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Analytical Approaches to Address Challenges in the Analysis of Cannabinoids in Vascular Matrices Using Mass Spectrometry

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ABSTRACT

Phytocannabinoids are bioactive metabolites derived from the *Cannabis sativa* plant. They have garnered attention due to their recreational uses and therapeutic potential. Although various analytical strategies have been employed for their analysis, mass spectrometry (MS) coupled to chromatographic separation is superior due to its sensitivity and selectivity. Various MS-based strategies, namely Gas chromatography (GC-MS) and liquid chromatography - MS (LC-MS) are reviewed with focus on the analysis of phytocannabinoids in vascular matrices. These include plasma, serum, whole blood, and dried blood spots (DBS). Applications, advantages and challenges associated with each MS strategy in vascular matrices are evaluated and critically discussed. In addition, the review outlines the challenges in DBS spot analysis, such as hematocrit bias, versus plasma/serum and whole blood processing, which involves protein removal, extraction and cleanup steps.

1 | Introduction

Cannabis sativa is a species of *Cannabaceae*, and it is one of the most controversial plants in the world in terms of its societal impact and legal status (Antunes et al. 2023). In some jurisdictions, it is considered an illicit drug (Nicolaou et al. 2021), but it has been recently legalized widely in Western nations. *Cannabis sativa* produces secondary plant metabolites called phytocannabinoids, a class of terpenophenolic compounds (ElSohly and Slade 2005; Duggan 2021). Phytocannabinoids can be classified into 11 distinct types as shown in Figure 1 (Montero et al. 2022; Kinghorn et al. 2017; Gülck and Møller 2020). Phytocannabinoids have captured scientific attention due to their therapeutic potential. They have shown neuroprotective properties (Sánchez and García-Merino 2012), in many neurodegenerative diseases, such as Parkinson's and Alzheimer's diseases (Varshney et al. 2023; Vallée et al. 2017), due to their anti-inflammatory and antioxidant effects (Chu et al. 2024; Casedas et al. 2022). Δ^9-tetrahydrocannabinol (THC), a major psychoactive component of the plant, and cannabidiol (CBD), a non-psychoactive component, are among the hundreds of identified cannabinoids (Pourseyed Lazarjani et al. 2020; Hanuš et al. 2016; Khalsa et al. 2022), and they have been widely investigated for their therapeutic use (Landmark and Brandl 2020; Cascio et al. 2017). THC is used as medicine for pain management and is strongly linked to the psychoactive effects of cannabis consumption (Malabadi et al. 2023). In Canada, THC is considered a third line option for pain treatment, especially for neuropathic pain (Moulin et al. 2014; Mu et al. 2017).

CBD is acknowledged for its numerus potential therapeutic properties, including antiseizure (Silvestro et al. 2019), analgesic, anti-inflammatory, and anxiolytic effects (Malabadi et al. 2023; Wright et al. 2020). In fact, the US Food and Drug Administration (FDA) has approved Epidiolex® (CBD isolate) for the treatment of seizures associated with tuberous sclerosis complex in patients 1 year of age and older (Rubin 2018). In addition, it was demonstrated that Epidiolex® is safe, and

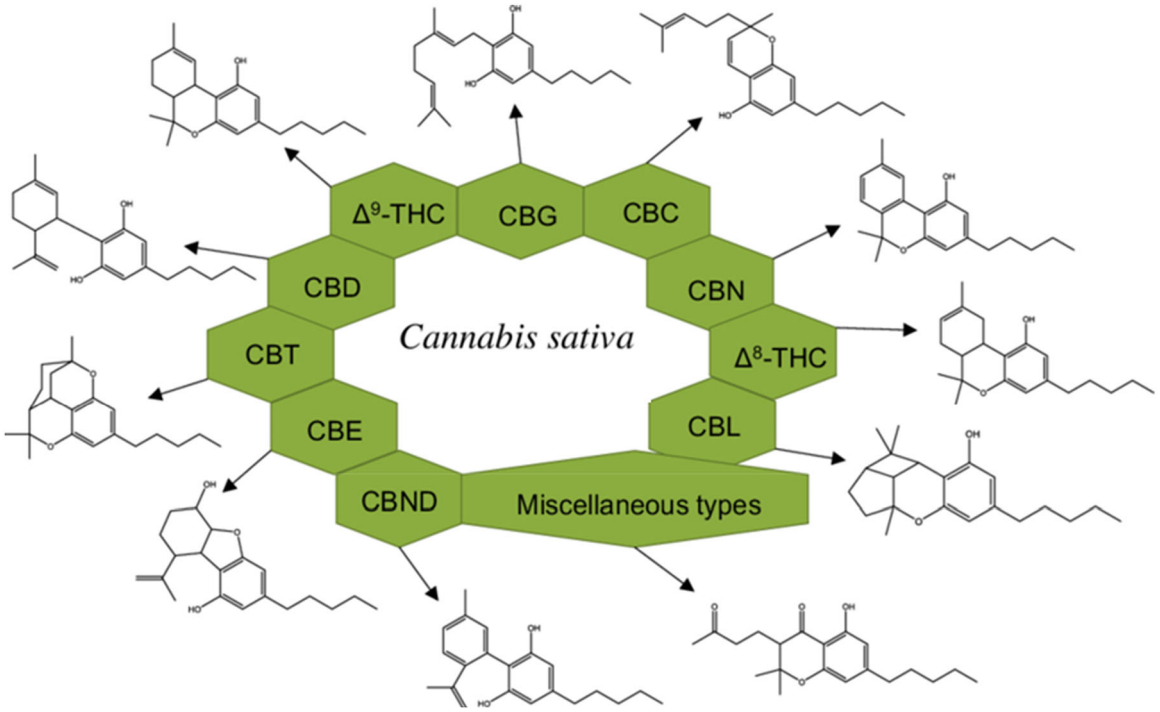

FIGURE 1 | Representative structures for the types of phytocannabinoids, Δ^9-trans-tetrahydrocannabinol (Δ^9-THC), Δ^8-trans-tetrahydrocannabinol (Δ^8-THC), cannabidiol (CBD), cannabigerol (CBG), cannabichromene (CBC), cannabinodiol (CBND), cannabielsoin (CBE), cannabicyclol (CBL), cannabinol (CBN), cannabitriol (CBT), and miscellaneous types, adapted from an open access article, which permits unrestricted use, distribution and reproduction in any medium (Montero et al. 2022).

effective for the treatment of autism-spectrum disorder (Ganesh and Shareef 2020). Another drug, Sativex® (CBD-THC in 1:1 w/w ratio), is prescribed to treat neuropathic pain linked to multiple sclerosis without causing adverse effects or affecting driving performance in patients (Khalsa et al. 2022; Celius and Vila 2018). The use of Sativex® has been approved in 28 countries, including Canada (Khalsa et al. 2022). Finally, cannabis may have an antidepressant-like actions because of cannabichromene (CBC), which is a major non-psychotropic biologically active phytocannabinoid (Davis and Hatoum 1983; El-Alfy et al. 2010).

Due to the global expansion of cannabis consumption, considerable research has been conducted to study cannabinoids and cannabis-based products (Micalizzi et al. 2021). These studies generally require robust analytical techniques to accurately identify, quantify, and characterize phytocannabinoids in various matrices. Analytical methods that have been employed to analyze phytocannabinoids includes nuclear magnetic resonance (NMR) (Ayakeme et al. 2024; Leite 2018; Powers and Siegel 2006), and chromatography, such as thin-layer chromatography (TLC), high-performance liquid Chromatography (HPLC) and gas chromatography (GC) (Correia et al. 2023; Formisano et al. 2024; Zulfqar et al. 2023; Galettis et al. 2021; Nahar et al. 2023). Each method has advantages and limitations; NMR, for example, can provide detailed structural information and is non-destructive, but it has a relatively low sensitivity and limited ability to quantify compounds at low concentrations (Letertre et al. 2021). Similarly, the chromatographic analysis with UV detection may lack selectivity and the needed sensitivity for biological samples. On the other hand, mass spectrometry (MS), when combined with separation techniques, can ensure accurate measurement at low concentrations in complex biological samples. In addition, MS provides structural information of tested cannabinoids, helping in their profiling and characterization. HPLC-MS and GC-MS can offer many opportunities for the effective analysis of cannabinoids. The utilization of GC-MS to identify and quantify acidic phytocannabinoids is, however, challenging due to the high temperature in a chromatography column leading to decarboxylation of acidic cannabinoids to their neutral forms (Pourseyed Lazarjani et al. 2020; Micalizzi et al. 2021; Kanabus et al. 2021). Derivatization overcomes such problems by transforming the acidic cannabinoids to more stable and volatile analogs (Pourseyed Lazarjani et al. 2020; Micalizzi et al. 2021; Kanabus et al. 2021). LC-MS, on the other hand, allows for the identification and quantification of both neutral and acidic cannabinoids without derivatization, which is an advantage over GC-MS (Chen and Rogers 2019). In addition, Matrix effects in mass spectrometry can significantly impact ionization efficiency and as a result quantitative accuracy, particularly in complex biological fluids. Whole blood, as expected, tends to produce more ion suppression compared to plasma due to the cellular content and higher endogenous compound load. Careful method development, however, including SPE cleanup and the use of stable isotope-labeled internal standards, is crucial to reduce such unfavorable effects.

Studying phytocannabinoids in vascular matrices, namely plasma, serum, whole blood, and dried blood spot (DBS), can enable health care providers, researchers, and law enforcement to document cannabis intake, monitor therapeutic drug levels in blood, and identify driving under the influence of drugs (DUID) cases (Schwope et al. 2011).

This paper will explore the fundamental principles of sample preparation, and analytical approaches associated with GC-MS and LC-MS methods to highlight the strengths, limitations and challenges related to each analytical approach used in phytocannabinoid research, aiming to enrich the literature pertaining to phytocannabinoid-based research.

2 | Vascular Matrices and Associated Challenges for MS-Based Analysis

Plasma and serum are used in pharmacokinetic studies and clinical monitoring because these matrices are less complex than whole blood (Schwilke 2009a). The absence of cellular components in plasma and serum allows accurate quantification without interference from blood cells. Analyzing phytocannabinoids in whole blood, on the other hand, offers simple handling without the need for plasma extraction, which reduces the loss of target analytes. Analysis of whole blood provides a complete measurement of both free analytes and those held within blood cells. Therefore, whole blood is frequently the only specimen utilized in DUID cases and forensic investigations (Schwilke 2009a). When the whole-blood/plasma cannabinoid ratios were measured in human blood and plasma samples, median (interquartile range) whole-blood/plasma ratios are found to be 0.39 (0.28–0.48) for THC, 0.56 (0.43–0.73) for the primary metabolite of THC 11-hydroxy-THC (11-OH-THC), and 0.37 (0.24–0.56) for the secondary metabolite of THC carboxy-THC (THC-COOH) (Schwilke 2009a). These results indicate differences in how cannabinoids are distributed, which is important for the interpretation of the physiological effects of forensic toxicology results. However, the analysis of phytocannabinoids in whole blood has a higher potential of matrix interference from endogenous compounds and cellular components in comparison to serum or plasma.

A vascular matrix that can be collected easily, and is less invasive in collection, is DBS that, as whole blood sample, reflects the complete composition of the blood. DBS sampling is simple, quick, minimally invasive, and cost-effective (Meikopoulos et al. 2024). Additionally, DBS can be stored at room temperature without notable degradation of the analytes (Mercolini et al. 2013). Applications of DBS in bioanalysis have increased over the years, including for newborn screening, therapeutic drug monitoring (TDM) (Edelbroek et al. 2009), forensic toxicology and preclinical drug development (Stove et al. 2012). DBS provides significant advantages over traditional blood sampling approaches. It requires a small volume of blood ($\leq 100\ \mu L$) from the fingertip or heel (Stove et al. 2012; Sadler Simões et al. 2018). Furthermore, DBS maintains the integrity of the sample during transportation and storage, making it an efficient sampling technique for long-term storage of samples for forensic and clinical purposes (EROL ÖZTÜRK 2023). Additionally, DBS has been applied for doping control (Thevis et al. 2023). However, the blood sample used for DBS should be placed and distributed properly on DBS cards; otherwise, the distribution of cannabinoids across the spot will be uneven, causing inaccurate quantification (Choi et al. 2014). The small volume of DBS needs a highly sensitive analytical technique to detect cannabinoids with low concentrations.

While GC-MS methods are well-established and widely accepted in forensic toxicology, such as the detection of THC in whole blood or plasma (Nicolaou et al. 2021; Hoffman et al. 2020), the increasing adoption of LC-MS in clinical and pharmacokinetic studies (Sempio et al. 2022; Manca et al. 2022; Sallam et al. 2023; Reisdorph et al. 2024; Pigliasco et al. 2020; Chan-Hosokawa et al. 2022) has highlighted the need for cannabinoid-specific validation protocols that are recognized in both forensic and clinical regulatory fields. In the forensic context, method validation is typically guided by recognized authorities, such as the Scientific Working Group for Forensic Toxicology (SWGTOX), which provide detailed criteria for cannabinoid analysis especially THC in biological matrices, including blood and urine (Scientific Working Group for Forensic Toxicology [SWGTOX] et al. 2013). Clinical applications, by contrast, rely on the general bioanalytical validation guidelines from the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA), most often applied to plasma or serum (Administration 2018; European Medicines agency EMA 2011). However, no harmonized validation standards currently exist for LC-MS methods specifically targeting cannabinoids, which presents a notable gap in current regulatory guidance.

Studying phytocannabinoids in vascular matrices has limitations. Firstly, the distribution of phytocannabinoids in blood does not represent the whole concentration of phytocannabinoids in the body as phytocannabinoids tend to partition into lipid-rich tissues, including adipose tissue and the brain (Grotenhermen 2003). Another limitation is the high plasma protein binding of phytocannabinoids. THC and CBD, for example bind plasma protein up to 95%–99% (Landmark and Brandl 2020; Huestis 2007). Therefore, effective extraction is needed to minimize matrix effects and to ensure efficient recovery. Efficient extraction is also critical due to low blood levels as, for instance, THC concentrations in the blood reach approximately 1–4 ng/mL within 3–4 h of consumption (Sharma et al. 2012). This low concentrations of phytocannabinoids in vascular matrices should be considered during the development of GC-MS or LC-MS methods.

Another major challenge for the analysis of phytocannabinoids in vascular matrices is the stability of the cannabinoids during storage. Blood and plasma samples can be stored at 4°C or −10°C for up to 4 months (Johnson et al. 1984) while storing samples at −20°C preserves cannabinoid for up to 6 months (Johnson et al. 1984; Schwilke et al. 2009b). Another factor affecting the stability of phytocannabinoids is the storage container (Christophersen 1986), as phytocannabinoids could be absorbed onto the walls of plastic containers due to their lipophilic nature (Christophersen 1986). When THC-containing whole blood samples are stored in polystyrene plastic containers at −20°C for 4–24 weeks, significant loss (60%–100%) of THC occurs, while the THC level remains unchanged in glass vials (Christophersen 1986). Exposure to light is the greatest factor in the loss of phytocannabinoids, therefore, samples should be stored in darkness (Fairbairn et al. 1976; Trofin et al. 2012). Based on these known challenges, phytocannabinoids are usually best stored in amber glass containers at −20°C.

During the analysis of cannabinoids in vascular matrices using MS, there are other unfavorable factors that need to be addressed, including decarboxylation, matrix effects and isomer separation. Decarboxylation of acidic cannabinoids to their

neutral counterparts occurs during GC-MS analysis due to high temperature, impacting the accuracy of the analytical method (Citti et al. 2020). Moreover, matrices can affect chromatographic separation and ionization efficiency, highlighting the importance of sample preparation (Williams et al. 2023). The presence of structural isomers, such as Δ^9-THC and CBD or positional isomers such as Δ^8-THC versus Δ^9-THC, represents another challenge for analysis. THC and CBD, shown in Figure 1, will co-elute and interfere in the analysis of each other (Reber et al. 2022). Therefore, their chromatographic separation is required for effective analysis. To address the above issues, many strategies can be employed at various steps of the analytical procedure, namely sample preparation, chromatographic conditions, and MS parameters, as discussed in this paper.

3 | Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS is considered the method of choice for the analysis of cannabinoids in forensic applications (Nicolaou et al. 2021), and is widely used for screening, after positive testing by immunochemical methods (Goodwin et al. 2006; Scurlock et al. 2006). However, choosing the correct stationary phase (polar or nonpolar) for GC-MS analysis is pivotal for achieving efficient separation. For example, the hydrophobicity of phytocannabinoids requires the use of nonpolar stationary phases for separation (Hoffman et al. 2020; Dawidowicz et al. 2024; Holowinski et al. 2022; Dybowski et al. 2020; Cannarozzi 2020; Dawidowicz et al. 2022; Frei et al. 2022), as detailed below. GC is mainly employed for the analysis of volatile analytes; however, nonvolatile compounds can sometimes be chemically derivatized to yield volatile and stable derivatives (Nahar et al. 2023; Dawidowicz et al. 2024). Derivatization of cannabinoids allows for the use of GC-MS to analyze cannabinoids in routine forensic investigations, albeit with the proper extraction approach.

In addition to extraction, choosing the column and the detector is crucial to analyze cannabinoids in vascular matrices. Both GC-flame ionization detector (FID), and GC-MS, including tandem mass spectrometry (GC-MS/MS), are successfully utilized in the analysis of phytocannabinoids not only within vascular matrices, but also from plant extracts (Wilson et al. 2022; De Prato et al. 2022; Attard et al. 2022; Amirav et al. 2022). While GC-FID is simple and reliable in the identification and quantification of naturally occurring cannabinoids (Nahar et al. 2020), GC-MS provides high-quality structural information. The most popular ionization mode in GC-MS for the analysis of cannabinoids is electron impact (EI) (Nahar et al. 2020), providing MS spectra with characteristic fragment ions that are crucial for the identification of the analytes.

3.1 | GC-MS for Plasma/Serum Analysis

In GC-MS, quick, easy, cheap, effective, rugged, and safe (QuEChERS) procedures, protein precipitation, and solid phase extraction (SPE) are used to extract cannabinoids from plasma and serum (Hoffman et al. 2020; Dawidowicz et al. 2024; Holowinski et al. 2022; Dybowski et al. 2020; Cannarozzi 2020; Dawidowicz et al. 2022; Frei et al. 2022). However, some extraction agents can impact the stability of cannabinoids during sample preparation. It was shown that the transformation of CBD to other cannabinoid structures depends on multiple factors, including the concentration of hydrogen ions in plasma, the type of the precipitation agent, the temperature in the GC injector, and the time of incubation of the studied sample with the precipitation agent (Dybowski et al. 2020). Acidic agents, such as trifluoroacetic acid (TFA) are added during protein precipitation targeting CBD in human blood/plasma (Holowinski et al. 2022; Dybowski et al. 2020) and it was found that a significant amount of CBD transforms to Δ^9-THC, Δ^8-THC, cannabinol (CBN), and other CBD transformation products, meaning that TFA negatively affected the stability of CBD (Holowinski et al. 2022; Dybowski et al. 2020). When GC-MS results are compared to LC-MS data in the same study, the TFA-mediated transformation of a CBD sample to Δ^9-THC and Δ^8-THC was confirmed (Dybowski et al. 2020). Therefore, it is recommended to use neutral precipitation agents, such as acetonitrile (ACN), methanol or acetone, for protein precipitation before GC-MS analysis for the accurate determination of CBD in plasma (Nahar et al. 2023).

In addition to the role of acidic additives, the temperature during analysis impacts the analytical results. While CBD transforms to other derivatives and isomers in GC-MS, it does not convert to other isomers during LC-MS because the kinetics of CBD transformation in room temperature is relatively slow (Dybowski et al. 2020). This difference in transformation of CBD to other derivatives obtained from the GC-MS and LC-MS, in the same study, suggests that the transformation of CBD to its derivatives occurs due to the high temperature in the GC injector, accelerating chemical transformation of CBD (Dybowski et al. 2020).

To stabilize and enhance the sensitivity during GC-MS analysis, derivatization is commonly used for analytes (Nahar et al. 2023; Dawidowicz et al. 2024). The efficacy of silylation or acylation for the GC-MS analysis of CBG was examined (Dawidowicz et al. 2024), showing enhanced sensitivity three times with an LOD of 0.93 ng/mL instead of 2.78 ng/mL in case of nonderivatized samples (Dawidowicz et al. 2024). In the same investigation, the cyclization of CBG (Figure 2) was superior to silylation or acylation as cyclization transforms CBG to its pyranic derivative, enhancing the limit of detection (LOD) 10-fold, without compromising the sensitivity of other cannabinoids (Dawidowicz et al. 2024). Similarly, it was shown that derivatization with N-Methyl-N-trimethylsilyl trifluoroacetamide (MSTFA) in hempseed oil prevents degradation and decarboxylation by capping hydroxyl and carboxyl groups on the analytes (Macherone 2020).

The type of columns and the detectors can impact chromatography with the most common capillary column for the analysis of phytocannabinoids is Zebron ZB-5® (Dawidowicz et al. 2024; Holowinski et al. 2022; Dybowski et al. 2020; Dawidowicz et al. 2022; Frei et al. 2022). This column has small diameter and is packed with nonpolar stationary phase (5% diphenyl and 95% dimethyl polysiloxane), providing good separation for a wide range of cannabinoids (Nahar et al. 2020). However, it does not have a sufficient level of polarity to separate and distinguish CBD from CBC (Nahar et al. 2023). To overcome this disadvantage, an intermediate polarity column such as 35% diphenyl in a dimethyl silicone is utilized, offering satisfactory

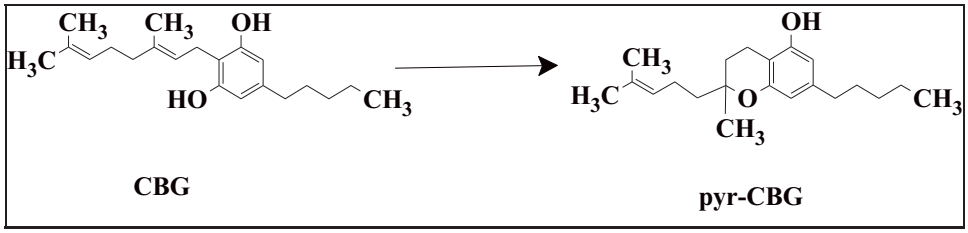

FIGURE 2 | Cyclization reaction of CBG to its pyranic form (Pyr-CBG).

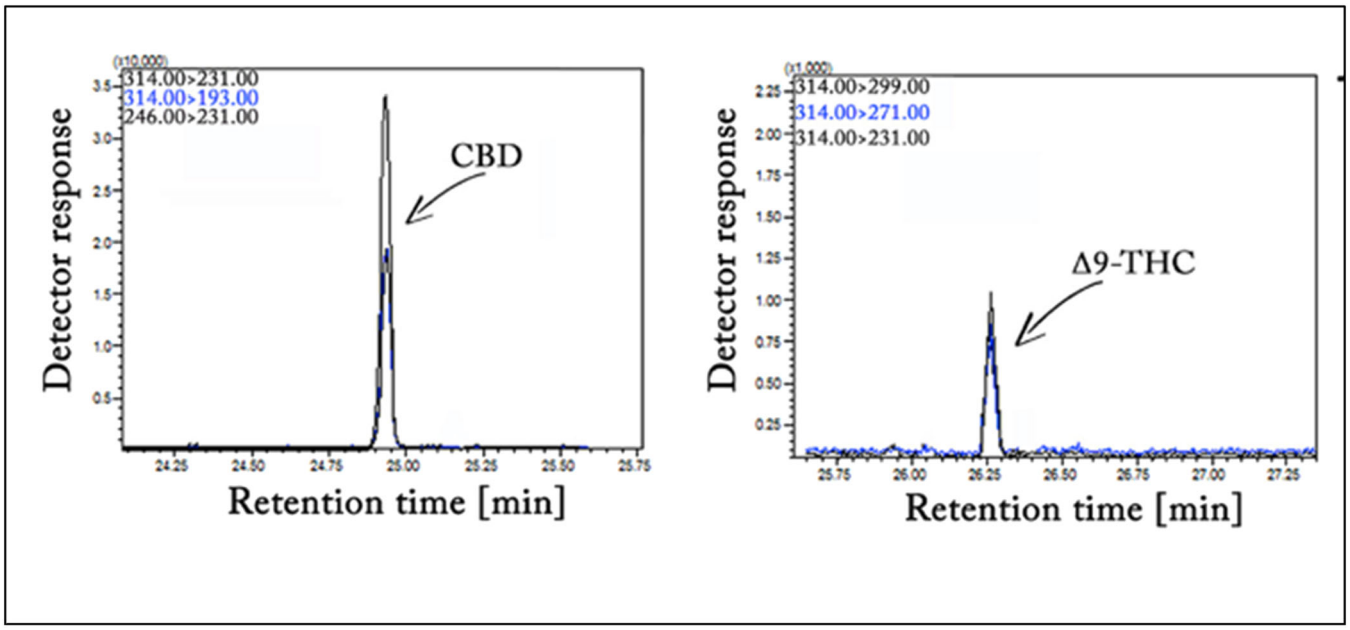

FIGURE 3 | MRM chromatograms after protein precipitation by TFA from samples of human plasma containing 50 ng/ml CBD. Three overlapping transitions for each compound were used, namely: 314 → 193, 314 → 231 and 246 → 231 for CBD and 314 → 299, 314 → 271 and 314 → 231 for THC. The figure adapted from Dybowski et al. (2020) with permission.

separation of CBD and CBC (Nahar et al. 2023). Another capillary column, which is a mid-polar one, can be used, namely Agilent DB-17ms^{LTM} as It allows for faster programming of temperature and reduces analysis time to 9.5 min (Cannarozzi 2020) instead of 30 min in case of Zebron ZB-5® columns (Hoffman et al. 2020; Dawidowicz et al. 2022). In addition to the column, the utilized detectors play a role during analysis for both identification and quantification. To evaluate the impact of the detector on the analysis of cannabinoids, Dawidowicz et al. analyzed CBG, CBD, CBC, Δ^9-THC, and CBN in human plasma sample using GC with both FID and MS (Dawidowicz et al. 2024). GC-FID and GC-MS show similar response for all studied cannabinoids, except for CBG (Dawidowicz et al. 2024). The use of MS results in a notably lower response for CBG, with one-twenty-fifth the signal of the other studied cannabinoids (Frei et al. 2022). This low response emphasizes previous observations of poor MS sensitivity to CBG (Mareck et al. 2021). The observed lower response to CBG is addressed by derivatizing it to its pyranic derivative, allowing for a LOD of 0.26 ng/mL instead of 2.78 ng/mL (Dawidowicz et al. 2024).

For quantification, multiple reaction monitoring (MRM) in GC-MS/MS is used to quantify various cannabinoids in different matrices, including human and pig plasma, human serum albumin (HSA) and egg white protein solution (Dawidowicz et al. 2024; Holowinski et al. 2022; Dybowski et al. 2020; Cannarozzi 2020). The utilization of multiple MRM transitions provides accurate and reliable identification and quantification of the analyte, reducing interference form the matrix and from other co-eluting compounds. As an example of this, 3 MRM transitions of CBD and THC of the highest intensity are presented in Figure 3: 314 →193, 314 → 231 and 246 → 231 for CBD and 314 → 299, 314 → 271 and 314 → 231 for THC (Dybowski et al. 2020).

GC-MS is widely applied in detecting cannabinoids like CBD, THC, CBN, 11-OH-THC, and THC-COOH in plasma and serum, revealing distinct distribution patterns across these matrices (Cannarozzi 2020). THC-COOH consistently appears in both blood and serum, while other cannabinoids are more readily detected in blood (Cannarozzi 2020). In forensic cases, GC-MS has been used to analyze THC and its metabolite THC-COOH in postmortem blood samples from accident victims. THC and its metabolites were found in three cases as the only detected substance, though toxicology findings alone were inadequate to conclude causation of death (Hoffman et al. 2020). These findings demonstrate the critical role of GC-MS in studying cannabinoid distribution and supporting forensic interpretations.

Additionally, GC-MS can be conducted in tandem with LC-MS. For example, targeted GC-MS and untargeted LC-MS analysis is applied to compare phytocannabinoids and terpenoids in *Cannabis sativa* plants, showing significant differences in the cannabinoid profiles of the two cultivars of cannabis grown indoors under artificial lights using artificial growth mediaversus to those grown outdoors in natural environments (Zandkarimi et al. 2023). Indoor plants have greater oxidized and degraded cannabinoids, which may have adverse or unknown biological effects. On the other hand, outdoor-grown samples have more primary phytocannabinoids (Zandkarimi et al. 2023). The comprehensive understanding of the composition of secondary metabolites, such as cannabinoids and terpenes grown in different environmental conditions, is crucial for the advancing agricultural practices and predicting biological effects upon consumption of cannabis.

3.2 | GC-MS for Whole Blood

Similar to serum and plasma, extraction of cannabinoids from whole blood for their analysis with GC-MS can be done utilizing extraction methods such as Liquid-liquid extraction (LLE), protein precipitation or SPE (Dawidowicz et al. 2022; Frei et al. 2022). In fact, QuEChERS was compared to LLE for the isolation of THC and its metabolites, 11-OH-THC and THC-COOH, from human whole blood (Dawidowicz et al. 2022). When QuEChERS is used, the approach includes a clean-up by dispersive solid-phase extraction (D-SPE) with a C18 sorbent (Dawidowicz et al. 2022). Subsequently, derivatization by silylation is done before the analysis. On the other hand, the LLE procedure requires extraction by n-hexane/ethyl acetate (9:1, v/v) followed by centrifugation and derivatization by silylation of the organic phase, which contains target cannabinoids. The results from both extraction methods were statistically compared by analysis of variance (ANOVA), showing that the QuEChERS method, in this case, has better extraction efficiency than LLE (Dawidowicz et al. 2022). The higher efficacy of QuEChERS could be attributed to the several steps in the extraction procedure that reduces matrix effects while increasing the recovery of phytocannabinoids. In another investigation, to extract THC, its metabolite 11-OH-THC, CBD and CBN, automated C18 SPE cartridge is used (Frei et al. 2022). The addition of acetic acid (0.1 M, 0.5 M, and 1.0 M) results in equal signal intensity of THC, 11-OH-THC, CBN, and CBD, while all acidic solutions increase the peak intensity of THC-COOH 10-fold in comparison with pure water (Frei et al. 2022). A comprehensive summary of published GC-MS methodologies employed for the analysis of phytocannabinoid in vascular matrices, detailing the chromatographic and MS conditions utilized in these studies, is presented in Table S1, Supporting Information.

In sum, despite its wide application for the analysis of phytocannabinoids in vascular matrices, GC-MS necessitates the derivatization of non-volatile phytocannabinoids to volatile derivatives with sufficient thermal stability. Otherwise, phytocannabinoids will undergo degradation or transformation to other derivatives, which can negatively impact the results. Although derivatization improves peak shape and resolution, the derivatization process is time-consuming and requires high temperatures. These limitations of GC-MS are not encountered when using LC-MS, which can analyze both volatile and non-volatile compounds without the need for derivatization.

4 | Liquid Chromatography-Mass Spectrometry (LC-MS)

LC-MS is widely utilized to identify and quantify cannabinoids in different biological matrices, including plasma and whole blood (Sempio et al. 2022; Manca et al. 2022; Sallam et al 2023; Reisdorph et al. 2024; Pigliasco et al. 2020; Chan-Hosokawa et al. 2022). In addition to not requiring derivatization, separation in LC occurs at mild temperatures (usually at room temperature). Thus, the analyte integrity is preserved, making LC-MS an ideal technique for the analysis of thermally labile analytes. Applications of MS for the analysis of phytocannabinoids are discussed below based on the type of the matrix.

4.1 | Plasma/Serum

LC-MS/MS has been applied for different purposes when studying cannabinoids in plasma. For example, LC- ESI-MS/MS in positive ionization was applied to study sex differences impacting the pharmacokinetics and behavioral effects of orally administered cannabis in mice (Sallam et al. 2023). In this study, THC, 11-OH-THC, and THC-COOH were analyzed in plasma, adipose, and brain samples. The findings indicate that female mice exhibited higher plasma levels of THC (C_{max}: 27.0 ng/mL) and 11-OH-THC (C_{max}: 24.3 ng/mL), comparing to male mice plasma level of THC (C_{max}: 12.6 ng/mL) and 11-OH-THC (C_{max}: 1.9 ng/mL). The higher levels in female mice were associated with stronger physiological and behavioral responses. Although THC and its metabolites existed in higher levels in brain and adipose tissues in males, female mice were more sensitive to the physiological effects of THC, indicating variation between sexes in THC pharmacokinetics and dynamics (Sallam et al. 2023). The various factors that may impact LC-MS analysis in plasma are sample preparations, chromatographic conditions and ionization in the MS instrument.

4.1.1 | Sample Preparation and Chromatographic Conditions

Various extraction techniques can be used for LC-MS with protein precipitation being one approach that is commonly applied due to its simplicity and the ability to isolate all examined cannabinoids (Manca et al. 2022; Sallam et al. 2023; Reisdorph et al. 2024). Protein precipitation, followed by online extraction via loading the samples onto a C8 analytical guard column, was utilized to extract 17 cannabinoids and their metabolites, including THC and CBD, from human plasma (Sempio et al. 2022). The eluted analytes were eluted into the analytical column to be to screened and quantified using LC-MS method According to the authors, protein precipitation requires only 20 min, which is less than SPE by around 10 min, and it has the potential to isolate all target cannabinoids and their metabolites (Sempio et al. 2022). Another study compared three extraction protocols, namely LLE, SPE and protein precipitation, followed by cleanup with a Phree™ column

(Mohamed et al. 2021). Both LLE (using hexane/acetate (8:2, v/v)) and hydrophilic-lipophilic balance (HLB) SPE extraction provided the highest extraction efficiency, with recovery ranging from 60.4% to 85.4% for both methods for the target phytocannabinoids, CBN, CBD, THC-COOH, 11-OH-THC and THC (Mohamed et al. 2021). LLE was preferred by many studies due to its simplicity, short time and cost-effectiveness (Mohamed et al. 2021; Joye et al. 2020; DeGregorio et al. 2020; Dumbraveanu et al. 2023). In addition to conventional LLE, a simplified mini-QuEChERS was used, namely salting-out assisted liquid-liquid extraction (SALLE) that involves using a high concentration of water-soluble salts namely, magnesium sulfate ($MgSO_4$), sodium chloride (NaCl) and sodium citrate dihydrate in the salting-out mixture. Adding the salts facilitates clear phase separation of the non-aqueous phase (i.e., ACN) that contains the target cannabinoids from the aqueous phase. On the other hand, hydrophilic compounds, such as proteins stay in the aqueous phase (da Silva et al. 2020). The method is successfully utilized to extract THC, 11-OH-THC, THC-COOH, CBN, and CBD from plasma, with extraction yields of 88.7%–97.3% (da Silva et al. 2020). Processing time with SALLE is 15 min, due to the absence of a solvent evaporation step (da Silva et al. 2020). Also, SALLE is a green method, using fewer organic solvents, compared to protein precipitation, SPE and LLE (Zhang et al. 2009). In sum, different extraction techniques have been employed successfully to clean up the sample and improve the analytical results, while at the same time being an effective eco-friendly (da Silva et al. 2020).

Another factor for effective analysis of phytocannabinoids is the chromatographic conditions, including the type of the stationary and mobile phases. To analyze cannabinoids using LC-MS in plasma and other vascular matrices, reverse-phase chromatography, typically C18 columns, are commonly used in many studies (Manca et al. 2022; Mohamed et al. 2021; Dumbraveanu et al. 2023; da Silva et al. 2020). These columns have strong hydrophobic properties, providing excellent retention and separation for a broad range of non-polar cannabinoids based on their variable hydrophobicities (Manca et al. 2022; Mohamed et al. 2021; Dumbraveanu et al. 2023; da Silva et al. 2020). Furthermore, C18 columns are highly compatible with the mobile phases that are used during the analysis of phytocannabinoids, such as methanol, ACN and water, resulting in optimal separation efficiency and resolution (Manca et al. 2022; Mohamed et al. 2021; Dumbraveanu et al. 2023; da Silva et al. 2020).

Another important variable that will impact separation is the composition of the mobile phases. The commonly used mobile phase consists of gradient elution of phase A (polar solvent, such as water with 0.1% formic acid) and Phase B (nonpolar solvents, such as acetonitrile and/or methanol with 0.1% formic acid) (Sempio et al. 2022; Manca et al. 2022; Sallam et al. 2023; Dumbraveanu et al. 2023; da Silva et al. 2020; Krämer et al. 2021; Pichini et al. 2020). Gradient elution helps to effectively separate mixtures of phytocannabinoids with different polarities (Sempio et al. 2022; Manca et al. 2022; Sallam et al. 2023; Dumbraveanu et al. 2023; da Silva et al. 2020; Krämer et al. 2021; Pichini et al. 2020). Although gradient systems are widely used, simple isocratic elution has been reported with a mobile phase composition of 0.2% acetic acid in water/ACN (35:65, v/v) (Mohamed et al. 2021), allowing for the separation of CBD and THC at retention times of 3.43 and 6.90 min, respectively (Mohamed et al. 2021). Separation of THC and CBD is important for their differentiation because they are structural isomers producing the same product ions during MS/MS (Mohamed et al. 2021; Cai et al. 2024). The use of additives, such as formic acid during LC-MS analysis maintained pH stability and results in a better peak shape, improving the resolution during chromatographic separations (Citti et al. 2016). Optimization of chromatographic parameters will not only separate structural isomers, such as THC and CBD (Manca et al. 2022; Reisdorph et al. 2024; da Silva et al. 2020; Pichini et al. 2020), but also separate positional isomers such as Δ^8-THC versus Δ^9-THC, which can be helpful for clinical applications (Manca et al. 2022; Reisdorph et al. 2024; da Silva et al. 2020; Pichini et al. 2020). As an example, optimal separation of Δ^8-THC and Δ^9-THC in human plasma was achieved with retention times of 6.1 and 6.75 min for Δ^8-THC versus Δ^9-THC by utilizing protein precipitation with ACN followed by UPLC-ESI-MS/MS in the positive ion mode using C18 column, with a mobile phase of 0.1% v/v formic acid in water and 0.1% v/v formic acid in ACN (Manca et al. 2022).

4.1.2 | Mass Spectrometric Parameters, Ionization

Several ionization methods have been used for the analysis of phytocannabinoids, including electrospray Ionization (ESI), atmospheric pressure chemical ionization (APCI) and matrix-assisted laser desorption ionization (MALDI). The most used ionization source is ESI (Manca et al. 2022; Sallam et al. 2023; Reisdorph et al. 2024; Mohamed et al. 2021; Dumbraveanu et al. 2023; da Silva et al. 2020; Krämer et al. 2021; Pichini et al. 2020) that can ionize phytocannabinoids by the protonation or deprotonation at the hydroxyl group in the positive or negative ion modes, respectively. APCI is another ionization technique that was also used successfully to analyze cannabinoids (Sempio et al. 2022) and despite its lower sensitivity in comparison to ESI, it has the advantage of reducing matrix effects. In fact, when APCI in positive ion mode is combined with online extraction process the background noise is decreased by avoiding ionization of non-volatile compounds, such as phosphatidylcholine lipids in the matrix, without the need to increase the HPLC run time (Klawitter et al. 2017). The utilization of LC-MS/MS equipped with APCI could effectively separate major metabolites, namely the hydroxylated forms of CBD (6α-OH-CBD, 6β-OH-CBD, and 7-OH-CBD), and identify CBD-glucuronide (CBD-gluc) in plasma (Sempio et al. 2022). While most studies have utilized either APCI or ESI, MALDI coupled with MS is also tested for the analysis of phytocannabinoids, albeit in hair samples (Beasley et al. 2016) rather than vascular matrices. Unlike APCI and ESI, MALDI requires the use of a matrix, such as 2,5-dihydroxybenzoic acid (DHB) and α-cyano-4-hydroxycinnamic acid (CHCA) that is mixed with the cannabinoid sample before analysis (Lewis et al. 2000). All reported methods used CHCA because it does not interfere with the analysis of cannabinoids (Kernalléguen et al. 2023). The LOD achieved by a MALDI-MS screening method was 0.1 ng/mg for both THC and CBD (Kernalléguen et al. 2023). To the best of our knowledge MALDI-MS has not been used for the analysis of phytocannabinoids in vascular matrices, but its successful use in hair samples indicates the possibility of using

the technique in other biological samples. MALDI-MS is, however, a key technology for imaging MS (i.e., MALDI-IMS) and has been used to study the distribution of cannabinoids in hair samples or herb mixtures (Kernalléguen et al. 2023; Kuwayama et al. 2014). IMS has the potential to complement analytical data obtained from vascular matrices allowing for comprehensive evaluation of cannabinoids biodistribution in a biological system. Overall, ESI provides high sensitivity and is the most commonly used ionization source in cannabinoid research, while APCI minimizes matrix effects with less sensitivity than ESI. The choice of the ionization source should be determined by the requirement of the study.

4.2 | Whole Blood

Whole blood is widely used in bioanalytical research of cannabinoids because it contains cannabinoids from both the cellular component and the plasma. However, collecting blood samples is an invasive procedure that requires skilled technicians (Mercolini et al. 2013). To collect blood with less invasiveness, volumetric absorptive micro-sampling (VAMS) has been used. VAMS only requires 30 μL of sample, followed by online SPE-LC-MS/MS to simultaneously determine THC and CBD levels (Pigliasco et al. 2020). The approach is a less invasive compared to traditional venous blood sampling for TDM of medical cannabis. Moreover, the VAMS device allows the collection of accurate volumes (Pigliasco et al. 2020). The VAMS-LC-MS/MS method is a valid alternative to TDM for patients treated with Epidiolex®, particularly pediatrics patients and/or newborns (Pigliasco et al. 2020). Although VAMS offers convenience and minimal invasiveness, it presents challenges related to the small nature of sample volumes, as the tip of the VAMS device absorbs a fixed volume of 10, 20, or 30 μL. Analysis of such small volumes requires high sensitivity to ensure accurate and reliable results. After the collection of blood samples, different extraction methods are used to extract phytocannabinoids from the complex blood matrix, such as to LLE and SPE, QuEChERS (Chan-Hosokawa et al. 2022; Joye et al. 2020; DeGregorio et al. 2020; ANSI/ASB 2019; Marín-San román et al. 2020; Ferrari Júnior and Caldas 2022; Orfanidis et al. 2021). Similar to the analysis of cannabinoids in plasma using LC-MS (Section 3.1) the same chromatographic conditions are used to analyze cannabinoids in whole blood (Pigliasco et al. 2020; Chan-Hosokawa et al. 2022; Reber et al. 2022; Joye et al. 2020; DeGregorio et al. 2020; Ferrari Júnior and Caldas 2022; Orfanidis et al. 2021; Hubbard et al. 2020). Although these chromatographic parameters could effectively separate and analyze phytocannabinoids, the presence of cannabinoid isomers still represents a challenge. The presence of isomers such as Δ^8-THC and Δ^8-THC-COOH overlaps with the peaks of Δ^9-THC and Δ^9-THC-COOH cannabinoids, impacting both identification and quantification (Kinghorn et al. 2017). Therefore, separation of these isomers is crucial for the selective analysis of cannabinoids in blood samples and all other matrices. An SPE-LC-MS/MS method was developed to quantify Δ^8-THC, Δ^9-THC, Δ^8-THC-COOH, and Δ^9-THC-COOH in blood and urine samples (Reber et al. 2022). Chromatographic separation of these isomers is achieved by optimizing the chromatographic para meters using a multi-step gradient. The gradient program involves a sudden increase in organic mobile solvents to prevent excessive peak broadening and to maintain a relatively short run time. The higher organic mobile phase (95%) is held for an extended time to wash the column of interfering phospholipids. The use of such an approach ensures effective separation with an acceptable run time of 10 min (Figure 4) (Reber et al. 2022). The method achieves baseline separation of Δ^8-THC and Δ^9-THC and offer quantitative data for Δ^8-THC and Δ^9-THC, with sensitivity of 1 ng/mL (Reber et al. 2022). A similar LC-MS/MS method was developed to separate Δ^8-THC and Δ^9-THC

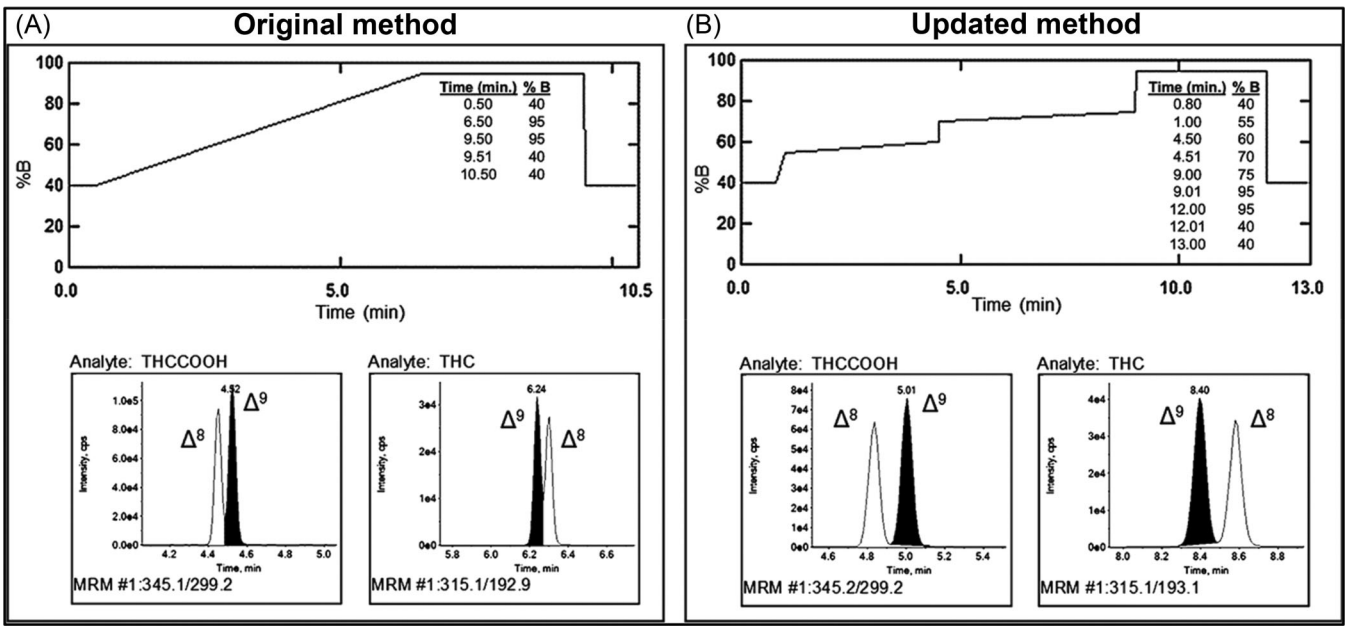

FIGURE 4 | Chromatography gradient settings with profiles and results. Extracted ion chromatograms showing improvements in resolving isomeric peaks when comparing the original laboratory method to the updated method, using mobile phases of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The figure adapted from an article that is not entitled to domestic copyright protection under U.S. law and is therefore in the public domain (Reber et al. 2022).

isomers and their metabolites in blood samples (Chan-Hosokawa et al. 2022). It was achieved in a 10 min run time and using a 100 mm C18 column instead of the initial use of a 50 mm C18 column with 5.5 min runtime. By employing a longer run time and a longer C18 column, Δ^9-THC (retention time = 8.27 min) and Δ^8-THC (retention time = 8.55 min) demonstrated complete baseline separation. Chromatographic separation of Δ^8-THC-COOH (retention time = 5.49 min) from Δ^9-THC-COOH (retention time = 5.71 min) was also attained (Chan-Hosokawa et al. 2022). Overall, the optimized chromatographic parameters were developed to separate different cannabinoids from their isomers to be identified and quantified.

In most LC-MS methods for whole blood analysis, the ionization source was ESI; however, APCI was also used. An LC-MS/MS method with APCI in the MRM mode was used to quantify THC and CBD. After online SPE extraction of the human blood sample, the APCI-MRM-QqQ instrument in positive ion mode quantified THC and CBD using the same MRM transition $[M+H]^+$ 315.2 → 193.1 along with their deuterated IS $[M+H]^+$ 318.2 → 196.1 m/z, respectively (Pigliasco et al. 2020). The retention times were 3.43 and 6.90 min for CBD and THC, respectively. The linearity range of THC and CBD is 1–100 ng/mL, with a run time of 7.5 min (Pigliasco et al. 2020). Other MRM transitions of different cannabinoids are shown in Table 1.

LC-HRMS with an Orbitrap-based instrument was used in the parallel reaction monitoring (PRM) mode as another targeted quantification technique to determine cannabinoids in human blood sample (Joye et al. 2020). The difference between MRM and PMR is that the former operates with each precursor-to-product ion transition is monitored sequentially, limiting the monitoring time for each transition. In contrast, PRM monitors all product ions of a selected precursor ion simultaneously using a high-resolution instrument, allowing for longer monitoring of the ions, improving sensitivity for low-abundance analytes (Joye et al. 2020; Rauniyar 2015). The method was developed to analyze THC, 11-OH-THC, THC-COOH and CBD utilizing LLE in acidic condition and LC-HRMS in PRM mode in a polarity switching setting with a negative polarity for 11-OH-THC and THC-COOH (Joye et al. 2020). The assay was linear in the concentration range of 0.4–20 ng/mL for THC, CBD and 11-OH-THC and of 2–100 ng/mL for THC-COOH. PRM-targeted methods showed a similar performance in terms of linearity, precision, and repeatability as MRM. Orbitrap-based PRM offered high specificity as the high-resolution nature of the instrument separated background ions from targeted molecules (Joye et al. 2020). The method was applied to 187 blood samples, and the results were compared with previously published MRM-based method, showing a strong correlation between PRM and MRM results (Fabritius et al. 2013), with correlation coefficients above 0.993 for all measured cannabinoids (Joye et al. 2020). Overall, LC-MS can be utilized in different modes for comprehensive analysis of phytocannabinoids in whole blood samples. A comprehensive summary of LC-MS methodologies used for the analysis of phytocannabinoids in vascular matrices is shown in Table S2, Supporting Information.

Since the cannabinoid levels in blood and plasma is low in the (ng/mL range; Huestis 2007), highly sensitive LC-MS/MS method is crucial for pharmacokinetics studies and phytocannabinoid research. The utilization of mobile phase additives, such as ammonium fluoride can enhance the sensitivity of LC-MS/MS to detect the very low concentrations of cannabinoids and their metabolites in blood and plasma. Ammonium formate is a mobile phase additive that has been utilized to enhance the sensitivity more than 10 folds of several compounds in previous studies (McFadden and Ames 2023; Takkis et al. 2017).

TABLE 1 | MRM transitions of common cannabinoids.

Analyte	Parent MRM [*m/z*]	Ion quantifier MRM [*m/z*]	Ion qualifier MRM [*m/z*]	Cone voltage [V]	Collision energy [V]
CBN	311	293	223	−25	−18
CBD	315	193	259	25	20
Δ^9 THC	315	193	259	25	20
Δ^8 THC	315	193	259	20	20
11-OH- THC	331	313	175	30	12
THC-COOH	345	299	327	25	15
THCA	357	313	245	−30	−30
CBDA	357	313	245	−36	−12
THC-COOH-glu	519	343	113	−25	−22
7-OH-CBD	331	175	201	25	12
THC-D3	318	196	262	36	20
CBD-D3	318	196	262	64	22
11-OH-THC-D3	334	316	178	35	25
THC-COOH-D3	348	302	330	25	30
THC-COOH-glu-D3	522	346	116	−26	−12

Note: The analytes detected in negative ionization presents negative. Adapted from Manca et al. (2022) with permission.

4.3 | Dried Blood Spots

DBS have many advantages in comparison to other vascular matrices, namely its convenience in collection, inexpensive nature, stability, and the easiness of transportation (EROL ÖZTÜRK 2023). To collect a sample, a few 100 microliters of capillary blood are taken by pricking the finger, heel, or toe with a lancet and are applied to a DBS filter paper. The paper is then dried overnight at room temperature (EROL ÖZTÜRK 2023). After drying, the DBS is cut out, and a mixture of methanol or ACN and deuterated internal standard in methanol is added (Meikopoulos et al. 2024; EROL ÖZTÜRK 2023; Protti et al. 2017). The recovery ranged from 84.6% to 106% for THC, CBN and CBD, 52%–63% for THC, and 87%–91% for THC-COOH (Meikopoulos et al. 2024; EROL ÖZTÜRK 2023). The variation in recovery is due to sample preparation methods or differences in the type of the filter paper, which can affect the homogeneity and extraction of targeted analytes.

In general, cannabinoids are analyzed using the same LC-MS/MS approaches discussed in the Section, 3.1 and 3.2 (Meikopoulos et al. 2024; EROL ÖZTÜRK 2023; Protti et al. 2017) DBS quantitative studies showed linearity ranges of 0.1–50 ng/mL for THC and THC-COOH and 0.1–100 for THC, with coefficient of determinations of at least 0.995 (EROL ÖZTÜRK 2023; Protti et al. 2017). To quantify cannabinoids using a DBS sample, LC-ESI-MS/MS in both positive and negative ionization is usually employed (Meikopoulos et al. 2024; EROL ÖZTÜRK 2023;

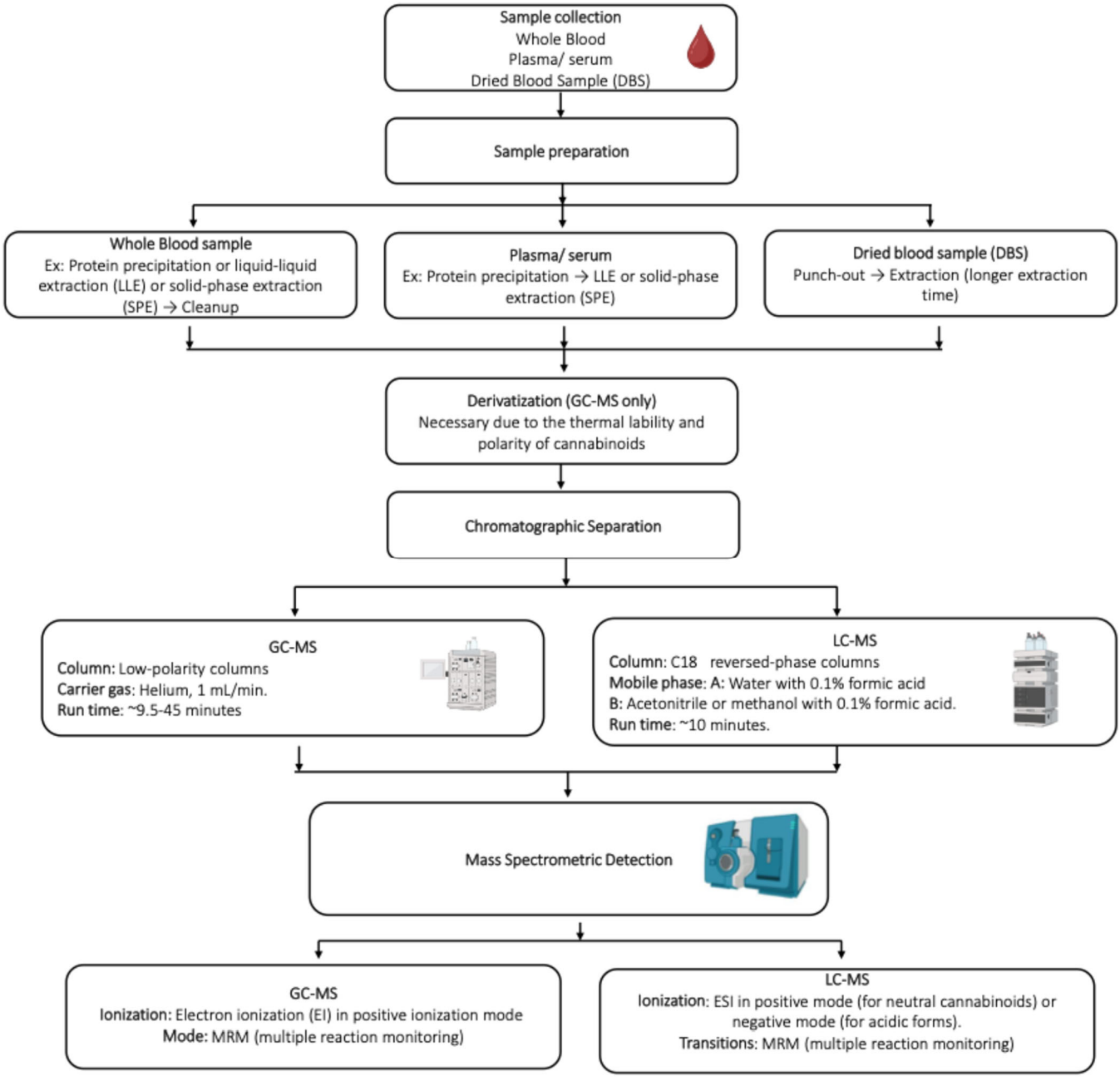

FIGURE 5 | A diagram illustrating the analytical workflow of common conditions and parameters for the analysis of phytocannabinoid using GC-MS and LC-MS in blood, plasma, and DBS matrices.

Protti et al. 2017). When cannabinoids levels were determined by DBS in 103 human samples, the results are found to be in agreement with whole blood quantification (EROL ÖZTÜRK 2023). In the study, all samples were positively identified in both whole blood sample and DBS samples without any false negatives (EROL ÖZTÜRK 2023). Thus, DBS provides a great promise for future applications in drug screening for both toxicological and forensic purposes due to the simplicity of sample collection (Meikopoulos et al. 2024).

Despite being accurate and patient friendly compared to venepuncture, DBS has limitations and needs highly sensitive instrumentation and methodologies. Sample volume is limited in DBS to merely 10–100 μL that can be insufficient for comprehensive analysis. Stability of phytocannabinoids should also be considered during the drying pre-analytical procedure and during storage of in a DBS matrix. Although THC, CBN, CBD show stability over 5 days under three different storage conditions, including room temperature (Meikopoulos et al. 2024), other factors may affect stability, such as light exposure and storage time. Another challenge during the analysis of phytocannabinoids in DBS using LC-MS/MS is hematocrit effect, which is the most significant parameters affecting spot characteristics, including drying time, homogeneity and diffusion; all of which may affect the reproducibility of the analytical method (Zakaria et al. 2016). In summary, DBS coupled with LC-MS/MS offers the detection and quantification of even trace amounts of cannabinoids, which is crucial in forensic and clinical applications. However, factors, such as stability of the phytocannabinoids and hematocrit effects should be monitored and considered.

Based on the above discussed methods, an analytical workflow summarizing the analysis of phytocannabinoids by GC-MS or LC-MS is shown in Figure 5. The workflow starts with sample preparation, that includes protein precipitation, LLE, or SPE, followed by chromatographic separation utilizing GC or LC with optimized conditions. The identification and/or quantification of target phytocannabinoids is then performed using different modes, including MRM-MS after the optimization of the mass spectrometric parameters. In addition, Table 2 shows a comparison summary of GC-MS and LC-MS for phytocannabinoids analysis in vascular matrices. The comparison highlights the respective advantages and limitations of each approach as well as practical considerations such as cost and throughput capabilities.

5 | Closing Remarks

The analysis of cannabinoids and their metabolites using mass spectrometry coupled with either GC or LC for vascular matrices is important due to the widespread consumption of cannabis products in many jurisdictions. GC-MS is highly effective for the separation and analysis of volatile and semi-volatile cannabinoids, offering excellent sensitivity and specificity. However, GC-

TABLE 2 | Comparative summary of GC-MS and LC-MS methods to analyze phytocannabinoids in vascular matrices.

Aspect	GC-MS	LC-MS
Suitability for various matrices	Well-established for plasma and blood; requires derivatization due to cannabinoid volatility and thermal sensitivity	Highly versatile; suitable for blood, plasma, and DBS without the need for derivatization
Sample preparation	Requires extensive sample preparation and derivatization, increasing time and potential risk for data reproducibility	Minimal preparation; direct injection after extraction, enhancing throughput
Sensitivity & specificity	High sensitivity; may be limited by derivatization efficiency and matrix interferences	High sensitivity and specificity in MRM mode; better performance in complex matrices like DBS
Matrix effects	Moderate; derivatization can reduce interferences, but ion suppression can persist	Significant matrix effects are possible, especially in whole blood and DBS; mitigated by internal standards
Total time of analysis	Higher mainly due to longer preparation times (9.5–45 min)	Lower with faster analysis run time (5–15 min) and simpler preparation
Cost & instrument availability	Generally lower instrument cost; derivatization reagents increase consumable cost	Higher instrument cost; widely used in clinical labs for multiplex assays
Regulatory acceptance	Established in forensic settings; accepted in clinical labs	Increased acceptance in regulatory contexts - forensic and clinical
Common pitfalls	Variability in derivatization, thermal degradation of analytes	Ion suppression, matrix complexity, mostly gradient elution is needed, and hematocrit effect (notably in DBS)
Mitigation strategies	Standardized derivatization protocols; use of internal standards	Optimized extraction and cleanup; optimized chromatographic and spectrometric parameters; matrix-matched calibration; use of internal standards

MS requires derivatization to enhance volatility, which makes the method expensive and time-consuming. On the other hand, LC-MS does not require derivatization, making it greatly preferred for the detection and quantification of phytocannabinoids, including non-volatile and thermally labile ones. Despite analytical advantages, the development of LC-MS/MS methods for the analysis of phytocannabinoids in biological samples still needs improvements in terms of sensitivity and the ability to separate and detect isomers.

There are many challenges in the analysis of the phytocannabinoids in vascular matrices that supersede the MS part of the analysis. Efficient extraction is crucial to isolate the very low concentrations of cannabinoids from the blood. Furthermore, the separation of THC isomers, such as Δ^8-THC from Δ^9-THC, is crucial to correctly identify compounds. Ongoing advancements in sample preparation and analytical methods are important in addressing current challenges so that effective analytical methods are applied for therapeutic and toxicological purposes. Continued research and development in the field will enhance the applicability and robustness of cannabinoid analysis across various biological matrices.

For future development, emerging technologies in analytical chemistry are poised to transform cannabinoid testing in vascular matrices. For example, ambient ionization mass spectrometry, such as direct analysis in real time (DART-MS) and desorption electrospray ionization (DESI-MS) are gaining interest for their ability to directly analyze complex biological matrices with minimal sample preparation, potentially reducing analysis time and preserving sample integrity (Rankin-Turner and Heaney 2021; Shi et al. 2024). This technique may be, however, more suitable for DBS that is already a dried sample. In addition, miniature portable MS platforms are being explored for field-based cannabinoid screening; however, these instruments currently face limitations in analytical sensitivity and specificity compared to conventional LC-MS and GC-MS systems. Advances in automation and miniaturization, particularly in sample preparation (e.g., microextraction, automated SPE) could advance their future use.

Despite the above promising developments, significant gaps remain in regulatory guidelines, particularly in validation protocols tailored to cannabinoids in forensic and clinical matrices. Current standards are often adapted from general bioanalytical standards, such as FDA or EMA guidelines and do not fully address the unique challenges associated with cannabinoids particularly their legal status. Establishing matrix-specific validation criteria and certified reference materials will be essential for ensuring reliable and comparable results of cannabinoid testing methods across different labs

Author Contributions

Radwa Mahmoud: conceptualization, formal analysis, investigation, visualization, writing – original draft preparation. **Robert B. Laprairie:** conceptualization, funding acquisition, supervision, writing – review and editing. **Anas El-Aneed:** conceptualization, funding acquisition, project administration, supervision, writing – review and editing. all authors agreed to the final submitted version of the paper.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Supplementary Table 1: Methods for the identification and quantification of cannabinoids in dried blood spots whole blood, plasma, and serum using GC-MS. **Supplementary Table 2:** Methods for the identification and quantification of cannabinoids in dried blood spots whole blood, plasma, and serum using LC-MS.