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Challenges and Opportunities for the Analysis of Terpenes in Cannabis

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Abstract

Cannabis is a complex plant with over 400 chemical entities of which more than 60 of them are cannabinoids. While cannabinoids are the primary psychoactive and medicinal components of cannabis, volatile terpenes contribute to the many significant fragrance attributes that ultimately influence consumer preference for cannabis. There are over 120 different terpene compounds that have been identified in the *Cannabis sativa* plant alone. Analysis of terpenes in cannabis is extremely important because they contribute to its potency and sensory perceptions. Current methods of quantifying terpenes in cannabis involve the use of chromatographic techniques. However, such techniques require sample preparation, are time-consuming, and the instrument involved can be expensive and requires a skilled operator. The use of Fourier Transform Infrared spectroscopy and chemometrics offer a fast, non-destructive, and affordable means of analyzing terpenes in cannabis. This manuscript will discuss challenges in cannabis terpene analysis using the aforementioned methods including method fragmentation and method multiplicity as well as issues related to its legal use. In general, the cannabis testing industry is poised for a breakthrough in the field of analytical science given the recent laws legalizing its medicinal use as well as advances in the field of spectroscopic miniaturization.

Keywords: Terpene, cannabis testing, Fourier Transform Infrared spectroscopy, chemometrics, chromatography

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1.Introduction

Cannabis is a genus of flowering plants belonging to the *Cannabaceae* family. It is most widely known for its psychoactive and medicinal properties. The genus includes a group of three plants namely *Cannabis sativa, Cannabis indica,* and *Cannabis ruderalis*, although all three may be treated as subspecies of the single species, *Cannabis sativa* (Figure 1).

1.1 Components of cannabis

Figure 1. Cannabis plants

There are approximately 500 natural components isolated and identified in cannabis (Table 1) [1]. *Cannabaceae* plants, *Cannabis sativa L.* and *Humulus lupulus L.* are rich in terpenes, amounting to 3-5% of the dry mass of the female inflorescence. These terpenes, known to be mono- and sesquiterpenes are derived from two or three isoprene units, respectively [2]. There are over 120 different terpenes identified in *Cannabis sativa* plant alone, and every strain is known to have a different composition and of unique type of terpenes [3]. Terpenes in cannabis contribute to fragrance attributes of the cannabis products [4]. Further, studies have shown that terpenes exhibit medicinal properties as supported by *in vitro*, animal and clinical trials such as anti-cancer, anti-tumor, analgesic, anticonvulsive, antidepressant, ant-inflammatory, anti-oxidant, antibiotic, neuroprotective, antidiabetic, and anti-mutagenic properties among others [2]. Some of the most important terpenes and their structures are shown in Figure 2. Besides terpenes, cannabis is also known to contain flavonoids, which also serve as phytochemicals similar to that of terpenes [5].

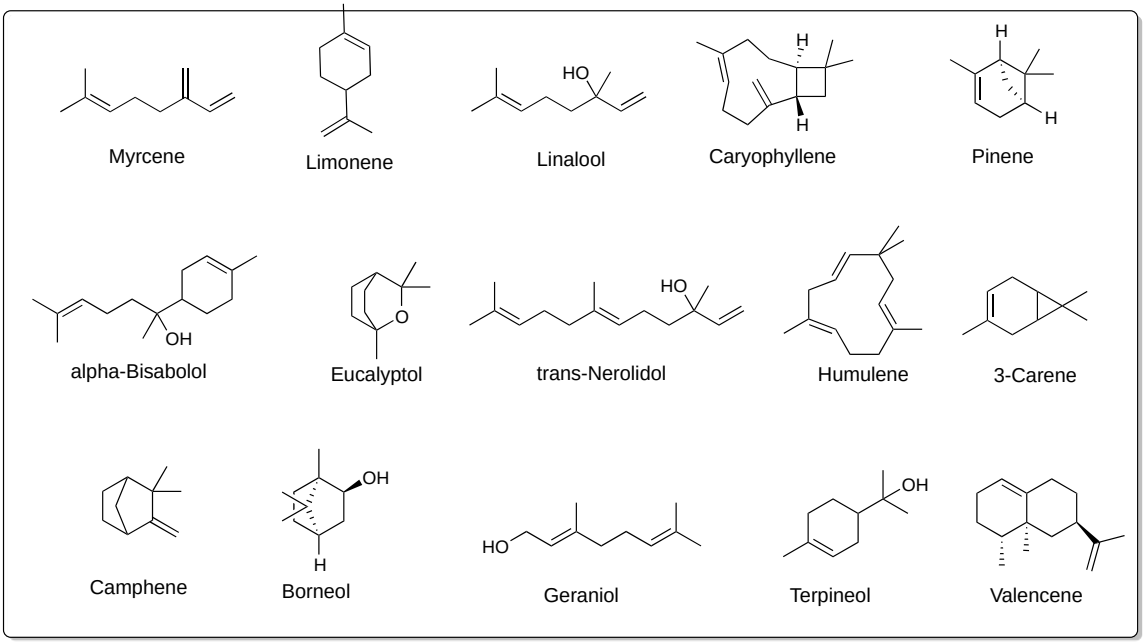

Figure 2. Important terpenes in cannabis

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Flavonoids possess anti-fungal, anti-oxidant, and anti-inflammatory properties among others [6]. In addition to other compounds such as terpenes and flavonoids, cannabis is also known to produce terpenophenolic compounds which are in general referred to as the cannabinoids [7]. While there are more than 120 cannabinoids that exists in nature, Δ-9 tetrahydrocannabinol (THC) and cannabidiol (CBD) are identified as the most important ones (Figure 3).

Terpene chemoprofiles are important to distinguish among different strains of cannabis with otherwise identical cannabinoid content. As stated earlier, the cannabis plant is considered to be complex and researchers are still trying to understand it completely [8]. Classifying different cannabis types according to their terpene content was found to be more useful than using their cannabinoid content. This is because terpenes are considered to be one of the major compounds found in cultivars that determine the nuanced effects of how a particular consumer feels [8]. In a recent study, the process involves using the expression levels of the different terpenes that are translated well based on the 1,400 single nucleotide polymorphisms that were used as markers [9].

Table 1. Components found in cannabis [1].

Chemical class	Number of known chemical entities
Amino acids	18
Cannabinoids	66
Elements	9
Fatty acids	22
Flavonoids	21
Hydrocarbons	50
Nitrogenous compounds	27
Non-cannabinoid phenols	25
Pigments	2
Proteins, glycoproteins, enzymes	11
Simple acids	21
Simple alcohols	7
Simple aldehydes	12
Simple esters and lactones	13
Simple ketones	13
Steroids	11
Sugars and related compounds	34
Terpenes	120
Vitamins	1
TOTAL	483

1.2 Molecular interactions of cannabis-related compounds

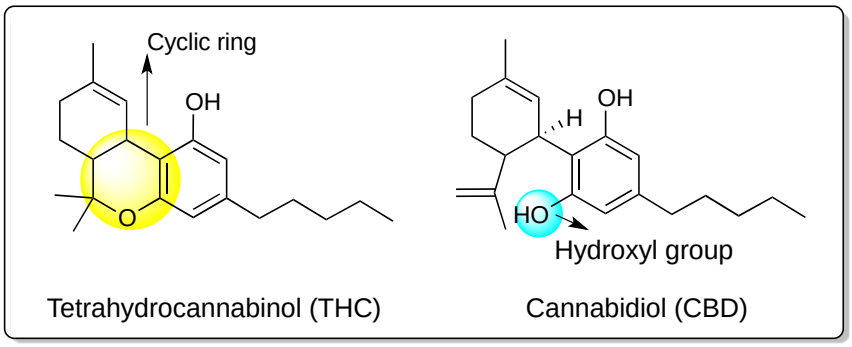

Figure 3. Structure of the important cannabinoids, Δ-9 tetrahydrocannabinol (THC) and cannabidiol (CBD)

According to the United Nations Office on Drugs and Crime, cannabis is the most widely cultivated, trafficked, and consumed drug worldwide. Cannabis varieties that are cultivated for non-drug use are often referred by the term 'hemp' or 'industrial hemp' and contain less than 0.3% of THC. While both THC and CBD share the same exact chemical formula, $C_{21}H_{30}O_2$, there is one distinct difference in their structure, the THC contains a

cyclic ring while the CBD has a hydroxyl group in its place (Figure 3). This difference provides each their unique pharmacological properties [10]. THC and CBD are known to interact with the cannabinoid receptor type 1 (CB1) and cannabinoid receptor type 2 (CB2) located in the endocannabinoid system of all mammals. CB1, a G protein-coupled cannabinoid receptor essential for a healthy functioning brain is primarily located in the central and peripheral nervous system with high abundance in the brain. CB2 receptors, on the other hand, are found in the cells of the immune system and are known to reduce inflammation and provide moderate immune response to pathogens [11]. Most cannabinoids including endocannobinoids such as anandamide, 2-arachidonoylglycerol, and THC possess the ability to bind to the aforementioned receptors. THC, a potent partial agonist of CB1 stimulates the receptors and overwhelms the endocannabinoid system and disrupts their function. On the other hand, CBD as a negative allosteric modulator modifies the receptor's ability to bind to cannabinoids while also changing its shape. It is for this same reason that CBD does not produce the same psychotropic effects as that of THC [1].

2. Cannabis distribution and usage

2.1 Abundance and origin

Considered to be one of the oldest cultivated plants, cannabis is grown primarily for grain and fiber and also for some recreational, medicinal, and religious purposes in East Asia. Wild cannabis contains very low amount of cannabinol (a mildly psychoactive cannabinoid found in trace amounts in cannabis) and grows across the mountain foothills from Caucasus to Western China and some parts of central Asia. While there is not much information on the prehistoric use of cannabis outside Eastern China where it was cultivated as an oil seed crop, photographed macro remains of cannabis have been recovered from burials in the Turpan Basin (ca. 800 to 400 BCE) in Northwest China [12,13].

2.2 Uses of cannabis

Cannabis has been used for medicinal and therapeutic applications for many years. Although by federal law, the possession of cannabis is illegal in the US, many states have legalized cannabis for medicinal use. While the U.S. Food and Drug administration has not approved cannabis for treatment of cancer or other medical conditions, some commercially available cannabinoids such as dronabinol and nabilone were proven to be effective in treating cancer related side effects. A recent meta-analysis conducted involving more than 3000 patents revealed that the use of cannabis and cannabinoids exhibited significant positive effects on spasticity as well as pain and bladder dysfunction in the population [14].

As a potential antiviral agent, three preclinical studies on CBD's role were examined. The first study showed a direct antiviral effect of CBD against *Hepatitis C*, the second study demonstrated an indirect viral action on Kaposi's sarcoma-associated herpesvirus, and the final study elucidated that CBD alleviated effects of neuroinflammation triggered by Theiler's murine encephalomyelitis virus. All these results suggest that CBD could be a good candidate for preclinical studies of coronavirus disease-19 [15-17].

2.3 Cannabis market analysis

Due to the wide range of legitimate medicinal and therapeutic applications, legalization of cannabis is gaining momentum worldwide. This has directly triggered a dramatic increase in demand in recent years unlocking several opportunities for companies operating in this sector. It has been estimated that cannabis market size could grow close to $97.35 billion USD by 2026. This acceleration in the cannabis market growth is sustained by the high investments in the research and development of cannabis infused drugs for therapeutic applications. Also, the present-day availability of cannabis products in various forms such as concentrates, infused products and topicals no longer require consumers to stick to the traditional joints and pipes [18].

By type

Cannabis flower or bud which contains about 15% to 30% THC, is the most popular consumable product in the global market. Owing to the general belief that vaporizing or ingesting cannabis concentrates is a healthier version than smoking cannabis buds, the concentrates are getting an overall consumer's acceptance and, hence, leads the global market. Further, the cannabis concentrates may contain more than 80% THC, which creates the euphoric "high" and comes in diverse flavors and textures [19].

By application

The expansion in the list of countries legalizing cannabis for medicinal uses accounts for the major share of the cannabis market. In the United States, where cannabis legalization is more popular, it comprises a bigger percentage of adult population in the age 50 years and older. This demographic profile is expected to increase owing to the increased risk in chronic diseases after 50 years of age. This is because cannabis-based drugs such as Sativex, Marinol, and Cesamet are gaining increased demand owing to their effectiveness in the treatment of chronic pian, multiple sclerosis, anxiety, epilepsy and cancer where conventional medicine has failed [20].

Regional analysis

The North American cannabis market is a dominating region in the world owing to the government-supported medical marijuana laws imposed in the US. In 2012, Colorado and Washington became the first two states to legalize recreational marijuana while in the year 2019, another eleven states were added to the list. With robust demands from the US, Canada, and European countries, the South American cannabis production is expected to increase in the coming years. Canada also plays a vital role in the cannabis market as it recently became the flag-bearer for recreational cannabis legalization [21].

3. Chemical analysis of cannabis

3.1 Chromatographic analysis of terpenes in cannabis

Sample preparation for cannabis starts with crushing the plant cells followed by extracting the crushed sample using an appropriate solvent (or through distillation). The desired terpene(s) is

then separated from the other unknown contents of the extracts, and analyzed and quantified using an appropriate method of analysis (e.g., thin layer chromatography (TLC), gas chromatography (GC), or liquid chromatography (LC)) [22]. Terpenes are most responsive to GC, due to their characteristic volatility. Since residual solvents used for extraction of terpenes are extremely volatile, they are not ideally analyzed by high performance liquid chromatography (HPLC) [23].

Many different methods have been refined to provide an improved and direct analysis of terpenes in cannabis. The conventional approach for terpene analysis in cannabis involves solvent extraction followed by GC with flame ionization detector (GC-FID) analysis. The FID is a good detector for GC quantification, but it does not provide any information, other than the retention time. Retention indices, which are based on retention/elution times from a particular GC stationary phase, are primarily the viable route for differentiating terpene species. The use of the FID as the primary detector of choice has several advantages including low cost, accuracy, and uncomplicated interface, which make it a potent tool for quality control. Despite this, there is still valid concern with the type of GC detector to use best for terpene analysis [24].

A research group at Phenomenex™ developed a GC-FID method with excellent resolution and peak-shape for 33 primary and secondary terpenes found in cannabis. The selectivity and high temperature limits of their Zebron™ ZB-5PLUS column allows for great resolution of the key terpenes and high bake out for the removal of low volatile matrix contaminants that may be present [25]. This allows simplicity in sample preparation prior to terpene analysis. Further, a GC-FID method for 20 terpenes from cannabis using their Zebron ZB-624 PLUS GC column was also developed. The Zebron ZB-624 PLUS GC column upper temperature limit is 300°C/320°C, which gives the flexibility to elute out high boiling terpene analytes [26]. In addition, Cardenia et al. developed a fast GC-FID routine method for the determination of main terpenes and total CBD present in hemp. Their study resulted in a fast detection of 29 different terpenes and CBD (with total analysis time of <16 min) without derivatization and with a satisfactory sensitivity (LOD=0.03 – 0.27 µg/mL, LOQ=0.10 – 0.89 µg/mL) and repeatability (interday RSD was <7.82 %, whereas the intraday RSD was <3.59 %) [27]. Overall, this GC-FID method is a primary choice for fast, robust and high-sensitive determination of main terpenes and total CBD present in hemp.

Another dependable way to quantify residual solvents in a cannabis sample is through headspace gas chromatography–flame ionization detection (HS-GC-FID). This serves as another technique that can be used for sample preparation of terpenes. Headspace sampling is based on heating the solid or liquid sample inside a sealed vial (thereby, driving the volatile compounds into a gas phase), equilibrating the system and then analyzing the air above it. The process is relatively interference-free because it allows only volatile analytes to be extracted from the solid/liquid sample into the gas phase [28]. An aliquot is then withdrawn from the headspace of the vial and analyzed by GC-FID in order to determine the volatile components of the sample. One approach for HS-GC-FID that is particularly useful for analyzing terpenes in cannabis concentrates is the full evaporation technique (FET). FET sample preparation involves the use of a miniscule sample amount (e.g., 20–50 mg), which effectively creates a single-phase gas system in the headspace vial at equilibrium [29]. FET is prime for problematic matrices like cannabis concentrates because it effectively eliminates matrix interferences that contribute to inaccurate quantification. Additionally, it has little to no manual sample handling and a very small sample size and high sensitivity can be achieved through the creation of a single-phase system in the headspace vial

[30]. In one study, an FET headspace GC-FID method was used to analyze a comprehensive suite of terpenes in hops that are also found in cannabis samples. Good chromatographic separation allowed quantification of critical compounds including α-pinene, β-myrcene, α-humulene, β-caryophyllene, and caryophyllene oxide. Their method utilized straightforward FET sample preparation. In addition, because it prevents nonvolatile material from entering the GC system, the method was found to contribute to column lifetime and also reduced inlet maintenance. The technique eliminated the need for additional capital investment for different instrumentation and/or columns [31].

Headspace (HS), Solid Phase Micro-extraction of Headspace (HS-SPME) or Split/Splitless Injection (SSI), on the other hand, are viable techniques and have advantages and disadvantages for terpene analysis. SPME uses a fiber coated with a liquid (polymer), a solid (sorbent), or a combination of both. The fiber coating extracts the compounds from the sample by absorption in the case of liquid coatings or absorption in the case of solid coatings. The SPME fiber is then inserted directly into the chromatograph for desorption and analysis. SPME can be performed by either direct immersion with the sample or headspace sampling. HS-SPME is considered an effective technique since this approach eliminates the complex oily matrix. Conventional HS as previously discussed also targets volatiles that include the terpenes, leaving the high molecular weight oils and cannabinoids behind [23]. In addition, the method does not require organic solvents, and with the use of an auto-sampler, is highly reproducible and requires little "hands-on" sample preparation time. Due to the technique's high sensitivity, very little sample is required. Moreover, coupling this with HS produces very clean chromatographic analysis with little to no background from the extracted matrix, which in turn will maintain the cleanliness of the GC system. Based on this, an HS-SPME method was developed that allowed an easy and accurate determination of terpene content in cannabis. The method was shown to be useful in the analysis of the three important terpenes found in cannabis: α-pinene, (R)-(+)-limonene, and linalool, but could also be used for other terpenes as well [32].

Besides the aforementioned techniques for the analysis of terpenes in cannabis, the use of GC-MS, another widely used technique, offers the added benefit of spectral peak authentication to warrant that identification is accurate with no co-eluting interferences [33]. The primary choice for a research setting is the mass spectrometer (MS) detector. It is more expensive and complicated than FID but it provides both good quantitative capabilities. It also provides the mass spectra for each species that elutes from the chromatograph. However, for terpene analysis, it may still not be the best detector of choice. Since terpene class molecules share many structural and functional similarities, even their fragmentation and sub-sequential identification by MS may lead to unpredictable results and should be backed by other chromatographic identification methods. Despite the possible complicated interpretation, MS is still a better qualitative analysis tool than the FID, especially for distinguishing non-isobaric terpenes [24]. Moreover, GC-MS provides a different level of sensitivity.

Coupling GC-MS with HS has been demonstrated in some studies. This combined with the Agilent Residual Solvent Analyzer, an Agilent VF-35 GC column and appropriate restrictors allowed total chromatographic separation of 22 targeted terpenes that were naturally occurring in *Cannabis sativa* plant material and wax samples that give the plant its distinctive aroma and character. The analysis used both FID detection for quantification and extended linear range, and mass selective

detection (MSD) for elaborate terpene identification. Using integrated Capillary Flow Technology to split the column effluent in a controllable and precise manner to the two detectors, this ultra-fast methodology almost quadrupled laboratory productivity compared to traditional terpene analysis and brought analysis time down from approximately 30 minutes per sample to just 6 minutes [34]. In another study, coupling of the GC-MS with a simple HS method was used in the analysis of the terpene/terpenoid profiles of both hops and cannabis. The method was able to detect the characteristic terpenes and terpenoids of both, and distinguished between different hops varieties [35].

Another GC-MS method was developed and validated for the quantification of terpenes in cannabis plant material, which included α- pinene, β-pinene, β-myrcene, limonene, terpinolene, linalool, α-terpineol, β-caryophyllene, α-humulene, and caryophyllene oxide. The concentration-response relationship for all analyzed terpenes using the developed method was linear with r^2 values > 0.99. The average recoveries for all terpenes in spiked indoor cultivated samples were between 95.0–105.7 %, with the exception of terpinolene (67–70 %). The measured repeatability and intermediate precisions (% relative standard deviation) in all varieties ranged from 0.32 to 8.47%. The limit of detection and limit of quantitation for all targeted terpenes were determined to be 0.25 and 0.75 μg/mL, respectively. The proposed method was found to be highly selective, reliable, and accurate, and was applied for the simultaneous determination of the aforementioned major terpenes in the *Cannabis sativa* biomass [36].

Other elaborate coupled GC systems such as the development of a novel Static Headspace Gas Chromatography Mass Spectrometry (SHS)-GC-MS-MS method for the simultaneous analysis and quantification of 93 terpenoids was also studied. This method was found to be especially valuable for the analysis of cannabis in inflorescences and extracts, as they possess a very rich repertoire of terpenoids, but could also be potentially extended to other plants [37]. The Pegasus BT 4D facilitated fast and confident terpene profiles of cannabis strains through enhanced two-dimensional chromatographic resolution and high-performance time of flight mass spectrometry (TOF-MS). Robust compound characterization was achieved through spectral similarity searches of large, well-established databases, and mass D values increased confidence in these determinations [38]. Recently, new technology based on vacuum ultraviolet spectroscopy (VUV) was developed as a new GC detector. The VUV detector probed in the 125-240 nm wavelength and since virtually all chemical compounds absorbed light in this range, the VUV enabled analysis of virtually all molecules; making it an essentially universal detector [15]. The VUV detector filled a need, which was complementary to MS detection in terms of the qualitative information it provided. Using the VUV, each compound presented a particular absorbance spectrum. Different species of terpene mixtures that can be difficult to be differentiated by their electron ionization mass spectra, can be well discriminated based on their VUV spectra [39]. Thus, it is no longer necessary for a baseline chromatographic separation of components in a mixture since analytes exhibit different spectra. Therefore, co-eluting peaks can be separated post-run through the use of library spectra and built-in instrument software. This process of "deconvolution" assumes that two co-eluting terpenes will give a peak with an absorbance spectrum equal to the sum of the two single absorbance spectra [28]. It is possible to fully separate the two peaks after the run due to their different absorbance spectra [39]. With the ability to deconvolute unresolved peaks, a lengthy temperature program to separate all terpenes (isomeric) is no longer necessary, thus, allowing rapid analysis times. In addition, the presence of co-eluting components in complex matrices, that elude

GC detectors, can be identified easily based on comparison of the measured spectra with pure reference spectra contained in the VUV spectral library. In a recent study demonstrating this, the vacuum ultraviolet absorption spectra of 41 different standard terpenes were investigated and compared. The spectra were found to be highly featured and easily differentiated. The technique was demonstrated to be a powerful tool for reliable and accurate qualitative and quantitative analysis of terpenes from complex natural mixtures [39].

The other issue in terpenes analysis is the extraction process itself. Terpenes can be extracted with the use of solvents (e.g. alcohols, n-alkanes among others), however the procedure is usually expensive and tedious. The plant needs to be manually crushed and then solvent is used to extract components from the plant, ideally at least 3 times and combined to achieve decent results. The problem is that some terpenes may be extracted better with a certain solvent, making their extraction easier and more optimized than others [40]. The choice of solvent can cause selectivity against some terpenes, which hinders the extent of analysis. HPLC is generally not recommended; since terpenes have very low ultraviolet (UV) or MS sensitivity [23]. In addition, co-elutions of the cannabinoids and terpenes are very likely when analyzing real cannabis samples by HPLC-UV methods. While HPLC may be tempting to use for terpenes analysis, a GC-FID or GC-MS is really the most straightforward and recommended way of analyzing terpenes in cannabis as mentioned earlier. Terpenes, being relatively volatile and neutral, and are better analyzed using GC in general. However, looking onward, laboratories performing routine testing of cannabis will need to test efficiency and, once regulations are established, test also for other things in the matrix as well (i.e. pesticides). LC-MS-MS (liquid chromatography tandem mass spectrometry) represents an ideal analytical platform to address all of these testing needs. As demonstrated recently showing the utility of the Triple Quad™ 3500 LC-MS-MS system for the analysis of terpenes in cannabis products, instrument performance was excellent, with precision within $< \pm$ 8% ($n = 3$) and signal-to-noise > 10 at 1 ppb for all target compounds. Spike recoveries of 80-120% showed the quantitative accuracy of the method in a variety of cannabis matrices [41]. On the other hand, a liquid chromatography-ultraviolet (LC-UV) analysis of terpenes in cannabis is not recommended and will likely cause more issues than it will provide solutions. A good solution to the co-elutions by LC-UV is to choose a good GC-HS method. Interferences from the complex sample matrix, as well as the much fewer volatile cannabinoids can be eliminated then. Table 2 summarizes various chromatographic techniques used in analyzing terpenes in the recent years.

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Table 2. Chromatographic methods of analyzing terpenes in cannabis in the recent years.

References	Method/ Instrumentation	Objectives	Terpenes analyzed	Results
[25]	GC-FID	To develop a GC-FID method with excellent resolution and peak-shape for 33 primary and secondary terpenes found in cannabis	Examples include α-Pinene, Camphene, Myrcene, α-Phellandren, 3-Carene, α-Terpinene, p-Cymene,Limonene, Ocimene-, Linalool Fenchol, Isoborneol	Great resolution of key terpenes and high bake out for the removal of low volatile matrix contaminants present.
[26]	GC-FID	To develop a GC-FID method for 20 terpenes from cannabis	Examples include α-Pinene, Camphene, β-Myrcene, (-)-β-Pinene, d-3-Carene, α-Terpinene, d-Limonene	The ZB-624PLUS was shown to have an upper temperature limit of 300/320 °C, which gives the flexibility to elute out high boiling terpenes analytes.
[27]	GC-FID	To validate a method for simultaneous determination of both terpenes and cannabidiol in hemp	Twenty-nine (29) different terpenes	The study resulted in a fast detection of 29 different terpenes and CBD (total analysis time <16 min) without derivatization and with satisfactory sensitivity and repeatability.
[31]	Full Evaporation Technique (FET)-HS-GC-FID	To demonstrate the viability of FET headspace injection and GC-FID analysis of residual solvents in cannabis concentrate method	Examples include α-pinene, β-myrcene, α-humulene, β-caryophyllene, and caryophyllene oxide	The study showed quantification without the use of matrix-matched standards by creating a single non-partitioning phase system in the headspace vial. Good chromatographic separation allowed quantification of critical

				compounds across the volatility range.
[39]	GC-VUV	To investigate and compare the vacuum ultraviolet absorption spectra of 41 different standard terpenes	Forty-one (41) different standard terpenes and four turpentine samples	The spectra were found to be highly featured and easily differentiated. The technique was demonstrated to be a powerful tool for reliable and accurate qualitative and quantitative analysis of terpenes from complex natural mixtures.
[42]	GC-MS	To conduct terpene analyses using a Shimadzu GCMS-QP2010SE single quadrupole mass spectrometer with the HS-20 headspace autosampler for sample introduction	Forty-one (41) different terpenes	It was demonstrated that different storage conditions can change terpene results over time and this should be taken into consideration when analyzing cannabis samples as the results show less than expected results.
[38]	GC-MS	To develop an analytical approach for the effective characterization of terpenes in different cannabis strains	Forty (40) terpene standards	The BT 4D facilitated fast and confident cannabis product “fingerprinting” through enhanced two-dimensional chromatographic resolution and high performance TOFMS. Robust compound characterization was achieved through spectral similarity searches of large, well-established databases. Mass values increased confidence in these determinations.

[36]	GC-MS	To develop and validate terpenes in cannabis plant material	Examples include α-pinene, β-pinene, β-myrcene, limonene, terpinolene, linalool, α-terpineol, β-caryophyllene, α-humulene, and caryophyllene oxide	The measured repeatability and intermediate precisions (% relative standard deviation) in all varieties ranged from 0.32 to 8.47%. The limit of detection and limit of quantitation for all targeted terpenes were determined to be 0.25 and 0.75 µg/ mL, respectively. The proposed method was found to be highly selective, reliable, and accurate and was applied for the simultaneous determination of these major terpenes in the *C. sativa* biomass.
[34]	HS-GC-MS	To provide full chromatographic separation of 22 targeted terpenes that naturally occur in *C. sativa* plant material and wax samples	Twenty-two (22) terpenes	The analysis can be completed in less than 6 minutes and uses both FID detection for quantification and extended linear range, and mass selective detection (MSD) for terpene speciation. This ultra-fast methodology almost quadrupled laboratory productivity compared to traditional terpene analysis, which takes approximately 30 minutes per sample.
[37]	(S)HS-GC-MS-MS	To develop a novel Static Headspace (SHS)-GC-MS-MS method for the	Ninety-three (93) terpenes	This method is especially valuable for the analysis of Cannabis inflorescences and extracts, as they possess a very

		simultaneous analysis and quantification of 93 terpenoids		rich repertoire of terpenoids, but could potentially be applied to other plants.
[43]	HS-SPME	To develop a simple headspace SPME-GC/MS method for the analysis of terpene/terpenoid profiles of both hops and cannabis	Examples include β-myrcene, caryophyllene, and humulene	A simple headspace SPME-GC/MS method was used in the analysis of the terpene/terpenoid profiles of both hops and cannabis. The method was able to detect the characteristic terpenes and terpenoids of both, and to distinguish between different hop varieties.
[32]	HS-SPME	To develop an HS-SPME method which allows for an easy and accurate determination of terpene content in cannabis	Examples include α-Pinene, (R)-(+) Limonene and Linalool and other terpenes as well	The quantitative analysis of selected terpenes was achieved using HS-SPME with a 100-µm PDMS fiber. Accuracies were >90% with precision of <3% RSD for spiked replicates. The method provided results comparable to a conventional solvent extraction procedure.
[41]	LC	To develop an LC-MS/MS method that uses atmospheric pressure chemical ionization (APCI) and the budget-friendly SCIEX Triple QuadTM 3500 LC- MS/MS system for the analysis of	Seven (7) terpenes found in cannabis	These results demonstrated the utility of the Triple Quad™ 3500 system for the analysis of terpenes in cannabis products. Instrument performance was excellent, with precision within < ± 8% (n = 3) and signal-to-noise > 10 at 1 ppb for all target compounds. Spike recoveries of 80-120% showed the quantitative accuracy of the

		terpenes in cannabis products		method in a variety of cannabis matrices.

3.2 Spectroscopy and cannabis terpene testing and research

Spectroscopic techniques have becoming more popular than the chromatographic methods in analyzing terpenes in cannabis. Spectroscopy is the science which deals with the interaction between matter and electromagnetic radiation as a function of the wavelength or frequency of the radiation [43]. In this regard, spectroscopy can be classified according to the nature of interaction between the energy and the material. These types include: absorption spectroscopy, emission spectroscopy, elastic scattering and reflection spectroscopy, impedance spectroscopy, inelastic spectroscopy, coherent or resonance spectroscopy and nuclear spectroscopy [44,45].

GC with FID is currently the foremost method for quantitating terpene mixtures as mentioned earlier. This process involves sending a sample through an effusion tube and separating the sample into differing density components. The components are then exposed to a flame in order to ionize them. Finally, a sensor detects the composition of the sample and sends the results to be processed into a data set. A previous study used GC-FID to extract the terpene trilactones from ginkgo leaves in order to provide a quick, effective, one step determination of the compound [46]. Despite the study being successful, there are still limitations to this technique such as cost, time, and certification. Moreover, recent literatures in Cannabis research have utilized nuclear, inelastic scattering (Raman) and absorption spectroscopy (e.g., FTIR).

Nuclear spectroscopy is a specific field of spectroscopy which deals with the properties of a nucleus in probing properties of materials [47]. Usually, the emission or absorption of radiation by the nucleus creates a signature by which an identity of a molecule can be recorded and identified. One equipment used in nuclear spectroscopy is the nuclear magnetic resonance (NMR) spectrometer. The NMR spectrometer records the electromagnetic signal with a frequency characteristic to a given nuclei when the nuclei is perturbed by a weak oscillating magnetic field [48,49]. In this aspect, the one-dimension proton (^{1}H) NMR is one of the most common NMR techniques. The proton is known to be the most sensitive nucleus that gives sharp signals in an electromagnetic field [50]. Cannabis secondary metabolites can be identified and quantified using their respective chemical shifts (i.e., signals) and their relative height against a known standard. Using NMR, it was found out that the substitution of the carboxylic acid on the cannabinoid nucleus has great effect on the chemical shifts of the said compound. This shows that changes in functional groups can yield specific chemical shifts which can be used for identification of various metabolites such as terpenes [51]. Moreover, various cultivars of cannabis have been differentiated based on the secondary metabolites they produced. NMR has allowed a non-destructive and noninvasive way to analyze these compounds without too much complicated prefractionation steps [52].

Another noninvasive and nondestructive technique to determine terpenes and secondary metabolites in cannabis is Raman spectroscopy [53]. Raman is a light scattering technique where a high intensity light source is bombarded onto a sample. The molecules in the sample would then scatter the incident light. Mostly scattered light, called Rayleigh scatter, is of the wavelength as the laser source. This in effect does not give useful information. On the other hand, Raman scatter which is a small amount of light are scattered at different wavelengths and are dependent on chemical structure of a given molecule. This signature scatter gives useful information on the

identity and nature of the analyte [54]. Raman spectroscopy was found to identify different cannabis species based on the secondary metabolites present [53]. Moreover, it was found to be useful to quantify terpenes and other secondary metabolites in a nondestructive method [55].

FTIR with attenuated total reflectance accessory (FTIR-ATR), on the other hand is a universally useful instrument, with information rich spectra. It is also considered as relatively fast and easy while also being inexpensive and is very sensitive when measuring samples. FTIR produces much higher quality spectra than could previously be obtained [56]. Despite FTIR-ATR not being able to detect some molecules, it still has a very wide range of samples that can be measured quickly, efficiently, and with much accuracy [57]. FTIR can provide a wide range of data points when compared to other spectroscopic methods, for example GC-FID. The cost alone for use of these machines pushes for one to purchase a machine of this sort. Using a novel approach, such as FTIR, to measure samples will save time and expense. Recent studies using gas chromatography – Fourier transform infrared spectroscopy (GC/FTIR) was conducted to analyze various terpenes, one of which included p-cymene [58]. Another study was performed using GC/FTIR; however, it also involved using a wall-coated open tubular Carbowax capillary column in order to increase sensitivity and efficiency [59]. Both studies provide an in-depth view of the advantages that FTIR can offer when analyzing terpene mixtures. Table 3. summarizes various spectroscopic methods used in analyzing terpenes in the recent years.

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Table 3. Spectroscopic methods of analyzing terpenes in cannabis in the recent years

References	Method/ Instrumentation	Objectives	Terpenes analyzed	Results
[60]	Coherent anti-Stokes Raman Scattering (CARS) microspectroscopy	To spatially map secondary metabolites	Essential oils in trichomes, fluorophores, cannabinoids, monoterpens, fatty acids	Mapped secondary metabolites through a label-free and non-destructive method and distinguish between cell and chemo-types.
[53]	Raman spectroscopy	To differentiate cannabis from hemp using a noninvasive and nondestructive method	Cannabigerol, cannabigerolic acid (CBGA), THC, delta-9-tetrahydrocannabinolic acid (THCA), CBD, and cannabidiolic acid (CBDA)	Developed a method which can accurately differentiate hemp, CBD-rich hemp and cannabis (THCA-rich hemp)
[61]	Raman spectroscopy	To characterize essential oils and differentiate fractions containing terpenes and terpenoids	Terpenes and terpenoids which include limonene, estragole, ocimene, carene, etc.	Characterized the hemp essential oil and its five fractions using a noninvasive technique.
[55]	Near-infrared spectroscopy and FT-NIR spectrometer	To develop a more accurate quantitative technique for cannabinoid analyses	Cannabinoids CBDV, Δ9-THCV, CBC, CBD, Δ8-THC, Δ9-THC, CBG and CBN	Quantitatively determined cannabinoids in dried and ground hemp samples using tandem-NIR spectroscopy and FT-NIR. A predictive model was also proposed.

[62]	FTIR Spectroscopy	To determine quantitatively cannabinoid levels in samples	THC, THCA, CBD	Potency levels of THC, THCA and total THC in distillate and concentrate samples were determined with high accuracy.
[52]	NMR spectroscopy	To discriminate Cannabis cultivars based on their secondary metabolite	Δ9-tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA)	Demonstrated differentiation of various cultivars without any pre-purification steps.
[63]	FTIR	To optimize Cannabis grows through secondary metabolite production	Cannabinoids, cannabidiolic acid and cannabidiol	Quantitatively mapped the effect of different light intensities on the secondary metabolite production of Cannabis.
[64]	Functional Near Infrared Spectroscopy	To determine the effects of Δ9-tetrahydrocannabinol on human prefrontal activity	Δ9-tetrahydrocannabinol	Cannabis intoxication was associated with an increase on hemodynamic blood flow at the prefrontal cortex of the human brain (*in vivo* study).
[55]	Near infrared Spectroscopy	To estimate the content of cannabinoids, terpenes and secondary metabolites	Cannabinoids and terpenes	NIRS and FT-IR predicted the contents of secondary metabolites in Cannabis using a nondestructive and cheaper method compared to GC.

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[65]	NMR spectroscopy	To quantify CBD in industrial products	CBD	^{1}H NMR was utilized to quantify CBD in products derived from *Cannabis* seeds. Cannabinoid was suggested as a potential molecular marker for food processing quality.

3.3 Chemometrics in cannabis terpene testing

Chemometrics is an interdisciplinary method of analytical chemistry in which trends are extracted from data sets. Trends are often extrapolated and applied based on statistical and mathematical methods combined with fields such as mathematical logic and chemistry. Chemometrics has also been proposed to be used in the area of forensic science as well as in the biotech industry [66]. The study of chemometrics has given rise to two commonly used predictive modeling methods: partial least squares (PLS) and principal components regression (PCR) [67,68]. PLS, is a technique primarily suited for situations in which constructs are measured by a very large number of indicators and where maximum likelihood covariance-based structural equation modelling and spectroscopic tools are utilized [69]. PCR, on the other hand, is a regression analysis technique for analyzing multiple regression data that suffer from multicollinearity [70]. In the comparison between PLS and PCR, no significant differences were reported in the prediction errors of either. PLS virtually always required fewer latent variables than PCR, however this did not appear to influence predictive ability [71].

The future of the chemometrics field is currently projected in the direction of modeling, calibration, and pattern recognition sequences [72]. Other areas that are focused upon include multivariate process modelling and monitoring [73]. Chemometrics is projected to become a critical tool in the future for qualitative analysis, especially when considering the approaching development of high-dimensional data [74]. The long-term hope of chemometrics will mature as a trustworthy science and change the way analytical methods are developed and subsequently applied [75].

Studies involving chemometrics as applied to cannabis terpene quantification typically require the use of GC-FID since terpenes are considered volatile compounds, especially compared to the cannabinoids. Most applications on the use of FTIR and chemometrics as applied to terpenes are in the analysis of cannabinoids. THC, a cannabinoid considered to be the main target for potency analysis has a boiling point of around 315°F whereas most volatile terpenes will start to evaporate at around 70°F. Thus, for terpenes, the type of analysis to be performed would typically be using the GC. The Cary 630 FTIR spectrometer offers a great tool to test strain samples of cannabis. This would allow growers to see the composition of cannabinoids and terpenes in its gas and solid forms. Many cannabinoids and terpenes have varying decarboxylation points, which implies that some methods of consumption can be better than others for different strains [76]. FTIR-ATR with PLS in particular, was shown to accurately quantify THCA and CBDA in dried cannabis flowers, providing a quick, convenient, and portable potency test for recreational and medicinal cannabis cultivars [63]. Whereas GC-MS and NMR require sample preparation, and a specialist analytical lab, which could consequently lead to delays, an FTIR analysis coupled with chemometrics can be performed in a rapid and accurate manner. Further, FTIR can be considered an inexpensive and an accurate method [77]. In another study, a novel mid-infrared spectrometer coupled with PLS was developed for general quantitative chemical analyses for cannabinoid and terpene profiling of cannabis oils [78].

4.Challenges, future directions, and recommendations and the maturity of the cannabis industry

4.1 Challenges and future outlook for analytical laboratory

The future of cannabis lab testing estimates is challenging to find. The primary numbers being floated around originate from a June 2015 market report published by GreenWave Advisors titled *Marijuana lab testing: An in depth analysis of investing in one of the industry's most attractive plays*. GreenWave suggested that if the U.S. were to quickly legalize cannabis at the federal level, lab testing revenues alone would be $553 million by 2020, $866 million including related activities such as data analysis and consulting [79-82]. Another forward-looking statement by Research and Markets in March 2017 suggested the cannabis testing market across the globe could be valued at $1.4 billion by 2021, affected positively by legalization of medical cannabis, laboratory growth, and information technology adoption, negatively by analytical instruments' high costs and a "dearth of skilled professionals" [82]. A more conservative number was offered by Coherent Market Insights in July 2018, suggesting a global market at $1.5 billion USD by 2026 [82,83].

The cannabis industry and cannabis testing are in their infancies. As the need for better quality control continues and standardization is introduced, it is likely that lower limits for the various cannabis contaminants will be established and regulations will be introduced. Mass spectrometry will likely play a greater role in quantitation as detection levels are lowered and confirmatory tests are required. The health benefits of terpenes present in cannabis will also provide a fertile area of scientific research. CBD, CBG and other compounds appear to have a synergistic relationship with each other as well as with various THC forms and terpenes. This field needs much more investigation to determine mechanisms of action, bioavailability and health benefits" [82,84,85].

Besides the aforementioned issues and challenges, it was demonstrated that different storage conditions can change terpene results over time, and this should be taken into consideration when analyzing cannabis samples as result underestimation can occur. Varying storage conditions and degradation experiments should be the next study in the ever-changing world of regulatory cannabis testing [42].

4.2. Challenges and future directions in analytical methods

Achieving the correct analytical result for terpene analysis is a challenging task that includes considering various critical factors such as equipment selection, instrument method parameters, and method extraction optimization whenever necessary. In order to achieve a clear path to establish a scientifically justified validation method for terpene analysis, it is recommended to have guidance from the US FDA-regulated Pharmaceutical Industry's cGMP framework. This itself will stand up to legal scrutiny [86].

Although it is believed that liquid chromatography (LC) and mass spectrometry (MS) will likely become power horses for cannabis testing, there are still opportunities for innovation especially as with regards to instrumentation since this directly affect how sample preparation is carried out [87]. For example, Big Sur Analytics in California has introduced a BSS 2000 instrument that

provides an easy-to-use and portable device for the analysis of terpene and cannabinoid profiles in just 2 minutes within the mid-IR region [87].

In the upcoming years, cannabis terpene testing will be more convenient with the use of FTIR techniques. For example, Agilent has recently released a Cary 630 for quick and real-time potency analysis of cannabinoids in cannabis. Although not terpenes, the application can be utilized in such compounds [62].

Despite that mass spectrometry techniques have been employed to some extent in cannabis research, it was suggested by Nie, et al. (2019) to use the technique widely by utilizing superior combination of selectivity and sensitivity to study diversity of cannabis and its products which include terpenes. Good Laboratory Practices (GLP) and strict adherence by testing labs with established regulations by well-established organizations such as the Association of Official Agricultural Chemists should be implemented. This would allow safe chemical monitoring and integrity of cannabis materials and products [88].

Moreover, the use of noninvasive and nondestructive techniques such as NMR, Raman and FT-IR are new alternative methods to the established techniques such as LC and GC-MS. These nondestructive techniques allow fewer processing steps and sample preparation, cheaper price, and high accuracy without sacrificing high-throughput analyses [89]. Moreover, these techniques allow samples to be recovered after analyses; hence, samples can be analyzed in real-time.

4.3 Challenges related to method fragmentation and method multiplicity

Method fragmentation is one of the key issues in analyzing terpenes and other analytes in cannabis. This happens when results differ due to variations in sample preparation techniques used. For example, different labs may use different quantities of plant materials, grind these differently, dissolve these in different solvents at varying concentrations, and store at different temperatures. This variability makes a huge difference in the analysis of the same cannabis samples. The presence of inexperienced people analyzing these cannabis samples can also contribute to the variability of the results. Thus, it is necessary to have at least one experienced analytical chemist in charge of the lab [87].

Cannabis may not only be analyzed for the presence of terpenes but also other parameters including moisture content, microbial enumeration, pesticides, cannabinoids, and heavy metals among others. Sample preparation techniques for these methods differ widely. For example, for water/moisture content analysis, a hand grinder is used; for bacteria testing, the sample is put in media solution, then plate and incubate for 24-48 hours. Extraction must be taken but taken into consideration not to miss any analyte that maybe needed for other analysis. Because cannabis is such a complex matrix, extracting mycotoxins and mycotoxins maybe a challenge [87].

In general, the cannabis testing industry is poised for a breakthrough in the field of analytical science given the recent laws legalizing its medicinal use. Since laws vary from state to state, the list of analytes and methods to be used are also changing [90]. However, in general, advances in research in cannabis continues to be not making great strides due to legal restrictions around the world including its uses and effects on humans [91]. The resins of cannabis are known to contain

hundreds of different terpene and cannabinoid metabolites. Many of these metabolites remain largely unexplored. Rigorous studies are needed in order to ensure reproducibility of terpene profiles in cannabis. This can be done using diverse cannabis genotypes grown under controlled environmental conditions and then consequently analyze such terpene profiles quantitatively and qualitatively over the course of the plant growth and development [91].

5. Conclusion

Chromatographic analysis of terpenes from cannabis employs gas chromatography as the main technique choice of analysis. Variability in this technique comes from sample introduction, using HS, SPME or the more novel FET. All these can be coupled with various detectors and detection modes. These include but are not limited to FID, MS, or VUV. Although LC is not really a popular choice for the analysis of terpenes from cannabis, it is slowly gaining attention. The combination of LC with MS is currently the more attractive option, although complex matrices still pose a great challenge in this mode of analysis.

Despite being a multi-billion-dollar global industry, advances in scientific research still continues to be lagging behind due to its legal restrictions associated with its use and adverse effects on humans. Besides the use of infrared spectroscopy for terpene analysis in cannabis, the role of genomic, molecular and biomolecular properties that define terpenes will be anticipated to grow in large numbers as the landscape restrictions for cannabis ease around the world.

Chemometrics is quickly becoming an important counterpart in definitively and qualitatively analyzing a variety of different compounds and species of substances. This field has also made appearances in various topics branched from disciplines including analytical chemistry, biology, forensics, computer science, etc. The groups of interest in chemometrics, particularly PLS and PCR, are anticipated to become instrumental methods of analysis within a wide range of subjects.

List of abbreviations

CARS: Coherent anti-Stokes Raman scattering
CBC: cannabichromene
CB1: cannaboid receptor type 1
CB2: cannaboid receptor type 2
CBD: cannabidiol
CBDA: cannabidiolic acid
CBDV: cannabidivarin
CBG: Cannabigerol
CBN: cannabinol
FET: full evaporation technique
FID: flame ionization detector
FTIR: Fourier transform infrared spectroscopy
FTIR-ATR: fourier transform infrared spectroscopy with attenuated total reflectance
GC: gas chromatography
GC-FID: gas chromatography–flame ionization detection
GLP: good laboratory practices

HPLC: high performance liquid chromatography
HS: Headspace
HS-GC-FID: headspace gas chromatography–flame ionization detection
HS-SPME: Solid Phase Micro-extraction of Headspace
LC: liquid chromatography
LC-MS-MS: liquid mass tandem-mass spectrometry
LC-UV: liquid chromatography-ultraviolet
MS: mass spectrometry
MSD: mass selective detection
NIR: near infrared spectrometry
NMR: Nuclear Magnetic Resonance
PCR: Principal Component Regression
PLS: Partial least squares
SHS-GC-MS-MS: Static Headspace Gas Chromatography Mass Spectrometry
SPME: Solid phase microextraction
SSI: Split/Splitless Injection
THC: tetrahydrocannabidiol
THCA: Tetrahydrocannabinolic acid
THCV: tetrahydrocannabivarin
TLC: thin layer chromatography
TOF-MS: Time of Flight Mass Spectrometry
UV: ultraviolet
VUV: vacuum ultraviolet spectroscopy

Compliance with Ethical Standards

Conflict of Interest

The authors have no conflict of interest.

Human and Animal Rights and Informed Consent

This study does not involve any human or animal subjects.

References

[1] Grotenhermen F, Russo E. *Cannabis and Cannabinoids: Pharmacology, Toxicology, and Therapeutic Potential*. Psychology Press, 2002.
[2] Nuutinen T. Medicinal properties of terpenes found in Cannabis sativa and Humulus lupulus. *European Journal of Medicinal Chemistry* 2018; 157: 198–228.
[3] Identifying Terpenes in Cannabis | List of Cannabis Terpenes, https://www.encore-labs.com/terpene-identification (accessed 23 December 2020).
[4] Booth JK, Bohlmann J. Terpenes in Cannabis sativa – From plant genome to humans. *Plant Science* 2019; 284: 67–72.

[5] Baron EP. Medicinal Properties of Cannabinoids, Terpenes, and Flavonoids in Cannabis, and Benefits in Migraine, Headache, and Pain: An Update on Current Evidence and Cannabis Science. *Headache: The Journal of Head and Face Pain* 2018; 58: 1139–1186.
[6] What are cannabis flavonoids and what do they do? *Leafly*, https://www.leafly.com/news/cannabis-101/what-are-marijuana-flavonoids (2018, accessed 23 December 2020).
[7] Adams IB, Martin BR. Cannabis: pharmacology and toxicology in animals and humans. *Addiction* 1996; 91: 1585–1614.
[8] Lab Cannabis Testing is Steering the Future of the Industry. *Digipath Inc. - When Science Matters*, https://digipath.com/news/cannabis-testing-is-steering-the-future-of-the-industry/ (2019, accessed 23 December 2020).
[9] Classifying Cannabis Types By Terpenes Rather Than Cannabinoids. *Digipath Labs*, https://digipathlabs.com/news/classifying-cannabis-types-by-terpenes-rather-than-cannabinoids/ (2019, accessed 23 December 2020).
[10] Page Robert L., Allen Larry A., Kloner Robert A., et al. Medical Marijuana, Recreational Cannabis, and Cardiovascular Health: A Scientific Statement From the American Heart Association. *Circulation* 2020; 142: e131–e152.
[11] Rahn EJ, Hohmann AG. Cannabinoids as pharmacotherapies for neuropathic pain: from the bench to the bedside. *Neurotherapeutics* 2009; 6: 713–737.
[12] Ren M, Tang Z, Wu X, et al. The origins of cannabis smoking: Chemical residue evidence from the first millennium BCE in the Pamirs. *Science Advances* 2019; 5: eaaw1391.
[13] Cannabinol CBN - A New Potential Therapeutic for Unmet Medical Needs. *InMed Pharmaceuticals*, https://www.inmedpharma.com/learn/what-is-cannabinol/ (accessed 24 December 2020).
[14] Torres-Moreno MC, Papaseit E, Torrens M, et al. Assessment of Efficacy and Tolerability of Medicinal Cannabinoids in Patients With Multiple Sclerosis: A Systematic Review and Meta-analysis. *JAMA Netw Open* 2018; 1: e183485.
[15] Lowe HIC, Toyang NJ, McLaughlin W. Potential of Cannabidiol for the Treatment of Viral Hepatitis. *Pharmacognosy Res* 2017; 9: 116–118.
[16] Maor Y, Yu J, Kuzontkoski PM, et al. Cannabidiol inhibits growth and induces programmed cell death in kaposi sarcoma-associated herpesvirus-infected endothelium. *Genes Cancer* 2012; 3: 512–520.
[17] Mecha M, Feliú A, Iñigo PM, et al. Cannabidiol provides long-lasting protection against the deleterious effects of inflammation in a viral model of multiple sclerosis: a role for A2A receptors. *Neurobiol Dis* 2013; 59: 141–150.
[18] Spindle TR, Bonn-Miller MO, Vandrey R. Changing landscape of cannabis: novel products, formulations, and methods of administration. *Current Opinion in Psychology* 2019; 30: 98–102.
[19] Atakan Z. Cannabis, a complex plant: different compounds and different effects on individuals. *Ther Adv Psychopharmacol* 2012; 2: 241–254.
[20] Notcutt WG. Clinical Use of Cannabinoids for Symptom Control in Multiple Sclerosis. *Neurotherapeutics* 2015; 12: 769–777.
[21] Queirolo R. The effects of recreational cannabis legalization might depend upon the policy model. *World Psychiatry* 2020; 19: 195–196.
[22] Jiang Z, Kempinski C, Chappell J. Extraction and Analysis of Terpenes/Terpenoids. *Curr Protoc Plant Biol* 2016; 1: 345–358.

[23] Herring T. Instrumentation Used for Terpene Analysis. *Cannabis Industry Journal*, https://cannabisindustryjournal.com/column/instrumentation-used-for-terpene-analysis/ (2017, accessed 23 December 2020).
[24] Leghissa A, Hildenbrand ZL, Schug KA. A review of methods for the chemical characterization of cannabis natural products. *J Sep Sci* 2018; 41: 398–415.
[25] Trass M, Anderson T, Krepich S. Analysis of 33 Primary and Secondary Terpenes Found in Cannabis by GC-FID. 2.
[26] Misa A, Dhandapani R. Analysis of 20 Terpenes in Cannabis by GC-FID using Zebron™ ZB-624PLUS™ GC Column. 2.
[27] Cardenia V, Bakro F, Wielgusz K, et al. Development and Validation of a Fast GC/FID Method for the Simultaneous Determination of Terpenes and CBD in Hemp (Cannabis sativa L.). 1.
[28] Hildenbr DZ, Leghissa A, Schug DKA. The Other Side of Cannabis: Terpenes. *Cannabis Industry Journal*, https://cannabisindustryjournal.com/column/the-other-side-of-cannabis-terpenes/ (2018, accessed 23 December 2020).
[29] Brault A, Agasse V, Cardinael P, et al. The full evaporation technique: a promising alternative for residual solvents analysis in solid samples. *J Sep Sci* 2005; 28: 380–386.
[30] restek. A Fast, Simple FET Headspace GC-FID Technique for Determining Residual Solvents in Cannabis Concentrates. *Restek GmbH - Chromatografie Produkte*, https://www.restekgmbh.de/aktuelles/fast-simple-fet-headspace-gc-fid-technique-determining-residual-solvents-cannabis (2014, accessed 23 December 2020).
[31] Rigdon A, Hilliard C, Cochran J. A Preliminary FET Headspace GC-FID Method for Comprehensive Terpene Profiling in Cannabis. 8.
[32] Stenerson KK, Halpenny MR. Analysis of terpenes in cannabis using headspace solid-phase microextraction and GC-MS. *LCGC North Am* 2017; 35: 28.
[33] Halpenny M, Stenerson KK. *Quantitative Determination of Terpenes in Cannabis Using Headspace Solid Phase Microextraction and GC-MS*. Gersten Application Note, 2017.
[34] Honnold R, Kubas R, Macherone A. Analysis of Terpenes in Cannabis Using the Agilent 7697A/7890B/5977B Headspace GC-MSD System: Faster Analysis Time = Greater Productivity. *Agilent Application Notes*; 10.
[35] Headspace SPME-GC/MS Analysis of Terpenes in Hops and Cannabis. *Sigma-Aldrich*, https://www.sigmaaldrich.com/technical-documents/articles/analytix-reporter/analysis-terpenes-hops-cannabis.html (accessed 23 December 2020).
[36] Ibrahim E, Wang M, Radwan M, et al. Analysis of Terpenes in Cannabis sativa L. Using GC/MS: Method Development, Validation, and Application. *Planta Medica* 2019; 85: 431–438.
[37] Shapira A, Berman P, Futoran K, et al. Tandem mass spectrometric quantification of 93 terpenoids in Cannabis using static headspace injections. *Analytical chemistry* 2019; 91: 11425–11432.
[38] LECO Corporation, Saint Joseph, Michigan USA. Determining Terpene Profiles of Cannabis Strains Using GC and GCxGC with High Performance TOFMS, https://knowledge.leco.com/component/edocman/application-note-cannabis-terpene-profiles-peg-bt-4d-558/viewdocument?Itemid=1749 (2019).
[39] Qiu C, Smuts J, Schug K. Analysis of Terpenes and Turpentines using Gas Chromatography with Vacuum Ultraviolet Detection. *Journal of separation science*; 40. Epub ahead of print 16 December 2016. DOI: 10.1002/jssc.201601019.

[40] Krill C, Rochfort S, Spangenberg G. A High-Throughput Method for the Comprehensive Analysis of Terpenes and Terpenoids in Medicinal Cannabis Biomass. *Metabolites*; 10. Epub ahead of print 6 July 2020. DOI: 10.3390/metabo10070276.
[41] Hyland KC, Borton C, Winkler P, et al. Quantitation of Terpenes in Cannabis Products Using APCI LC-MS/MS. *Planta Medica* 2016; 82: PA34.
[42] Shimadzu. Simplified Cannabis Terpene Profiling by GCMS, https://www.shimadzu.fr/sites/shimadzu.seg/files/gcms-1604-terpeneprofilingcannabis.pdf (2016).
[43] Stenerson K. Headspace SPME-GC/MS Analysis of Terpenes in Hops and Cannabis. *Sigma-Aldrich*, https://www.sigmaaldrich.com/technical-documents/articles/analytix-reporter/analysis-terpenes-hops-cannabis.html (accessed 23 December 2020).
[44] Skoog DA, Crouch SR, Holler FJ. *Principles of instrumental analysis.* Belmont, CA: Thomson Brooks/Cole, 2007.
[45] Mariani Z, Strong K, Wolff M, et al. Infrared measurements in the Arctic using two Atmospheric Emitted Radiance Interferometers. *Atmospheric Measurement Techniques* 2012; 5: 329–344.
[46] Lang Q, Kwang Yak H, Wai CM. Selective dissolution and one step separation of terpene trilactones in ginkgo leaf extracts for GC-FID determination. *Talanta* 2001; 54: 673–680.
[47] Garrett PE, Rodríguez TR, Varela AD, et al. Multiple Shape Coexistence in $^{110,112}\mathrm{Cd}$. *Phys Rev Lett* 2019; 123: 142502.
[48] Hoult DI, Bhakar B. NMR signal reception: Virtual photons and coherent spontaneous emission. *Concepts in Magnetic Resonance* 1997; 9: 277–297.
[49] Rabi II, Zacharias JR, Millman S, et al. A New Method of Measuring Nuclear Magnetic Moment. *Phys Rev* 1938; 53: 318–318.
[50] Govindaraju V, Young K, Maudsley AA. Proton NMR chemical shifts and coupling constants for brain metabolites. *NMR in Biomedicine* 2000; 13: 129–153.
[51] Choi YH, Hazekamp A, Peltenburg-Looman AMG, et al. NMR assignments of the major cannabinoids and cannabiflavonoids isolated from flowers of Cannabis sativa. *Phytochemical Analysis* 2004; 15: 345–354.
[52] Choi YH, Kim HK, Hazekamp A, et al. Metabolomic Differentiation of Cannabis s ativa Cultivars Using 1H NMR Spectroscopy and Principal Component Analysis. *Journal of natural products* 2004; 67: 953–957.
[53] Sanchez L, Baltensperger D, Kurouski D. Raman-Based Differentiation of Hemp, Cannabidiol-Rich Hemp, and Cannabis. *Anal Chem* 2020; 92: 7733–7737.
[54] Tu AT. *Raman Spectroscopy in Biology: Principles and Applications*. Wiley, 1982.
[55] Sánchez-Carnerero Callado C, Núñez-Sánchez N, Casano S, et al. The potential of near infrared spectroscopy to estimate the content of cannabinoids in Cannabis sativa L.: A comparative study. *Talanta* 2018; 190: 147–157.
[56] Starsinic M, Taylor RL, Walker PL, et al. FTIR studies of Saran chars. *Carbon* 1983; 21: 69–74.
[57] Smith BC. *Fundamentals of Fourier Transform Infrared Spectroscopy*. CRC Press, 2011.
[58] Kalasinsky VF, McDonald JT Jr. GC/FTIR Spectra of Terpenes*. *Journal of Chromatographic Science* 1983; 21: 193–200.
[59] Kalasinsky VF, Pechsiri S, Kalasinsky KS. Analysis of Terpenes by Capillary Gas Chromatography/Fourier Transform Infrared Spectroscopy (GC/FTIR). *Journal of Chromatographic Science* 1986; 24: 543–548.

[60] Ebersbach P, Stehle F, Kayser O, et al. Chemical fingerprinting of single glandular trichomes of Cannabis sativa by Coherent anti-Stokes Raman scattering (CARS) microscopy. *BMC Plant Biology* 2018; 18: 275.
[61] Hanif MA, Nawaz H, Naz S, et al. Raman spectroscopy for the characterization of different fractions of hemp essential oil extracted at 130°C using steam distillation method. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 2017; 182: 168–174.
[62] Mainali D, Clara S. Quick and Real-Time Potency Determination of Cannabinoids Using FTIR Spectroscopy. *Agilent Application Notes*; 4.
[63] Smith BC, Lewis MA, Mendez J. Optimization of cannabis grows using Fourier transform mid-infrared spectroscopy. *Application Note, Perkin Elmer Inc, San Jose/CA*.
[64] Keles HO, Radoman M, Pachas GN, et al. Using Functional Near-Infrared Spectroscopy to Measure Effects of Delta 9-Tetrahydrocannabinol on Prefrontal Activity and Working Memory in Cannabis Users. *Front Hum Neurosci*; 11. Epub ahead of print 2017. DOI: 10.3389/fnhum.2017.00488.
[65] Siciliano C, Bartella L, Mazzotti F, et al. 1H NMR quantification of cannabidiol (CBD) in industrial products derived from Cannabis sativa L.(hemp) seeds. In: *IOP Conference Series: Materials Science and Engineering*. IOP Publishing, 2019, p. 012010.
[66] Kumar R, Sharma V. Chemometrics in forensic science. *TrAC Trends in Analytical Chemistry* 2018; 105: 191–201.
[67] Frank lldiko E, Friedman JH. A Statistical View of Some Chemometrics Regression Tools. *Technometrics* 1993; 35: 109–135.
[68] Rathore AS, Bhushan N, Hadpe S. Chemometrics applications in biotech processes: A review. *Biotechnology Progress* 2011; 27: 307–315.
[69] Haenlein M, Kaplan AM. A Beginner's Guide to Partial Least Squares Analysis. *Understanding Statistics* 2004; 3: 283–297.
[70] Principal Components Regression. In: *NCSS Statistical Software*.
[71] Wentzell PD, Vega Montoto L. Comparison of principal components regression and partial least squares regression through generic simulations of complex mixtures. *Chemometrics and Intelligent Laboratory Systems* 2003; 65: 257–279.
[72] Brown SD, Blank TB, Sum ST, et al. Chemometrics. *Anal Chem* 1994; 66: 315–359.
[73] Wold S, Sjöström M. Chemometrics, present and future success. *Chemometrics and Intelligent Laboratory Systems* 1998; 44: 3–14.
[74] Szymańska E, Gerretzen J, Engel J, et al. Chemometrics and qualitative analysis have a vibrant relationship. *TrAC Trends in Analytical Chemistry* 2015; 69: 34–51.
[75] Frank IE, Kowalski BR. Chemometrics. *Anal Chem* 1982; 54: 232–243.
[76] Cannabis Testing for Potency and Terpenes, https://www.cannabistech.com/articles/gas-liquid-chromotography-ftir-spectroscopy-testing-terpenes/ (accessed 23 December 2020).
[77] On-Site Analysis of Cannabis Strength Using FT-IR Spectrosocopy. *AZoM.com*, https://www.azom.com/article.aspx?ArticleID=13308 (2016, accessed 23 December 2020).
[78] Smith BC. Determination of Cannabinoid and Terpene Profiles in Cannabis Oils by Mid-Infrared Spectroscopy: 1. Cannabinoids. 6.
[79] DigiPath-Investor-Presentation-11.3.pdf, https://digipath.com/wp-content/uploads/2016/11/DigiPath-Investor-Presentation-11.3.pdf (accessed 23 December 2020).

[80] Signal Bay Makes Strategic Acquisition in the $850M Cannabis Testing Market. *CannabisFN*, https://www.cannabisfn.com/signal-bay-makes-strategic-acquisition-in-the-850m-cannabis-testing-market/ (2015, accessed 23 December 2020).
[81] Why Are Cannabis Labs Important and What Makes Them a Great Investment?, http://finance.yahoo.com/news/why-cannabis-labs-important-makes-133100676.html (accessed 23 December 2020).
[82] Markets R and. Cannabis Testing Market to Reach $1.4 Billion by 2021 - Driven by Growing Number of Cannabis Testing Laboratories - Research and Markets, https://www.prnewswire.com/news-releases/cannabis-testing-market-to-reach-14-billion-by-2021---driven-by-growing-number-of-cannabis-testing-laboratories---research-and-markets-300432416.html (accessed 23 December 2020).
[83] https://www.coherentmarketinsights.com CMI. Cannabis Testing Market Estimated to be Worth US$ 1.5 Billion by 2026, https://www.coherentmarketinsights.com/press-release/cannabis-testing-market-estimated-to-be-worth-us-15-billion-by-2026-64 (accessed 23 December 2020).
[84] Kuzdzal S, Clifford R, Winkler P, et al. Cannabis testing. *Cannabis: A Clinician's Guide* 2018; 133.
[85] Shimadzu_Whitepaper_Emerging_Cannabis_Industry.pdf, https://www.ssi.shimadzu.com/sites/ssi.shimadzu.com/files/Industry/Literature/Shimadzu_Whitepaper_Emerging_Cannabis_Industry.pdf (accessed 23 December 2020).
[86] admin. Agricor Laboratories Advanced Terpene Analysis for Cannabis Products. *Agricor Laboratories*, https://agricorlabs.com/terpene-analysis/ (accessed 23 December 2020).
[87] Overcoming Challenges of Cannabis Sample Prep. *Lab Manager*, https://www.labmanager.com/product-focus/overcoming-challenges-of-cannabis-sample-prep-1127 (accessed 23 December 2020).
[88] Nie B, Henion J, Ryona I. The Role of Mass Spectrometry in the Cannabis Industry. *J Am Soc Mass Spectrom* 2019; 30: 719–730.
[89] Hazekamp A, Choi Y, Verpoorte R. Quantitative Analysis of Cannabinoids from Cannabis sativa Using 1H-NMR. *Chemical & pharmaceutical bulletin* 2004; 52: 718–21.
[90] Cannabis Testing Opens Up a Whole New Market - ProQuest, https://search.proquest.com/openview/00c2ecbf0b692a0d8d55754409a406f7/1?pq-origsite=gscholar&cbl=2032040 (accessed 23 December 2020).
[91] Booth JK, Bohlmann J. Terpenes in Cannabis sativa – From plant genome to humans. *Plant Science* 2019; 284: 67–72.