raman, FT-NIR, NIR, and FT-IR are only used for structural clarification, there has been a noticeable movement in recent years toward their use for quick quantitative phytocannabinoid profiling. These vibrational spectroscopy techniques are fast, cheap, non-destructive, and require little to no sample preparation (e.g., drying, grinding), even though they can offer a rapid, versatile, and non-invasive approach to qualitative and quantitative profiling and growth staging of cannabis plants and extracts. They also demonstrate far higher LOD and LOQ than the described chromatography-'wet' methods. Even though the instruments are expensive and highly specialized personnel are needed, NMR is thought to be a very accurate, fast, and repeatable technique that provides a quantitative assessment of cannabis without the need for chromatographic separation, certified reference standards, or a pre-purification step. Many well-established methods are currently available for chemical analyses of phytocannabinoids; however, these methods still need to be refined and modified in light of new scientific data regarding the plant and its metabolites, especially concerning the pharmacological activity and its potential applications in medicine, the connection between potency and favorable and unfavorable health effects, the interaction between specific phytocannabinoids and other active ingredients, quality assurance, and stability studies of cannabis and cannabis-derived products. The field of phytocannabinoid profiling should advance to the point where orthogonal analytical techniques are applied to examine cannabis plant material and cannabis-derived products in an unbiased manner. It is expected that cheminformatics approaches for small molecule identification and MSLs will be used in the near future to identify a large number of novel phytocannabinoids and other chemicals. This will make precise and thorough phytocannabinoid and terpene profiles accessible.

REFERENCES

1. Whiting PF, Wikff RF, Deshpande S, et al. Cannabinoids for medical use: a systematic review and meta-analysis. JAMA., 2015; 313(24): 2456-2473.
2. UNODC. Recommended Methods for the Identification and Analysis of Cannabis and Cannabis Products. New York, NY: UNODC., 2009. https: //www.unodc.org/documents/scientific/ST-NAR-40- Ebook_1.pdf. Accessed, 2020; July 10.

3. Citti C, Braghiroli D, Vandelli MA, Cannazza G. Pharmaceutical and biomedical analysis of cannabinoids: a critical review. J Pharm Biomed Anal., 2018; 147: 565-579.
4. Andre CM, Hausman JF, Guerriero G. Cannabis sativa: the plant of the thousand and one molecules. Front Plant Sci., 2016; 7: 19. 10.3389%2Ffpls. 2016; 00019.
5. Fischedick JT, Hazekamp A, Erkelens T, Choi YH, Verpoorte R. Metabolic fingerprinting of Cannabis sativa L., cannabinoids and terpenoids for chemotaxonomic and drug standardization purposes. Phytochemistry, 2010; 71: 2058-2073.
6. Brenneisen R. Chemistry and analysis of phytocannabinoids and other cannabis constituents. In: ElSohly M, ed.Marijuana and the Cannabinoids Forensic Science and Medicine. New York: Humana Press, 2006; 17- 49.
7. Burstein S. Cannabidiol (CBD) and its analogs: a review of their effects on inflammation. Bioorg Med Chem., 2015; 23(7): 1377-1385
8. Corroon J, Phillips JA. A cross-sectional study of cannabidiol users. Cannabis Cannabinoid Res., 2018; 3(1): 152-161. 9. Hädener M, König S, Weinmann W. Quantitative determination of CBD and THC and their acid precursors in confiscated cannabis samples by HPLC-DAD. Forensic Sci Int., 2019; 299: 142-150.
9. Villamor JL, Bermejo AM, Tabernero MJ, Fernández P. Determination of cannabinoids in human hair by GC/MS. *Anal. Lett.,* 2004; **37**(3): 517–528. doi: 10.1081/AL-120028624.
10. Karschner EL, Barnes AJ, Lowe RH, Scheidweiler KB, Huestis MA. Validation of a two-dimensional gas chromatography mass spectrometry method for the simultaneous quantification of cannabidiol, Δ9-tetrahydrocannabinol (THC), 11-hydroxy-THC, and 11-nor-9-carboxy-THC in plasma. *Anal. Bioanal. Chem.,* 2010; **397**(2): 603–611. doi: 10.1007/s00216-010-3599-6.
11. Dussy FE, Hamberg C, Luginbühl M, Schwerzmann T, Briellmann TA. Isolation of Δ9-THCA-A from hemp and analytical aspects concerning the determination of Δ9-THC in cannabis products. *Forensic Sci., Int.,* 2005; **149**(1): 3–10. doi: 10.1016/j.forsciint.2004.05.015.
12. Deidda R, et al. Analytical quality by design: Development and control strategy for a LC method to evaluate the cannabinoids content in cannabis olive oil extracts. *J. Pharm. Biomed. Anal.,* 2019; **166**: 326–335. doi: 10.1016/j.jpba.2019.01.032.
13. Sherma J, Rabel F. Thin layer chromatography in the analysis of cannabis and its components and synthetic cannabinoids. *J. Liq. Chromatogr. Relat. Technol.,* 2019; **42**(19–20): 613–628. doi: 10.1080/10826076.2019.1663529.

14. Aizpurua-Olaizola O, Omar J, Navarro P, Olivares M, Etxebarria N, Usobiaga A. Identification and quantification of cannabinoids in *Cannabis sativa* L. plants by high performance liquid chromatography-mass spectrometry. *Anal. Bioanal. Chem.,* 2014; **406**(29): 7549–7560. doi: 10.1007/s00216-014-8177-x.
15. Isaac G, et al. Ultra-high performance supercritical fluid chromatography applications for natural products analysis. *Planta Med. Int. Open,* 2017; **4**(S01): S1–S202. doi: 10.1055/s-0037-1608335.
16. Lebel P, Waldron KC, Furtos A. Rapid determination of 24 synthetic and natural cannabinoids for LC-MS-MS screening in natural products and drug inspection applications. *Curr. Trends Mass Spectrom. Suppl. LCGC N. Am.,* 2015; **13**(1): 8–14.
17. Hazekamp, A. *A general introduction to cannabis as medicine. In: Cannabis: extracting the medicine.* PhD diss., Institute of Biology Leiden (IBL), Faculty of Science, Leiden University (2007).
18. Layton CE, Aubin AJ. Method validation for assay determination of cannabidiol isolates. *J. Liq. Chromatogr. Relat. Technol.,* 2018; **41**(3): 114–121. doi: 10.1080/10826076.2018.1424637.
19. Mudge EM, Murch SJ, Brown PN. Leaner and greener analysis of cannabinoids. *Anal. Bioanal. Chem.,* 2017; **409**(12): 3153–3163. doi: 10.1007/s00216-017-0256-3.
20. Layton, C. & Reuter, W. M. Analysis of Cannabinoids in Hemp Seed Oils by HPLC using PDA detection. Application Note. PerkinElmer, Shelton, CT, Unites States. (2015). https: //cdn.technologynetworks.com/tn/Resources/pdf/analysis-of-cannabinoids-in-hemp-seed-oils-by-hplc-using-pda-detection.pdf. (accessed 13 May 2019). [Ref list]
21. Aubin, A. J., Layton, C. & Helmueller, S. Separation of 16 cannabinoids in cannabis flower and extracts using a reversed phase isocratic HPLC method, waters application note 720006426EN (2018).
22. Saingam W, Sakunpak A. Development and validation of reverse phase high performance liquid chromatography method for the determination of delta-9-tetrahydrocannabinol and cannabidiol in oromucosal spray from cannabis extract. *Rev. Bras. Farmacogn.,* 2018; **28**(6): 669–672. doi: 10.1016/j.bjp.2018.08.001.
23. Zgair A, et al. Development of a simple and sensitive HPLC-UV method for the simultaneous determination of cannabidiol and Δ(9)-tetrahydrocannabinol in rat plasma. *J. Pharm. Biomed. Anal.,* 2015; **114**: 145–151. doi: 10.1016/j.jpba.2015.05.019.

24. Guideline I. H. T. Validation of analytical procedures: Text and methodology Q2 (R1) version 4. In *Proceedings of the International Conference for Harmonization; Geneva, Switzerland* (2005).
25. Hazekamp A, Simons R, Peltenburg-Looman A, Sengers M, van Zweden R, Verpoorte R. Preparative isolation of cannabinoids from Cannabis sativa by centrifugal partition chromatography. J Liq Chromatogr Relat Technol., 2009; 27(15): 2421–39. https://doi.org/10.1081/jlc-200028170.
26. Leghissa A, Hildenbrand ZL, Schug KA. A review of methods for the chemical characterization of cannabis natural products. J Sep Sci., 2018a; 41(1): 398–415. https://doi.org/10.1002/jssc.201701003
27. Citti C, Braghiroli D, Vandelli MA, Cannazza G. Pharmaceutical and biomedical analysis of cannabinoids: a critical review. J Pharm Biomed Anal., 2018; 147: 565–79. https://doi.org/10.1016/j.jpba.2017.06.003.
28. Hazekamp A, Simons R, Peltenburg-Looman A, Sengers M, van Zweden R, Verpoorte R. Preparative isolation of cannabinoids from Cannabis sativa by centrifugal partition chromatography. J Liq Chromatogr Relat Technol., 2009; 27(15): 2421–39. https://doi.org/10.1081/jlc-200028170.
29. Dussy FE, Hamberg C, Luginbuhl M, Schwerzmann T, Briellmann TA. Isolation of Delta9-THCA-A from hemp and analytical aspects concerning the determination of Delta9-THC in cannabis products. Forensic Sci Int., 2005; 149(1): 3–10. https://doi.org/10.1016/j.forsciint.2004.05.015.
30. Leghissa A, et al. Determination of cannabinoids from a surrogate hops matrix using multiple reaction monitoring gas chromatography with triple quadrupole mass spectrometry. J Sep Sci., 2018b; 41(2): 459–68.
31. Leghissa A, et al. Detection of cannabinoids and cannabinoid metabolites using gas chromatography with vacuum ultraviolet spectroscopy. Sep Sci Plus., 2018c; 1(1): 37–42.
32. Hazekamp, A.; Peltenburg, A.; Verpoorte, R.; Giroud, C. Chromatographic and Spectroscopic Data of Cannabinoids from Cannabis sativa L. J. Liq. Chromatogr. Relat. Technol., 2005; 28: 2361–2382.
33. Mandrioli, M.; Tura, M.; Scotti, S.; Gallina, T. Fast Detection of 10 Cannabinoids by RP-HPLC-UV Method in Cannabis sativa L. Molecules, 2019; 24: 2113.
34. Namdar, D.; Mazuz, M.; Ion, A.; Koltai, H. Variation in the compositions of cannabinoid and terpenoids in Cannabis sativa derived from inflorescence position along the stem and extraction methods. Ind. Crops Prod., 2018; 113: 376–382.

35. Sherma, J.; Fried, B. (Eds.) Handbook of Thin-Layer Chromatography, 3rd ed., rev. expanded, Marcel Dekker: New York, NY, USA, 2003.
36. Sherma, J.; Rabel, F. Thin layer chromatography in the analysis of cannabis and its components and synthetic cannabinoids. J. Liq. Chromatogr. Relat. Technol., 2019; 42: 613–628.
37. Ramirez, C.L.; Fanovich, M.A.; Churio, M.S. Cannabinoids: Extraction Methods, Analysis, and Physicochemical Characterization. In Studies in Natural Products Chemistry; Elsevier: Amsterdam, The Netherlands, 2019; 143–173.
38. Kowalska, T.; Sajewicz, M.; Sherma, J. Planar Chromatography—Mass Spectrometry; CRC Press: Boca Raton, FL, USA., 2020.
39. Mandrioli, M.; Tura, M.; Scotti, S.; Gallina, T. Fast Detection of 10 Cannabinoids by RP-HPLC-UV Method in Cannabis sativa L. Molecules, 2019; 24: 2113.
40. Fischedick, J.T.; Glas, R.; Hazekamp, A.; Verpoorte, R. A qualitative and quantitative HPTLC densitometry method for the analysis of cannabinoids in Cannabis sativa L. Phytochem. Anal., 2009; 20: 421–426.
41. Galand, N.; Ernouf, D.; Montigny, F.; Dollet, J.; Pothier, J. Separation and Identification of Cannabis Components by Different Planar Chromatography Techniques (TLC, AMD, OPLC). J. Chromatogr. Sci., 2004; 42: 130–134.
42. Fan, X.-H.; Cheng, Y.-Y.; Ye, Z.-L.; Lin, R.-C.; Qian, Z.-Z. Multiple chromatographic fingerprinting and its application to the quality control of herbal medicines. Anal. Chim. Acta., 2006; 555: 217–224.
43. Cheng, Y.; Chen, M.; Tong, W. An Approach to Comparative Analysis of Chromatographic Fingerprints for Assuring the Quality of Botanical Drugs. J. Chem. Inf. Comput. Sci., 2003; 43: 1068–1076.
44. Trigg, S. Development of Gas and Liquid Chromatographic Methods for the Separation and Quantification of 11 Cannabinoids. Bachelor Thesis, Murdoch University, Perth, Australia, 2017.
45. Gambaro, V.; Dell'Acqua, L.; Farè, F.; Froldi, R.; Saligari, E.; Tassoni, G. Determination of primary active constituents in Cannabis preparations by high-resolution gas chromatography/flame ionization detection and high-performance liquid chromatography/UV detection. Anal. Chim. Acta., 2002; 468: 245–254.
46. Kalinová, J.P.; Vrchotová, N.; Tˇríska, J.; Hellerová, Š. Industrial hemp (Cannabis sativa L.) as a possible source of cannabidiol. J. Cent. Eur. Agric., 2021; 22: 110–118.

47. Geschäftsstelle der Arzneibuch-Kommissionen. Bundesinstitut fur Arzneimittel und Miedizinprodukte, Monografie Cannabis extractum normatum (Eingestellter Cannabis-Extrakt). In German Pharmacopoeia; Geschäftsstelle der Arzneibuch-Kommissionen, Bundesinstitut fur Arzneimittel und Miedizinprodukte: Bern, Switzerland, 2020.
48. Carcieri, C.; Tomasello, C.; Simiele, M.; de Nicolò, A.; Avataneo, V.; Canzoneri, L.; Cusato, J.; di Perri, G.; D'Avolio, A. Cannabinoids concentration variability in cannabis olive oil galenic preparations. J. Pharm. Pharmacol., 2018; 70: 143–149.
49. Gul, W.; Gul, S.W.; Radwan, M.M.; Wanas, A.S.; Mehmedic, Z.; Khan, I.I.; Sharaf, M.H.M.; ElSohly, M.A. Determination of 11 Cannabinoids in Biomass and Extracts of Different Varieties of Cannabis Using High-Performance Liquid Chromatography. J. AOAC Int., 2015; 98: 1523–1528.
50. Patel, B.; Wene, D.; Fan, Z. Qualitative and quantitative measurement of cannabinoids in cannabis using modified HPLC/DAD method. J. Pharm. Biomed. Anal., 2017; 146: 15–23.
51. Burnier, C.; Esseiva, P.; Roussel, C. Quantification of THC in Cannabis plants by fast-HPLC-DAD: A promising method for routine analyses. Talanta., 2019; 192: 135–141.
52. Chandra, S.; Lata, H.; Mehmedic, Z.; Khan, I.; ElSohly, M. Assessment of Cannabinoids Content in Micropropagated Plants of Cannabis sativa and Their Comparison with Conventionally Propagated Plants and Mother Plant during Developmental Stages of Growth. Planta Med., 2010; 76: 743–750.
53. Brighenti, V.; Licata, M.; Pedrazzi, T.; Maran, D.; Bertelli, D.; Pellati, F.; Benvenuti, S. Development of a new method for the analysis of cannabinoids in honey by means of high-performance liquid chromatography coupled with electrospray ionisation-tandem mass spectrometry detection. J. Chromatogr., A 2019; 1597: 179–186.
54. Meng, Q.; Buchanan, B.; Zuccolo, J.; Poulin, M.-M.; Gabriele, J.; Baranowski, D.C. A reliable and validated LC-MS/MS method for the simultaneous quantification of 4 cannabinoids in 40 consumer products. PLoS ONE, 2018; 13: e0196396.
55. Mutje P, et al. Full exploitation of Cannabis sativa as reinforcement/filler of thermoplastic composite materials. Compos A: Appl Sci Manuf., 2007; 38(2): 369–77.
56. Hazekamp A, Choi YH, Verpoorte R. Quantitative analysis of cannabinoids from Cannabis sativa using 1H-NMR. Chem Pharm Bull., 2014; 52(6): 718–21.
57. Casiraghi A, et al. Extraction method and analysis of cannabinoids in cannabis olive oil preparations. Planta Med., 2018; 84(04): 242–9.
58. L. Nováková, L. Matysová, and P. Solich, *Talanta.,* 2006; **68**(3): 908-918.

59. A. Bala, S. Rademan, K.N. Kevin, V. Maharaj, and M.G. Matsabisa, *Nat. Prod. Commun.* 2019; **14**(8): 1934578X19872907.
60. S. Fekete, V. Sadat-Noorbakhsh, C. Schelling, I. Molnár, D. Guillarme, S. Rudaz, and J.L. Veuthey, *J. Pharm. Biomed. Anal.*, 2018; **155:** 116-124.
61. A.C. Elkins, M.A. Deseo, S. Rochfort, V. Ezernieks, and G. Spangenberg, *J. Chromatogr. B: Biomed. Sci. Appl.* 2019; **1109:** 76-83.
62. V. Brighenti, F. Pellati, M. Steinbach, D. Maran, and S. Benvenuti, *J. Pharm. Biomed. Anal.,* 2017; **143:** 228-236.
63. N. Hidayati, A. Saefumillah, and A.H. Cahyana, in IOP Conference Series: Materials Science and Engineering, 2020; **902**(1): 012063.
64. W. Dong, J. Liang, I. Barnett, P.C. Kline, E. Altman, and M. Zhang, *Anal. Bioanal. Chem., 2019;* **411**(30): 8133-8142.
65. D.R. Grijó, D.L. Bidoia, C.V. Nakamura, I.V. Osorio, and L. Cardozo-Filho, *J. Supercrit. Fluids,* 2019; **149:** 20-25.
66. R. Pavlovic, G. Nenna, L. Calvi, S. Panseri, G. Borgonovo, L. Giupponi, and A. Giorgi, *Molecules, 2018;* **23**(5): 1230
67. C. Citti, D. Braghiroli, M.A. Vandelli, and G. Cannazza, *J. Pharm. Biomed. Anal.,* 2018; **147:** 565–579.
68. L.L.Romano and A. Hazekamp, *Cannabinoids, 2013;* **1**(1): 1-11.
69. R. Deidda, H.T. Avohou, R. Baronti, P.L Davolio, B. Pasquini, M. Del Bubba, and S. Furlanetto, *J. Pharm. Biomed. Anal., 2019;* **166:** 326-335.
70. A. Bettiol, N. Lombardi, G. Crescioli, V. Maggini, E. Gallo, A. Mugelli, and A. Vannacci, *Front. Pharmacol., 2019;* **9:** 1543.
71. L.A. Ciolino, T.L. Ranieri, and A.M. Taylor, *Forensic Sci., Int.,* 2018; **289:** 438-447.
72. E.M. Mudge, S.J. Murch and P.N. Brown, *Anal. Bioanal. Chem.,* 2017; **409**(12): 3153-3163.
73. A. Nemeškalová, K. Hájková, L. Mikulů, D. Sýkora, and M. Kuchař, *Talanta.,* 2020; **219:** 121250.
74. J.F. Araneda, T. Chu, M.C. Leclerc, S.D. Riegel, and N. Spingarn, *Analytical Methods,* 2020; **12**(40): 4853-4857.