Progress in the analysis of phytocannabinoids by HPLC and UPLC (or UHPLC) during 2020-2023

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Abstract

Introduction:

Organic molecules that bind to cannabinoid receptors are known as cannabinoids. These molecules possess pharmacological properties similar to those produced by *Cannabis sativa* L. High-performance liquid chromatography (HPLC) and ultra-performance liquid chromatography (UPLC, also known as ultra-high-performance liquid chromatography, UHPLC) have become the most widely used analytical tools for detection and quantification of phytocannabinoids in various matrices. HPLC and UPLC (or UHPLC) are usually coupled to an ultraviolet (UV), photodiode array (PDA) or mass spectrometric (MS) detector.

Objective:

To critically appraise the literature on the application of HPLC and UPLC (or UHPLC) methods for the analysis of phytocannabinoids published from January 2020 to December 2023.

Methodology:

An extensive literature search was conducted using Web of Science, PubMed and Google Scholar, and published materials including relevant books. In various combinations, using cannabinoid in all combinations, cannabis, hemp, hashish, *Cannabis sativa*, marijuana, analysis, HPLC, UHPLC, UPLC, quantitative, qualitative and quality control were used as the keywords for the literature search.

Results:

Several HPLC and UPLC (or UHPLC)-based methods for the analysis of phytocannabinoids were reported. While simple HPLC-UV or HPLC-PDA-based methods were common, the use of HPLC-MS, HPLC-MS/MS, UPLC (or UHPLC)-PDA, UPLC (or UHPLC)-MS and UPLC (or UHPLC)-MS/MS, was also reported. Applications of mathematical and computational models for optimization of protocols were noted. Pre-analyses included various environmentally friendly extraction protocols.

Conclusion:

During the last four years, HPLC and UPLC (or UHPLC) remained the main analytical tools for phytocannabinoid analysis in different matrices.

Keywords

Cannabis sativa; cannabinoid; phytocannabinoid; psychotropic drugs; HPLC, UPLC, UHPLC, cannabis; hashish; weed; marijuana; HPLC-MS; HPLC-PDA; HPLC-MS/MS; analysis; detection; quality control

Short abstract

HPLC and UPLC (or UHPLC) are versatile analytical tools for the analysis of phytocannabinoids. This review critically appraises the literature on the application of HPLC and UPLC (or UHPLC) methods for the analysis of phytocannabinoids published from January 2020 to December 2023. During this period, HPLC and UPLC (or UHPLC) remained the main analytical tools for phytocannabinoid analysis. Applications of mathematical and computational models for optimization of protocols were noted. Pre-analyses included various environmentally friendly extraction protocols.

1 INTRODUCTION

Organic molecules of plant origin that bind to the cannabinoid receptors (endocannabinoid system) are known as phytocannabinoids[1-6]; these molecules display similar pharmacological properties as produced by *Cannabis sativa* L. Major phytocannabinoids are presented in Figure 1. There are well over a hundred phytocannabinoids discovered to date, and Δ^9-tetrahydrocannabinol (**14**, Δ^9-THC or THC) and cannabidiol (**5**, CBD) are two main phytocannabinoids found in *C. staiva*[2]. The psychoactive property of *C. sativa* is mainly owing to the presence of Δ^9-THC (**14**), but cannabidiol (**5**) is the main compound that offers antipsychoactive properties of this plant[1-6]. Phytocannabinoids are accumulated in viscous resins produced mainly in the glandular trichomes of *C. sativa* and can be structurally classified into eight major classes[1-4]: cannabichromenes (**1**, CBC), cannabicyclols (**3**, CBCL), cannabidiols (**5**, CBD), cannabigerols (**8**, CBG), cannabinols (**10**, CBN) and tetrahydrocannabinols (**14**, THC), cannabielsoins (**20**, CBE), *iso*-tetrahydrocannabinols (**13**, Δ^8-THC) and cannabicitrans (**22**, CBT)[1-4]. Phytocannabinoids are known for their therapeutic and cosmeceutical values; cannabis is often indicated for the treatment of pain, glaucoma, nausea, depression, and neuralgia, and is used in various cosmeceutical products[1-6]. Cannabis can be of two types: medicinal cannabis and recreational cannabis. Medicinal cannabis has a higher level of CBD (>20%, **5**) than THC (~1%, **14**), and recreational cannabis possesses a higher amount of THC (>20%, **14**) than CBD (~2%, **5**). The pharmacological properties of phytocannabinoids vary considerably between cultivars[1-4,7]. It is not only *C. sativa* that produces phytocannabinoids, but there are several other plant species including *Acmella oleraceae, Echinacea angustifolia, E. purpurea, Helichrysum umbraculigerum* and *Radula marginata* that produce phytocannabinoids[1-4]. However, phytocannabinoids produced by other species than *C. sativa* are of different structural classes, e.g., alkamides.

Since the discovery of the first phytocannabinoid (cannabinol, **10**, CBN) in 1940 by Robert S. Cann and subsequently, tetrahydrocannabinol (**14**, THC) and many other phytocannabinoids, many analytical tools and methods have been applied for the detection, identification, quantification and analysis of phytocannabinoids in plants as well as in various biological matrices like human blood, urine, hair and nails, often involving pharmacokinetic studies and/or forensic analysis[1-4,7]. LC-based techniques using HPLC and UPLC (or UHPLC)[2], hyphenated with several detection methods, e.g., UV-Vis (ultraviolet-visible), PDA

(photodiode array) and MS (mass spectrometry), have become popular over the years and are preferred techniques for the analysis of phytocannabinoids in various matrices[1-4].

Since the publication of the review article[2] on the application of LC methods in the analysis of phytocannabinoids covering the period 2010-2019, there has been a large number of publications published on this topic, and this has prompted the need for the publication of another review article. Therefore, this review article critically appraises the publications on HPLC and UPLC (or UHPLC)-based analysis of phytocannabinoids related to forensic, pharmaceutical, food, pharmacokinetic and phytochemical analysis published during 2020-2023. This review also captures the advancement of computational tools, MS databases, and various advanced detection technologies for LC-based analysis of phytocannabinoids.

2 HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) AND ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY (UPLC)/ULTRA-HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (UHPLC)

High-performance liquid chromatography or high-pressure liquid chromatography (HPLC), is a popular, modern, powerful and versatile chromatographic separation technique. This analytical technique has been routinely used for the separation, identification and quantification of various compounds present in complex mixtures like an herbal extract or product, and also to obtain chemical profiles or fingerprints of crude mixtures[2,8,9]. Usually in a standard HPLC operation, a column is of 2.0-4.6 mm in diameter, and 20-250 mm in length, packed with a stationary phase, e.g., reversed-phase C_{18} silica (2-10 μm particle size). While UV-Vis and photodiode array (PDA) detectors are the two most widely used detectors in HPLC, hyphenation with more sophisticated detection techniques, *e.g.,* mass spectrometer (MS) and nuclear magnetic resonance (NMR) spectrometer, has become popular in recent years, as these hyphenations enhance detection efficiency and precision, particularly for unknown phytochemicals[8]. Mass spectrometric (MS) detection has emerged as the most efficient detection technology for the analysis of natural products, e.g., phytocannabinoids, as it offers useful structural information for unambiguous identification of compounds.

For MS detection, the most common ionization technique used in HPLC-MS for phytochemical analysis is a soft ionization technique like electrospray ionization mass spectrometry (ESI-MS) that reveals the molecular ion species with only a few fragment ions[8,9].

Tandem mass spectrometry (MS/MS) provides fragments through collision-induced dissociation of the molecular ions, and the use of LC-MS/MS has considerably increased over the years for the detection of phytochemicals in different matrices[10]. NMR, albeit the least sensitive of all detection techniques, is coupled with an HPLC to obtain detailed structural information that cannot be obtained by any other detection technologies[2,8,9]. There are also other detectors that can be hyphenated to an LC system, and the examples could include evaporative light scattering detector (ELSD), infrared (IR) detector, electrochemical detector, and fluorescent detector[2].

Aiming at the improvement of separation efficiency and shorter run time in an HPLC operation, UPLC was introduced in 2004 by Waters based on sub 2-micron porous particles[2]. This advanced liquid chromatographic technique could offer a remarkably short analysis time and require a small amount of solvent(s) as a mobile phase[11]. A much better separation efficiency and resolution of analyte mixtures could also be achieved by UPLC. As UPLS is a trademark registered by Waters, similar advanced LC systems using sub 2-micron particle size and smaller columns that were introduced by other manufactures could not be called UPLC, and therefore, the term UHPLC emerged. So, UHPLC and UPLC are essentially the same techniques, and should never be confused as different techniques[2]. In fact, UPLC and UHPLC are synonyms.

One should remember that the fundamental characteristic feature of UHPLC and UPLC instruments is the sub 2-micron particles as opposed to particle size between 2.0-10 microns in conventional HPLC systems[2]. As the column particles are quite small (<2 μm), they generate a significantly higher back pressure, and consequently, UHPLC or UPLC systems must be able to cope with a pressure above 6,000 psi, which may be the upper limit of classical HPLCs. UPLC (or UHPLC) could adequately address analytical challenges linked to separation, detection and quantification of secondary metabolites from various matrices more quickly than was previously possible. It can be noted that in a UPLC (or UHPLC), the run time could be up to three and nine fold shorter than that of conventional HPLC systems using 3 and 5 μm columns, respectively[2]. Owing to several advantages of UPLC (or UHPLC) over conventional HPLC, UPLC (or UHPLC) has been increasingly becoming a routine technique for chemical, biomedical and pharmaceutical analysis as well as for the analysis of phytochemicals including phytocannabinoids. However, despite a series of advantages, the major disadvantage of UPLC

(or UHPLC) is the higher back pressure compared to conventional HPLC, which decreases the life of the columns, and the particles of less than 2 µm cannot be regenerated and, therefore, have a limited use[2]. The detection methods that can be hyphenated with a UPLC (or UHPLC) system are similar to those of HPLC methods as outlined above. However, because of the richness of structural information that one could have from MS data, UPLC (or UHPLC) is often coupled with an MS detector.

In the following sections/subsections various specific HPLC and UPLC (or UHPLC)-based analytical methods for the analysis of phytocannabinoids in various matrices published during 2020-2023 are discussed.

3 HPLC and UPLC (or UHPLC) ANALYSIS OF PHYTOCANNABINOIDS

Since the publication of the review article[2] on the application of HPLC and UPLC (or UHPLC) methods in the analysis of phytocannabinoids covering the period 2010-2019, several publications have appeared in the literature on this topic[12-185]. All these publications highlight the importance of HPLC and UPLC (or UHPLC) techniques as well as showcase remarkable advancements in sensitivity and versatility associated with these techniques in relation to phytocannabinoid analysis[12]. While the use of conventional HPLC methods still dominate this area of analysis, a steady increase in the methods using UPLC (or UHPLC) in the analysis of phytocannabinoids in *C. sativa* L. plant parts, commercially available cannabis products, and in forensic samples of human origins. Publications during the period covered by this review article also demonstrate that despite the availability of several detection technologies that could be hyphenated with an HPLC or a UPLC (or UHPLC) system, the PDA and the MS/MS technologies are still the two most popular detection methods for phytocannabinoid analysis. The introduction and application of different mathematical and computation modelling methods as well as chemometric tools have enhanced the quality of HPLC and UPLC (or UHPLC)-based analysis of phytocannabinoids, while the impact of artificial intelligence and robotics in HPLC and UPLC (or UHPLC) has begun to emerge.

There are different types of columns available for HPLC and UPLC (or UHPLC) analysis, but reversed-phase C_{18} packed columns are still the most popular choice, with some use of C_8 or phenyl columns, for phytocannabinoid analysis. Like the previous years, acetonitrile (ACN), methanol (MeOH) and water, with small percentage, usually 0.1%, of formic acid (HCOOH) or

acetic acid (CH_3COOH), or various formate or acetate buffers, most often form the mobile phase, running with a flow rate ranged from 0.2-1.5 mL/min, depending on the use of HPLC or UPLC (or UHPLC). The applications of HPLC and UPLC (or UHPLC)-based methods in the analysis of phytocannabinoids are discussed in the following subsections under different classes of matrices.

3.1 HPLC and UPLC (or UHPLC) analysis of cannabinoids in *Cannabis sativa* L. plant samples and cannabis consumer products

A large volume of HPLC and UPLC (or UHPLC) methods that have been reported for the analysis of cannabinoids in *C. sativa* L. plant samples[13-94] and cannabis consumer products, e.g., hashish, marijuana and cannabis oils[95-137], since 2020 are presented in Tables 1 and 2, and discussed in the following subsections.

3.1.1 *Cannabis sativa* L. plant samples

Both HPLC and UPLC (or UHPLC) methods have been applied for the separation, identification and quantitation of cannabinoids in *C. sativa* L. samples, mainly including aerial parts, biomass, flowers, inflorescences and leaves[13-93] (Table 1). However, simple HPLC-based methods have remained more dominant than UPLC (or UHPLC)-based methods for the analysis of *C. sativa* plant samples during 2020-23. While UV-Vis detectors (usually set at 210-220 nm) including photodiode array detector (PDA) or simply diode array detectors (DAD) are extensively used in plant analysis for phytocannabinoids, the use of various MS detectors has increased in recent years. Various Agilent, Dionex (Thermofisher), Waters and Shimadzu LC-systems have appeared as the most popular choice among the researchers. However, there were a couple of reports on the use of a Jasco LC system in the analysis of phytocannabinoids. It has also been noted that instead of conventional 5 μm particle size, much smaller particle sizes (<5 μm) have been used in HPLC operations. Some of the methods in the analysis of plant samples, despite being termed as UPLC or UHPLC methods, were strictly HPLC methods as the particle size of the column used in those experiments were not sub-2 μm. A classic example could be the work published by Deidda et al.[13], where they used a Poroshell 120 EC-C_{18} column (150 mm × 2.1 mm, 2.7 μm) coupled with a Poroshell 120 EC-C_{18} guard column (5 mm × 2.1 mm, 2.7 μm) to detect CBC (**1**), CBD (**5**), CBDA (**6**), CBG (**8**), CBGA (**11**), CBN (**10**), Δ^8-THC (**13**), Δ^9-THC (**14**) and Δ^9-THCA (**15**) in the aerial parts of *C. sativa*, but the separation

was claimed as a UHPLC separation. The confusion might have come from the fact that they performed the analysis on an Acquity UPLC H-class system coupled with a PDA detector. The main objective of their study was to compare the HPLC method with a less frequently used UHPSFC (ultra-high-performance supercritical-fluid chromatography) method for the analysis of phytocannabinoids. Recently, an Agilent 1200 series HPLC coupled with DAD and Poroshell column 120 SB-C_{18} (150 mm x 4.6 mm, 2.7 μm), eluted with a mobile phase gradient comprising 0.1% formic acid (HCOOH) in water and acetonitrile (ACN), have been used for the straightforward detection of CBD (**5**), CBG (**8**), CBN (**10**), Δ^9-THC (**14**) and Δ^9-THCA (**15**) in the aerial parts[14] (Table 1). There have been several other HPLC-based analyses of cannabinoids in the aerial parts of this plant, either using a DAD or an MS detector or in combination of both, reported in the literature[15-23] (Table 1). In all those studies[15-22], the column length, diameter and particle size ranged, respectively, 100-250 mm, 2.1-4.6 mm and 2.7-5 μm. Several acidic phytocannabinoids, *e.g.,* CBCA (**2**), CBDA (**6**), CBGA (**9**), and CBNA (**11**), were identified in the aerial parts using a Shimadzu Nexera ×2 UHPLC system coupled with an SPD-M20A PDA in series with a Shimadzu LCMS8040 triple quadrupole system with an ESI source employing an Ascentis Express RP-C_{18} column (150 mm × 2.1 mm, 2.7 μm) and a simple water-ACN gradient (both containing 0.1% HCOOH)[16]. Treyer et al.[18] have recently reported a similar HPLC method, but incorporating multiple detectors, e.g., PDA, ELSD (evaporative light scattering detector) and ESI-MS, for the detection of CBC (**1**), CBD (**5**), CBDA (**6**), CBG (**8**), Δ^9-THC (**14**) and Δ^9-THCA (**15**). A simple isocratic HPLC method using 80% ACN in 25 mM ammonium formate solution at a flow rate of 1 mL/min and a total run time of 15 min afforded separation and identification of CBC (**1**), CBD (**5**), CBDA (**6**), CBG (**8**), CBGA (**9**), Δ^9 -THC (**14**) and Δ^9-THCA (**15**)[22]. A Luna C_{18} column (150 mm × 4.6 mm, 5 μm) and PDA detection at 228 nm were employed in this study. The aerial parts from the *in vitro* cultivated *C. sativa* plant have recently been analysed by an HPLC-DAD method, where a Phenomenex Kinetex C_{18} column (100 mm × 2.1 mm, 5 μm) was used employing a gradient elution with a mobile phase comprising 0.1% HCOOH in water and MeOH[23]. This method was found to be simple, efficient and cost-effective, and could successfully separate and identify CBD (**5**), CBDA (**6**), CBN (**10**), Δ^8-THC (**13**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).

In addition to HPLC methods as discussed above there have been at least four UPLC or UHPLC methods applied for the cannabinoid analysis involving the aerial parts of *C. sativa*[24-

[27]. In those analyses, the column length was 100 or 150 mm, and the particle size was 1.7 μm in all cases[25-27] except one, where it was 1.8 μm[24] (Table 1). A MeOH-water (containing 0.1% HCOOH) gradient was employed for successful separation and PDA-based identification of CBD (**5**), CBDA (**6**), CGB (**8**), CBGA (**9**), CBN (**10**), Δ^9-THC (**14**) and Δ^9-THCA (**15**)[24], while a similar HPLC method, but using an ESI-MS/MS detection, was used to analyse the aerial parts of *C. sativa* revealing the presence of CBD (**5**), CBG (**8**), CBN (**10**) and Δ^9-THC (**14**)[26]. Maly et al.[25] have recently reported a UHPLC method using both a DAD (monitored at 220 nm) and an HRESI-MS (both positive and negative ion modes) detection for the analysis of aerial part samples (Table 1). Using a Dionex Ultimate 3000 UHPLC system, an Acquity UPLC BEH C_{18} column (150 mm ×2.1 mm, 1.7 μm) and a gradient elution with a mobile phase comprising 5% MeOH in water, and isopropanol-MeOH-water (65:30:5), both containing 5 mM ammonium formate and 0.1% HCOOH, afforded an excellent separation and unambiguous identification of CBC (**1**), CBCA (**2**), CBCL (**3**), CBLA (**4**), CBD (**5**), CBDA (**6**), CBGA (**9**), CBN (**10**), CBNA, CBDV (**18**), CBDVA (**19**), Δ^8-THC (**13**), Δ^9-THC (**14**), Δ^9-THCA (**15**), Δ^9-THCA-COOH and Δ^9-THCVA (**17**). A similar LC-MS method using an Acquity UPLC I-Class coupled with a Xevo TQ-S Micro™ triplequadrupole mass spectrometer (MS/MS), but a mobile phase comprising 0.1% HCOOH in water and MeOH:ACN (50:50) provided detection of CBC (**1**), CBCL (**3**), CBD (**5**), CBDA (**6**), CBG (**8**), CBGA (**9**), CBN (**10**), CBDV (**18**), Δ^8-THC (**13**), Δ^9-THC (**14**), Δ^9-THCA (**15**), Δ^9-THCA-COOH and Δ^9-THCV (**16**) in the aerial parts of industrial hemp[27].

During the period covered by this review article, cannabis biomass has been almost exclusively analysed by HPLC, not UPLC or UHPLC[28-31]. CBC (**1**), CBCA (**2**), CBD (**5**), CBDA (**6**), CBDV (**18**), CBDVA (**19**), CBG (**8**), CBGA (**9**), Δ^9-THC (**14**) and Δ^9-THCA (**15**) were successfully identified in cannabis biomass using an Agilent 1290 Infinity II LC System, and combination of an isocratic and a linear gradient in a 17 min run[28,29]. A simple isocratic elution with 75% ACN in water containing 0.1% HCOOH, using a Luna C_{18} column afforded PDA-based detection (220 nm) of several phytocannabinoids, *e.g.,* CBC (**1**), CBCA (**2**), CBCL (**3**), CBD (**5**), CBDA (**6**), CBDV (**18**), CBDVA (**19**), CBG (**8**), CBGA (**9**), CBN (**10**), Δ^8-THC (**13**) Δ^9-THC (**14**), Δ^9-THCA (**15**), THCV (**16**) and THCVA (**17**)[30]. A water-MeOH (both containing 0.1% HCOOH) gradient could separate CBC (**1**), CBCA (**2**), CBD (**5**), CBDA (**6**), CBDV (**18**), CBDVA (**19**), CBG (**8**), CBGA (**9**), CBN (**10**), Δ^9-THC (**14**), Δ^9-THCA (**15**), THCV (**16**) and THCVA (**17**) in cannabis biomass samples extracted with a pressurized solvent extraction method[31], where an Accucore C_{18} Polar Endcapped

column (100 mm × 2.1 mm, 2.6 µm) was used. A PDA detector set at 230 and 306 nm, together with a heated electrospray ionization mass analyzer (HESI-MS) in positive ion mode was used with a Vanquish core LC system and a Poroshell EC-C_{18} column (100 mm x 3 mm, 2.7 µm) for the analysis of phytocannabinoids in biomass samples. This method differentially identified isomeric phytocannabinoids (Table 1) together with other cannabinoids, *e.g.,* CBC (**1**), CBCA (**2**), CBD (**5**), CBDA (**6**), CBG (**8**), CBGA (**9**), Δ^9-THC (**14**), Δ^9-THCA (**15**), *Cis*-Δ^9-THC and *Cis*-Δ^9-THCA[32].

While flowers or inflorescences of *C. sativa* are generally analysed for phytocannabinoids (Table 1), it is not common to separate buds from flowers in such an analysis. It can be clarified here that an inflorescence is a cluster of flowers arranged on a stem that is composed of a main branch or a system of branches, while a flower is simply without any stem or branches with it. In some studies flowers were separated from the stem and then analysed. Botanically, an inflorescence is defined as a cymose panicle, formed in the axil of the leaves on several stem segments. Chen et al.[33] achieved simultaneous separation and identification of CBC (**1**), CBD (**5**), CBDA (**6**), CBDV (**18**), CBG (**8**), CBGA (**9**), CBN (**10**), Δ^8 -THC (**13**), Δ^9 -THC (**14**), Δ^9-THCA (**15**) and THCV (**16**) in buds. In this study, over one hundred bud samples from 20 different varieties of *C. sativa*, collected from the state of Maryland, were analyzed. Using a NexLeaf CBX for Potency C_{18} column (150 mm x 4.6 mm, 2.7 µm) coupled with a Zorbax Eclipse Plus-C_{18} narrow bore guard column (12.5 mm x 2.1 mm, 5 µm), eluted with gradient run with 0.085% phosphoric acid in water and MeOH, the order of elution of these phytocannabinoids were as follows (as detected by DAD set at 220 nm): CBDV (**18**), THCV (**16**), CBD (**5**), CBG (**8**), CBDA (**6**), CBGA (**9**), CBN (**10**), Δ^9 -THC (**14**), Δ^8 -THC (**13**), CBC (**1**) and Δ^9-THCA (**15**). CBDV (**18**) was the first eluted at *ca.* 5 min, while Δ^9-THCA (**15**) was the last to come off the column (t_R = *ca.* 17 min). Later, a simple isocratic HPLC-PDA method was described for the determination of CBD (**5**) and Δ^9-THC (**14**) content in dried female flower buds of *C. sativa*; the isocratic elution was performed with 80% MeOH in water with a flow rate of 1 mL/min[34].

Flowers or inflorescences of *C. sativa* are one of the most significant plant parts for cannabinoid contents[1-4]. Several HPLC methods have been described in the literature for the identification of cannabinoids from flowers[35-44] (Table 1). A straightforward isocratic elution with 0.1% HCOOH/0.5 mM ammonium formate in water: 0.1% HCOOH/0.5 mM ammonium

formate in ACN (1:3) at a flow rate of 1.5 mL/min afforded an excellent separation and PDA-based identification of CBC (**1**), CBCA (**2**), CBCL (**3**), CBG (**8**), CBN (**10**), CBNA (**11**), Δ^9-THC (**14**), THCV (**16**) and THCVA (**17**) in hemp flowers[36]. This was the first study that aimed at direct comparison of the cannabinoid composition of floral extracts from unpollinated and pollinated flowers from hemp plants grown under identical conditions; it was concluded that pollination did not cause the total loss of measurable cannabinoids like CBD (**5**), albeit there were some differences between the phytocannabinoid composition of extracts from pollinated and nonpollinated flowers. CBC (**1**), CBD (**5**), CBDA (**6**), CBDV (**18**), CBG (**8**), CBGA (**9**), Δ^9-THC (**14**) and Δ^9-THCA (**15**) were identified using a simple gradient HPLC method utilising water and MeOH (with 0.07% phosphoric acid) and monitoring by UV detection at 220 nm; a Shim-pack XR-ODSII C_{18} chromatographic column (150 mm x 3 mm, 2.2 μm) was used (Table 1)[37]. It can be noted that the column diameter (3 mm) and the particle size (2.2 μm) were almost like UPLC or UHPLC columns and thus increased efficiency in separation in this method. A similar gradient HPLC-PDA method (25 mM ammonium acetate solution-MeOH as the mobile phase), but with a standard column diameter of 4.6 mm and 5 μm particle size, has recently been reported for the identification of CBD (**5**), CBDA (**6**), CBN (**10**), Δ^9-THC (**14**), and Δ^9-THCA (**15**) in flower samples[38]. Interestingly, the detection wavelength was set pretty low (at 205 mm) in this HPLC run. Earlier, a much shorter column [Shim-pack XR ODS-II (75 mm x 3 mm, 2.2 μm)] eluted with a gradient comprising 0.07% phosphoric acid in water and MeOH was used for the analysis of flower samples identifying CBC (**1**), CBD (**5**), CBDA (**6**), CBDV (**18**), CBG (**8**), CBGA (**9**), CBN (**10**), Δ^8-THC (**13**), Δ^9-THC (**14**), Δ^9-THCA (**15**), THCV (**16**)[39].

While the use of PDA or DAD in the HPLC analysis of *C. sativa* flower samples is quite common[40,42,43], MS detection was applied in the quantitative determination of CBC (**1**), CBCA (**2**), CBCL (**3**), CBCLA (**4**), CBD (**5**), CBDA (**6**), CBDVA (**19**), CBG (**8**), CBGA (**9**), CBN (**10**), CBNA (**11**), Δ^9-THC (**14**), Δ^9-THCA (**15**), THCV (**16**) and THCVA (**17**) in flowers[41]. In this study, an Agilent 1290 Infinity UHPLC system coupled with a TSQ Quantiva triple quadrupole mass spectrometer, an Ace-3, C_{18}-amide column (100 mm × 2.1 mm, 3 μm) with a guard column (Ace-3, C_{18}-amide, 10 mm × 2.1 mm, 3 μm) and a gradient elution with water-ACN (both containing 0.1% HCOOH) over 21 min were used. For the detection of phytocannabinoids, an ESI-MS/MS was used in positive ion mode. The unique feature of this study was the use of an

amide column. Recently, Correia et al.[44], have reported a simple isocratic HPLC method (75% ACN in water with 0.1% HOCOOH; flow rate = 0.5 mL/min; run time = 30 min, column temperature = 20 °C and injection volume = 10 μL) for the simultaneous identification of CBD (**5**), CBN (**10**), CBDA (**6**), Δ^8-THC (**13**), Δ^9-THC (**14**) and Δ^9-THCA (**15**) in flower tops of *C. sativa*. An Agilent 1100 series LC chromatographic system with PDA detector, and a Kinetex C_{18} column (150 mm x 2.1 mm, 2.6 μm) equipped with a C_{18} guard column (2.1 mm) were used in this study. The small column diameter and small particle size afforded significant efficiency and precision in this HPLC method.

There are at least four UPLC (or UHPLC) methods for the analysis of cannabinoids in *C. sativa* flowers available in the literature[45-48]. Of them, three used UV-based detection[46-48], while one used ESI-MS/MS detection[45]. Two of these studies used column particle size of 1.6 μm[46,47], whereas the other two used 1.7 μm particle size[45,48]. All columns were 100 mm in length and the diameter was 2.1-3.0 mm (Table 1). Three of these analyses were UPLC[45-47], i.e., the instrument was from Waters, and one UHPLC (an Agilent instrument)[48]. Eleven phytocannabinoids, e.g., CBC (**1**), CBD (**5**), CBDA (**6**), CBDV (**18**), CBG (**8**), CBGA (**9**), CBN (**10**), Δ^8 -THC (**13**), Δ^9 -THC (**14**), Δ^9 -THCA (**15**) and THCV (**16**), were successfully determined, as a part of the quality control of hemp products (flowers), using a UPLC-MS/MS method using ESI in both positive and negative ion modes.[45] This UPLC-MS/MS method was fast (only 13 min run time) sensitive, accurate and precise. An Acquity Classic UPLC coupled with a Xevo TQ MS detector, an Acquity UPLC BEH C_{18} column (100 mm x 2.1 mm, 1.7 mm) connected to an Acquity UPLC BEH C_{18} VanGuard precolumn (5 mm x 2.1 mm) and a water-ACN (containing 0.1% HCOOH) gradient were employed in this study. Among the three PDA-based identification methods of phytocannabinoids using a UPLC or UHPLC[46-48], two used an isocratic[46,47] and one used a gradient elution method[48] using a mobile phase composed of water and ACN (Table 1). The method described by Mudge and Brown[48], was quite comprehensive, albeit it was a simple isocratic elution, as it successfully identified CBC (**1**), CBD (**5**), CBDA (**6**), CBG (**8**), CBGA (**9**), CBN (**10**), Δ^9-THC (**14**), Δ^9-THCA (**15**), THCV (**16**) and THCVA (**17**) in dried flower samples.

Inflorescences are the most studied parts of the plant *C. sativa*[49-76], and HPLC-based methods with DAD or PDA detection remain the most sought after method for analysis of phytocannabinoids in inflorescences (Table 1). An effective HPLC-PDA method, although it

was described as a UPLC-PDA method, for the determination of the principal non-psychoactive cannabinoids, *e.g.,* CBD (**5**), CBDA (**6**) and Δ^9-THC (**14**), in the inflorescences of fibre-type *C. sativa* was reported where an Acquity UPLC-PDA, a Fortis Speed Core C_{18} PFP column (100 mm x 2.1 mm, 2.6 μm) and a gradient elution (flow rate = 0.4 mL/min) with water-ACN (both containing 0.1% HCOOH) were used (Table 1)[49]. As the particle size of the column was not sub-2 μm, this method is an HPLC method. An isocratic HPLC method with UV detection and using a Raptor ARC-18 column (150 mm × 4.6 mm, 2.7 μm) afforded precise identification of CBC (**1**), CBCA (**2**), CBD (**5**), CBDA (**6**), CBG (**8**), CBGA (**9**), Δ^9-THC (**14**) and Δ^9-THCA (**15**) in the inflorescences of hemp[50]. A similar isocratic method was also described for the identification of CBC (**1**) and Δ^9-THC (**14**) in the inflorescences of *C. sativa*[51]. Both DAD and ESI-MS detection were applied in the gradient HPLC method for the analysis of acidic and neutral phytocannabinoids, *e.g.,* CBD (**5**), CBDA (**6**), Δ^9-THC (**14**) and Δ^9-THCA (**15**)[52]. ESI-MS was used in both positive and negative ion modes, respectively, for neutral cannabinoids and acidic cannabinoids (Table 1). The mobile phase was composed of three different solvents, 0.1% HCOOH in water, ACN and MeOH. Jokic et al.[53] used a similar UHPLC method, but with UV detection at 201 and 230 mm, and an InfinityLab Poroshell 120-C_{18} column (4.6 mm × 150 mm, 4 μm) to identify CBC (**1**), CBCA (**2**), CBD (**5**), CBDA (**6**), CBG (**8**), CBGA (**9**), CBN (**10**), CBDV (**18**) and CBDVA (**19**), Δ^9-THC (**14**), Δ^9-THCA (**15**) and THCVA (**17**). The use of 4 μm particle size was rather unusual for HPLC-based phytocannabinoid analysis. There are several similar HPLC-based analyses of inflorescences of *C. sativa* using UV, DAD or PDA detection[54-64,66,69-71,73-76], which are summarized in Table 1, where various columns, *e.g.,* Accucore C_{18} column (100 mm × 2.1 mm, 2.6 μm), ACE 3 C_{18}-PFP column (150 mm × 3.0 mm, 3 μm), Ascentis Express C_{18} column (150 mm x 3.0 mm, 2.7 μm), Agilent XDB column (250 mm × 4.6 mm, 5.0 μm), Discovery C_{18} column (250 mm × 4.6 mm, 5 μm), Dr Maisch ReproSil XR 120 (3.5 mm × 50 mm, 3 μm) column, Fortis Speed Core C_{18} PFP column (100 mm x 2.1 mm, 2.6 μm), Infinity Lab Poroshell 120 EC-C_{18} column (150 mm x 3 mm, 2.7 μm), Phenomenex Luna C_{18} column (150 × 4.6 mm, 5 μm), Phenomenex Kinetex C_{18} column (100 mm x 2.1 mm, 2.6 μm) and Restek Raptor ARC-18 column (150 mm x 4.6 mm, 2.7 μm), were used. In all those methods, the use of 0.1% HCOOH has been quite common, with occasional use of phosphoric acid and trifluoro acetic acid (TFA) with the main solvents of the mobile phase, *e.g.*, water, ACN and MeOH.

While the use of reversed-phase HPLC is wide-spread in the analysis of phytocannabinoids in various matrices, De Luca et al.[60] investigated the effect of polarity of stationary and mobile phases in the retention behaviour of phytocannabinoids in normal-phase HPLC. The extracts from inflorescences of *C. sativa* were examined. Four normal-phase columns: 150 mm × 4.0 mm Eurospher II 100-5 columns packed with 5 µm fully porous particles: (i) NH_2, (ii) Diol, (iii) CN and iv) Si (bare silica) were employed. Similarly, four different mobile phase compositions 95:5 were used, isocratic: (i) Hept/IPA, (ii) Hept/EtOH, (iii) Hex/IPA and (iv) Hex/EtOH. The analyses were conducted on an AZURA HPLC system, and CBC (**1**), CBD (**5**), CBDV (**18**), CBG (**8**), CBN (**10**) and Δ^9-THC (**14**) were analyzed in those normal-phase conditions. CBC (**1**), CBD (**5**), CBDA (**6**), CBG (**8**), CBN (**10**), Δ^9-THC (**14**) and Δ^9-THCA (**15**) were identified in inflorescence samples using both UV and ESI-MS detections[61]. For the HPLC-MS analysis, a tertiary solvent system with multistep gradient was used (Table 1). CBD (**5**), CBDA (**6**), CBN (**10**), Δ^9-THC (**14**) and Δ^9-THCA (**15**) were analysed by HPLC-DAD and HPLC-MS, where two different HPLC systems were used: Agilent 1200 Series Gradient HPLC System and Surveyor HPLC system coupled to a linear ion-trap mass spectrometer (Linear Trap Quadropole)[62]. Two different columns, Luna C_{18} column (150 × 4.6 mm, 5 µm) and Discovery C_{18} column (250 mm × 4.6 mm, 5 µm) were used, respectively, for the HPLC-DAD and HPLC-MS analyses. A similar dual detection approach (UV and MS) using HPLC was also adopted previously to identify CBD (**5**) and CBDA (**6**)[64]. A Dionex Ultimate 3000 UHPLC coupled with a UV 6000LP Photodiode array UV/vis detector and MS detector Finnigan LQT (XL) equipped with Heated Electro Spray Ion source (HESI II) was used in that study.

CBD (**5**), CBG (**8**), cannabidihexol (CBDH) and Δ^9-THC (**14**) were identified in inflorescences of *C. sativa* by a HPLC-MS method, where a heated ESI-MS (HESI) in positive and negative ion modes was used[65], while CBC (**1**), CBD (**5**) and Δ^9-THC (**14**) were simultaneously identified using an HPLC-MS method, where an ESI was used on negative ion mode and a tertiary multistep gradient with the mobile phase comprising water (with 0.1% acetic acid), ACN and MeOH was used[67]. In a similar HPLC-MS study of inflorescences, CBD (**5**), Δ^9-THC (**14**) and Δ^9-tetrahydrocannabiphorol (Δ^9-THCP) were successfully identified using a Shimadzu Nexera XR HPLC coupled with a Shimadzu triple-quadrupole LCMS-8040, with an electrospray ionization (ESI) source and a Restek Raptor ARC C_{18} column (150 mm x 2.1 mm, 2.7 µm) coupled with a guard column (5 mm × 2.1 mm, 2.7µm)[68]. In this experiment, a

gradient elution was conducted with a mobile phase composed of water with 5 mM ammonium formate and MeOH (Table 1). CBC (**1**), CBCA (**2**), CBD (**5**), CBDA (**6**), CBDV (**18**), CBDVA (**19**), CBG (**8**), CBGA (**9**), CBN (**10**), CBNA (**11**), Δ^9-THC (**14**) and Δ^9-THCA (**15**) were identified by a heated ESI-MS detector used with a Thermo Fisher Surveyor HPLC; a Phenomenex Synergi Hydro RP column (150 mm x 2 mm, 4 μm) with a C_{18} guard column (4 mm x 3 mm) was used[72]. An HPLC-UV-MS-based enantioseparation of chiral phytocannabinoids in medicinal cannabis has recently been reported by Russo et al.[73]. A Vanquish Core HPLC System was used, and two different detectors, DAD and ESI operating in both positive and negative ion modes were used. For the DAD-based analyses, four different columns, CHIRALCEL OD-R [cellulose tris (3,5-dimethylphenylcarbamate)] (250 mm × 4.6 mm, 10 μm), CHIRALCEL OB-H [cellulose tribenzoate] (250 mm × 4.6 mm, 5 μm), CHIRALCEL OJ-H [cellulose tris (4-methylbenzoate)] (250 mm × 4.6 mm, 5 μm), CHIRALPAK AD [amylose tris (3,5-dimethylphenylcarbamate)] (250 mm × 4.6 mm, 10 μm), and CHIRALPAK AD-RH [amylose tris (3,5-dimethylphenylcarbamate)] (150 mm × 4.6 mm, 5 μm) columns, were employed and the mobile phase comprised ACN, EtOH and isopropanol. The use of EtOH in HPLC runs is rather rare nowadays. The enantiomers of *trans*-CBD (**5**) and *trans*-Δ^9 -THC (**14**) were separated with 60% ACN, column temperature at 30◦C and a flow rate of 1.5 mL/ min. However, the HPLC-MS operation utilized an usual solvent system comprising water-ACN (both containing 0.1% HCOOH).

Several UPLC or UHPLC-based methods have been presented in the literature for the analysis of cannabinoids in inflorescences of *C. sativa*[77-84]. Like the HPLC analyses outlined above, both PDA or DAD and MS detection technologies have been used with UPLC or UHPLC systems. However, the use of MS detection appears to be more popular with UPLC or UHPLC systems for the analysis of inflorescences for phytocannabinoids. CBCA (**2**), CBDA (**6**), CBGA (**9**), Δ^9-THC (**14**) and Δ^9-THCA (**15**) were identified with an isocratic run with 30% HCOOH and 20 mM ammonium formate buffer at pH 2.9 and 70% ACN for a 16 min run at a flow rate of 0.3 mL/min and PDA detection at 228 nm[77,78]. An Acquity Arc FTN-R UPLC system and a Kinetex 1.7-μm XB-C_{18} column (150 mm x 2.1 mm, 1.7 μm) were used in this analysis. Two other UHPLC-PDA methods were quite similar[82,84], which employed a gradient elution with water-ACN, both containing 0.1% TFA to identify CBC (**1**), CBD (**5**), CBDA (**6**), CBDV (**18**), CBG (**8**), CBN (**10**), Δ^9-THC (**14**) and Δ^9-THCA (**15**). A Titan C_{18} column (100 mm × 3 mm, 1.9 μm) was

used in both studies. There are at least four UHPLC-MS methods available in the literature published using 2020-23[79-81,83]. An Agilent 1290 UHPLC system coupled with a 6530 accurate mass quadrupole time-of-flight mass spectrometer was used for the identification of CBD (**5**), CBDA (**6**), CBE (**20**), CBEA (**21**) and Δ^9-THC (**14**) in inflorescence extracts[79]. A Luna Omega polar C_{18} column (100 mm × 2.1 mm, 1.6 µm) was employed with a gradient elution with 0.1% HCOOH in water and 10% MeOH in ACN. This method was developed to study the thermal decarboxylation of acidic cannabinoids in *Cannabis* species and to identify transformed cannabinoids based on their MS/MS data. CBD (**5**) and CBG (**8**) could easily be identified and quantified using simple gradient elution with water-ACN (both containing 0.1% HCOOH) and A Hypersil Gold RP column (100 mm × 3 mm, 1.8 µm) on an Agilent 1290 UHPLC coupled to an Agilent 6410 triple quadrupole mass spectrometer[80]. A more comprehensive UHPLC-MS method was developed on a Dionex Ultimate 3000 Thermo Fisher UHPLC coupled with a Q-Exactive Plus quadrupole Orbitrap MS allowing the identification of CBDA (**6**), CBDV (**18**), CBG (**8**), CBGA (**9**), CBNA (**11**), Δ^9-THC (**14**) and THCV (**16**) in inflorescences[81]. This method also employed principal component analysis (PCA) in the analysis of data. An Acquity UPLC BEH C_{18} column (150 mm x 2.1 mm, 1.7 µm) was used with a water-ACN (both containing 0.1% HCOOH) gradient elution. A series of less-common and common phytocannabinoids, *e.g.,* butyl-cannabidiolic acid (CBDA-C4), CBC (**1**), CBCA (**2**), CBD (**5**), CBDA (**6**), CBDV (**18**), CBDVA (**19**), cannabidicannabidiorcolic acid (CBDOA), cannabifuranic acid (CBFA), CBN (**10**), tetrahydrocannabinolic acid B (THCA-B), Δ^9-THC (**14**), Δ^9-THCA (**15**) and Δ^9-THCVA (**17**), were successfully identified by a UHPLC-TOF-MS/MS, Luna Omega C_{18} column (50 mm × 2.1 mm, 1.6 µm) and a gradient elution with water-ACN (both containing 0.1% TFA)[83].

There have been several HPLC-based studies performed with the leaves of *C. sativa* for the presence of cannabinoids during 2020-23[85-90]. UV detection has been found to be most common in leaf analysis by HPLC (Table 1), with only one case of the use of MS detection[90]. CBD (**5**) and CBDA (**6**), and unusual cannabinoids like α-cannabispirol (**25**), β-cannabispirol (**26**), cannabiripsol (**27**) and cannabitriol (**28**) were successfully identified in the extracts of leaves of *C. sativa* using a simple isocratic elution with 30% MeOH in water and 0.1% TFA at a flow rate of 1.0 mL/min on a Luna C_{18} (250 mm × 4.6 mm, 5 µm), where a UV detector at 225 was used[86]. A similar isocratic HPLC-UV method (0.1% CH_3COOH in both water and ACN (1:1) at a flow rate of 1 mL/min) afforded identification of CBD (**5**) and Δ^9-THC (**14**) (Table 1)[87].

However, the run time was unusually quite long (65 min). A Waters Bondapak C_{18} column (300 mm x 3.9 mm, 10.0 μm), an unusual column in the sense of length and particle size for an analytical column, was used to identify CBC (**1**), CBD (**5**), CBDV (**18**) and Δ^9-THC (**14**) using a Shimadzu HPLC-UV system[88], while an isocratic elution with 80% MeOH in water at a flow rate of 1 mL/min over 55 min afforded detection of CBD (**5**) and CBDA (**6**), CBG (**8**), CBN (**10**), Δ^8-THC (**13**) and Δ^9-THC (**14**) on the leaves in the vegetative stage, inflorescence in the flowering stage, inflorescence of seeds in the seeding stage and the mature seeds[89]. Leaves, roots, stem, inflorescences and seeds from industrial hemp were analyzed by a Hewlett-Packard HPLC HP 1050 coupled with an LCQ Accela Fleet atmospheric pressure chemical ionization (APCI), using a Phenomenex Luna C_{18} (2) column (150 mm x 2 mm, 3 μm) eluted with an isocratic mobile phase comprising 5% ACN (containing 0.1% *o*-phosphoric acid) as solvent A and 80% ACN in water (containing 0.1% *o*-phosphoric acid) as solvent B, running 83% B in A at a flow rate of 0.25 mL/min[90]. Both UV and APCI-MS detection were used, but for the MS detection instead of phosphoric acid HCOOH was used. This method afforded the identification of CBD (**5**), CBDA (**6**) and CBGA (**9**).

There are only three reports available on the UHPLC analysis of leaves, leaves and flowers, and hemp microgreens published during 2020-23[91-93]. Li et al.[91] conducted an LC-MS-based untargeted metabolomics which revealed chemical differences of cannabis leaves from different regions of China. An Agilent 1290 infinity UHPLC system coupled with a 6530 QTOF system with a Dual AJS ESI source operating in positive ionization mode was used in this study, a Zorbax Eclipse Plus C_{18} column (100 mm x 2.1 mm, 1.8 μm) was employed and gradient elution with water-CAN (both containing 0.1% HCOOH) afforded identification of CBD (**5**) and Δ^9-THC (**14**). A similar UHPLC-MS analysis was performed on hemp microgreens using a Dionex Ultimate 3000 UHPLC coupled with an Orbitrap Q-Exactive mass spectrometer[93]. CBDA (**6**), CBG (**8**), CBGA (**9**), CBN (**10**), CBNA (**11**), Δ^9-THC (**14**) and Δ^9-THCA (**15**) were successfully identified using a Luna Omega column (50 mm x 2.1 mm, 1.6 μm) eluted with water-ACN (both containing 0.1% HCOOH). Toloza et al.[92] have recently reported the only UHPLC-PDA-based analysis cannabinoids, CBD (**5**) and CBN (**10**), in leaves during 2020-23. It was a simple isocratic elution with a mixture of 0.1% HCOOH in water and ACN (49:51) at a flow rate of 0.45 mL/min and a total run time of 25 min; a Hypersil Gold RP column (150 mm × 2.1 mm × 1.9 μm) was employed.

There is only one study on cannabinoids in seeds of *C. sativa* reported in the literature during 2020-23[94]. In this study, *in situ* decarboxylation-pressurized hot water extraction for selective extraction of phytocannabinoids from the seeds of this plant was examined, and a part of that an HPLC-MS analysis was performed identifying CBD (**5**), CBN (**10**) and Δ^9-THC (**14**). Dionex UltiMate 3000 HPLC and UHPLC System coupled with a Bruker-Compact-QQTOF-MS/MS equipped with an electrospray ionization (ESI) and an Agilent C_{18} Wate column (100 mm x 4.6 mm, 3.5 µm) were employed. The mobile phase comprised water and ACN, both containing 0.1% HCOOH.

3.1.2 HPLC and UPLC (or UHPLC) analysis of cannabis consumer products

Various HPLC- and UPLC (or UHPLC)-based analytical techniques have become popular in the analysis of phytocannabinoids in cannabis consumer products[2] (Table 2). These consumer products include smoking products, *e.g.,* hashish and marijuana, cannabidiol oil, cannabis oil, cannabis olive oil, infusions and liquids, tinctures, cannabis food products and cannabis-containing cosmeceuticals (Table 2)[95-137]. In the HPLC and UPLC (or UHPLC) methods, the use of PDA or MS detection technologies has become routine for the analysis of these consumer products[2]. The use of HPLC as opposed to UPLC (or UHPLC) has remained the method of choice during 2020-23, but the use of UPLC (or UHPLC) has increased considerably over the last few years (Table 2). Appropriate quality control methods are essential for ensuring the quality of cannabis consumer products and thus optimizing the therapeutic outcome in certain cases[2].

A large quantity of illicit hashish is seized every year in Algeria[95]. As a result, it is necessary to investigate the total content of major cannabinoids. In a recent investigation involving 3265 hashish samples, a standard HPLC-DAD method (Thermo Finnigan Surveyor HPLC system with DAD) was applied for the successful simultaneous detection of CBD (**5**), CBDA (**6**), CBN (**10**), CBNA (**11**), Δ^9-THC (**14**) and Δ^9-THCA (**15**) in investigated samples[95]. A Phenomenex C_{18} analytical column (150 mm x 4.6 mm, 5 µm) and mobile phase comprising ACN, MeOH and 0.1% aqueous *o*-phosphoric acid were used. (THC + CBN)/CBD ratio revealed two hashish chemotypes: the traditional one (chemotype II) included most of the studied samples and could be characterized by the THC:CBD:CBN proportions of about 60:35:5, whereas, chemotype I included all hashish samples that possessed high THC and low CBD content, characterized by THC:CBD:CBN proportions of about 85:13:2. An identical HPLC-DAD

study was also conducted in the following year which identified CBD (**5**) and Δ^9-THC (**14**) in seized hashish samples[96].

Johnson et al.[97] have recently compared the advertised versus actual CBD (**5**) contents in various consumer products including CBD oils, aqueous tinctures, e-liquids and drinks sold in the UK. An Agilent 1260 HPLC-DAD was employed with a Raptor ARC-18 LC reversed-phase column (150 mm × 4.6 mm, 2.7 µm) and an isocratic elution with 75% of ACN containing 0.1% of HCOOH in aqueous ammonium formate (5mM) containing 0.1% HCOOH. CBD (**5**) and CBDA (**6**) could successfully be quantified in the investigated products. In most of these products over-labelling of CBD concentration was observed, which highlighted the fact that in the UK, there is a need for improved product standards, and clear legislative guidance on permitted tolerance limits for advertised concentrations.

Cannabis-based products like edible extracts, infusions and liquids were studied by Viviers et al.[98] and Elhendawy et al.[30] using HPLC-DAD methods (Agilent Infinity 1260 HPLC) involving a simple isocratic run with 75% ACN in 75% ACN in water (containing 0.1% HCOOH). In one study[30], several phytocannabinoids, e.g., CBC (**1**), CBCA (**2**), CBCL (**3**), CBD (**5**), CBDA (**6**), CBDV (**18**), CBDVA (**19**), CBG (**8**), CBGA (**9**), CBN (**10**), Δ^8-THC (**13**), Δ^9-THC (**14**), Δ^9-THCA (**15**), THCV (**16**) and THCVA (**17**), could be detected in the investigated products (Table 2), whereas the other study[98] detected only CBD (**5**) and Δ^9-THC (**14**). In recent years, there has been a steady increase in the availability of formulations with high concentration of Δ^8-THC (**13**), which is only about 33% as active as Δ^9-THC (**14**)[99]. The World Antidoping Agency prohibited the use of Δ^8-THC (**13**), and as such, the need for determining the composition of doping is obvious. An investigation of doping samples by Agilent 1290 HPLC coupled with a QTRap 6500 MS employing an Eclipse XDB-C_8 column (100 mm x 2.1 mm, 3.5 µm), and Eclipse XDB-C_{18} column (100 mm x 2.1 mm, 3.5 µm) and a gradient elution revealed the presence of Δ^8-THC (**13**), Δ^9-THC (**14**) and Δ^9-THCA (**15**) in the investigated samples. In this study, ES-MS/MS was used in negative ion mode and utilized multiple reaction monitoring (MRM) option. It can be noted that, because of structural similarities, in many LC-based analyses, Δ^8-THC (**13**) and Δ^9-THC (**14**) coelute, but the method presented opportunities for differentiating between these two. A simple HPLC-PDA gradient method was developed for the detection of CBD (**5**), CBDA (**6**), CBN (**10**), Δ^9-THC (**14**) and Δ^9-THCA (**15**) in cannabis light preparations containing dried female inflorescences of *C. sativa* chemotype III[100], while cannabis seized products were

analysed by an isocratic run with MeOH and 10 mM ammonium acetate at pH 5.2 (80:20) at a flow rate of 0.5 mL/min and a column temperature of 30°C, employing a Kinetex C_{18} (100 mm × 4.6 mm, 2.6 μm) column, and identified CBD (**5**), CBDA (**6**), CBG (**8**), CBGA (**9**), CBN (**10**), Δ^8-THC (**13**), Δ^9-THC (**14**) and Δ^9-THCA (**15**)[101].

Cannabis oil and/or cannabidiol (CBD, **5**)-based oil preparations have become one of the most popular consumer products because of a variety of beneficial effects of CBD (**5**), which is not a controlled substance, on human health[1-4]. There are several studies on the HPLC-based analysis of cannabis oil or CBD (**5**)-rich oil available in the literature for the period covered in this article[44,97,102-112]. However, the analysis of cannabis oil using UHPL or UPLC has been limited to a few studies reported in the literature (Table 2).

A simple UV-based detection was successful in identifying CBD (**5**), CBDA (**6**), Δ^9-THC (**14**), Δ^9-THCA (**15**) in pharmaceutical grade cannabis oil, where Poroshell 120 EC-C_{18} column (50 mm x 3 mm, 2.7 μm) column was used with gradient elution comprising 0.1% HCOOH in water and 0.05% HCOOH in MeOH and a run time of only 11 min[102]. While the use of particle sizes much smaller than the usual particle size of 5 μm in analytical HPLC has increased in recent years, the use of columns of various lengths with 5 μm particle size appears to be quite common in cannabis oil analysis[103-106,108] (Table 2). In cannabis oil analysis, the most commonly used detector has been a UV detector including a PDA, but the use of MS detector[104] has also been observed. Analakkattillam et al.[108] reported an isocratic HPLC run (20 min) for the detection of CBD (**5**) and Δ^9-THC (**14**) in cannabis oil-infused products, while a gradient elution was conducted with water and MeOH, both containing 0.1% HCOOH and 2 mM ammonium formate, to identify CBC (**1**), CBD (**5**), CBDA (**6**), CBDVA (**18**), CBG (**8**), CBGA (**9**), cannabigerovarinic acid (CGBVA), CBN (**10**), cannabivarin (CBV), Δ^9-THC (**14**), Δ^9-THCA (**15**), THCV (**16**) and THCVA (**17**) in cannabis oil and oil-containing capsules using an ESI-MS detector operated in positive ion mode[109]. The use of the isocratic mobile phase in HPLC-UV (or PDA) analysis of CBD-oil has been quite popular as the analysis generally targets the detection of CBD (**5**) and a few other cannabinoids[44,97,111]. Li et al.[111] have recently reported the analysis of CBD (**5**) full spectrum oil revealing the presence of CBD (**5**), CBDA (**6**), CBDV (**18**), CBDVA (**19**), CBGVA, CBG (**8**), CBGA (**9**), CBN (**10**) and CBT (**22**) in the oil sample; a simple isocratic elution with 87% MeOH in water at a flow rate of 0.2 mL/min and a column temperature of 35^{o}C was used along with a MultoHigh 100 RP C_{18} column (125 mm x 4.6 m, 3

μL). They used both the UV detection at 212 nm (Agilent 1260 HPLC system coupled with a UV-vis), and the ESI-MS in positive ion mode (Dionex Ultimate 3000 UHPLC coupled with a UV/vis detector and a TSQ Quantum Access MAX triple quadrupole (TQD) mass spectrometer detector). Similarly, CBD (**5**) was quantified in powder and sunflower oil samples containing CBD (**5**) using both a DAD and an MS detector[112] (Table 2). Analysis of essential oil from inflorescences of *C. sativa* is not that common, but Piani et al.[110] analysed cannabis essential oil and detected CBD (**5**), CBDA (**6**), CBN (**10**), Δ^9-THC (**14**) and Δ^9-THCA (**15**) in the samples using a simple UV detector.

Reddy et al.[113] employed a simple isocratic HPLC-UV method for the quantification of CBD salt, and two other HPLC-based methods have been reported for the quantification and profiling of commercially available cannabinoids[114,115] (Table 2). An isocratic elution with 80% ACN in water over 8 min at a flow rate of 1 mL/min on a reversed-phase C_{18} column afforded the identification of CBD (**5**) and Δ^9-THC (**14**) in commercial industrial hemp products[116]. CBD-containing e-liquids has become quite popular recently, and often they contain other minor cannabinoids or contaminants prompting the need for HPLC-based quality control of the product. Johnson et al.[97] reported a straightforward isocratic HPLC-UV method using 75% ACN/HCOOH (0.1%) in aqueous ammonium formate (5mM)/HCOOH (0.1%) for the quantification of CBD (**5**) and CBDA (**6**) in e-liquid.

The use of phytocannabinoids, particularly CBD (**5**) or CBD oil, in cosmetics and cosmeceutical products has gained remarkable popularity over the past decade, and CBD (**5**) or CBD oil has routinely been used in many mainstream cosmetic products. The increase in popularity of using CBD (**5**) in such products comes with the risk of adulteration and associated quality issues of these consumer products. CBD (**5**), CBDA (**6**), CBN (**10**), Δ^9-THC (**14**) and Δ^9-THCA (**15**) were detected in hemp cosmetic products using an HPLC-UV method[117]. A HPLC-DAD-HRMS isocratic method was developed for the detection of CBG (**8**), cannabigerobutol (CBGB) and cannabigerovarin (CBGV) in hemp products[118]. A similar HPLC method with dual detection, i.e., UV and MS, was employed for the identification of CBD (**5**), CBDA (**6**), CBN (**10**), Δ^9-THC (**14**) and Δ^9-THCA (**15**) in various CBD-infused consumer products like cream, lip balm, lotion and shampoo[119] (Table 2). The particle size of 3.5 μm used in the column in this study was quite unusual. MS detection was in multiple reaction monitoring

(MRM) mode. An isocratic HPLC-UV method was reported for the detection and quantification of CBD (**5**), CBN (**10**) and Δ^9-THC (**14**) in lotion samples[120].

There are at least three reports on the analysis of phytocannabinoids in medical cannabis oil samples published since 2020 [57,69,121]. One used a UV[57], one used a PDA[69] and one an MS/MS[121] detection method with the HPLC method for the analysis of various phytocannabinoids in medical cannabis oil samples. Among these methods, the method described by Hall et al., 2022[69], simultaneously identified at least two phytocannabinoids, i.e., CBC (**1**), CBD (**5**), CBDA (**6**), CBDV (**18**), CBG (**8**), CBGA (**9**), CBN (**10**), Δ^8-THC (**13**), Δ^9-THC (**14**) and Δ^9-THCA (**15**) in the medical oil samples (Table 2).

CBD (**5**), CBDA (**6**), CBN (**10**), Δ^9-THC (**14**) and Δ^9-THCA (**15**) were identified in medicinal cannabis products using an ACE C_{18} column (150 mm x 4.6 mm, 5 μm) on a Shimadzu HPLC-UV system[122]. A mobile phase composed of water (A) and ACN (B), both containing 0.1% HCOOH and a gradient elution: 0-16 min 5-95% B in A, and then coming back to 5% B in 16-17 min at a flow rate of 0.6 mL/min, a column temperature of 25°C and a run time of 18 min were used. A more comprehensive isocratic HPLC-UV method affording identification of seventeen phytocannabinoids, *i.e.,* CBC (**1**), CBCA (**2**), CBCL (**3**), CBCLA (**4**), CBD (**5**), CBDA (**6**), CBDV (**18**), CBDVA (**19**), CBG (**8**), CBGA (**9**), CBN (**10**), CBNA (**11**), Δ^8-THC (**13**), Δ^9-THC (**14**), Δ^9-THCA (**15**), THCV (**16**) and THCVA (**17**) in medical cannabis products including flos and oil was reported by Galetis et al.[123] (Table 2).

Nonprescription commercial CBD products were subjected to HPLC-DAD analysis affording successful quantification of CBD (**5**), CBN (**10**) and Δ^9-THC (**14**) in the samples[124,125]. Similarly, CBD (**5**), CBDA (**6**), Δ^9-THC (**14**), Δ^9-THCA (**15**) were detected in the nonpsychotropic cannabis oil using a HPLC-UV method that employed a ZORBAX Eclipse XDB C_{18} column (250 mm x 3 mm, 5 μm) on a Dionex UltiMate 3000 system coupled with a UV-Vis detector[126] (Table 2).

Cannabis resin is a compressed sticky brown solid made from the resinous parts (stalked resin glands called trichomes) of the plant, and it is commonly known as hashish. Cannabis resin has been the subject to HPLC analysis, and there are at least three reports on the HPLC analysis of cannabis resin published in the literature since 2020 [38,127,128] (Table 2); all used a UV or PDA detection method and 5 μm particle size for C_{18} column packing.

The use of UPLC (or UHPLC) along with PDA and MS detections in the analysis of cannabis oil and other cannabis products has been on the increase in recent years (Table 2). The particle sizes of the column ranged from 1.6 to 1.8 μm. Duzan et al.[45] reported the UPLC-MS analysis of cannabis extract, tincture and cream revealing the presence of CBC (**1**), CBD (**5**), CBDA (**6**), CBDV (**18**), CBG (**8**), CBGA (**9**), CBN (**10**), Δ^8 -THC (**13**), Δ^9 -THC (**14**), Δ^9 -THCA (**15**) and THCV (**16**) in the samples; the mobile phase comprised water and ACN, both containing 0.1% HCOOH, and an Acquity UPLC BEH C_{18} column (100 mm x 2.1 mm, 1.7 mm) connected to an Acquity UPLC BEH C_{18} VanGuard precolumn (5 mm x 2.1 mm) were used.

While a simple UV-Vis detector was coupled to a UPLC system to determine CBD (**5**) and Δ^9-THC (**14**)[129], a DAD was used for the identification of CBC (**1**), CBD (**5**), CBDA (**6**), CBG (**8**), CBGA (**9**), CBN (**10**), Δ^9-THC (**14**), Δ^9-THCA (**15**), THCV (**16**) and THCVA (**17**)[48] in cannabis oil samples. Manca et al.[130] used an UHPLC-MS for the identification of CBD (**5**), CBDA (**6**), CBN (**10**), Δ^9-THC (**14**) and Δ^9-THCA (**15**); in this piece of work, the noticeable things were the length of the column, which was just 30 mm, and the use of isopropanol in the mobile phase, in addition to water and ACN and HCOOH (Table 2). Almost an identical UPLC-MS method was reported for the detection of CBD (**5**), CBDA (**6**), Δ^9-THC (**14**) and Δ^9-THCA (**15**) in cannabis oil galenic preparations[131]; these researchers also used a UHPLC-MS system but only using 0.1% HCOOH in water and 0.1% HCOOH in ACN in the gradient mobile phase. CBC (**1**), CBD (**5**), CBDV (**18**), CBG (**8**), CBN (**10**) and *cis*- and *trans*-Δ^9-THC (**14**) were successfully determined in CBD oil samples using a UHPLC-DAD system where a Shimadzu Nexera UHPLC system, a Phenomenex XB-C_{18} column (50 mm × 2.1 mm, 1.7 μm) and a gradient elution with 0.1% HCOOH in water (A) and 0.1% HCOOH in ACN (B), starting with 5% B, increasing to 100% B over 18 min were employed[132]. A UPLC-MS method was applied for the detection of CBD (**5**), CBDA (**6**), Δ^9-THC (**14**), Δ^9-THCA (**15**) and Δ^9-THC conjugates in cannabis decoction and oil samples[133], while a UHPLC-HRMS/MS was used for the identification of CBD (**5**), CBG (**8**), CBN (**10**) and Δ^9-THC (**14**) in cannabis resins and cannabis oil samples[26] (Table 2).

There have been at least two UHPLC-based methods published since 2020 for the simultaneous analysis of commercially available phytocannabinoids, one used dual detection (UV and MS)[134], and one used a PDA (Table 2). Sixteen phytocannabinoids, CBC (**1**), CBCA (**2**), CBD (**5**), CBDA (**6**), CBDV (**18**), CBDVA (**19**), CBG (**8**), CBGA (**9**), CBCLA (**4**), CBN (**10**), CBT (**22**), Δ^8-THC (**13**), Δ^9-THC (**14**), Δ^9-THCA (**15**), THCV (**16**) and THCVA (**17**) were successfully analysed

by a UHPLC-MS method[134], while the UV-based detection allowed simultaneous identification of at least ten phytocannabinoids including CBC (**1**), CBD (**5**), CBDA (**6**), CGB (**8**), CBGA (**9**), CBN (**10**), Δ^8-THC (**13**), Δ^9-THC (**14**), Δ^9-THCA (**15**) and THCV (**16**)[135]. The latter[135] study also used normal phase isocratic elution with *n*-hexane/isopropanol/ethanol/trifluoroacetic acid (TFA) (96: 3: 1: 0.1), at a flow rate of 0.21 mL/min at 25°C with an injection volume of 0.5 μL, as well as a reversed phase system comprising water/ACN/TFA (45: 55: 0.1), isocratic elution at a flow rate of 0.8 mL/min at 25°C with an injection volume of 0.5 μL. Various column packings were exploited in this analysis.

A UPLC-PDA (UV detection at 228 nm)method was described for the identification of CBD (**5**), CBDA (**6**), CBGA (**9**), CBN (**10**), Δ^9-THC (**14**) and Δ^9-THCA (**15**) in ground cannabis inflorescences samples packaged in tamper-proof cartridges (Syqe cartridges)[136]. The gradient mobile phase comprised water and ACN, both containing 0.1% HCOOH, and an Acquity UPLC C_{18} column (150 mm x 2.1 mm, 1.7-μm) was used. The presence of CBD (**5**), 7-OH-CBD, 6α-OH-CBD, CBDV (**18**) and CBE (**20**) was detected by a UHPLC-MS method where ESI-MS interface was used both in positive and negative ion modes [137] (Table 2).

3.2 HPLC and UPLC (or UHPLC) analysis of cannabinoids in biological samples

Marijuana or hashish, a cocktail of at least 30 different major cannabinoids, generally prepared from crushing the leaves, flowers (inflorescences) and even stem of *C. sativa*, is one of the oldest recreational and addictive natural products used by the humans for centuries[1-4] In many countries, however, the non-medical consumption of cannabis or marijuana or hashish is illegal. This demands the use of appropriate analytical tools, like HPLC and UPLC (or UHPLC), for the analysis of biological and forensic samples, e.g., blood, oral liquid, hair and urine, to confirm marijuana usage (Tables 3-7)[133, 138-167]. After consumption of marijuana, THCA (**13**), is converted to THC (**12**) by heat during smoking, but after metabolism, this compound is excreted in the urine mainly as its glucuronide conjugate[1-4].

3.2.1 Human blood samples

Among the biological and forensic samples, the blood (whole blood, plasma or serum) is one of the most favoured samples for cannabinoid analysis[1,2]. Human blood, plasma and serum samples are quite extensively used in forensic analysis to detect the consumption of cannabinoids[1,2]. HPLC and UPLC methods are commonly used for the analysis of

phytocannabinoids and their metabolic products in blood samples[133, 138, 139, 141-152]. Table 3 lists various HPLC and UPLC (or UHPLC)-based methods for the analysis of phytocannabinoids and their metabolic products in human blood samples. During 2020-23, all reported HPLC or UPLC/UHPLC analyses of phytocannabinoids almost exclusively used C_{18} columns of various dimensions (Table 3), with the exception of one, where an Ascentis Express RP-Amide analytical column (150 mm x 3 mm, 2.7 μm)[139] was used. A clear shift from the use of conventional HPLC to UPLC/UHPLC could be observed during this period. Plasma still remains the major blood component that is routinely used for the analysis of phytocannabinoids in blood. In human blood samples, various unaltered phytocannabinoids and their conjugates derived from Phase I and phase II metabolism, e.g., 11-OH-THC (**12**), THC-COOH (**29**), THC-glucuronide, THCA-glucuronide and THC-COOH-glucuronide have been detected by various LC-based methods using, almost exclusively, an MS detector employing ESI-MS/MS method. It can be noted that glucuronidation is an established phase II metabolic pathway for several drugs including phytocannabinoids and endogenous substances[140]. Despite being a secondary metabolic pathway for a compound preceded by phase I hydroxylation, glucuronidation alone can serve as the main metabolic pathway for compounds, including some highly hydrophilic compounds.

CBD (**5**), CBDA (**6**), CBN (**10**), 11-OH–Δ^9-THC (**12**), Δ^8-THC (**13**), Δ^9-THC (**14**), Δ^9-THCA (**15**), 11-nor-9-carboxy-Δ^9-THC and Δ^9-THCA-glucuronide were successfully determined in human plasma samples using an Acquity H-Class PLUS coupled with a XEVO TQ-S micro MS detector with an ESI interface and a Phenomenex Kinetex C_{18} column (100 mm x 2.1 mm, 2.6 μm) (Table 3)[138]. The particle size of the column was close to UHPLC or UPLC columns and afforded excellent separation of these phytocannabinoids and their conjugates. A similar HPLC method, but instead of using an ESI-MS/MS, an atmospheric pressure chemical ionization (APCI) in combination with multiple reaction monitoring in positive ion mode, was applied for the separation and identification of CBC (**1**), CBD (**5**) and its metabolites including CBD-glucuronide (CBD-glu), CBDA (**6**), 6α-hydroxy-CBD (CBD-6α-OH), 7-hydroxy-CBD (CBD-7-OH), CBN (**10**), Δ^9-THC (**14**) and its metabolites including Δ^9-THCA-glucuronide, 11-OH-Δ^9-THC (**12**), and THCVA (**17**)[139] . The mobile phase used in this study was also different; instead of a gradient elution using a mixture of water-ACN (both containing 0.1% HCOOH), 0.04% formic acid in water and ACN:MeOH:isopropanol (3:1:1) was used (Table 3). In both these HPLC

analyses, the total run time was 10 min. Dybowski et al.[141] have recently reported an HPLC-MS/MS method using a column with a slightly larger particle size (3 μm instead of 2.6 or 2.7 μm as in the previous methods) and an isocratic elution, and affording identification of CBD (**5**), CBG (**8**), CBN (**10**) and Δ^9-THC (**14**). These are the only three HPLC-based analyses of human blood samples (plasma) reported in the literature during the period covered by this review. However, the use of UPLC/UHPLC for the analyses of phytocannabinoids in human blood has been well documented in at least 12 different published papers during 2020-23.[133,142-152]. Among them, there are four reports on the analysis of blood plasma samples for phytocannabinoids and their conjugates (Table 3).

Blood plasma samples are quite often used in forensic analysis for the detection of illegal cannabinoid consumption[1,2]. An Acquity UPLC H-Class system coupled to the Xevo® TQ-D tandem quadrupole MS equipped with a Z-spray source and a Coreshell Kinetex C_{18} UPLC column (100 mm x 2.1 mm, 1.7 μm) were used detect CBD (**5**) and Δ^9-THC (**14**) in the human plasma samples[146]. An isocratic elution with 5 mM ammonium acetate and ACN with 0.1% formic acid, at a flow rate of 0.3 mL/min and a column temperature of 40°C afforded successful analysis of these phytocannabinoids (Table 3). However, the main highlight of this study was the use of an in-tube solid-phase microextraction with a dummy molecularly imprinted monolithic capillary coupled to UPLC-MS/MS; the novel MIP monolithic capillary synthesized with hydrogenated CBD (**5**) as dummy template presented adequate mechanical resistance meaning long lifetime and good tolerance to organic solvents and used a small mass of template molecule. The method was found to be easy to use, cost-effective, high reproducibility and high selectivity. The coupling of the sample preparation step to main UPLC operation enhanced the efficiency of analysis and reduced the potential sources of errors, and thus could be applied for the analysis of plasma samples from patients receiving CBD as a part of their treatment. A similar UPLC-MS/MS method was also published where instead of an isocratic elution, a gradient elution for less than 10 min as the run time was used for the detection of the same phytocannabinoids, CBD (**5**) and Δ^9-THC (**14**) in human plasma samples[147]. Two UHPLC methods, one using a Dionex Ultimate 3000 UHPLC system coupled with a TSQ Quantiva™ Triple Quadrupole MS detector and the other using a Shimadzu Nexera X2 UHPLC system coupled with a tandem MS detector, were reported; the first one detected CBD (**5**) and Δ^9-THC (**14**)[148], and the second one identified 7-COOH-CBD and Δ^9-THC

metabolites, both using an Acquity UPLC BEH C_{18} column (50 mm x 2.1 mm, 1.7 μm), eluted with a water/ACN gradient[149] (Table 3).

Phytocannabinoids, e.g., CBD (**5**), CBG (**8**), CBN (**10**) and Δ^9-THC (**14**) and Δ^9-THCA (**15**) and their conjugates, e.g., 7-COOH-CBD (**7**), 7-OH-CBD, 6α-OH-CBD, 6β-OH-CBD, Δ^9-THC (**14**), 11-OH-Δ^9-THC and (11-COOH-THC (or THC-COOH, **29**), were analysed in human whole blood samples using an Acquity UPLC H-Class System coupled with an MS detector (Table 3)[142,143]. An Acquity BEH C_{18} column (150 mm × 2.1 mm, 1.7 μm), with or without, a guard column was used. In a recent study, a Sciex Exion LC system coupled with a QTRAP® 6500+ triple quadrupole linear ion trap MS equipped with a TurboIonSpray interface has been used for the detection of Δ^9-THC (**14**), Δ^9-THC-OH and Δ^9-THC-COOH (**29**), where an Acquity UPLC HSS T3 C_{18} column (100 mm x 2.1 mm, 1.8 μm) was used[144]. The MS detection was in two modes, MRM and MS^3. Similarly, a UHPLC-MS/MS method using a Dionex Ultimate 3000 UHPLC coupled to a TSQ Quantiva™ Triple Quadrupole and an atmospheric pressure chemical ionization (APCI) in the positive ion mode was successful in analyzing CBD (**5**) and Δ^9-THC (**14**) in human capillary blood samples[145]. In this study, a shorter column (50 mm) was used.

Since 2020, there have been four UPLC (or UHPLC) methods published in the literature for the analysis of human serum samples for phytocannabinoids and their conjugates.[150-152]. In all those studies, ESI-MS/MS detection technology was utilized. Pichini et al.[150] used an Acquity UPLC I-Class coupled with a Waters Xevo TQ-S micro mass spectrometer (triple quadrupole) equipped with an electrospray ionization source operating in negative-ion mode (ESI-) and an Acquity UPLC BEH C_{18} column (100 mm x 2.1 mm, 1.7 μm), eluted with a gradient comprising ammonium formate 5 mM at pH 7.5 and ACN to determine CBD (**5**), CBDA (**6**), 7-hydroxycannabidiol (CBD-7-OH) and 6α-hydroxycannabidiol (CBD-6α-OH). Earlier, the same group reported the detection of CBD (**5**), CBDA (**6**), Δ^9-THC (**14**), Δ^9-THCA (**15**) and Δ^9-THC conjugates in human serum samples using the same UPLC system but employing a shorter column (50 mm)[133] (Table 3). Both methods could potentially be applied to clinical trials on the use of different medical cannabis preparations. CBD (**5**), CBDA (**6**), 7-COOH-CBD (**7**), 6α-OH-CBD, 6β-OH-CBD and 7-OH-CBD were successfully analysed in human serum samples using the same Acquity UPLC-MS system and using the same column [Acquity UPLC BEH C_{18} column (100 mm x 2.1 mm, 1.7 μm)][151]. In all three cases an elevated column temperature was used (50°C). A UHPLC-MS/MS method using a Phenomenex Onyx monolithic C_{18} column

(100 mm x 3 mm, 1.5 μm) and a gradient elution with 2% MeOH in water containing 10 mM ammonium formate and 0.1% formic acid, and MeOH/CAN/isopropanol (80:10:10) containing 8 mM ammonium formate and 0.08% formic acid to identify CBD (**5**) and 7-OH-CBD in human serum samples[152].

3.2.2 Human sweat, oral fluid and breast milk samples

Human breast milk is a highly complex biological fluid, and is not routinely used for forensic analysis of phytocannabinoids, but is used to detect cannabinoids to protect breast-fed infants from possible toxicities of cannabinoids[1,2]. Extraction of phytocannabinoids and their conjugates from this matrix is challenging owing to its high lipid and protein contents (up to 5% by weight). However, it is essential for monitoring cannabinoids in breast milk resulting from passive or nonrecent active maternal exposure. CBD (**5**), CBG (**8**), 11-OH-Δ^9-THC (**12**), Δ^9-THC (**14**), Δ^9-THCA (**15**) and Δ^9-THC-glucuronide were successfully identified in human breast milk samples using an Agilent 1200 series LC system coupled with a Sciex API5000 tandem mass spectrometer and employing a Poroshell Eclipse C_{18} column (50 mm x 4.6 mm, 2.7 μm), eluted with a mobile phase comprising isopropanol, MeOH and water containing 0.1% HCOOH[153] (Table 4). The reported validated method was efficient and sensitive; it was found to be able to simultaneously quantify several phytocannabinoids and their conjugates in human breast milk using a sample volume of only 200 μL, and to support both clinical studies and routine screening analysis of donated breastmilk. It was assumed that this LC-MS/MS method could significantly improve the safety of donated breastmilk and provide a suitable platform to quantify cannabinoids in pharmacokinetic studies in breast milk[153].

The analysis of human oral fluid samples for phytocannabinoids and their metabolites by HPLC or UPLC/UHPLC is quite common[2]. Human oral fluid samples are routinely used in forensic analysis to ascertain illegal cannabis consumption. However, during the period covered in this review article, there have been only one HPLC[154] and three UPLC (or UHPLC)[133, 155, 156] methods appeared in the literature (Table 4). An HPLC-MS/MS method for the analysis of phytocannabinoids along with several other drugs of abuse was validated where CBD (**5**), CBN (10), Δ^9-THC (**14**), and Δ^9-THC-COOH (**29**) were successfully detected using an Agilent 1100 series equipped with a Sciex API4000 TurboIonSpray interface for pneumatically assisted

electrospray, and employing an Agilent Pursuit XRs Ultra 2.8 C_{18} column (100 mm × 2.0, 2.8 μm)[154]. The mobile phase used in this study comprised 10 mM formic acid in water and 5% ACN in MeOH with 10 mM formic acid, and a gradient elution was performed (Table 4). A significant relative matrix effect was observed but this effect was minimised by adopting a sample pretreatment step and prior evaluation of the matrix protein content. This method was found to be sensitive, simple, reliable, and rapid with a simple sample preparation step, and could be used in the analysis of forensic samples.

A Cortecs C_{18} UPLC column (50 mm x 2.1 mm, 1.6 μm) was used with the Acquity UPLC coupled with Xevo TQ-S instrument for the LC-MS/MS analysis of human oral fluid samples for the determination of CBC (**1**), CBD (**5**), CBDA (**6**), CBG (**8**), CBN (**10**), Δ^8-THC (**13**), Δ^9-THC (**14**), 11-OH-Δ^9-THC (**12**), Δ^9-THCA (**15**), THCV (**16**), CBDV (**18**) and cannabidiorcol (CBD-C1), where a water-ACN (both containing 0.1% HCOOH) gradient was employed (Table 4)[155]. A solid-phase extraction (SPE) step was used prior to UPLC operation, and a total of 200 human oral fluid samples, collected within the States of Michigan, Indiana, Wisconsin, Ohio and Illinois, were analysed by this method, where all the above cannabinoids were detected on at least one sample, Δ^8-THC (**13**), was confirmed in 11 samples, with or without the presence of Δ^9-THC (**14**), a high concentration (>400 ng/mL) of 11-OH-Δ^9-THC (**12**) or THC-COOH (**29**) was found in three samples, CBD (**5**), CBG (**8**), CBN (**10**), Δ^9-THCA (**15**), THCV (**16**) were confirmed, respectively, in 74, 39, 44, 107 and 112 of the 179 confirmed Δ^9-THC (**14**)-positive samples. Apparently, this published method was the first method for oral fluid analysis for simultaneous detection of Δ^8-THC (**13**) along with several other cannabinoids and their metabolites. As simultaneous quantitation of multiple phytocannabinoids and metabolites in human oral fluid offers valuable information related to cannabinoid consumption and interpreting cannabinoid-induced driving impairment, the validated method described by Lin et al.[155] could be used in forensic analysis of human oral fluids. Samples of oral fluid after repeated intake of CBD-rich cannabis smoking were analysed by a UHPLC-MS method employing an Agilent Infinity 1290 UHPLC system coupled with a 4500 QTRAP hybrid triple quadrupole/linear ion trap MS with a Turbo V ion source and an Ace Excel Super C_{18} column (75 mm × 2.1 mm, 2 μm) linked to a C_{18} guard column to efficiently detect CBD (**5**), Δ^9-THC (**14**) and Δ^9-THCA (**15**) within a short run time of only 5 min (Table 4)[156]. Earlier, Pichini et al.[133] reported a UPLC-MS/MS analysis of CBD (**5**), CBDA (**6**), Δ^9-THC (**14**), Δ^9-THCA (**15**) and Δ^9-THC

conjugates in samples of human oral fluid and sweat. While oral fluid is a routinely used sample for such analysis, the use of human sweat samples for the analysis of phytocannabinoids is quite unusual. When dealing with sweat, phytocannabinoids and their metabolites were extracted from the sweat patch absorbent pad samples with MeOH spiked with ISs. The UPLC-MS/MS method reported here allowed the identification and quantification of phytocannabinoids and their metabolites in conventional and nonconventional human biological samples.

Although the use of human saliva samples for the analysis of cannabinoids is not that common, an online SPE-UHPLC-MS/MS method for the therapeutic monitoring of CBD (**5**) and 7-OH-CBD in human saliva was reported by Franco et al.[152] (Table 4). In this study, a UHPLC ExionLC 100 integrated system coupled with a 3200 QTRAP® triplequadrupole linear ion trap mass spectrometer fitted with a TurboIonSpray interface was used, and a Phenomenex Onyx monolithic C_{18} column (100 mm x 3 mm, 1.5 μm), eluted with a gradient comprising ACN, MeOH and water was employed (Table 4). This method was demonstrated as a simple, selective, sensitive and accurate for the analysis of the aforementioned cannabinoids in a low volume of saliva sample (50 μL). This method was also found to be easy to implement in a clinical setting as it required a minimum processing and hands-on time, there was no evaporation or concentration step, and it involved a direct injection of the sample in the UHPLC-MS/MS system after a simple protein precipitation step with extremely low matrix effect and automated online SPE.

3.2.3 Human hair and nail samples

Human hair samples are one of the most commonly used samples for forensic analysis for drugs of abuse or illegal drugs like cannabis[1,2]. Hair analysis is usually performed to monitor and detect long-term usage of drugs. While the use of nail samples in forensic analysis for illegal drugs is not that common, these samples can also provide useful information like hair samples in the case of long-term drug usage. Both hair and nail samples are keratinized biological matrices that offer notable advantages over traditional matrices, particularly, wider time window, non-invasive sampling and good stability of the analytes over time. However, there are some difficulties or disadvantages in using hair or nail samples for the analysis of phytocannabinoids and their conjugates, which mainly include low

concentrations of the major metabolite, THC-COOH (**29**). So, it is necessary to employ an effective extraction method prior to any LC or GC-based analysis of human hair or nail samples. In recent years, solid-phase microextraction (SPME) has enhanced the extraction efficiency from small samples. During the period of 2020-23, only four publications dealing with hair and nail samples using HPLC and UPLC (or UHPLC) have been published[156-159].

Among the two HPLC-based analyses, while the publication by Faro et al.[157] reported the analysis of human hair samples collected from patients treated with medical cannabis identifying CBD (**5**) and its metabolites, 11-OH-THC (**12**) 7-COOH-CBD (**7**), 7-hydroxycannabidiol (7-OH-CBD), 6α-hydroxycannabidiol (6α-OH-CBD) and 6β-hydroxycannabidiol (6-β-OH-CBD), Δ^9-THC (**14**) and Δ^9-THCA (**15**) in the samples, Mannocchi et al.[158], analysed both hair and nail samples for the detection of Δ^9-THC (**14**) along with several other drugs of abuse using rather an Oasis HLB column with an unusual column length of only 20 mm and at an elevated column temperature of 50^{o}C (Table 5). In both cases, ESI-MS was used for detection and identification of phytocannabinoids in the samples. It is interesting to note that despite the method described by Faro et al.[00] (2022) was mentioned as a UHPLC-MS method, a column with 2.6 μm particle size was used (Table 5). So, technically it was not a UHPLC method as UHPLC or UPLC columns should have a particle size of 2 μm or less. In a similar fashion, Mannocchi et al.[158] also claimed that their method was a UPLC-MS/MS method (Acquity I Class coupled with a Waters XEVO TQ-S Micro tandem quadrupole mass spectrometer) but used a column with 5 μm particle size [Oasis HLB column (20 mm x 4.6 mm, 5 μm]. It appears that clearly there are some misunderstandings among researchers about the definition of UPLC or UHPL.

Since 2020, only two UPLC (or UHPLC) methods have been published for the determination of phytocannabinoids, one in human hair samples, and the other in human nail samples[156,159]. CBD (**5**), Δ^9-THC (**14**) and Δ^9-THCA (**15**) were successfully identified in human hair samples collected after repeated intake of CBD-rich cannabis smoking[156] (Table 5). In this study, an Agilent Infinity 1290 UHPLC system coupled with a 4500 QTRAP hybrid triple quadrupole/linear ion trap MS with a Turbo V ion source was used with an Ace Excel Super C_{18} column (75 mm × 2.1 mm, 2 μm) linked to a C_{18} guard column, eluted with a gradient elution comprising water/formic acid 5 mM and ACN. ESI-MS was in negative ion mode, and MS/MS (MS^2) selected reaction monitoring (SRM) acquisition was employed. The highlight of

this method was its short run time (5 min). Head and pubic hair collected one week after the exposure revealed the presence of CBD (**5**) at concentrations of 312 and 178 pg/mg, respectively, but the same samples were found to be negative for THC (**14**) and THC-COOH (**29**). This study demonstrated that hair analysis is effective in the discriminating between the repeated consumption of CBD-rich cannabis and THC-prevalent products. A UPLC-MS/MS with high-throughput sample preparation step was applied for the analysis of 106 different drug samples including Δ^9-THC (**14**) in human nail samples[159]. The sample preparation step in this study involved efficient cryogenic grinding coupled with room temperature zircon beads grinding for the extraction of drugs from nail samples. An Acquity UPLC coupled to a Waters Xevo TQ-XS triple quadrupole spectrometer (QQQ-MS/MS) with an ESI source, and an Acquity UPLC HSS column (150 mm x 2.1 mm, 1.8 μm) were used. ESI-MS in positive ion operating in multiple reaction monitoring (MRM) mode was employed. This quantitative data evaluation of authentic nail samples collected from drug addicts under withdrawal treatment in community rehabilitation centers could detect drugs in the lower pg/mg range, and thus, this nail analysis method could be a useful tool for the large-scale monitoring of drugs abuse including phytocannabinoids.

3.2.4 Human urine samples

Human urine samples are extensively used in forensic toxicological analysis for the determination of illegal drugs, including phtocannabinoids, and their metabolites[1,2]. Table 6 presents the applications of HPLC and UPLC-based analytical methods for the analysis of phytocannabinoids and their biotransformation products in human urine samples[133, 150, 156, 160-162]. ESI-MS/MS methods have been used almost exclusively for the detection of phytocannabinoids in these LC operations. When it comes to urine analysis for the presence of phytocannabinoids and their conjugates, during the last three years, there has been a clear shift from the use of standard HPLC to UPLC or UHPLC instrumentation, and to the use of MS-based detection technology. It can be noted that the use of MS detectors has become routine in most of the laboratories dealing with cannabinoids analysis in human urine samples, because of the increased availability and reduced cost, and obviously owing to the richness of structural information that this detection may provide[2]. There is only one report on the use of an HPLC method for the detection of phytocannabinoids, e.g., Δ^9-THCA (**15**) along with several other drugs of abuse, in human urine samples, where an Agilent Infinity 1290 II LC

system coupled with a 6470 Triple Quadrupole MS (UHPLC-QQQ-MS) was used[160]. A standard reversed-phase C_{18} column (150 mm x 4.6 mm), but with smaller particle size (3 μm), and a Jet Stream Technology Ion Source (AJS) ESI in positive and negative ion modes were employed in this study. The mobile phase combination used in this work was slightly different because instead of 0.1% HCOOH, only 0.05% HCOOH was used with 10 mmol/L ammonium formate solution as solution A, where MeOH was used as solution B in the gradient elution over 17 min total run time (Table 6). However, additionally Liu et al.[160] used a Dionex Ultimate 3000 UHPLC coupled with Q Exactive Plus Hybrid Quadrupole-Orbitrap MS (UHPLC-QE Plus-HRMS) system and a Hypersil GOLD column (100 mm × 2.1 mm, 1.9 μm) to analyze human urine samples for the detection and quantification of ^{9}-THCA (**15**). In both cases, prior to HPLC and UHPLC analyses, a liquid-liquid extraction protocol was introduced for sample preparation.

There are at least five different UPLC (or UHPLC) methods reported for the analysis of phytocannabinoids and their metabolic products during 2020-23[133, 150, 156, 160-162]. Waters Acquity UPLC system appears to be the most popular choice, followed by Agilent UHPLC and Dionex UHPLC systems (Table 6). Pichini et al.[133] used a short UPLC column (50 mm x 2.1 mm, 1.7 μm) for the detection of CBD (**5**), CBDA (**6**), Δ^9-THC (**14**), Δ^9-THCA (**15**), 11-OH-THC (**12**), THC-COOH (**29**) and Δ^9-THC conjugates, e.g., Δ^9-THC-glucuronate and THC-COOH-glucuronate, in human urine samples (Table 6). The UPLC run was conducted with an elevated column temperature at 50^{o}C and at a flow rate of 0.4 mL/min. The use of a short column reduced the total run time to only 10 min. Among the detected cannabinoids, THC-COOH-glucuronate was the first metabolite eluted from the column (t_R = 4.27 min), whereas Δ^9-THCA (**15**) was the last emerge from the column (t_R = 6.29 min)[133]. In this study, urine samples were collected prior to experiment and then each 2 h for the next 24 h. Each single analyte concentration measured in the different urine samples multiplied for the collected volume and summed together represented the 0–24 h amount excreted in urine. In this experiment. A year later, the same group of researchers reported the comprehensive identification of CBD (**5**), CBDA (**6**), 7-carboxycannabidiol (CBD-7-OH), 7-hydroxycannabidiol (CBD-7-OH) and 6α-hydroxycannabidiol (CBD-6α-OH) in human urine samples obtained from an individual treated with medicinal cannabis using a similar Waters UPLC-MS system, but using a longer column (100 mm)[150]. However, the total run time was only 8 min despite a longer column. The ESI ionization was performed in negative mode to reach the sensitivity required to detect

trace amounts, with limits of quantification ranging from 0.05 to 0.1 ng/mL. This method was found to be accurate, precise and rapid for the detection of phytocannabinoids and their conjugates in human urine samples.

Analysis of phytocannabinoids, e.g., CBD (**5**), Δ^8-THC (**13**), Δ^9-THC (**14**), Δ^{10}-THC, Δ^8-THC-COOH, Δ^9-THC-COOH (**29**), in human urine samples in forensic toxicology casework by isomer-selective UPLC-MS/MS has recently been reported, where a Waters Cortecs UPLC C_{18} Plus analytical column (50 mm x 2.1 mm, 1.6 μm) was used[161]. In this study, instead of a linear gradient, a step gradient for near isocratic separation of cannabinoids was used (Table 6). All these cannabinoids were eluted between 1,94 and 4.87 min. This method was proved to be a simple, and rapid method for routine and high-volume testing of forensic urine samples. Gerace et al.[156] used an Agilent Infinity 1290 UHPLC system coupled with a 4500 QTRAP hybrid triple quadrupole/linear ion trap MS with a Turbo V ion source for the analysis of CBD (**5**), Δ^9-THC (**14**), Δ^9-THCA (**15**) in human urine samples obtained after repeated intake of CBD-rich smoking. An Ace Excel Super C_{18} column (75 mm × 2.1 mm, 2 μm) linked to a C_{18} guard column was used, and the gradient mobile phase comprised 5 mM formic acid in water and ACN. With a flow rate of 0.6 mL/min and a column temperature of 45°C, the total run time was impressively short (5 min). In this study, ESI-MS/MS was in negative ion mode, and MS/MS (MS^2) selected reaction monitoring (SRM) acquisition was used. A similar method, but utilizing a longer column (100 mm) and multiple reaction monitoring (MRM) transitions, has recently been published for the analysis of several phytocannabinoids and their conjugates including CBD (**5**), 6-OH-CBD, 7-OH-CBD, 7-COOH-CBD (**7**), Δ^8-THC (**13**), Δ^9-THC (**14**), Δ^9-11-OH-THC, Δ^8-THC-COOH, Δ^9-THC-COOH (11-COOH-THC, **29**) and Δ^9-THC-COOH-glucuronide, in urine samples from laboratory staff with no prior experience of cannabis consumption[162]. However, the run time was a bit long (19 min). In this method, an automated micro solid-phase extraction (MSPE) was used online for sample cleanup prior to LC-MS analysis. Seventy eight forensic urine samples were analysed with this method and CBD (**5**), Δ^8-THC (**13**) and Δ^9-THC (**14**) metabolites were effectively detected in 54 samples. Δ^9-THC (**14**) metabolites were also identified in 15 samples containing Δ^8-THC-COOH. This piece of work established an analytical method that could offer continuous treatment of specimens and the detection for metabolites of CBD (**5**), Δ^8-THC (**13**) and Δ^9-THC (**14**) in urine samples by incorporating an

online SPME-UHPLC-MS/MS. This method has the potential for use in the determination of legal limits for CBD (**5**), Δ^8-THC (**13**) and Δ^9-THC (**14**).

3.2.5 Miscellaneous biological samples from human

There are at least five reports published during 2020-23 on HPLC and UPLC (or UHPLC)-based analysis of phytocannabinoids in various nonconventional biological samples obtained from humans, including dry bone, liver fraction, cerumen (ear wax) and cell free matrices (Table 7). Giordano et al.[163] reported the analysis of 11-COOH-THC or THC-COOH (**29**) in human dry bone samples from cranium, ribs and vertebrae using a Thermo-Fisher HPLC system coupled with a Q-Exactive Orbitrap–mass spectrometer with a heated ESI ionization source and a Synergi Hydro-RP column (150 mm x 2 mm, 4 μm) coupled to a C_{18} guard column (4 mm x 3 mm, 4 μm). A gradient elution with ammonium formate in water and MeOH, at a flow rate of 0.7 mL/min and a run time of 22 min was employed in this study. This study opened up a new possibility in forensic analysis of decomposed or skeletonized human remains, where conventional matrices like blood, oral fluid or urine are no longer available. Prior to LC analysis, accelerated solvent extraction (ASE) was carried out to extract cannabinoids from bone samples. Earlier. A similar HPLC-MS method, but using a column with smaller particle size (3 μm), and a gradient elution (flow rate = 0.3 mL/min) with ammonium acetate buffer containing 0.1% formic acid and 0.1% HCOOH in ACN were used for the analysis of S9 liver fraction leading to successful identification of 11-OH-THC (**12**) and its glucuronide, 11-COOH-THC or THC-COOH (**29**) and its glucuronide, and Δ^9-THC-glucuronic acid[164] (Table 7). In this study, the HESI-MS detector was used in both positive and negative ion modes, and investigated phase II metabolism of 11-OH-THC (**12**) *in vitro* and *in vivo*.

A sensitive, reliable and efficient UHPLC-ESI-MS/MS method has recently been reported for the analysis of CBC (**1**), CBD (**5**), CBDV (**18**), CBG (**8**) and CBN (**10**) in cell free or extracellular matrices[165]. Positive electrospray ionization and multiple reaction monitoring (MRM) modes were used. This method showed good linearities over a concentration range of 0.025 – 40 μg/mL and was shown to be applicable to the analysis of hemp ethanolic extracts. An optimized protein precipitation step was introduced in this study to achieve efficient cleanup and high recoveries of the phytocannabinoids, i.e., CBC (**1**), CBD (**5**), CBDV (**18**), CBG (**8**) and CBN (**10**), from different matrices, and to avoid matrix composition that

could potentially interfere with the chromatographic separations and ESI operation. Overall, this method was found to be highly efficient because of the less complicated preparation process, small sample size, short run time (14 min), enhanced detection sensitivity, accuracy and broad application range.

Another nonconventional and less frequently exploited biological sample for forensic analysis is cerumen, which is commonly known as earwax. In recent years, this biological matrix has been used for biomonitoring, aiming at the detection of psychotropic drugs including cannabinoids[166,167]. Several phytocannabinoids and their metabolites, e.g., CBD (**5**), CBN (**10**), Δ^9-THC (**14**), 11-OH-THC (**12**) and 11-COOH-THC or THC-COOH (**29**), were effectively detected and quantified in cerumen samples by using an Acquity I-Class UPLC coupled with a Xevo TQ-XS triple quadrupole MS, establishing the fact that cerumen samples could be successfully used as an alternative forensic sample for the analysis of drugs of abuse[166]. A short column (Acquity UPLC BEH C_{18} column; 50 mm x 2.1 mm, 1.7 µm) and a gradient elution with water and ACN, both containing 0.1% HCOOH, were applied in this study. One of the main advantages of using a cerumen sample is that unlike hair samples, it is less prone to external contamination caused by ambient air and cosmetics, and therefore, is more reliable for detection of drugs of abuse. Prior to UPLC analysis, cerumen samples were extracted with 1% acetic acid in ACN, by centrifugation. Later, almost an identical method was reported for the determination of phytocannabinoids, i.e., CBC (**1**), CBD (**5**), CBG (**8**), CBN (**10**), Δ^9-THC (**14**), and 11-COOH-THC or THC-COOH (**29**), in human cerumen samples obtained from 14 cannabis users using UPLC-MS/MS[167]. Δ^9-THC (**14**) was detected in twelve samples, and CBG (**8**), CBN (**10**) and Δ^9-THC (**14**) were quantified at concentrations 0.02–0.21, 0.01–0.24 and 0.01–4.86 µg/g, respectively, in six samples. A significant inter-individual variation in detected cannabinoid concentration dependent on age, sex and time of use was observed.

3.3 UPLC (or UHPLC) analysis of cannabinoids in animal samples

Table 8 displays the summary of all LC-based analytical methods for the quantification of phytocannabinoids and their conjugates in various animal samples published during 2020-2023[168-174]. There were only seven reports on the analysis of phytocannabinoids in various animal samples available in the literature during this period, and all these studies involved the use of UPLC (or UHPLC) rather than standard HPLC methods, and ESI-MS as the detection

technology. Almost exclusively all studies used a Waters UPLC-MS/MS system, except one, where a Vanquish UHPLC Q-Exactive system coupled with a Hybrid Quadrupole-Orbitrap MS was used. Cattle plasma samples following oral administration of hemp were analysed by a Waters Acquity H UPLC coupled with a TQ-S triple quadrupole MS detector for the detection of cannabinoids and their conjugates derived from metabolism, including CBC (**1**), CBCA (**2**), CBD (**5**), CBDV (**18**), CBDVA (**19**), CBG (**8**), CBN (**10**), 11-OH-Δ^9-THC (**12**), Δ^8-THC (**13**) Δ^9-THC (**14**), Δ^9-THC-COOH (**29**), Δ^9-THC-glucuronic acid and, Δ^9-THCV (**16**), Δ^9-THCA (**15**), aiming at establishing plasma pharmacokinetics of phytocannabinoids[168]. This study demonstrated that acidic cannabinoids, e.g., CBDA (**6**), were rapidly absorbed from the rumen and distributed in the body. A twelve minute gradient using the mobile phase ACN-water (both containing 0.1% HCOOH) at an elevated column temperature of 55^{o}C, and a flow rate of 0.5 mL/min was used. A similar UPLC method to establish pharmacokinetics involving blood samples from healthy dogs after intranasal, intrarectal and oral administration of CBD (**5**) was reported by Polidoro et al.[169]; instead of ACN, methanol (MeOH) was used in the mobile phase. In this study, a Xevo TQ-XS triple quadrupole mass spectrometer was used to obtain ESI-MS/MS data in positive ion mode. It was observed that intranasal, intrarectal and oral administration of, respectively, 20, 100 and 100 mg CBD (**5**) was well tolerated by the dogs. This study recommended the use of intranasal delivery of CBD (**5**) in veterinary medicine over oral administration because of faster absorption of CBD (**5**). Earlier, a UPLC-MS based analysis of dog serum and urine samples successfully detecting 11-OH-Δ^9-THC (**12**), Δ^9-THC (**14**), Δ^9-THCA (**15**), and Δ^9-THC-glucuronide in serum, and CBD (**5**), 11-OH-Δ^9-THC (**12**), Δ^9-THC (**14**), Δ^9-THCA (**15**), Δ^9-THC-glucuronide and Δ^9-THCA-glucuronide in urine[170]. A shorter column (50 mm) was used with an ACN-water based linear gradient elution at a 0.4 mL/min flow rate and column temperature of 40^{o}C. This piece of work demonstrated that a UPLC-MS/MS method using multiple reaction monitoring (MRM) could be useful in the diagnosis of marijuana toxicosis in dogs. Plasma samples from donkeys were analysed by UPLC-MS/MS using an Acquity UPLC HSS-T3 column (100 mm x 2.1 mm, 1.8 μm) for the separation and identification of 11-OH-Δ^9-THC (**12**), Δ^9-THC (**14**) and Δ^9-THCA (**15**)[171]. This was the first report on marijuana toxicosis in donkeys.

There are at least three reports[172-174] on the analysis of mice samples for detecting cannabinoids during the period covered in this review article, and all of these articles were published in 2023 (Table 8). Upadhyay et al.[172] used an Acquity BEH C_{18} column (50 mm × 2.1

mm, 1.7 μm) and an ACN-water (both containing 0.1% HCOOH) gradient at a flow rate of 0.6 mL/min for the detection of Δ^9-THC (**14**) and Δ^9-THCA (**15**) in the brain and peripheral tissues of mice after intranasal instillation of a nanoformulation ,while Δ^8-THC (**14**), Δ^9-THC (**14**), 11-OH-Δ^9-THC (**12**) and Δ^9-THC-COOH (**29**) were successfully determined in the brain and plasma samples from mice after being exposed to cannabis smoke establishing differential pharmacokinetics of Δ^9-THC (**14**) based on sex, age and strain[173]. In both cases, ESI-MS/MS was used for the detection of cannabinoids. Δ^9-THC (**14**), Δ^9-THCA (**15**) and Δ^9-THCV (**16**) and their metabolites were successfully detected in primary hepatocytes of mice using a Vanquish UHPLC Q-Exactive system coupled with a Hybrid Quadrupole-Orbitrap MS[174]. In this study, an HSS T3-C_{18} column (100 mm x 2.1 mm, 1.8 μm) and a gradient elution with ACN-water (0.1% HCOOH) were employed with a flow rate of 0.3 mL/min. This study established the link between metabolism and biological activity of phytocannabinoids.

3.4 HPLC and UPLC (or UHPLC) analysis of cannabinoids in dietary supplements, food and beverages

Cannabinoids may be present in food and beverages, either as contaminants or as food additives[2,175,176] (Table 9). Cannabis seeds have long been used as a food source and there are several food products available in the marker that contain purified cannabinoids or cannabis extracts[175-181]. Therefore, the qualitative and quantitative analysis of cannabinoids in food and food supplements, and beverages is essential for quality assurance and the control of dietary intake of cannabinoids-containing food items.[2] An HPLC-DAD method, employing a simple isocratic elution with 80% ACN in water (both containing 0.1% formic acid, HCOOH) at 1 mL/min, has recently been reported for the analysis of cannabinoids, particularly CBD (**5**) and THC (**14**) in cannabis-infused ice cream[103] (Table 9). The retention times for CBD (**5**) and THC (**14**), respectively, were *ca.* 3 and 6.5 min. This study also looked at the stability of these cannabinoids in ice cream over period of as long as 270 days. A similar isocratic HPLC-DAD method was used for the determination of CBD (**5**) in various food supplements that contain CBD (**5**)[178] (Table 9). Dawson and Martin[177] investigated chocolate matrix interference on cannabinoid analytes, e.g., CBD (**5**), CBG (**8**), CBN (**10**) and THC (**14**), using an HPLC-DAD method with a much shorter run time (7 min), using an ACN-water gradient and at a slightly higher temperature (40^{o}C) than the room temperature (Table 9). In this study, a reversed-phase column (150 mm x 4.6 mm) packed with 2.7 mm C_{18} silica was used. It is well-known

that chocolate is an extremely complex food matrix for analyte extraction and quantification, mainly because of high fat content and presence of polyphenolic compounds[177]. However, the method described by these authors was found to be successful in the analysis of cannabinoids in three different chocolate matrices, i.e., milk chocolate, dark chocolate and unsweetened non-Dutch process cocoa powder. It was observed that chocolate matrix could inhibit the complete extraction of cannabinoids in solution.

An reversed-phase HPLC-PDA (set at 228 nm for monitoring the chromatogram) together with HPLC-MS has recently been used for the comprehensive identification of a series of phytocannabinoids, e.g., CBD (**5**), CBDA (**6**), CBDV (**18**), CBDVA (**19**), CBGA (**9**), CBN (**10**), Δ^9-THC (**14**), Δ^9-THCA (**15**), Δ^9-THCV (**16**), Δ^9-THCVA (**17**), in hemp-seed based food products, such as, oil, flour, flour by-product[179]. It can be noted that the term hemp, industrial hemp or fibre-type cannabis is only used to describe some *Cannabis sativa* varieties, where CBD (**5**) and its acid precursor CBDA (**6**) are the main components. An ESI-MS detector was employed in positive ion mode.

During the past three years, there appears to be only two true UPLC (or UHPLC) methods reported for the analysis of cannabinoids in dietary supplements, food and beverages[180,181] (Table 9). A Waters Acquity UPLC coupled with an MS detector (negative ion mode using multiple reaction monitoring, MRM) has been employed in a recent study to detect CBD (**5**), CBDA (**6**), CBDV (**18**), CBG (8), CBN (**10**), D^9-THC (**14**), D^9-THCA (**15**), THCV (**16**), and D^9-THC-COOH (**29**) in cannabinoids containing food products including chocolate, fondant, biscuits, beverages, cookies and baijiu[180]. In this study, an ACN-water (both containing 0.1% HCOOH) gradient was used at a flow rate of 0.3 mL/min and a run time of 12 min and Waters Acquity UPLC BEH Shield C_{18} column (100 mm x 3 mm, 1.7 mm) was used as the stationary phase. A tandem ESI-MS using MRM in negative ion mode was applied. Three moka coffee drinks, enriched with shredded inflorescences of different cultivars of hemp, marketed in southern Italy, were analysed by a UHPLC-HRMS (Shimadzu Nexera UHPLC) method for qualitative and quantitative phytocannabinoid profiles[181]. The reversed-phase C_{18} silica column used in this study was much shorter than the columns mentioned earlier; the length of the column was just 50 mm and the diameter was 2.1mm with a particle size of 1.6 μm. Cannabidiolic acid (CBDA, **6**) and THCA (**15**) emerged as the major phytocannabinoids present in those coffees. Interestingly, no trace of CBN (**10**) and THC (**14**) could be detected

in the samples examined. This study pointed a new direction for the exploitation of hemp processing by-product to obtain high value-added drinks. In this study, an ESI probe was used in both positive and negative ion modes and the Atmospheric Pressure Chemical Ionization (APCI) probe was used for fully automatic mass calibration using the Calibrant Delivery System (CDS). It can be mentioned that the fragmentation pattern of each detected metabolite was investigated, not only in terms of *m/z* ratio but also taking into consideration of the fragments' intensity, obtained during the collision-induced dissociation (CID) of the precursor ions as it would be helpful in the case of constitutional isomers that usually produce qualitatively similar MS/MS spectra. The use of QqTOF (quadrupole-quadrupole-time-of-flight) mass analyzer was found to be effective in this study.

3.5 HPLC and UPLC (or UHPLC) analysis of cannabinoids in waste water and sewage

LC-based methods are routinely used for the detection of phytocannabinoids present in various other matrices, *e.g.,* waste water samples[2]. It can be noted that waste water based epidemiological studies are generally conducted to monitor the presence of cannabis-derived psychoactive substance, e.g., Δ^9-THC (**14**) or other illicit drugs, and the use of LC-based analysis often encounters various problems, especially related to a limit of quantification at low ng/L concentration[182]. Only four research papers have been published during 2020-23 on the LC-based analysis of waste water samples for the detection of phytocannabinoids, two involving HPLC and two other using UPLC (or UHPLC) systems[182-185] (Table 10). In all those cases, ESI-MS/MS was used for the detection of cannabinoids. Δ^9-THC (**14**) was successfully detected from unfiltered sewage with an LLQ of 5.17 ng/L as quantified by an HPLC-MS/MS system using a standard analytical HPLC column with particle size of 5 μm[182]. This piece of work demonstrated the real-word application of LC-MS/MS methods for illicit drug surveillance in waste water-dependent epidemiology. Foppe et al.[183] reported the analysis of 39 different drugs and metabolites, including Δ^9-THCA-glucuronide and THC-COOH-glucuronide, in an upstream waste water network in Massachusetts, using an HPLC-MS/MS method. A reversed-phase biphenyl column with much smaller particle size (2.6 μm) was used in that study and a solid-phase-extraction (SPE)-based pretreatment of waste water samples was incorporated in the protocol. Waste water collected from the waste water treatment plant, located along the Febros River shoreline in Vila Nova de Gaia, Porto, was analysed for the presence of cannabinoids by a UPLC-MS/MS using a multiple reaction monitoring (MRM)

mode; an SPE-based pretreatment was also used[184]. Several phytocannabinolids, e.g., CBDV (**18**), CBN (**10**), CBD (**5**), CBG (**8**), THCV (**16**), CBDA (**6**), Δ^9-THC (**14**), Δ^9-THCA (**15**) and THC-COOH (**29**) (in the order of increasing retention time between 6.58 and 7.75 min), could successfully be detected in urban effluent waste water samples using this method. A similar UPLC-based method[185] has recently been reported for the detection of Δ^9-THC-COOH (**29**) in waste water collected from urban waste water treatment plants in China, where a reversed-phase C_{18} column with 1.7 μm particle size and a pretreatment step involving magnetic solid-phase extraction (MSPE) were employed. It can be noted that a new type of SPE using magnetic nanoparticles as sorbents was used in this study, which allowed enhance separation of cannabinoids from a complex matrix in the presence of an external magnetic field. This method appears to be a simple, rapid and sensitive method for the analysis of cannabinoids and their metabolites or conjugates in waste water.

4. CONCLUSIONS

During the period 2020-23, HPLC and UPLC (or UHPLC)]-based analytical methods, particularly LC-MS/MS techniques, have continued to dominate the analytical methods for phytocannabinoids in various matrices, mainly biological matrices. However, HPLC-PDA methods are still popular for the analysis of cannabinoids in plant matrices. The ever-increasing popularity of UPLC (or UHPLC) is mainly because of better precision, shorter run time, less use of solvent (mobile phase), and increased affordability. Besides, the price difference between a UPLC (or UHPLC) and a standard HPLC system has decreased considerably over the last few years. While water and ACN, both containing 0.05-0.1% HCOOH or CH_3COOH or phosphoric acid, are still the most widely used mobile phase combination, the use of MeOH instead of ACN has also been observed. Most of the methods reported for the analysis of phytocannabinoids are gradient methods, but the use of isocratic methods, especially for the analysis of a few phytocannabinoids, is still observed. Application of various mathematical models and computational techniques for process optimization and accurate quantification of phytocannabinoids in different matrices has been observed.

ACKNOWLEDGEMENT

L Nahar gratefully acknowledges the financial support of the European Regional Development Fund - Project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868).

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REFERENCES

1. Nahar L, Guo M, Sarker SD. Gas chromatographic analysis of naturally occurring cannabinoids: A review of literature published during the past decade. *Phytochem Anal.* 2020; 31: 135-146.
2. Nahar L, Onder A, Sarker SD. A review on the recent advances in HPLC, UHPLC and UPLC analyses of naturally occurring cannabinoids (2010-2019). *Phytochem Anal.* 2020; 31: 413-457.
3. Nahar L, Uddin, SJ, Alam MA and Sarker SD. Extraction of naturally occurring cannabinoids: An update, *Phytochemical Anal.* 2021; 32: 228-241.
4. Nahar L, Gavril G-L, Sarker SD. Application of gas chromatography in the analysis of phytocannabinoids, *Phytochemical Anal.* 2023; 34: 903-924.
5. Lafaye G, Karila L, Blecha L, et al. Cannabis, cannabinoids, and health. *Dialogues Clin Neurosci.* 2017; 19(3): 309-316.
6. Pertwee RG. Cannabinoids. Springer-Verlag, Berlin, 2005.
7. Nahar L, Sarker SD. Guest Editorial: UPLC in phytochemical analysis. *Trends in Phytochem Res.* 2019; 3: 1-2.
8. Sarker SD, Nahar L. Natural Products Isolation, 3rd Edition, Humana Press –Springer-Verlag, USA. 2012.
9. Sarker SD, Nahar L. In "Evidence-Based Validation of Herbal Medicine: Farm to Pharma" (Editor: Mukherjee, P.), Applications of high performance liquid chromatography in the analysis of herbal products, Elsevier, USA. 2015.
10. Tiscione NB, Miller R, Shan X, Sprague J, Yeatman DT. An efficient, robust method for the determination of cannabinoids in whole blood by LC-MS-MS. *J Anal Toxicol*. 2016; 40(8): 639-648.

11. Nováková L, Matysová L, Solich P. Advantages of application of UPLC in pharmaceutical analysis. *Talanta*. 2006; 68: 908-918.
12. Catani M, Felletti S, Buratti A, De Luca C, Cavazzini A. Perspectives and pitfalls in potency testing of cannabinoids by high performance liquid chromatography (HPLC). *LC GC North Am*. 2022; 40: 78-81.
13. Deidda R, Schelling C, Roussel J-M, Dispas A, De Bleye C, Ziemons E, Hubert P, Veuthey J-L. The analysis of cannabinoids in cannabis samples by supercritical fluid chromatography and ultra-high-performance liquid chromatography: A comparison study. *Anal Sci Adv.* 2021; 2: 2-14.
14. Salazar-Bermeo J, Moreno-Chamba B, Martinez-Madrid MC, Valero M, Rodrigo-Garcia J, Hosseinian F, Martin-Bermudo F, Aguado M, de la Torre R, Marti N, Saura D. Preventing mislabeling: A comparative chromatographic analysis for classifying medical and industrial cannabis. *Molecules.* 2023; 28: article number 3552.
15. Durante C, Anceschi L, Brighenti V, Caroli C, Afezolli C, Marchetti A, Cocchi M, Salamone S, Pollastro F, Pellati F. Application of experimental design in HPLC method optimisation for the simultaneous determination of multiple bioactive cannabinoids. GC vs. HPLC in quantitation of CBD, CBG, Δ9-THC and CBN in plasma using different sample preparation methods. *J Pharm Biomed Anal.* 2022; 221: article number 115037.
16. Mastellone G, Marengo A, Sgorbini B, Scaglia F, Capetti F, Gai F, Peiretti PG, Rubiolo P, Cagliero C. Characterization and biological activity of fiber-type *Cannabis sativa* L. aerial parts at different growth stages. *Plants.* 2022; 11: article number 419.
17. Milanez PR, da Silva FMR, Scussel R, de Melo ME, Martins AB, Machado-de-Avila RA, Barlow JW, Feuser PE, Rigo FK, Amaral PDA. Cannabis extracts and their cytotoxic effects on human erythrocytes, fibroblasts, and murine melanoma*. Rev. Bras. Farmacog.* 2021; 21: 750-761.
18. Treyer A, Reinhardt JK, Eigenmann DE, Oufir M, Hamburger M. Phytochemical comparison of medicinal cannabis extracts and study of their CYP-mediated interactions with coumarinic oral anticoagulants. *Med Cannabis Cannabinoids*. 223; 6: 21-31.
19. Krizman M. A simplified approach for isocratic HPLC analysis of cannabinoids by fine tuning chromatographic selectivity. *Eur Food Res Technol.* 2020; 246: 315-322.

20. Yao S, Ball, C, Miyagusuku-Cruzado G, Giusti MM, Aykas DP, Rodriguez-Saona LE. A novel handheld FT-NIR spectroscopic approach for real-time screening of major cannabinoids content in hemp. *Talanta.* 2022; 247: article number 123559.
21. Mastellone G, Marengo A, Sgorbini B, Rubiolo P, Cagliero C. Development of a dispersive solid-liquid microextraction method using natural eutectic solvents for a greener extraction of phytochemicals from fiber-type *Cannabis* sp. *Indust Crops Prod.* 2022a; 187: article number 115476.
22. Hammami N, Prove J-P, Joly DL, Moreau G. Associations between cannabinoids and growth stages of twelve industrial hemp cultivars grown outdoors in Atlantic Canada. *Indust Crops Prod.* 2021; 172: article number 113997.
23. Mano-Sousa BJ, Alves BC, Pedrosa AM, Lima PL, de Andrade FP, Duarte-Almeida JM. Validation of analytical method of cannabinoids: Novel approach using turbo-extraction. *Talanta.* 2023; 254: 124108.
24. Deville M, Dubois N, Denooz R, Charlier C. Validation of an UHPLC/DAD method for the determination of cannabinoids in seized materials: Analysis of 213 samples sold in Belgian CBD shops. *Forensic Sci Int.* 2020; 310: article number 110234.
25. Maly M, Benes F, Binova Z, Zlechovcova M, Kastanek P, Hajslova J. Efective isolation of cannabidiol and cannabidiolic acid free of psychotropic phytocannabinoids from hemp extract by fast centrifugal partition chromatography. *Anal Bioanal Chem.*2023; 415: 4827-4837.
26. Benes F, Fenclova M, Peukertova P, Binova Z, Dzuman Z, Hajslova J. Determination of seventeen phytocannabinoids in various matrices by UHPLC-HRMS/MS. *LCGC Europe.* 2020; 33: 8-16.
27. Berthold EC, Yang R, Sharma A, Ka,ble SH, Kanumuri SR, King TI, Popa R, Freeman JH, Brym ZT, Avery BA, McCurdy CR. Regulatory sampling of industrial hemp plant samples (*Cannabis sativa* L.) using UPLC-MS/MS method for detection and quantification of twelve cannabinoids. *J Cannabis Res.* 2020; 2: article number 42.
28. Giese MW, Lewis MA, Giese L, Smith KM. Development and validation of a reliable and robust method for the analysis of cannabinoids and terpenes in cannabis. *J AOAC Int.* 2015; 98: 1503-1522.

29. Zheljazkov VD, Maggi F. Valorization of CBD-hemp through distillation to provide essential oil and improved cannabinoids profile. Sci Rep. 2021; 11: article number 19890.
30. Elhendawy MA, Radwan MM, Ibrahim EA, Wanas AS, Chandra S, Godfrey M, ElSohly MA. Validation and quantification of fifteen cannabinoids in *Cannabis* and marketed products using high-performance liquid chromatography/photodiode away method. *Cannabis Cannabinoid Res.* 2023; article number 0335.
31. Olejar KJ, Kinney CA. Evaluation of thermo-chemical conversion temperatures of cannabinoid acids in hemp (*Cannabis sativa* L.) biomass by pressurized liquid extraction. *J Cannabis Res.* 2021; 3: article number 40.
32. Tolomeo F, Russo F, Kaczorova D, Vandelli MA, Bioagini G, Lagana A, Capriotti AL, Paris R, Fulvio F, Carbone L, Perrone E, Gigli G, Cannazza G, Citti C. *Cis*-Δ^9 - tetrahydrocannabinolic acid occurrence in *Cannabis sativa* L. *J Pharm Biomed Anal.* 2022; 219: article number 114958.
33. Chen X, Deng H, Heise JA, Puthoff DP, Bou-Abboud N, Yu H, Peng J. Contents of cannabinoids in hemp varieties grown in Maryland. *ACS Omega.* 2021; 6: 32186-32197.
34. Dehnavi MM, Ebadi A, Peirovi A, Taylor G, Salami SA. THC and CBD fingerprinting of an elite cannabis collection from Iran: Quantifying diversity to underpin future cannabis breeding. *Plants.* 2022; 11: article number 129.
35. Schwabe Al, Johnson V, Harrelson J, McGlaughlin ME. Uncomfortably high: Testing reveals inflated THC potency on retail Cannabis labels. *Plos One.* 2023; 18: article number e0282396.
36. Todd J, Song H, Acker RV. Does pollination alter the cannabinoid composition and yield of extracts from hemp (*Cannabis sativa* L. cv. *Finola*) flowers? *Indust Crops Prods.* 2022; 183: article number 114989.
37. Qamar S, Torres YJM, Parekh HS, Falconer JR. Fractional factorial design study for the extraction of cannabinoids from CBD-dominant cannabis flowers by supercritical carbon dioxide. *Processes.* 2022; 10: article number 93.
38. Sedan D, Vaccarini C, Demetrio P, Morante M, Montiel R, Sauri A, Andrinolo D. Cannabinoid Content in Cannabis Flowers and Homemade Cannabis-Based Products Used for Therapeutic Purposes in Argentina. *Cannabis Cannabinoid Res.* 2023; 8: 197-206.

39. Qamar S, Manrique YJ, Parekh HS, Falconer JR. Development and optimization of supercritical fluid extraction setup leading to quantification of 11 cannabinoids derived from medicinal cannabis. *Biology.* 2021; 10: article number 481.
40. Kovalchuk O, Li DP, Rodriguez-Juarez R, Golubov A, Hudson D, Kovalchuk I. The effect of cannabis dry flower irradiation on the level of cannabinoids, terpenes and anti-cancer properties of the extracts. *Bioanalysis Agric Biotechnol.* 2020; 29: article number 101736.
41. McRae G, Melanson JE. Quantitative determination and validation of 17 cannabinoids in cannabis and hemp using liquid chromatography-tandem mass spectrometry. *Anal Bioanal Chem.* 2020 ; 412 : 7381-7393.
42. Gilmore AM, Elhendawy MA, Radwan MM, Kidder LH, Wanas AS, Godfrey M, Hilreth JB, Robinson AE, ElSohly MA. Absorbance-transmittance excitation emission matrix method for quantification of major cannabinoids and corresponding acids: A rapid alternative to chromatography for rapid chemotype discrimination of *Cannabis sativa* varieties. *Cannabis and Cannabinoid Res.* 2023; 8: 911-922.
43. Carvalho VM, de Almeida FG, de Aguilar AFL, Pereira FCDS, Rocha ED. Facing the forensic challenge of cannabis regulation: A methodology for the differentiation between hemp and marijuana samples. *Braz J Anal Chem.* 2022; 9: 162-176.
44. Correia B, Ahmad SM, Quintas A. Determination of phytocannabinoids in cannabis samples by ultrasound-assisted solid-liquid extraction and high-performance liquid chromatography with diode array detector analysis. *J Chromatog A.* 2023; 1705: article number 464191.
45. Duzan A, Reinken D, Basti MM. Quality Control of 11 Cannabinoids by ultraperformance liquid chromatography coupled with mass spectrometry (UPLC-MS/MS). *J Anal Methods Chem.* 2023; 2023: article number 3753083.
46. De Leersnijder C, Duchateau C, De Braekeleer K, Deconinck E. Relative response factors and multiple regression models in liquid chromatography to quantify low-dosed components using alternative standards—proof of concept: total Δ9-THC content in cannabis flowers using CBD as reference. *Anal Bioanal Chem.* 2022; 414: 6507-6520.
47. Duchateau C, De Leersnider C, Barhdadi S, Canfyn M, De Braekeleer K, Deconinck W. Discrepancies between validated GC-FID and UHPLC-DAD methods for the analysis of Δ-9-THC and CBD in dried hemp flowers. *Drug Test Anal.* 2022; 14: 1732-1743.

48. Mudge EM, Brown PN. Determination of cannabinoids in *Cannabis sativa* dried flowers and oils by LC-UV: Single-laboratory validation, first action 2018.10. *J AOAC Int.* 2020; 103: 489-493.

49. Tzimas PS, Petrakis EA, Halabalaki M, Skaltsounis LA. Effective determination of the principal non-psychoactive cannabinoids in fiber type *Cannabis sativa* L. by UPLC-PDA following a comprehensive design and optimization of extraction methodology. *Anal Chim Acta*. 2021; 1150: article number 338200.

50. Erzen M, Kosir IJ, Ocvirk M, Kreft S, Cerenak A. Metabolomic analysis of cannabinoid and essential oil profiles in different hemp (*Cannabis sativa* L.) phenotypes. *Plants.* 2021; 10: article number 966.

51. Anis O, Vinayaka AC, Shalev N, Namdar D, Nadarajan S, Anil SM, Cohen O, Belausov E, Ramon J, Gati EM, Koltai H. Cannabis-derived compounds cannabichromene and Δ^9-tetrahydrocannabinol interact and exhibit cytotoxic activity against urothelial cell carcinoma correlated with inhibition of cell migration and cytoskeleton organization. *Molecules.* 2021; 26: article number 465.

52. Mazzara E, Torresi J, Fico G, Papini A, Kulbaka N, Dall'Acqua S, Sut S, Garzoli S, Mustafa AM, Cappellacci L, Fiorini D, Maggi F, Giuliani C, Petrelli R. A Comprehensive phytochemical analysis of terpenes, polyphenols and cannabinoids, and micromorphological characterization of 9 commercial varieties of *Cannabis sativa* L. *Plants.* 2022; 11: article number 891.

53. Jokic S, Jerkovic I, Pavic V, Aladic K, Molnar M, Kovac MJ, Vladimir-Knezevic S. Terpenes and cannabinoids in supercritical CO2 extracts of industrial hemp inflorescences: Optimization of extraction, antiradical and antibacterial activity. *Pharmaceuticals.* 2022; 15: article number 1117.

54. Szalata M, Dreger M, Zielinska A, Banach J, Szalata M, Wielgus K. Simple extraction of cannabinoids from female inflorescences of hemp (*Cannabis sativa* L.). *Molecules.* 2022; 27: article number 5868.

55. Gjorgievska VS, Karanfilova IC, Trajkovska A, Karapandzova M, Petrovska BB, Kulevanova S, Stefkov G. Monitoring of cannabis cultivar technological maturity by trichome morphology analysis and HPLC phytocannabinoid content. *Pharmacog Res.* 2023; 15: 94-100.

56. Gallo M, Formato A, Ciaravolo M, Formato G, Naviglio D. Study of the kinetics of extraction process for the production of hemp inflorescences extracts by means of conventional maceration (CM) and rapid solid-liquid dynamic extraction (RSLDE). *Separations.* 2020; 7: article number 20.

57. Sofras FMS, Alonso R, Retta DS, Lira PDL, Desimone MF, van Baren CM. Development and validation of a simple, fast, and accessible HPLC-UV method for cannabinoids determination in *Cannabis sativa* L. extracts and medicinal oils. *Curr Pharm Des.* 2023; 29: 1918-1928.

58. Brighenti V, Marchetti L, Anceschi L, Protti M, Verri P, Pollastro F, Mercolini L, Bertelli D, Zanardi C, Pellati F. Separation and non-separation methods for the analysis of cannabinoids in *Cannabis sativa* L. *J Pharm Biomed Anal.* 2021; 206: article number: 114346.

59. Monton C, Tanpao T, Navakul C, Pengkum T, Santasanasuwan S, Suksaeree J, Chaoenchai L, Songsak T. Cannabidiol, Δ^9-tertrahydrocannabinol and cannabinol contents of *Cannabis sativa* L. inflorescences claimed to be Hang Rog Phu Phan cultivar cultivated outdoors in various locations of Thailand. *Phytochem Lett.* 2023; 57: 126-132.

60. De Luca C, Buratti A, Krauke Y, Stephan S, Monks K, Brighenti V, Pellati F, Cavazzini A, Catani M, Felletti S. Investigating the effect of polarity of stationary and mobile phases on retention of cannabinoids in normal phase liquid chromatography. *Anal Bioanal Chem.* 2022b; 414: 5385-5395.

61. Anceschi L, Codeluppi A, Brighenti V, Tassinari R, Taglioli V, Marchetti L, Roncati L, Alessandrini A, Corsi L, Pellati F. Chemical characterization of non-psychoactive *Cannabis sativa* L. extracts, *in vitro* antiproliferative activity and induction of apoptosis in chronic myelogenous leukaemia cancer cells. *Phytotherap Res.* 2022; 36: 914-917.

62. Acquavia MA, Tesoro C, Pascale R, Ostuni A, Matera I, Bianca G, Scrano, L., Bufo, SA, Ciriello, R, Di Capua, A, Lelario F. Legal *Cannabis sativa* L. dried inflorescences: Cannabinoids contents and cytotoxic activity against human HepG2 cell line. *Appl Sci.* 2023; 13: article number 4960.

63. Mazuz M, Tiroler A, Moyal L, Hodak E, Nadarajan S, Vinayaka AC, Gorovitx-Haris B, Lubin I, Drori A, Drori G, Van Cauwenberghe O, Faigenboim A, Namdar D, Amitay-Laish I, Koltai H. Synergistic cytotoxic activity of cannabinoids from *Cannabis sativa* against cutaneous T-cell lymphoma (CTCL) *in vitro* and *ex vivo*. *Oncotarget.* 2020; 11: 1141-1156.

64. Martinenghi LD, Jonsson R, Lund T, Jenssen H. Isolation, purification and antimicrobial characterization of cannabidiolic acid and cannabidiol from *Cannabis sativa* L. *Biomolecules.* 2020; 10: article number 900.

65. Linciano P, Citti C, Russo F, Tolomeo F, Lagana A, Capriotti AL, Luongo L, Iannotta M, Belardo C, Maione S, Forni F, Vandelli MA, Gigli G, Cannazza G. Identification of a new cannabidiol n-hexyl homolog in a medicinal cannabis variety with an antinociceptive activity in mice: cannabidihexol. *Sci Reports.* 2020; 10: 22019.

66. Dreger M,Szalata M, Gorska-Paukszta M, Mankowska G, Oleszak G, Kwiatkowska E, Ozarowski M. Content of cannabinoids in clonally propagated industrial hemp. *J Nat Fibers.* 2023; 20: article number 2245968.

67. Blal K, Besser E, Procaccia S, Schwob O, Lerenthal Y, Tair JA, Meiri D, Benny O. The effect of cannabis plant extracts on head and neck squamous cell carcinoma and the quest for cannabis-based personalized therapy. *Cancers* 2023; 15: article number 407.

68. Bueno J, Greenbaum EA. (−)-*trans*-Δ^9-Tetrahydrocannabiphorol content of *Cannabis sativa* inflorescence from various chemotypes. *J Nat Prod.* 2021; 84: 531-536.

69. Hall DR, Sinclair JS, Bhuyan DJ, Khoo C, Li CG, Sarris J, Low M. Quality control of cannabis inflorescence and oil products: Response factors for the cost-efficient determination of ten cannabinoids by HPLC. *Talanta Open.* 2022; 5: article number 100112.

70. De Souza MR, Koetz M, Limberger RP, Henriques AT. DoE-assisted development and validation of a stabilityindicating HPLC-DAD method for simultaneous determination of five cannabinoids in *Cannabis sativa* L. based on analytical quality by design (AQbD) concept. *Phytochem Anal.* 2022; 33: 999-1017.

71. Fernandez S, Castro R, Lopez-Radcenco A, Rodriguez P, Carrera I, Garcia-Carnelli C, Moyna G. Beyond cannabinoids: Application of NMR-based metabolomics for the assessment of *Cannabis sativa* L. crop health. Front Plant Sci. 2023; 14: article number 1025932.

72. Giupponi L, Leoni V, Pavlovic R, Giorgi A. Influence of altitude on phytochemical composition of hemp inflorescence: A metabolomic approach. *Molecules.* 2020; 25: article number 1381.

73. Russo F, Tolomeo F, Vandelli MA, Biagini G, Lagana A, Capriotti AL, Cerrato A, Carbone L, Perrone E, Cavazzini A, Maiorano V, Gigli G, Cannazza G, Citti C. Enantioseparation of

chiral phytocannabinoids in medicinal cannabis. *J Chromatog B.* 2023; 1221: article number 123682.

74. Tzimas PS, Petrakis EA, Halabalaki M, Skaltsounis LA. Extraction solvent selection for *Cannabis sativa* L. by efficient exploration of cannabinoid selectivity and phytochemical diversity. *Phytochem Anal*. 2023; 35: 163-183.
75. Stasilowicz-Krzemien A, Sip S, Szulc P, Walkowiak J, Cielecka-Piontek J. The antioxidant and neuroprotective potential of leaves and inflorescences extracts of selected hemp varieties obtained with $scCO_2$. *Antioxidants* 2023; 12: article number 1827.
76. Fucak T, Kreft S, Svedruzic ZM, Tavcar E. Mechanism and kinetics of CBDA decarboxylation into CBD in hemp. *J Plant Biochem Biotechnol.* 2023; 32: 608-621.
77. Birenboim M, Chalupowicz D, Maurer D, Barel S, Chen Y, Fallik E, Paz-Kagan T, Rapaport T, Sadeh A, Kenigsbuch D, Shimshoni JA. Multivariate classification of cannabis chemovars based on their terpene and cannabinoid profiles. *Phytochem.* 2022; 200: article number 113215.
78. Birenboim M, Kenigsbuch D, Shimshoni JA. Novel fluorescence spectroscopy method coupled with N-PLS-R and PLS-DA models for the quantification of cannabinoids and the classification of cannabis cultivars. *Phytochem Anal.* 2023; 34: 280-288.
79. Seo C, Jeong M, Lee S, Kim EJ, Rho S, Cho M, Lee YS, Hong J. Thermal decarboxylation of acidic cannabinoids in Cannabis species: identifcation of transformed cannabinoids by UHPLC-Q/TOF-MS. *J Anal Sci Technol.* 2022; 13: article number 42.
80. Cheng Y, Ning K, Chen Y, Hou C, Yu H, Yu H, Chen S, Guo X, Dong L. Identification of histone acetyltransferase genes responsible for cannabinoid synthesis in hemp. *Chinese Med.* 2023; 18: article number 16.
81. Cicaloni V, Salvini L, Vitalini S, Garzoli S. Chemical profiling and characterization of different cultivars of *Cannabis sativa* L. inflorescences by SPME-GC-MS and UPLC-MS. *Separations.* 2022; 9: article number 90.
82. Spano M, Di Matteo G, Ingallina C, Botta B, Quaglio D, Ghirga F, Balducci S, Cammarone S, Campiglia E, Giusti AM, Vinci G, Rapa M, Ciano S, Mannina L, Sobolev AP. A multimethodological characterization of *Cannabis sativa* L. inflorescences from seven dioecious cultivars grown in Italy: the effect of different harvesting stages. *Molecules.* 2021; 26: article number 2912.

83. Piccolella S, Formato M, Picoraro MT, Crescente G, Pacifico S. Discrimination of CBD-, THC- and CBC-type acid cannabinoids through diagnostic ions by UHPLC-HR-MS/MS in negative ion mode. *J Pharm Biomed Anal.* 2021; 201: article number 114125.

84. Ingallina C, Sobolev AP, Circi S, Spano M, Fraschetti C, Filippi A, Di Sotto A, Di Giacomo S, Mazzoccanti G, Gasparrini F, Quaglio D, Campiglia E, Carradori S, Locatelli M, Vinci G, Rapa M, Ciano S, Giusti AM, Botta, Ghirga F, Capitani D, Mannina L. *Cannabis sativa* L. inflorescences from Monoecious cultivars grown in central Italy: An untargeted chemical characterization from early flowering to ripening. *Molecules.* 2020; 25: article number 1908.

85. Erukainure OL, Matsabisa MG, Salu VF, Oyedemi SO, Oyenihi OR, Ibeji CU, Islam MS. *Cannabis sativa* L. (var. *indica*) exhibits hepatoprotective effects by modulating hepatic lipid profile and mitigating gluconeogenesis and cholinergic dysfunction in oxidative hepatic injury. *Front Pharmacol.* 2021; 12: article number 705402.

86. De Vita S, Finamore C, Chini MG, Saviano G, De Felice V, De Marino S, Lauro G, Casapullo A, Fantasma F, Trombetta F, Bifulco G, Iorizzi M. Phytochemical analysis of the methanolic extract and essential oil from leaves of industrial hemp futura 75 cultivar: Isolation of a new cannabinoid derivative and biological profile using computational approaches. *Plants*. 2022; 11: article number 1671.

87. Kargili U, Aytac E. Supercritical fluid extraction of cannabinoids (THC and CBD) from four different strains of cannabis grown in different regions. *The J Supercritical Fluids.* 2022; 179: article number: 105410.

88. Kim AL, Yun YJ, Choi HW, Hong C-H, Shim HJ, Lee JH, Kim Y-C. Profiling cannabinoid contents and expression levels of corresponding biosynthetic genes in commercial cannabis (*Cannabis sativa* L.) cultivars. *Plants.* 2022; 11: article number 11223088.

89. Abdollahi M, Sefidkon F, Peirovi A, Calagari M, Mpusavi A. Assessment of the cannabinoid content from different varieties of *Cannabis sativa* L. during the growth stages in three regions. *Chem Biodivers.* 2021; 18: article number e2100247.

90. Kalinova J, Vrchotova N, Triska J, Hellerova S. Industrial hemp (*Cannabis sativa* L.) as a possible source of cannabidiol*. J Central Eur Agric.* 2021; 22: 110-118.

91. Li CR, Yang LX, Guo ZF, Yang H, Zhang Y, Wang YM, Zhang GZ, Li P, Gao W. LC-MS-based untargeted metabolomics reveals chemical differences of cannabis leaves from different regions of China. *Indust Crops Prod.* 2022; 176: article number 114411.

92. Toloza H, Buitrago OY, Orjuela A, Santaella MA, Hurtado AM, Arturo DE. Solvent extraction of *Cannabis sativa* under cryogenic conditions. *Sep Pur Technol.* 2024; 329: article number 124906.
93. Pannico A, Kyriacou MC, El-Nakhel C, Graziani G, Carillo P, Corrado G, Ritieni A, Rouphael Y, De Pascale S. Hemp microgreens as an innovative functional food: Variation in the organic acids, amino acids, polyphenols, and cannabinoids composition of six hemp cultivars. *Food Res Int.* 2022; 161: article number 111863.
94. Nuapia Y, Maraba K, Tutu H, Chimuka L, Chukrowska E. *In situ* decarboxylation-pressurized hot water extraction for selective extraction of cannabinoids from *Cannabis sativa*. Chemometric approach. *Molecules.* 2021; 16: article number 3343.
95. Boumrah Y, Baroudi S, Kecir M, Bouanani S. Characterization of Algerian-seized hashish over eight years (2011–2018). Part II: Chemical categorization. *J Forensic Scs.* 2020; 65: 1845-1851.
96. Boumrah Y, Kermouche W, Zarita B, Drardia E, Bouanani S. Hashish seizures in Algeria over the 2019–2020 period: The rise of potent hashish hybrids. *J Forensic Scs.* 2022; 67: 889-898.
97. Johnson DA, Hogan M, Marriot R, Heaney LM, Bailey SJ, Clifford T, James LJ. A comparison of advertised versus actual cannabidiol (CBD) content of oils, aqueous tinctures, e-liquids and drinks purchased in the UK. *J Cannabis Res.* 2023; 5: article number 58.
98. Viviers HJ, Petzer A, Gordon R. An assessment of the potency related to Cannabis-based products in the South African market. *Forensic Sci Int.* 2021; 322: article number 110754.
99. Rzeppa S, Grose J, Rautenberg C, Thieme D, Lepp T, Vallimae H, Keiler AM. Emergence of the less common cannabinoid Δ^8-tetrahydrocannabinol in a doping sample. *Drug Test Anal.* 2021; 13: 1936-1943.
100. Dei Cas M, Casagni E, Saccardo A, Arnoldi S, Young C, Scotti S, de Manicor EV, Gambaro V, Roda G. The Italian panorama of cannabis light preparation: Determination of cannabinoids by LC-UV. *Forensic Sci Int.* 2020; 307: article number 110113.
101. Buttenbender S, Carlos G, Steppe M, Ortiz RS, Limberger RP, Mendez ASL. Fast and reliable profiling of cannabinoids in seized samples using the method of HPLC–DAD followed by chemometrics. *Forensic Toxicol.* 2022; 40: 407-413.

102. Bongiorno P, Lopalco A, Casiraghi A, Spennacchio A, Pitruzzella A, Lopedota AA, Minghetti P, Denora N. Digital technologies applied to control the one-step process of cannabis olive oil preparations. *Pharmaceuticals.* 2023; 15: article number 870.
103. Raslan-Jaramillo JJ, Rios-Gajardo GA, Avello MA, de Diego MG. Determination of cannabinoids in *Cannabis sativa* oil and infused ice cream by LC-DAD method. *J AOAC Int.* 2024; 107: 140-145.
104. Ternelli M, Brighenti V, Ancheschi L, Poto M, Bertelli D, Licata M, Pellati F. Innovative methods for the preparation of medical Cannabis oils with a high content of both cannabinoids and terpenes. *J Pharm Biomed Anal.* 2020; 186: article number 113296.
105. Madej K, Kozka G, Winiarski M, Piekoszewski W. A simple, fast, and green oil sample preparation method for determination of cannabidioloic acid and cannabidiol by HPLC-DAD. *Separations*. 2020; 7: article number 60.
106. Madej K, Chmiolek A, Szlachta K, Piekoszewski W. HPLC-DAD analysis of hemp oil supplements for determination of four cannabinoids: Cannabidiol, cannabidiolic acid, cannabinol, and delta-9-tetrahydrocannabinol. *Separations*. 2021; 8: article number 227.
107. Blebea NM, Rambu D, Costache T, Negres S. Very fast RP–UHPLC–PDA method for identification and quantification of the cannabinoids from hemp oil. *Applied Sci.* 2021; 11: article number 9414.
108. Analakkattillam S, Langsi VK, Hanrahan JP, Moore E. Analytical method validation for assay determination of cannabidiol and tetrahydrocannabinol in hemp oil infused products by RP-HPLC. *Sci Reports.* 2022; 12: article number 12453.
109. Galant N, Czarny J, Powierska-Czarny J, Piotrowska-Cyplik A. Development and validation of the LC-MS/MS method for determination of 130 natural and synthetic cannabinoids in cannabis oil. *Molecules.* 2022; 27: article number 8601.
110. Piani B, Ferfula C, Bortolomeazzi R, Verardo G, Baldini M. Development and optimization of an HPLC-PDA method for the determination of major cannabinoids in hemp (*Cannabis sativa* L.) essential oil obtained by hydrodistillation. *Food Anal Methods.* 2022; 15: 1677-1686.
111. Li H, Zhao Q, Chang S, Wang L, Zhao B. Phytochemical analysis and bioactivity of different ethanolic extracts from cannabidiol full-spectrum oil. *J Mol Liq.* 2023; 372: article number 121173.

112. Kosovic E, Sykora D, Kuchar M. Stability Study of Cannabidiol in the Form of Solid Powder and Sunflower Oil Solution. *Pharmaceutics.* 2021; 13: article number 412.
113. Reddy PG, Bar-Hai A, Hoffman A, Feldmann SR, Domb AJ. Novel phenolate salts of bioactive agents: Cannabidiol phenolate salts. *Biorg Chem.* 2023; 141: article number 106914.
114. De Luca C, Buratti A, Umstead W, Franco P, Cavazzini A, Felletti S, Catani M. Investigation of retention behavior of natural cannabinoids on differently substituted polysaccharide-based chiral stationary phases under reversed-phase liquid chromatographic conditions. *J Chromatog A.* 2022; 1672: article number 463076.
115. Roussel JM, Schelling C, Righezza M, Veuthey JL. Application of prediction intervals to the interpretation of the robustness study of a UHPLC method for the separation of cannabinoids. *J Pharm Biomed Anal.* 2022; 220: article number 114977.
116. Jaren C, Zambrana PC, Perez-Roncal C, Lopez-Maestresalas A, Abrego A, Arazuri S. Potential of NIRS technology for the determination of cannabinoid content in industrial hemp (*Cannabis sativa* L.). *Agronomy.* 2022; 12: article number 938.
117. Sun DM, Song L, Wang HY, Yu CC, Chu Q, Lan T, Zhang WB. Establishment of detection methods for five cannabinoids in hemp cosmetics based on HPLC. *Anal Sci.* 2021; 37: 1821-1824.
118. Tolomeo F, Russo F, Vandelli MA, Biagini G, Capriotti AL, Lagana A, Carbone L, Gigli G, Cannazza G, Citti C. HPLC-UV-HRMS analysis of cannabigerovarin and cannabigerobutol, the two impurities of cannabigerol extracted from hemp. *J Pharm Biomed Anal.* 2021; 203: article number 114215.
119. Quinones R, Moreno S, Smythers AL, Sullins C, Pijor H, Brwon G, Trouten A, Richards-Waugh LL, Siddig A. Quantification of cannabis in infused consumer products and their residues on skin. *ACS Pharmacol Transl Sci.* 2022a; 5: 642-651.
120. Quinones R, Casino-Negroni A, Pijor H, Moreno S, Suarez K, Westfall TD, Sullins C, Ivey S, Buxo JA. Analysis of cannabinoids in lotions using high-performance liquid chromatography. *J Chem Edu.* 2022; 99: 3558-3565.
121. Maggini V, Calvi L, Pelagatti T, Gallo ER, Civati C, Privitera C, Squillante F, Maniglia P, Di Candia D, Spampatti R, Firenzuoli F. An optimized terpene profile for a new medical cannabis oil. *Pharmaceuticals.* 2022; 14: article number 298.

122. Dantas ASCL, de Souza MN, de Lima PC, Lima MED, Santana DS, Maranho RLN, Colonese A, Ochs SM, Monteirao MA. Development of a methodology for the determination of cannabinoids in cannabis-based products for medicinal purposes. *Quim Nova.* 2023; 46: 282-289.
123. Galetis P, Williams M, Gordon R, Martin JH. A simple isocratic HPLC method for the quantification of 17 cannabinoids. *Aust J Chem.* 2021; 74: 453-462.
124. Patel B, Wene D, Fan Z. Qualitative and quantitative measurement of cannabinoids in cannabis using modified HPLC/DAD method. *J Pharm Biomed Anal.* 2017; 146: 15-23.
125. Miller OS, Elder Jr EJ, Jones KJ, Gidal BE. Analysis of cannabidiol (CBD) and THC in nonprescription consumer products: Implications for patients and practitioners. *Epilepsy and Behavior.* 2022; 127: article number 108514.
126. Mastinu A, Ascrizzi R, Ribaudo G, Bonini SA, Premoli M, Aria F, Maccarinelli G, Gianoncelli A, Flamini G, Pistelli L, Memo M. Prosocial effects of nonpsychotropic *Cannabis sativa* in mice. *Cannabis Cannabinoid Res.* 2022; 7: article number 0017.
127. Santos-Alvarez I, Perez-Lloret P, Gonzalez-Soriano J, Perez-Moreno M. An approach to the evaluation of the potency of cannabis resin in Madrid: A health hazard? *Addicciones.* 2023; 35: 279-288.
128. Carvalho VM, de Aguilar AFL, Baratto LC, Souza FLC, Rocha ED. Cannabinoids quantification in medicinal cannabis extracts by high performance liquid chromatography. *Quim Nova.* 2020; 43: 90-97.
129. Lajoie C, Doyen A, Feutry P, Gagnon D, Brisson G. Impact of emulsifiers for the nanoencapsulation with maltodextrin of cannabis oil by spray drying on the physicochemical properties and bioaccessibility of cannabinoids. *Food Func.* 2022; 13: 10320-10332.
130. Manca A, Palermiti A, Mulu J, De Vivo ED, Zeaiter S, Simiele M, De Nicolo A, Cantu M, Cusato J, D'Avolio A. A description of cannabinoid levels in cannabis oil by high-performance liquid chromatography-mass spectrometry in a reference laboratory of north Italy. *Phytomed.* 2022; 102: article number 154218.
131. Palermiti A, Cafaro A, Barco S, Bucchioni P, Franceschini P, Cusato J, De Nicolo A, Manca A, De Vivo ED, Russo E, Cechi F, Pigliasco F, Lillo F, Tripodi G, D'Avolio A, Cangemi G. Analysis of cannabinoids concentration in cannabis oil galenic preparations:

harmonization between three laboratories in northern Italy. *Pharmaceuticals.* 2021; 14: article number 462.

132. Ohtsuki T, Friesen JB, Chen S-N, McAlpine JB, Pauli GF. Selective preparation and high dynamic-range analysis of cannabinoids in "CBD Oil" and other *Cannabis sativa* preparations. *J Nat Prod.* 2022; 85: 634-646.
133. Pichini S, Mannocchi G, Gottardi M, Perez-Acevedo AP, Poyatos L, Papaseit E, Perez-Mana C, Farre M, Pacifici R, Busardo FP. Fast and sensitive UHPLC-MS/MS analysis of cannabinoids and their acid precursors in pharmaceutical preparations of medical cannabis and their metabolites in conventional and non-conventional biological matrices of treated individual. *Talanta*. 2020; 209: article number 120537.
134. Song L, Carlson S, Valenzuela G, Chao M, Pathipaka SB. Development of a validated method for rapid quantification of up to sixteen cannabinoids using ultra-high-performance liquid chromatography diode-array detector with optional electrospray ionization time-of-flight mass spectrometry detection. *J Chromatog A.* 2022; 1670: article number 462953.
135. Onishi T, Umstead WJ. The separation of cannabinoids on sub-2 μm immobilized polysaccharide chiral stationary phases. *Pharmaceuticals*. 2021; 14: article number 1250.
136. Aviram J, Atzmony D, Frenklakh A, Kroll A, Zaks I, Hazekamp A. THC degradation does not impair the accuracy of THC doses aerosolized by the metered-dose SyqeAir inhaler: a 24-month stability trial. *J Cannabis Res.* 2022; 4: article number 55.
137. Schwarzenberg A, Carpenter H, Wright C, Bayazeid O, Brokl M. Characterizing the degradation of cannabidiol in an e-liquid formulation. *Sci Reports.* 2022; 12: article number 20058.
138. Manca A, Chiara F, Mula J, Palermiti A, Maiese D, Zeaiter S, De Nicolo A, Imperiale D, De Filippis G, Vischia F, De Cori D, Cusato J, D'Avolio A. A new UHPLC-MS/MS method for cannabinoids determination in human plasma: A clinical tool for therapeutic drug monitoring. *Biomed Pharmacotherap.* 2022a; 156: article number 113899.
139. Sempio C, Almaraz-Quinones N, Jackson M, Zhao W, Wang GS, Liu Y, Leehy M, Knupp K, Klawitte J, Christians U, Klawitter J. Simultaneous quantification of 17 cannabinoids by LC–MS-MS in human plasma. *J Anal Toxicol.* 2022; 46: 383-392.

140. Yang G, Ge S, Singh R, Basu S, Shatzer K, Zen M, Liu J, Tu Y, Zhang C, Wei J, Shi J, Zhu L, Liu Z, Wang Y, Gao S, Hu M. Glucuronidation: Driving factors and their impact of glucuronide disposition. *Drug Metab Rev.* 2017; 49: 105-138.
141. Dybowski MP, Dawidowicz AL, Rombel M, Typek R. *J Pharm Biomed Anal.* 2023; 234: article number 115563.
142. Orfanidis A, Gika HG, Theodoridis G, Mastrogianni O, Raikos N. A UHPLC MS-MS method for the determination of 84 drugs of abuse and pharmaceuticals in blood. *J Anal Toxicol.* 2020; 45: 28-43.
143. Pigliasco F, Malaca S, Faro AFL, Tini A, Cangemi G, Cafaro A, Barco S, Riva A, Pisati A, Amadori E, Striano P, Tagliabracci A, Huestis MA, Busardo FP. Cannabidiol, Δ^9-tetrahydrocannabinol, and metabolites in human blood by volumetric absorptive microsampling and LC-MS/MS following controlled administration in epilepsy patients. *Front Pharmacol.* 2022; 13: article number 1038754.
144. Proenca P, Teixeira HM, Martinho B, Monteiro C, Franco J, Corte-Real F. LC–MS-MS-MS3 for the determination and quantification of Δ^9-tetrahydrocannabinol and metabolites in blood samples. *J Anal Toxicol.* 2023; 47: 606-614.
145. Pigliasco F, Barco S, Dubois S, Marchese F, Striano P, Lomonaco T, Mattioli F, Tripodi G, Cangemi G. Cannabidiol determination on peripheral capillary blood using a microsampling method and ultra-high-performance liquid chromatography tandem mass spectrometry with on-line sample preparation. *Molecules.* 2020; 25: article number 3608.
146. Marchioni C, Viera TM, Crotti AEM, Crippa JA, Queiroz MEC. In-tube solid-phase microextraction with a dummy molecularly imprinted monolithic capillary coupled to ultra-performance liquid chromatography-tandem mass spectrometry to determine cannabinoids in plasma samples. *Anal Chim Acta.* 2020; 1099: 145-154.
147. Cruz JC, Rosa MA, Mores L, Carasek E, de Souza Crippa JA, Figueiredo EC, Queriroz MEC. Magnetic restricted-access carbon nanotubes for SPME to determine cannabinoids in plasma samples by UHPLC-MS/MS. *Anal Chim Acta.* 2022; 1226: article number 340160.
148. Gherzi M, Milano G, Fucile C, Calevo MG, Mancardi MM, Nobili L, Astuni P, Marini V, Barco S, Cangemi G, Manfredini L, Mattioli F, De Grandis E. Safety and pharmacokinetics of medical cannabis preparation in a monocentric series of young patients with drug resistant epilepsy. *Complement Therap Med.* 2020; 51: article number 102402.

149. Montilla CAP, Schaiquevich PS, Guido PC, Caraballo RH, Valenzuela GR, Cruz CV, Bournissen FG. An ultrafast ultrahigh-performance liquid chromatography coupled with tandem mass spectrometry method for cannabidiol monitoring in pediatric refractory epilepsy. *Therapeutic Drug Monitoring.* 2021; 43:712-717.
150. Pichini S, Malaca S, Gottardi M, Perez-Acevedo AP, Papaseit E, Perez-Mana C, Farre M, Pacifici R, Tagliabracci A, Mannochi G, Busardo FP. UHPLC-MS/MS analysis of cannabidiol metabolites in serum and urine samples. Application to an individual treated with medical cannabis. *Talanta.* 2021; 223: article number 121772.
151. Malaca S, Gottardi M, Pigliasco F, Barco S, Cafaro A, Amadori E, Riva A, Marcenaro M, Striano P, Cangemi G, Pacifici R, Pichini S, Bisardo FP. UHPLC-MS/MS analysis of cannabidiol and its metabolites in serum of patients with resistant epilepsy treated with CBD formulations. *Pharmaceuticals.* 2021; 14: article number 630.
152. Franco V, Palmisani M, Marchiselli R, Crema F, Fattore C, De Giorgis V, Varesio C, Rota P, Dibari VF, Perucca E. On-line solid phase extraction high performance liquid chromatography method coupled with tandem mass spectrometry for the therapeutic monitoring of cannabidiol and 7-hydroxy-cannabidiol in human serum and saliva. *Front Pharmacol*. 2022; 13: article number 915004.
153. Sempio C, Wymore E, Palmer C, Bunik M, Henthorn TK, Christians U, Klawitter J. Detection of cannabinoids by LC–MS-MS and ELISA in breast milk. *J Anal Toxicol.* 2021; 45: 686-692.
154. Calo L, Anzilloti L, Maccari C, Cecchi R, Andreoli R. Validation of bioanalytical method for the determination of synthetic and natural cannabinoids (new psychoactive substances) in oral fluid samples by means of HPLC-MS/MS. *Front Chem.* 2020; 8: article number 439.
155. Lin L, Amaratunga P, Reed J, Huang P, Lemberg BL, Lemberg D. Quantitation of Δ^8 -THC, Δ^9 -THC, cannabidiol and 10 other cannabinoids and metabolites in oral fluid by HPLC–MS-MS. *J Anal Toxicol.* 2022; 46: 76-88.
156. Gerace E, Bakanova SP, Di Corcica D, Salomone A, Vincenti M. Determination of cannabinoids in urine, oral fluid and hair samples after repeated intake of CBD-rich cannabis by smoking. *Forensic Sci Int.* 2021; 318: article number 110561.
157. Faro AFL, Venanzi B, Pilli G, Ripani U, Basile G, Pichini S, Busardo FP. Ultra-high-performance liquid chromatography-tandem mass spectrometry assay for quantifying

THC, CBD and their metabolites in hair. Application to patients treated with medical cannabis. *J Pharm Biomed Anal.* 2022; 217: article number 114841.

158. Mannocchi G, Di Trana A, Tini A, Zaami S, Gottardi M, Pichini S, Busardo FP. Development and validation of fast UHPLC-MS/MS screening method for 87 NPS and 32 other drugs of abuse in hair and nails: application to real cases. *Anal Bioanal Chem.* 2020; 412: 5125-5145.
159. Liu P, Liu W, Qiao H, Jiang S, Wang Y, Chen J, Su M, Si B. Simultaneous quantification of 106 drugs or their metabolites in nail samples by UPLC-MS/MS with high-throughput sample preparation: Application to 294 real cases. *Anal Chim Acta.* 2022; 1226: article number: 340170.
160. Liu Y, Dong T, Yan K, Wang Z, He G, Zhang Y, Ma C, Liu L, Chang W, Zhang L. A comprehensive and high-throughput screening method for multiple prohibited substances by UPLC-QE Plus-HRMS and HPLC-QQQ-MS in human urine for doping control. *Anal Methods.* 2023; 15: 3206-3224.
161. Rosano TG, Cooper JA, Schplz KL, Wood M. Confirmation of cannabinoids in forensic toxicology casework by isomer-selective UPLC–MS-MS analysis in urine. *J Anal Toxicol.* 2023; 47: 709-718.
162. Sim YE, Kim JW, Ko BJ, Kim JY, Cheong JC, Pyo J. Determination of urinary metabolites of cannabidiol, Δ8 -tetrahydrocannabinol, and Δ^9 -tetrahydrocannabinol by automated online μSPE–LC–MS/MS method. *J Chromatograph B.* 2023; 1214: article number 123568.
163. Giordano G, Biehler-Gomez L, Seneci P, Cattaneo C, Di Candia D. Detecting drugs in dry bone: a pilot study of skeletal remains with a post-mortem interval over 23 years. *Int J Legal Med.* 2021; 135: 457-463.
164. Hassenberg C, Calusen F, Hoffmann G, Studer A, Schurenkamp J. Investigation of phase II metabolism of 11-hydroxy-Δ-9-tetrahydrocannabinol and metabolite verification by chemical synthesis of 11-hydroxy-Δ-9-tetrahydrocannabinol-glucuronide. *Int J Legal Med.* 2020; 134: 2105-2119.
165. Zhao Y, Sepehr E, Vaught C, Yourick J, Sprando RL. Development and validation of a fit-for-purpose UHPLC-ESI-MS/MS method for the quantitation of cannabinoids in different matrices. *J Chromatog.* 2023; 1218: article number 123629.

166. Nicolaou AG, Stavrou IJ, Louppis AP, Constantinou MS, Kapnissi-Christodoulou C. Application of an ultra-performance liquid chromatography-tandem mass spectrometric method for the detection and quantification of cannabis in cerumen samples. *J Chromatograph A.* 2021; 1642: article number 462035.

167. Christodoulou MC, Constantinou MS, Louppis AP, Christou A, Stavrou IJ, Kapnissi-Christodoulou CP. Determination of cannabinoids in human cerumen samples by use of UPLC-MS/MS as a potential biomarker for drug use. *J Pharm Biomed Anal.* 2023; 231: article number 115412.

168. Kleinhenz MD, Magnin G, Lin Z, Griffin J, Kleinhenz KE, Montgomery S, Curtis A, Martin M, Coetzee JF. Plasma concentrations of eleven cannabinoids in cattle following oral administration of industrial hemp (*Cannabis sativa*). *Sci Reports.* 2020; 10: article number 12753.

169. Polidoro D, Temmerman R, Devreese M, Charalambous M, Van Ham L, Cornelis I, Broeckx BJG, Mandigers PJJ, Fischer A, Storch J, Bhatti SDM. Pharmacokinetics of cannabidiol following intranasal, intrarectal and oral administration in healthy dogs. *Front Vet Sci.* 2022; 9: article number 899940.

170. Fitzerald AH, Zhang Y, Fritz S, Whitehouse WH, Brabson T, Pohlman L, Cernicchiaro N, Tonozzi C, Ensley S. Detecting and quantifying marijuana metabolites in serum and urine of 19 dogs affected by marijuana toxicity. *J Vet Diagnostic Investigation.* 2021; 33: 1002-1007.

171. Fitzerald AH, Magnin G, Pace E, Bischoff K, Pinn-Woodcock T, Vin R, Myhre M, Comstock E, Ensley S, Coetzee JF. Marijuana toxicosis in 2 donkeys. *J Vet Diagnostic Investigation.* 2022; 34: 539-542.

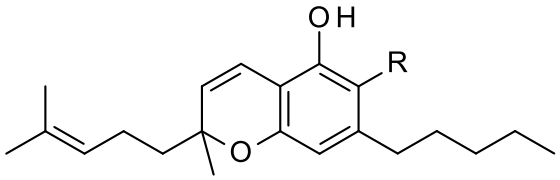
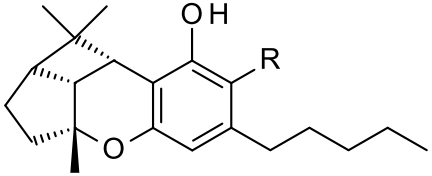
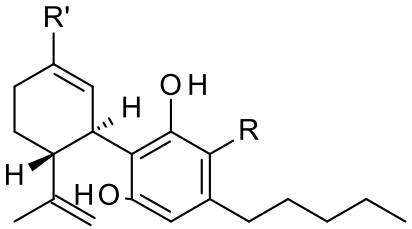
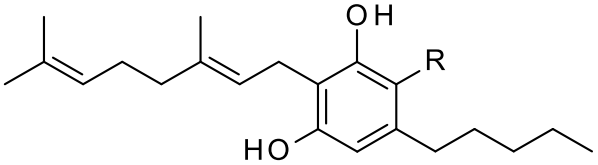
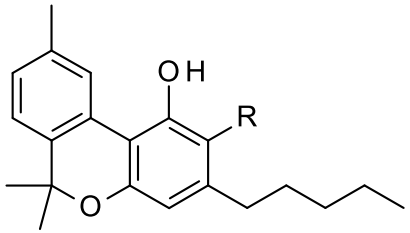
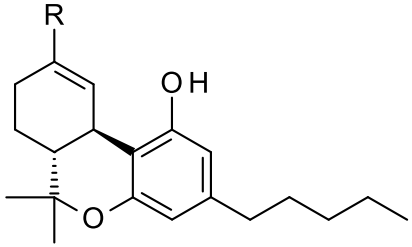
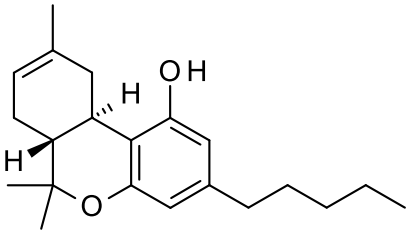
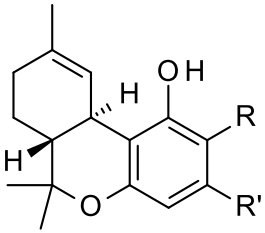
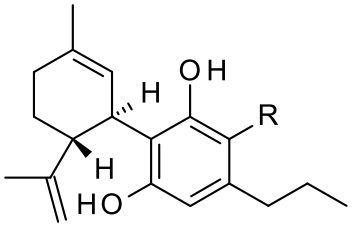
172. Upadhyay G, Fihurka O, Habecker C, Patel P, Sanchez-Ramos J. Measurement of Δ^9-THC and metabolites in the brain and peripheral tissues after intranasal instillation of a nanoformulation. *J Cannabis Res.* 2023; 5: article number 3.

173. Gazarov EA, Zequeira S, Senetra AS, Howard J, Sharma A, McCurdy CR, Lewis J, Bizon JL, Setlow B. Pharmacokinetics of delta-9-tetrahydrocannabinol following acute cannabis smoke exposure in mice; effects of sex, age and strain. *Front Pharmacol.* 2023; 14: article number 1227220.

174. Rao Q, Zhang T, Pu Q-L, Li B, Zhao Q, Yan D-M, Wu ZE, Li F. Comparative metabolism of THCA and THCV using UHPLC-Q-Exactive Orbitrap-MS. *Xenobiotica.* 2023; 53: 46-59.

175. Kanabus J, Bryla M, Roszko M, Modrzewska M, Pierzgalski A. Cannabinoids – Characteristics and potential for use in food production. *Molecules.* 2021; 26: article number 6723.
176. Kruger M, van Eeden T, Beswa D. *Cannabis sativa* cannabinoids as functional ingredients in snack foods – Historical and developmental aspects. *Plants.* 2022; 11: article number 3330.
177. Dawson DD, Martin RW. Investigation of chocolate matrix interference on cannabinoid analytes. *J Agric Food Chem.* 2020; 68: 5699-5706.
178. Andonova L, Ivanov E, Pencheva I, Konstantinov S. Elaboration and validation of HPLC/DAD method for quality control of products containing cannabidiol. *Curr Pharm Anal.* 2021; 17: 1218-1223.
179. Trovato E, Arena K, La Tella R, Rigano F, Vinci RL, Dugo P, Mondello L, Guarnaccia. Hemp seed-based food products as functional foods: A comprehensive characterization of secondary metabolites using liquid and gas chromatography methods. *J Food Composition Anal.* 2023; 117: article number 105151.
180. Shao M, Yu X, Huang L, Yao H, Li S. Determination of eight cannabinoids in foods with enhanced matrix removal liquid adsorbent by ultra performance liquid chromatography-tandem mass spectroscopy. *Chinese J Chromatog.* 2023; 41: 426-433.
181. Piccolella S, Crescente G, Formato M, Pacifico S. A cup of hemp coffee by moka pot from southern Italy: An UHPLC-HRMS Investigation. *Foods.* 2020; 9: article number 1123.
182. Zhu Z, Wang L, Jia Y, Duan S, Li S, Jiang L, Lin X, Yan F, Hou C, Hu C, Di B. Magnetic liposomes infused with GPCR-expressing cell membrane for targeted extraction using minimum organic solvent: An investigative study of trace THC in sewage. *Anal Chem.* 2023; 95: 12613-12622.
183. Foppe KS, Kujawinski EB, Duvallet C, Endo N, Erikson TB, Chai PR, Matus M. Analysis of 39 drugs and metabolites, including 8 glucuronide conjugates, in an upstream wastewater network via HPLCMS/MS. *J Chromatog B Analyt Technol Biomed Life Sci.* 2021; 1176: article number 122747.
184. Milan S, Lelario F, Scrano L, Ottati C, Bufo SA, Alpendurada FDM. Detection of eight cannabinoids and one tracer in wastewater and river water by SPE-UPLC–ESI-MS/MS. *Water*. 2022; 14: article number 588.

185. Chen S, Qie Y, Hua Z, Zhang H, Wang Y, Di B, Su M. Preparation of poly(methacrylic acid-co-ethylene glycol dimethacrylate)-functionalized magnetic polydopamine nanoparticles for the extraction of six cannabinoids in wastewater followed by UHPLC-MS/ MS*. Talanta.* 2023; 264: article number 124752.

 Cannabichromene (**1**, CBC) R = H Cannabichromenic acid (**2**, CBCA) R = COOH	 Cannabicyclol (**3**, CBCL) Cannabicyclolic acid (**4**, CBCLA)	 Cannabidiol (**5**, CBD) R = H, R' = Me Cannabidiolic acid (**6**, CBDA) R = COOH, R' = Me 7-Carboxy-cannabidiol (**7**, 7-COOH-CBD) R = H, R' = COOH
 Cannabigerol (**8**, CBG) R = H Cannabigerolic acid (**9**, CBGA) R = COOH	 Cannabinol (**10**, CBN) R = H Cannabinolic acid (**11**, CBNA) R = COOH	 11-Hydroxy-tetrahydrocannabinol (**12**, 11-OH-THC; R = CH_2OH) 11-Nor-9-carboxy-THC (**29,** 11-COOH-THC or THC-COOH; R = COOH)
 *trans*-(8)-Tetrahydrocannabinol (**13**, Δ^8-THC)	 Δ^9-Tetrahydrocannabinol	 Cannabidivarin (**18**, CBDV) R = H Cannabidivarinic acid (**19**, CBDVA) R = COOH

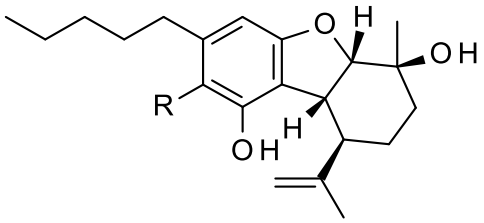
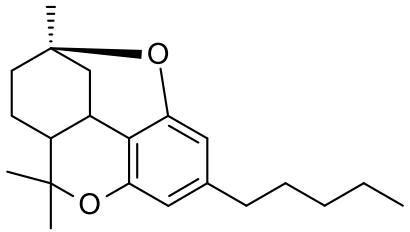
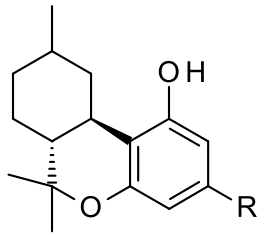
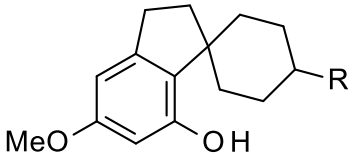
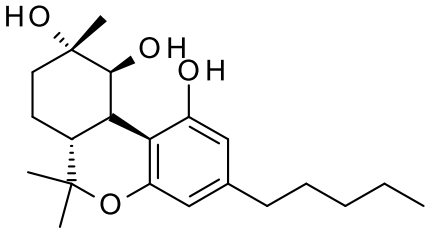
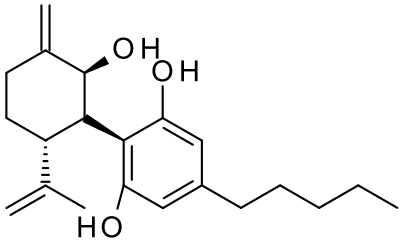
	(**14**, Δ^9-THC or THC) R = H, R ' = C_5H_{11} Δ^9-Tetrahydrocannabinolic acid (**15**, THCA) R = COOH, R' = C_5H_{11} Tetrahydrocannabivarin (**16**, THCV) R = H, R' = C_3H_7 Tetraydrocannabivarinic acid (**17,** THCVA) R = COOH, R' = C_3H_7	
 Cannabielsoin (**20,** CBE) R = H Cannabielsoic acid (**21,** CBEA) R = COOH	 Cannabicitran (**22**, CBT)	 Hexahydrocannabinol (**23**, HHC) R = C_5H_{11} Hexahydrocannabiphorol (**24**, HHCP) R = C_7H_{15}
 α-Cannabispirol (**25**) R = α-OH β-Cannabispirol (**26**) R = β-OH	 Cannabiripsol (**27**)	 Cannabitriol (**28**)

FIGURE 1 Major phytocannabinoids in different matrices analysed by HPLC

TABLE 1 HPLC and UPLC (or UHPLC) methods for the analysis of cannabinoids in *Cannabis sativa* L. plant samples

Plant parts	Instrumentation	Column	Mobile phase	Detection	Cannabinoids analysed/detected
HPLC methods					
Aerial parts	Acquity UPLC H-class system	Poroshell 120 EC-C_{18} column (150 mm × 2.1 mm, 2.7 μm) coupled with a Poroshell 120 EC-C_{18} guard column (5 mm × 2.1 mm, 2.7 μm)	Column temperature = 30°C; injection volume = 1 μL.	PDA set at 214 nm	CBC (**1**), CBD (**5**), CBDA (**6**), CBG (**8**), CBGA (**11**), CBN (**10**), Δ^8 -THC (**13**), Δ^9 -THC (**14**) and Δ^9-THCA (**15**)[13]
	Agilent 1200 series HPLC coupled with a DAD	Poroshell column 120 SB-C_{18} (150 mm x 4.6 mm, 2.7 μm)	Mobile phase: A = 0.1% HCOOH in water and B = 0.1% HCOOH in ACN. Gradient elution: 0–8 min, 65% B in A; 8–12 min, 65–95% B in A; and 12–13 min, 95% B in A. After each run, a 5 min column re-equilibration was carried out. Flow rate = 0.5 mL/min; injection volume = 2 μL	DAD set at 214 nm	CBD (**5**), CBG (**8**), CBN (**10**), Δ^9-THC (**14**) and Δ^9-THCA (**15**)[14]

	Hewlett-Packard 1100 HPLC	Ascentis Express C_{18} column (150 mm × 3.0 mm, 2.7 μm)	A mobile phase composed of 0.1% formic acid in water with ammonium formate at different concentrations (2, 5 and 8 mM) (A) and 0.1% HCOOH in ACN (B). Column temperature = 25^0C and injection volume 6 μL.	DAD at 210 and 220 nm using (Design of Experiment) DoE	CBC (**1**), CBCA (**2**), cannabichromevarin (CBCV), CBD (**5**), CBDA (**6**), CBDV (**18**), CBG (**8**), CBGA (**9**), cannabigerovarin (CBGV), CBN (**10**), cannabinerol (CBNR), Δ^8-THC (**13**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[15]
	Agilent Technologies 1200 series LC system equipped with an Agilent Technologies 6410 series triple quadrupole mass analyzer			ESI-MS/MS (negative ion mode) using DoE	
	Shimadzu Nexera ×2 UHPLC system coupled with an SPD-M20A PDA in series with a Shimadzu LCMS8040 triple quadrupole system with an ESI source	Ascentis Express RP-C_{18} column (150 mm × 2.1 mm, 2.7 μm)	Mobile phase A = water/HCOOH (99.9:0.1) and B = ACN/HCOOH (99.9:0.1). Gradient elution: 0 to 2 min 15% B in A, 2 to 52 min 15% to 86% B, and 52 to 55 min 86% B. The total analysis time, including pre- and	DAD set at 220 nm. ESI-MS in positive and negative ion modes	CBCA (**2**), CBD (**5**), CBDA (**6**), CBG (**8**), CBGA (**9**), CBNA (**11**) and Δ^9-THC (**14**)[16]

			post-running, was 67 min. Flow rate = 0.4 mL/min; column temperature = 30°C.		
	Shimadzu HPLC	Ascentis C_{18} column (250 mm × 2.1 mm, 5 µm)	The mobile phase consisted of water and MeOH. The column was equilibrated with 68% MeOH and then eluted with a linear gradient, increasing from 68 to 90.5% MeOH in water over 35 min, then 95% over 1 min. After 3 min, the column was adjusted to the initial conditions and re-equilibrated under that condition for 16 min. Run time = 56 min; flow rate =	UV detection at 230 nm	CBD (**5**) and Δ^9 -THC (**14**)[17]

			0.3 mL/min; column temperature = 30°C; injection volume = 30 μL.		
	Shimadzu HPLC	SunFire™ C_{18} column (150 mm x 3 mm, 3.5 μm) connected to a guard column (10 mm x 3 mm)	Mobile phase: water containing 0.1% formic acid (A), CAN containing 0.1% formic acid (B); 70% B (0–2 min), 70–77% B (2–20 min), 77–100% B (20– 25 min), 100% B (25–29 min) at a flow rate of 0.3 mL/min. Injection volume 2 μL.	HPLC-PDA-ELSD-ESIMS detection. PDA set at 225 nm	CBC (**1**), CBD (**5**), CBDA (**6**), CBG (**8**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[18]
Aerial parts (Hemp)	Finnigan Surveyor HPLC system equipped with a PDA detector	Luna C_{18} (2) column (150 mm × 3 mm, 3 μm)	The mobile phase was isocratic and consisted of water/ACN (9:31), with 0.1% HCOOH and 10 mM ammonium formate. Column temperature =	PDA set at 275 nm	CBC (**1**), CBD (**5**), CBDA (**6**), CBDV (**18**), CBG (**8**), CBGA (**9**), CBN (**10**), Δ^8-THC (**13**), Δ^9-THC (**14**), Δ^9-THCA (**15**) and THCV (**16**).[19]

			37°C; flow rate = 0.8 mL/min; injection volume = 5 μL; run time = 8.5 min.		
	Shimadzu Nexera-i LC-2040C UHPLC coupled to an LCMS-8040 tandem mass spectrometry detection with an ESI source	Raptor ARC-18 column (150 mm × 3.0 mm, 2.7 μm) connected to a Restek guard column	The mobile phase comprised A = water with 0.1% HCOOH and 4 mM ammonium formate and B = ACN with 0.1% HCOOH. Gradient elution: 0–15 min, 75% B in A; 15–18 min, 75–100% B in A; 18–20 min, 100–75% B in A; 20–23 min, 75% B in A. Column temperature = 30°C; injection volume = 10 μL; flow rate = 0.4 mL/min; run time = 23 min.	ESI-MS/MS in positive and negative ion mode	CBD (**5**), CBDA (**6**), Δ^9-THC (**14**), Δ^9-THC-d_3 and Δ^9-THCA (**15**).[20]

Aerial parts (fibre-type *Cannabis* sp.)	Shimadzu UHPLC XR coupled to a PDA	Ascentis Express RP-C_{18} column (100 mm × 2.1 mm, 2.7 μm)	Mobile phase A = 0.1% HCOOH in water and B = 0.1% HCOOH in ACN. Gradient elution: 0–3 min 5% B in A, 3–20 min 5–15% B in A, 20–30 min 15–25% B in A, 30–42 min 25–75% B in A, 42–52 min 75–100% B in A, 52–53 min 100% B with a flowrate of 0.4 mL/min.	PDA at 220 nm	CBD (**5**) and Δ^9-THC (**14**).[21]
Aerial parts of the hemp plant comprising the inflorescences, maturing seed heads and some upper leaves at three growth stages	Shimadzu HPLC-PDA	Luna C_{18} column (150 mm × 4.6 mm, 5 μm)	The mobile phase comprised an 80% ACN in 25 mM ammonium formate solution, and an isocratic run was performed. Flow rate = 1 mL/min; run time = 15 min; column	PDA set at 228 nm	CBC (**1**), CBD (**5**), CBDA (**6**), CBG (**8**), CBGA (**9**), Δ^9 -THC (**14**) and Δ^9-THCA (**15**).[22]

			temperature = 30°C.		
Aerial parts from *in vitro* cultivation	Shimadzu Proeminence UFLC coupled with an SPD-M20A PDA	Kinetex C_{18} column (100 mm × 2.1 mm, 5 µm)	Mobile phase: A = 0.1% formic acid in water and B = MeOH. Gradient elution: 0–1 min, 60% B in A; 1–3 min, 80% B in A; 3–5 min, 85% B in A; 5–6.5 min, 90% B in A, 6.5–7.0 min, 60% B in A. Flow rate = 0.5 mL/min; injection volume = 5 µL.	DAD set at 220 nm	CBD (**5**), CBDA (**6**), CBN (**10**), Δ^8-THC (**13**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[23]
Biomass	Agilent 1290 Infinity II LC System	Poroshell 120 EC-C_{18} column (150 mm × 2.1 mm, 2.7 mm) linked to a Poroshell 120 EC-C_{18} guard column (5 mm × 2.1 mm, 2.7 µm)	Mobile phase: 0.1% HCOOH in water (A) and 01.% HCOOH in ACN (B). HPLC run commenced with isocratic elution with 66% B for 8 min, a linear gradient to 95% B over the next 4 min, maintained at 95%	DAD at 214 nm	CBC (**1**), CBCA (**2**), CBD (**5**), CBDA (**6**), CBDV (**18**), CBDVA (**19**), CBG (**8**), CBGA (**9**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[28,29]

			B for 1 min, and then the column was re-equilibrated at 66% B for 4 min. Injection volume = 1.5 μL; flow rate = 0.5 mL/min; run time = 17 min.		
	Agilent 1260 HPLC	Reversed-phase Luna C_{18}(2) column	Isocratic elution. 75% ACN and 0.1% HCOOH in water.	PDA set at 220 nm	CBC (**1**), CBCA (**2**), CBCL (**3**), CBD (**5**), CBDA (**6**), CBDV (**18**), CBDVA (**19**), CBG (**8**), CBGA (**9**), CBN (**10**), Δ^8-THC (**13**) Δ^9-THC (**14**), Δ^9-THCA (**15**), THCV (**16**) and THCVA (**17**).[30]
	Dionex Ultimate 3000 HPLC-DAD system	Accucore C_{18} Polar Endcapped column (100 mm × 2.1 mm, 2.6 μm)	The mobile phase comprised 0.1% HCOOH in both water (A) and methanol (B). Gradient elution: started at 62% B, increasing to 66% B at 13.75 min, followed by an increase to 80% B at	DAD set at 210 and 220 nm	CBC (**1**), CBCA (**2**), CBD (**5**), CBDA (**6**), CBDV (**18**), CBDVA (**19**), CBG (**8**), CBGA (**9**), CBN (**10**), Δ^9-THC (**14**), Δ^9-THCA (**15**), THCV (**16**) and THCVA (**17**).[31]

			20 min. This was maintained for 4 min before returning to 62% B and equilibrating for 3 min. Column temperature = 50°C; flow rate 0.45 mL/min; injection volume = 2 µL; run time = 24 min.		
	Vanquish core system coupled with an Exploris 120 Orbitrap mass spectrometer	Poroshell EC-C_{18} column (100 mm x 3 mm, 2.7 µm)	A gradient elution starting from 95% solvent A (0.1% aqueous HCOOH) and 5% B (ACN with 0.1% HCOOH), linearly increased to 95% B in 20 min and held for 3 min; after a washing step at 98% B for 7 min, the system was re-equilibrated at the initial condition for 6 min. The flow rate	PDA set at 230 and 306 nm. HESI-MS in positive ion mode	CBC (**1**), CBCA (**2**), CBD (**5**), CBDA (**6**), CBG (**8**), CBGA (**9**), Δ^9-THC (**14**), Δ^9-THCA (**15**), *Cis*-Δ^9-THC and *Cis*-Δ^9-THCA.[32]

			= 0.5 mL/min, run time of 36 min and column temperature 30°C.		
Buds	Shimadzu Prominence-i LC-2030C Plus system	NexLeaf CBX for Potency C_{18} column (150 mm x 4.6 mm, 2.7 μm) coupled with a Zorbax Eclipse Plus-C_{18} narrow bore guard column (12.5 mm x 2.1 mm, 5 μm)	Mobile phase: 0.085% Phosphoric acid in water (A) and 0.085% phosphoric acid in MeOH (B). Gradient: 0−9 min, isocratic 68% B; 9.0−16.0 min, 68−92% B; 16.0−17.0 min 92−100% B. Column temperature = 50°C; flow rate = 1.6 mL/min; run time = 18 min.	DAD detection at 220 nm	CBC (**1**), CBD (**5**), CBDA (**6**), CBDV (**18**), CBG (**8**), CBGA (**9**), CBN (**10**), Δ^8 -THC (**13**), Δ^9 -THC (**14**), Δ^9-THCA (**15**) and THCV (**16**).[33]
Dried female flower buds	Smartline model HPLC system Knauer	Reversed-phase C_{18} column, coupled with a KNAUER guard column and pre-column	Isocratic elution with 80% MeOH in water with a flow rate of 1 mL/min. Injection volume =	PDA set at 220 nm	CBD (**5**) and Δ^9-THC (**14**).[34]

			20 μL; column temperature 30°C.		
Flowers	Agilent 1260 Infinity II HPLC system equipped with DAD	Reversed-phase C_{18} silica column (150 mm x 4.6 mm, 5 μm)	Gradient elution with water and ACN, both containing 0.1% HCOOH.	DAD set at 240 nm	Δ^9-THC (**14**), and Δ^9-THCA (**15**).[35]
	Agilent 1260 Infinity HPLC system equipped with VWD detector	Restek Raptor ARC_{18} reversed-phase column (150 mm x 4.6 mm, 2.7 μm)	Isocratic elution with 0.1% HCOOH/0.5 mM ammonium formate in water: 0.1% HCOOH/0.5 mM ammonium formate in ACN (1:3) at a 1.5 mL/min flow rate.	UV-PDA at 220 nm	CBC (**1**), CBCA (**2**), CBCL (**3**), CBG (**8**), CBN (**10**), CBNA (**11**), Δ^9-THC (**14**), THCV (**16**) and THCVA (**17**).[36]
	Shimadzu HPLC	Shim-pack XR-ODSII C_{18} chromatographic column (150 mm x 3 mm, 2.2 μm)	Mobile phases A and B were a mixture of water and MeOH, with phosphoric acid (99.93/0.07%). It started with 65% B (for 1 min, then, it gradually increased to 72% over 25 min,	UV detection at 220 nm	CBC (**1**), CBD (**5**), CBDA (**6**), CBDV (**18**), CBG (**8**), CBGA (**9**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[37]

			and after that, it increased to 95% for 5 min. After maintaining these conditions for 2 min, the initial ratio of mobile phases was adjusted and the column was re-equilibrated for 12 min. Flow rate = 1 mL/min; run time = 45 min; injection volume = 10 μL; column temperature = 50°C		
	Shimadzu LC-20A coupled with PDA	Hypersil BDS C_{18} column (150 mm x 4.6 mm, 5 μm)	The mobile phase comprised A = MeOH and B = 25 mM ammonium acetate solution. Gradient elution: 75% A: 1 min, 75% to 95% A in 15 min, 95% A: 2 min, 95% to 75% A in 2 min,	DAD set at 205 nm	CBD (**5**), CBDA (**6**), CBN (**10**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[38]

			and 75% A: 5 min. Total run time was 25 min. Flow rate = 1mL/min; run time = 25 min		
	Shimadzu Prominence-I LC2030 C3D	Shim-pack XR ODS-II (75 mm x 3 mm, 2.2 µm)	Mobile phase: A = 0.07% phosphoric acid in water and B = 0.07% phosphoric acid in MeOH. Gradient elution: started with 65% B, increased to 72% B in 25 min, then to 95% in 5 min, and after maintaining these conditions for 2 min the initial ratio of mobile phases was adjusted and re-equilibrated the column for 12 min. Column temperature = 50°C; flow rate = 1	DAD set at 224 nm	CBC (**1**), CBD (**5**), CBDA (**6**), CBDV (**18**), CBG (**8**), CBGA (**9**), CBN (**10**), Δ^8-THC (**13**), Δ^9-THC (**14**), Δ^9-THCA (**15**) and THCV (**16**).[39]

			mL/min; injection volume = 10 μL; run time = 45 min.		
Dried flowers	Agilent 1200 Series HPLC	Kinetex Evo C_{18} column (100 mm × 2.1 mm, 5 μm) connected to a Security Guard Ultra guard column	The mobile phase comprised 50 mM ammonium formate in water (A) and 100% MeOH (B). Injection volume = 2 μL; flow rate = 0.3 mL/min.	UV-DAD at 230 and 280 nm	CBD (**5**), CBDA (**6**), CBG (**8**), CBGA (**9**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[40]
	Agilent 1290 Infinity UHPLC system coupled with a TSQ Quantiva triple quadrupole mass spectrometer	Ace-3, C_{18}-amide column (100 mm × 2.1 mm, 3 μm) with a guard column (Ace-3, C_{18}-amide, 10 mm × 2.1 mm, 3 μm)	Mobile phase: A = 0.1% HCOOH in water and B = 0.1% HCOOH in ACN. Gradient elution: 0–5 min, 57–70% B in A; 5.0–11.0 min, 70– 75% B in A; 11.0–13.0 min, 75–80% B in A; 13.0–14.0 min, 80–95% B in A, followed by a 4.0-min wash at 98% B in A and column re-equilibration at 57% B in A for 4.0. Column temperature = 40°C; injection volume = 1 μL; flow	ESI-MS/MS in positive ion mode	CBC (**1**), CBCA (**2**), CBCL (**3**), CBCLA (**4**), CBD (**5**), CBDA (**6**), CBDVA (**19**), CBG (**8**), CBGA (**9**), CBN (**10**), CBNA (**11**), Δ^9-THC (**14**), Δ^9-THCA (**15**), THCV (**16**) and THCVA (**17**).[41]

			rate = 0.5 mL/min; run time = 21 min.		
	Waters 2695 separations HPLC module coupled to a Waters 2996PDA detector	Luna C_{18}(2) column (150 mm x 4.6 mm, 3 μm)	Isocratic elution with ACN–water–HCOOH 77:23:0.1. Flow rate = 1.2 mL/min; injection volume 10 μL and run time = 20 min.	UV-PDA at 220 nm	CBD (**5**), CBDA (**6**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[42]
Dried flowers and leaves	Thermo Fisher Scientific HPLC-PDA	C_{18} column (250 mm × 4.6 mm, 5.0 μm) from Advanced Chromatography Technologies	Mobile phase A = 50 mM ammonium formate buffer, pH 5.19, and B = MeOH. A gradient elution: a linear increase of 68%-85% B (0-10 min), 85%-92% B (10-20 min) and finally 95% B over 5 min. After 25 min, the column was set to the initial condition and re-equilibrated for 5 min. Run time = 30 min; flow rate = 1	UV-PDA at 240 nm	CBD (**5**), CBDA (**6**), CBN (**10**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[43]

			mL/min; column temperature = 30°C		
Flower tops	Agilent 1100 series LC chromatographic system	Kinetex C_{18} column (150 mm x 2.1 mm, 2.6 µm) equipped with a C_{18} guard column (2.1 mm)	Isocratic elution: 75% ACN in water with 0.1% HCOOH. Flow rate = 0.5 mL/min; run time = 30 min; column temperature = 20 °C and injection volume = 10 µL.	PDA detection at 230 nm	CBD (**5**), CBN (**10**), CBDA (**6**), Δ^8-THC (**13**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[44]
Inflorescences	Acquity UPLC-PDA	Fortis Speed Core C_{18} PFP column (100 mm x 2.1 mm, 2.6 µm)	Mobile phase = water (A) and ACN (B) both containing 0.1% HCOOH. Gradient elution: started with 60% B in A maintained for 0.5 min, then increased to 72% B in A in 3 min, further increased to 90% B in A in 2 min and maintained for 1.5 min, and finally decreased to 60% B	PDA at 210 and 225 nm	CBD (**5**), CBDA (**6**) and Δ^9-THC (**14**).[49]

			in A in 0.1 min. The solvent composition was held at the initial conditions until 10.0 min for re-equilibration of the column. Column temperature = 25°C; flow rate = 0.4 mL/min; injection volume = 0.3 μL.		
	Agilent 1200 HPLC	Raptor ARC-18 column (150 mm × 4.6 mm, 2.7 μm)	The mobile phase comprised 5 mM ammonium formate and 0.1% HCOOH in water (A) and 0.1% HCOOH in ACN (B). Isocratic elution with 75% B at a 1.5 mL/min flow rate. Injection volume = 5 μL; run time = 15 min.	UV-Vis detection at 228 nm	CBC (**1**), CBCA (**2**), CBD (**5**), CBDA (**6**), CBG (**8**), CBGA (**9**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[50]

	Agilent 1260 Infinity II HPLC system	Raptor ARC-18 column (150 mm x 4.6 mm, 2.7 μm)	Isocratic elution with 20% ACN in water with 5 mM ammonium formate and 0.1% HCOOH at a flow rate of 1.5 mL/min. Injection volume = 20 μL.	UV detection at 220 nm	CBC (**1**) and Δ^9-THC (**14**).[51]
	Agilent 1260 LC coupled with a Varian MS 500 ion trap mass spectrometer	Agilent XDB (250 mm × 4.6 mm, 5 μm)	Gradient elution: 1% HCOOH in water (A), ACN (B) and MeOH (C). The gradient started with 30% A and 70% B, and in 20 min arrived at 70% B and 30% C; in 23 min it reached 100% C and stayed isocratic up to 33 min.	DAD at 299-400 nm, and ESI-MS in positive (for neutral cannabinoids) and negative ion (for acidic cannabinoids) modes	CBD (**5**), CBDA (**6**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[52]
	Agilent 1260 Infinity II	InfinityLab Poroshell 120-C_{18} column (4.6 mm × 150 mm, 4 μm)	Phase A = 0.1% HCOOH in water and phase B = 0.05% HCOOH in MeOH. Gradient	UV at 210 and 230 nm	CBC (**1**), CBCA (**2**), CBD (**5**), CBDA (**6**), CBG (**8**), CBGA (**9**), CBN (**10**), CBDV (**18**) and CBDVA (**19**), Δ^9-THC (**14**), Δ^9-THCA (**15**) and THCVA (**17**).[53]

			elution: 0.00–7.05 min 40% A; 7.05–49.37 min 23% A; 49.37–67.00 min 5% A, followed by a post-run time of 10 min during which conditions were reset to baseline. Flow rate = 1 mL/min; injection volume = 35 µL; column temperature = 50°C. run time = 67 min.		
		Accucore C_{18} column (100 mm × 2.1 mm, 2.6 µm)	Phase A = 0.01% HCOOH in ACN and B = 0.01% HCOOH in water. Gradient elution: start when 35% A, after 8 min decrease to 20% A, and 4 min later decrease to 0% A. Column	PDA set at 230 nm	CBD (**5**), CBDA (**6**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[54]

			temperature = 50^{o}C; injection volume 1 μL; flow rate = 0.3 mL/min.		
	Agilent 1200	Poroshell 120 EC-C_{18} column (150 mm x 3 mm, 2.7 μm)	Aqueous *o*-phosphoric acid (8.64 g/L) (solvent A) and ACN (solvent B). A gradient elution: 0 – 16 min from 36% to 18% A linear gradient, 16 – 17 min 18% to 36% A linear gradient and from 17 to 30 min re-equilibration of the column with a flow rate of 0.7 mL/min. Column temperature = 40^{O}C	PDA set at 225 and 306 nm	Δ^9-THC (**14**).[55]
	Agilent HPLC with DAD	Agilent XDB column (250 mm × 4.6 mm, 5.0 μm)	The mobile phase comprised water (A) and ACN (B), both containing 0.1% trifluoro acetic acid (TFA). Gradient	DAD set at 215 nm	CBD (**5**), CBDA (**6**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[56]

			elution: 80% B in A for 3 min; 100% B at 20 min; held for 5 min. Rune time = 20 min; flow rate = 1 mL/min.		
	Agilent 1260 HPLC	ACE 3 C_{18}-PFP column (150 mm × 3.0 mm, 3 μm)	Isocratic elution. 75% ACN and 0.1% HCOOH acid in water.	UV detection at 228 nm	CBC (**1**), CBD (**5**), CBDA (**6**), CBG (**8**), CBN (**10**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[57]
	Agilent 1260 Infinity II HPLC system	Ascentis Express C_{18} column (150 mm x 3.0 mm, 2.7 μm)	Mobile phase: 0.1% HCOOH in water (A) and 0.1% HCOOH in ACN (B) Gradient elution: 0–13 min 60% B, 13–17 min from 60% to 80% B, 17–22 min from 80% to 90% B, which was kept for 8 min. The post-running time = 10 min; flow rate = 0.4 mL/min; injection volume = 3 μL	DAD detection at 210 and 220 nm	CBD (**5**), CBDA (**6**), CBG (**8**), CBGA (**9**), CBN (**10**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[58]
	Agilent 1260 Infinity	ACE 3 C_{18}-PFP column (150 mm × 3.0 mm, 3 μm)	Isocratic elution.	UV detection at 222 nm	CBD (**5**), CBN (**10**) and Δ^9-THC (**14**).[59]

			Water and Methanol (17:83). Flow rate = 0.4 mL/min; temp = 25°C; injection volume = 5 μL; run time 20 min.		
	AZURA HPLC system	Four normal-phase columns: 150 mm × 4.0 mm Eurospher II 100-5 columns packed with 5 μm fully porous particles (100 A pore size) have been ˚ tested: (i) NH_2, (ii) Diol, (iii) CN and iv) Si (bare silica).	Four mobile phase compositions 95:5 were used, isocratic: (i) Hept/IPA, (ii) Hept/EtOH, (iii) Hex/IPA and (iv) Hex/EtOH. Column temperature = 25°C and injection volume 5 μL.	UV detection at 228 nm	CBC (**1**), CBD (**5**), CBDV (**18**), CBG (**8**), CBN (**10**) and Δ^9-THC (**14**).[60]
	Agilent 1260 Infinity II LC system coupled with a variable wavelength UV detector	Ascentis Express C_{18} column (150 mm x 3.0 mm, 2.7 μm)	Mobile phase: 1% HCOOH in water (A) and 1% formic acid in ACN (B). Gradient elution: 0–13 min 60% B in A, 13–17 min from 60 to 80% B in A, 17–22 min	UV detection at 210 and 220 nm	CBC (**1**), CBD (**5**), CBDA (**6**), CBG (**8**), CBN (**10**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[61]

			from 80 to 90% B in A, which was kept for 8 min. Flow rate = 0.4 mL/min; run time = 15 min; injection volume = 3 μL.		
	Dionex Ultimate 3000 UHPLC coupled with a Q-Exactive Orbitrap mass spectrometer with a heated electro-spray ionization (HESI) source		A ternary (A/B/C) multistep gradient (solvent A: 0.1% HCOOH in water, solvent B: 0.1% HCOOH in ACN and solvent C: MeOH). Gradient programme: 0–2 min from 50 to 67% B in A, kept for 4 min, 6–10 min from 67 to 90% B in A, kept for 4 min,14–15 min from 90% to 50% B in A, kept for 5 min for re-equilibration. Solvent C was	ESI-MS in positive and negative ion mode	

			constant at 5% throughout the run. Flow rate 0.3 mL/min; column temperature = 30°C; injection volume = 1 μL.		
	Agilent 1200 Series Gradient HPLC System	Luna C_{18} column (150 × 4.6 mm, 5 μm)	Linear gradient elution. A = water containing 0.1% HCOOH and B = ACN. 0–17 min (35–5% A), 17–22 min (5% A), 22–24 min (5–35% A), and 24–28 min (35% A). Column temp.= 25 °C; flow rate = 1 mL/min; injection volume = 20 μL	DAD set at 210 and 220 nm	CBD (**5**), CBDA (**6**), CBN (**10**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[62]
	Surveyor HPLC system coupled to a linear ion-trap mass spectrometer	Discovery C_{18} column (250 mm × 4.6 mm, 5 μm)	A = water containing 0.1% HCOOH and B = ACN containing 0.1% HCOOH. Gradient elution. 0–	ESI(+) was for the detection of CBD (**5**), THC (**14**), and CBN (**10**), and ESI(−) was for	

	(Linear Trap Quadrupole)		17 min from 35:65 (A:B) to 5:95, 17–22 min from 5:95 to 5:95, and 22–24 min from 5:95 to 35:65. The flow rates were 1 mL/min in the column and 200 µL/min in the electrospray ionization (ESI) source (split ratio 4:1). Column temp.= 45 °C; injection volume = 200 µL	CBDA (**6**), and THCA (**15**).	
	Dionex Ultimate 3000 UHPLC coupled with a DAD	Restek Raptor ARC-18 column (150 mm x 4.6 mm, 2.7 µm)	Mobile phase: A = 5 mM ammonium formate and 0.1% HCOOH, and B = 0.1% HCOOH in ACN. Isocratic elution with 75% B in A. Run time = 9 min; flow rate = 1.5	UV-DAD at 220 and 280 nm	CBD (**5**), CBG (**8**) and Δ^9-THC (**14**).[63]

			mL/min; Injection volume = 5 μL.		
	Dionex Ultimate 3000 UHPLC coupled with a UV 6000LP Photodiode array UV/vis detector and MS detector Finnigan LQT (XL) equipped with Heated Electro Spray Ion source (HESI II)	Kinetex C_{18} column (100 mm x 2.1 mm, 2.6 μm)	Mobile phase: A = 89% water, 1% HCOOH and 10% ACN and B = 0.1% HCOOH, 99.1% ACN. Gradient elution: started with 2% B in A; increased to 95% B in A in 35 min, kept for 5 min; re-equilibrated under initial conditions resulting in a total runtime of 40 min. Flow rate = 0.35 – 0.5 mL/min; column temperature = 45°C; injection volume = 2 μL.	DAD set at 220 nm; heated ESI-MS (HESI) in positive and negative ion modes	CBD (**5**) and CBDA (**6**).[64]
	Dionex Ultimate 3000 UHPLC coupled with a heated ESI source	Poroshell 120 SB-C_{18} column (100 mm x 3 mm, 2.7 μm)	The mobile phase was composed of 0.1% HCOOH in both water (A) and	Heated ESI-MS (HESI) in positive and	CBD (**5**), CBG (**8**), cannabidihexol (CBDH) and Δ^9-THC (**14**).[65]

	and a Q-Exactive Orbitrap mass spectrometer		ACN (B). Isocratic elution with 70% B in A for 10 min, then 95% B in A for 5 min and re-equilibration of the column for 2 min. Run time = 17 min; flow rate = 0.5 mL/min.	negative ion modes	
	Dionex Ultimate 3000	Dr Maisch ReproSil XR 120 (3.5 mm × 50 mm, 3 µm) column	A = water + 0.1% HCOOH, B = MeOH + 0.05% HCOOH. The elution: 1–6.8 min, 60% B→73% B; 6.8–9.3 min, 73% B→95% B; 9.3–9.4 min, 95% B→60% B; 9.4–10 min, 60% B. Injected sample: 1 µL; flow rate = 1 mL/min; the column temperature = 40°C.	DAD with detection at 228 nm	CBC (**1**), CBD (**5**), CBDA (**6**), CBG (**8**), CBN (**10**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[66]

	Dionex Ultimate 3000 coupled with a coupled with a Q Exactive™ Hybrid Quadrupole-Orbitrap MS	Kinetex C_{18} core-shell column (150 mm × 2.1 mm, 2.6 µm) with a guard column (0.5 µm depth filter × 0.1 mm)	A ternary multistep gradient (solvent A: 0.1% acetic acid (CH_3COOH) in water, solvent B: 0.1% CH_3COOH in ACN, and solvent C: MeOH). Solvent C was kept constant at 5% throughout the run. Multistep gradient program: initial conditions were 50% B raised to 67% B until 2 min, held at 67% B for 4min, and then raised to 90% B until 10 min, held at 90% B until 14 min, decreased to 50% B over the next min, and held at 50% B until 20 min for re-equilibration of the system prior to the	ESI in negative ion mode.	CBC (**1**), CBD (**5**) and Δ^9-THC (**14**).[67]

			next injection at a flow rate of 0.3 mL/min. Comin temperature = 30°C and injection volume = 1 mL.		
	Shimadzu Nexera XR HPLC coupled with a Shimadzu triple-quadrupole LCMS-8040, with an electrospray ionization(ESI) source	Restek Raptor ARC C_{18} column (150 mm x 2.1 mm, 2.7 µm) coupled with a guard column (5 mm × 2.1 mm, 2.7µm)	Mobile phase A = water with 5 mM ammonium formate, and B = MeOH. Gradient elution: 20% B in A at 0 min with a 1 min hold, 70% B in A at 17.5 min, 98% B in A at 17.5 min with a 5 min hold, and 20% B in A at 23.75 min with a 4.25 min hold. Flow rate = 0.5 mL/min; column temperature = 45°C; injection volume = 5 µL	ESI-MS in positive ion mode	CBD (**5**), Δ^9-THC (**14**) and Δ^9-tetrahydro-cannabiphorol (Δ^9-THCP).[68]

	Shimadzu Prominence-i LC-2030C 3D Plus HPLC-PDA	Luna C_{18}(2) column (150 mm × 4.6 mm × 5 µm) coupled who a Security C_{18} guard column (20 mm × 4.6 mm × 5 µm)	Mobile phase: A = water buffered with 20 mM ammonium formate and 0.1% formic acid, B = ACN and C = MeOH buffered with 10 mM ammonium formate and 0.05% HCOOH. Gradient elution: started with 50% B in A, increased to 53% B in A in 17 min, 17-30 min 25% A, 30% B and 45%C, 30.01 min 10% A, 45% B and 45%C, held until 32.00 min, 32.01 min 50% B in A, and held until 34 min. Column temperature = 40°C; flow rate = 2.5 mL/min;	PDA detection at 232 nm	CBC (**1**), CBD (**5**), CBDA (**6**), CBDV (**18**), CBG (**8**), CBGA (**9**), CBN (**10**), Δ^8-THC (**13**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[69]

			injection volume 10 µL.		
	Shimadzu Prominence coupled with a Shimadzu LC-2030C DAD	Kinetex C_{18} column (150 mm x 4.6 mm. 2.6 µm) connected to a Phenomenex Security Guard Ultra C_{18} guard-column	Mobile phase A = 0.05% CH_3COOH in water adjusted to pH 4.40 ± 0.05 with ammonium hydroxide (30% solution), and mobile phase B = ACN. Gradient elution: 0–15 min, 56–61% B; 15–19 min, 61% B; 19–23 min, 61–75% B; 23–30 min, 75% B; 30-31 min 56% B, followed by 4 min equilibration. Total run time = 35 min, injection volume 5 mL, flow rate = 0.8 mL/min and column temperature = 25°C.	UV-PDA at 220 nm	CBD (**5**), CBDA (**6**), CBN (**10**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[70]

	Shimadzu DGU-205R Ultra HPLC-PDA system	Hypersil C_{18} (150 mm × 4.6 mm, 3.0 μm) column linked to a Hypersil BDS C_{18} (10 mm × 4 mm, 3 3.0 μm) guard column	Solvent gradient composed of 1.0 mM HCOOH buffer at pH 3.53 (solvent A) and ACN (solvent B) at a flow rate of 1 mL/min. The gradient started with 70% B for 7 min, increased to 83.5% B and held constant for 1 min, then increased to 88.5% B over a 3 min period, one additional min to reach 99% B, then kept constant for 2 min followed by a 2 min to lower B to 70%, and finally held constant for 2 min. Run time = 18 min, column temp = 25°C,	PDA set at 220 nm	CBC (**1**), CBCA (**2**), CBD (**5**), CBDA (**6**), CBDV (**18**), CBDVA (**19**), CBG (**8**), CBGA (**9**), CBN (**10**), Δ^8-THC (**13**), Δ^9-THC (**14**), Δ^9-THCA (**15**), THCV (**16**) and THCVA (**17**).[71]

			injection volume = 10 μL.		
	Thermo Fisher Surveyor HPLC coupled to a Q-Exactive-Orbitrap MS analyser	Synergi Hydro RP column (150 mm x 2 mm, 4 μm) with a C_{18} guard column (4 mm x 3 mm)	Mobile phase comprised water and ACN, both containing 0.1% HCOOH. Gradient elution: started with 5% B in A; increased to 95% B in A in 30 min; returned to initial conditions at 35 min, followed by a 5-min re-equilibration period. Flow rate = 0.3 mL/min; column temperature = 30°C.	Heated electrospray ionization (HESI)	CBC (**1**), CBCA (**2**), CBD (**5**), CBDA (**6**), CBDV (**18**), CBDVA (**19**), CBG (**8**), CBGA (**9**), CBN (**10**), CBNA (**11**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[72]
	Vanquish Core System coupled to a interfaced to an Exploris 120 Orbitrap HRMS	CHIRALCEL OD-R [cellulose tris (3,5-dimethylphenylcarbamate)] (250 mm × 4.6 mm, 10 μm), CHIRALCEL OB-H [cellulose tribenzoate] (250 mm × 4.6 mm, 5 μm), CHIRALCEL OJ-H [cellulose tris (4-methylbenzoate)] (250 mm ×	ACN, EtOH and isopropanol (i-PrOH) (all with 0.1% of HCOOH); their percentage with respect to water was selected according to the	DAD set at 274 and 306 nm.	CBD (**5**), CBDA (**6**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[73]

		4.6 mm, 5 µm), CHIRALPAK AD [amylose tris (3,5-dimethylphenylcarbamate)] (250 mm × 4.6 mm, 10 µm), and CHIRALPAK AD-RH [amylose tris (3,5-dimethylphenylcarbamate)] (150 mm × 4.6 mm, 5 µm) columns	lipophilicity of the compound. The enantiomers of *trans*-CBD (**5**) and *trans*-Δ^9 -THC (**14**) were separated with 60% ACN, column temperature at 30◦C and a flow rate of 1.5 mL/ min.		
		Poroshell 120 EC-C_{18} (100 mm × 3.0 mm, 2.7 µm) with guard column (5 mm × 3 mm, 2.7 µm)	Water and ACN (both with 0.1% HCOOH) as mobile phase in a linear gradient from 60% to 95% ACN (with 0.1% formic acid) in 15 min, followed by isocratic elution at 95% ACN, washing step at 98% ACN for 3 min and re-equilibration with the initial	ESI-MS in both positive and negative ion modes	

			conditions. Run time = 26 min, flow rate = 0.5 mL/min and injection volume = 5 μL.		
Inflorescences (Hemp)	Acquity UPLC-PDA	Fortis Speed Core C_{18} PFP column (100 mm x 2.1 mm, 2.6 μm)	Mobile phase = water (A) and ACN (B) both containing 0.1% HCOOH. Gradient elution: started with 60% B in A until 0.5 min; increased to 68% B in A in 2.9 min and maintained there until 5.5 min; increased again to 90% B in A in 5.5-7.8 min and kept until 9 min; the initial composition was restored in 0.1 min and maintained over 1.9 min for column re-equilibration.	PDA set at 210 and 225 nm	CBC (**1**), CBD (**5**), CBDA (**6**), CBE (**20**), CBGA (**9**), CBN (**10**), CBT (**22**), CBDV (**18**), CBDVA (**19**) Δ^9-THC (**14**), *cis*-Δ^9-THC and Δ^9-THCA (**15**).[74]

			Column temperature = 25°C; flow rate = 0.4 mL/min; injection volume = 0.3 μL; run time = 11 min.		
Inflorescences and leaves	Shimadzu Ultra Fast LC Prominence System	Cortecs Shield RP18 column (150 mm x 4.6 mm, 2.7 μm)	0.1% TFA (41%), and ACN (41:59), isocratic, flow rate = 2 mL/min. Temp. = 35°C; injection volume: 10 μL.	DAD set at 228 nm	CBD (**5**) and CBDA (**6**).[75]
	Shimadzu 20AD XR UFCL XR system	Kinetex C_{18} column 2.6, 100A, (100 mm × 4.6 mm, 2.7 μm)	A = 2% ACN and 0.1% 3-fuoro-acetic acid; B = ACN with 2% water and 0.1% 3-fuoro-acetic acid. Gradient elution. 0.01–0.20 min 61% B, 0.20–0.21 min 61–43% B, 0.21–50 min 43–56% B. Flow rate = 2 mL/min; temp. =	PDA set at 220 and 280 nm	CBD (**5**) and CBDA (**6**).[76]

			40°C; injection volume: 10 μL.		
Leaves	Agilent 1100 series HPLC	Luna C_{18} column (150 mm × 4.6 mm, 5 μm)	Mobile phase: 0.1% of HCOOH in water (A) and ACN (B). Gradient elution: initial ratio 5% B in A, maintained for 10 min, changed to 10% B in A in 10 min, changed to 30% B in A in 10 min, to 50% B in A in 10 min, maintained for 0.5 min, and back to initial ratio in 0.5 min. Flow rate = 1 mL/min, column; injection volume = 20 μL; temperature = 30°C	PDA set at 254 nm	CBD (**5**) and Δ^9-THC (**14**).[85]
	Shimadzu HPLC	Luna C_{18} (250 mm × 4.6 mm, 5 μm)	Isocratic elution with 30% MeOH in water and 0.1% TFA at a flow rate of 1.0 mL/min.	UV detector set at 225 nm.	CBD (**5**) and CBDA (**6**), α-cannabispirol (**25**), β-cannabispirol (**26**), cannabiripsol (**27**) and cannabitriol (**28**).[86]

	Waters HPLC	ACE-5 C_{18} column (250 mm × 4.6 mm, 5 μm)	Isocratic run with 0.1% CH_3COOH in both water and ACN (1:1). Flow rate = 1 mL/min; run time = 65 min; injection volume = 20 μL; Column temperature = 30°C.	UV detector set at 210 nm	CBD (**5**) and Δ^9-THC (**14**).[87]
Leaves and vegetative shoots	Shimadzu HPLC	Bondapak C_{18} column (300 mm x 3.9 mm, 10.0 μm)	0.1% HCOOH in water (phase A) and 0.1% HCOOH in ACN (phase B). A gradient elution: 25% B for 5 min, increased to 50% B and maintained for 10–15 min, increased to 70% B and maintained for 20–30 min, increased to 100% B, maintained for 33–37 min, and subsequently	UV detector at 225 nm	CBC (**1**), CBD (**5**), CBDV (**18**) and Δ^9-THC (**14**).[88]

			restored to 25% B and maintained for 10–45 min. Injection volume = 10 µL, and the flow rate = 2 mL/min.		
Leaves in the vegetative stage, inflorescent in the flowering stage, inflorescent of seeds in the seeding stage and the mature seeds	HPLC System Knauer	MZ-C_{18} ODS column (250 mm × 4.6 mm, 3.5 µm)	Isocratic elution with 80% MeOH in water. Flow rate = 1 mL/min; run time = 55 min.	UV detector set at 220 nm	CBD (**5**) and CBDA (**6**), CBG (**8**), CBN (**10**), Δ^8-THC (**13**) and Δ^9-THC (**14**).[89]
Leaves, roots, stem, inflorescences and seeds from industrial hemp	Hewlett-Packard HPLC HP 1050 coupled with a LCQ Accela Fleet atmospheric pressure chemical ionization (APCI) interface and PDA detector.	Luna C_{18} (2) column (150 mm x 2 mm, 3 µm)	Mobile phase: A = 5% ACN + 0.1% *o*-phosphoric acid, and B = 80% ACN in water + 0.1% *o*-phosphoric acid. Isocratic elution with 83% B in A at a flow rate of 0.25	PDA at 220 nm, and APCI-MS	CBD (**5**), CBDA (**6**) and CBGA (**9**).[90]

			mL/min. For LC-MS, HCOOH was used instead of *o*-phosphoric acid.		
Seeds	Dionex UltiMate 3000 HPLC and UHPLC System coupled with a Bruker-Compact-QQTOF-MS/MS equipped with an electrospray ionization (ESI)	Agilent C_{18} Wate column (100 mm x 4.6 mm, 3.5 µm)	Mobile phase comprised water and ACN, both containing 0.1% HCOOH. Flow rate = 0.3 mL/min; run time = 15 min; injection volume 10 µL; column temperature = 30°C.	ESI-MS in positive ion mode	CBD (**5**), CBN (**10**) and Δ^9-THC (**14**).[94]
UPLC (or UHPLC) methods					
Aerial parts	Acquity UPLC-PDA	Acquity UPLC HSS C_{18} SB column (100 mm x 2.1 mm, 1.8 µm)	Mobile phase: A = MeOH and B = 0.1% HCOOH in water. Gradient elution: started with 65% of A and held for 1 min, linearly increased to 78% of A in 8 min, then 95% of A in 1 min,	PDA set at 211 and 220 nm	CBD (**5**), CBDA (**6**), CGB (**8**), CBGA (**9**), CBN (**10**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[24]

			held for 2 min. Finally, the gradient returned to the initial condition and was maintained for 3.5 min before the next injection. Column temperature = 45°C; flow rate = 0.35 mL/min; run time = 16 min		
	Dionex Ultimate 3000 UHPLC coupled with a DAD	Acquity UPLC BEH C_{18} column (150 mm ×2.1 mm, 1.7 µm)	Flow rate = 0.5 mL/min; column temperature = 45°C	DAD set at 220 nm	CBC (**1**), CBCA (**2**), CBCL (**3**), CBLA (**4**), CBD (**5**), CBDA (**6**), CBGA (**9**), CBN (**10**), CBNA, CBDV (**18**), CBDVA (**19**), Δ^8-THC (**13**), Δ^9-THC (**14**), Δ^9-THCA (**15**), Δ^9-THCA-COOH and Δ^9-THCVA (**17**).[25]
	Dionex Ultimate 3000 UHPLC coupled with an HRMS/MS		Mobile phase: A = 5% MeOH in water, and B = isopropanol-MeOH-water (65:30:5), both containing 5 mM ammonium formate and 0.1% HCOOH. Gradient elution: 60% B in A in	HRESI-MS in positive and negative ion modes	

			1 min at a flow rate of 0.3 mL/min, followed by a gradual change to 70% B in A in 10 min, and change to 100% B in 0.5 min simultaneously with a flow rate increase to 0.4 mL/min. The column was then washed with 100% B for 5 min and reconditioned to initial conditions for 2.5 min. Run time = 19 min; injection volume = 3 μL.		
	Dionex Ultimate 3000 UHPLC system coupled with a Q-Exactive Plus orbital trap mass spectrometer	Acquity UPLC BEH C_{18} column (100 mm × 2.1 mm, 1.7 μm)	Mobile phase: A = 5% MeOH in water and B = isopropanol-MeOH-water (65:30:5); both A and B with 5 mM ammonium formate and 0.1% HCOOH. Gradient elution: started with 5% B,	ESI-MS/MS in positive ion mode	CBD (**5**), CBG (**8**), CBN (**10**) and Δ^9-THC (**14**).[26]

			increasing to 60% B within the first minute, followed by a gradual change to 70% B in 10 min, rising sharply to 100% B in 0.5 min, washing with 100% B for 5 min and reconditioning to initial conditions for 2.5 min. Run time = 19 min; flow rate = 0.3 and 0.4 mL/min; injection volume 3 μL		
Aerial parts (industrial hemp)	Acquity UPLC I-Class coupled with a Xevo TQ-S Micro™ triplequadrupole mass spectrometer (MS/MS)	Acquity UPLC BEH C_{18} column (100 mm x 2.1 mm, 1.7 μm)	Mobile phase: 0.1% HCOOH in water (A) and MeOH + ACN (50:50) (B). Gradient elution: started with 89% B in A, held for 30 s, linearly increased to 100% B until 5.5 min, and then	ESI-MS in positive and negative ion modes	CBC (**1**), CBCL (**3**), CBD (**5**), CBDA (**6**), CBG (**8**), CBGA (**9**), CBN (**10**), CBDV (**18**), Δ^8-THC (**13**), Δ^9-THC (**14**), Δ^9-THCA (**15**), Δ^9-THCA-COOH and Δ^9-THCV (**16**).[27]

			sharply decreased back to the initial conditions for the final 30 s to re-equilibrate the column. Flow rate = 0.35 mL/min; injection volume = 2 μL; column temperature = 40^0C.		
Flowers	Acquity Classic UPLC coupled with a Xevo TQ MS detector	Acquity UPLC BEH C_{18} column (100 mm x 2.1 mm, 1.7 mm) connected to an Acquity UPLC BEH C_{18} VanGuard precolumn (5 mm x 2.1 mm)	Mobile phase comprised water (A) and ACN (B), both containing 0.1% HCOOH. Gradient elution: 50% B in A for 1 min; 100% B for 8 min; 50% B in A for 3 min; and equilibration of the column for 1 min. Injection volume = 5 μL; flow rate = 0.5 mL/min; column	ESI-MS/MS in positive and negative ion modes	CBC (**1**), CBD (**5**), CBDA (**6**), CBDV (**18**), CBG (**8**), CBGA (**9**), CBN (**10**), Δ^8 -THC (**13**), Δ^9 -THC (**14**), Δ^9 -THCA (**15**) and THCV (**16**).[45]

			temperature = 45°C.		
	Acquity UPLC-PDA	Cortecs UPLC Shield RP18 column (100 mm x 2.1 mm, 1.6 µm)	Isocratic elution at a flow rate of 0.7 mL/min, with mobile phase consisting of 48% water containing 0.1% formic acid and 52% ACN. Injection volume = 5 µL; column temperature = 35°C; run time = 18 min.	PDA set at 228 nm	CBD (**5**), CBDA (**6**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[46]
Flowers (hemp)	Acquity UPLC-PDA	Cortecs UPLC Shield RP18 column (100 mm x 2.1 mm, 1.6 µm)	Isocratic elution at a flow rate of 0.7 mL/min, with mobile phase consisting of 48% water containing 0.1% formic acid and 52% ACN. Injection volume = 5 µL; column	PDA set at 228 nm	CBD (**5**) and Δ^9-THC (**14**).[47]

			temperature = 35°C.		
Dried flowers	Agilent 1200 RRLC system coupled to a diode array detector (DAD)	Kinetex C_{18} column (100 mm × 3.0 mm, 1.7 µm)	Mobile phase: A = 10 mM ammonium formate and B = ACN. Gradient elution: 0–8 min, 52–66% B in A; 8–8.5 min, 66–70% B in A; 8.5–13 min, 70–80% B in A; 13–15 min, 80% B in A; a 7-min column equilibration after each run. Flow rate = 0.6 mL/min; injection volume = 5 µL.	DAD at 220 nm	CBC (**1**), CBD (**5**), CBDA (**6**), CBG (**8**), CBGA (**9**), CBN (**10**), Δ^9-THC (**14**), Δ^9-THCA (**15**), THCV (**16**) and THCVA (**17**).[48]
Inflorescences	Acquity Arc FTN-R UPLC	Kinetex 1.7-µm XB-C_{18} LC column (150 mm x 2.1 mm, 1.7 µm)	Isocratic elution with 30% HCOOH and 20 mM ammonium formate buffer at pH 2.9 and 70% ACN for a 16 min run at a flow rate of 0.3 mL/min.	UV-PDA at 228 nm	CBCA (**2**), CBDA (**6**), CBGA (**9**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[77,78]

			Injection volume = 2 µL and column temperature = 25°C.		
	Agilent 1290 UHPLC system coupled with a 6530 accurate mass quadrupole time-of-fight mass spectrometer	Luna Omega polar C_{18} column (100 mm × 2.1 mm, 1.6 µm)	Mobile phase comprised 0.1% HCOOH in water (A) and 10% MeOH in ACN (B). Gradient elution: 50% B in A for 0.0–15.0 min, 50–75% B in A for 15–34.5 min, 75–95% B in A for 34.5–35.0 min, 95% B in A for 35–37 min, 95–50% B in A for 37–37.5 min, and 50% B in A for 37.5–40 min. Flow rate = 0.4 mL/min; injection volume = 2 µL; column temperature = 40°C.	HR-ESI-MS/MS in positive ion mode	CBD (**5**), CBDA (**6**), CBE (**20**), CBEA (**21**) and Δ^9-THC (**14**).[79]

	Agilent 1290 UHPLC coupled to a Agilent 6410 triple quadrupole mass spectrometer	Hypersil Gold RP column (100 mm × 3 mm, 1.8 µm)	Mobile phases A (0.1% HCOOH in water) and B (0.1% HCOOH in ACN) were used for separation using the following gradient elution program: 0–14 min, 70%–80% B; 14–17 min, 80–100% B; 17–20 min, 100% B. Column temperature = 30°C, flow rate = 0.25 mL/min and injection volume = 1 µL.	ESI operating in both positive and negative ion modes	CBD (**5**) and CBG (**8**).[80]
	Dionex UltiMate 3000 Thermo Fisher UHPLC coupled with a Q-Exactive Plus quadrupole Orbitrap MS	Acquity UPLC BEH C_{18} column (150 mm x 2.1 mm, 1.7 µm)	Mobile phase = water (A) and ACN (B), both containing 0.1% HCOOH. Gradient elution: started with 30% B in A, maintained constant for 3 min,	ESI-MS in positive and negative ion modes and principal component analysis (PCA)	CBDA (**6**), CBDV (**18**), CBG (**8**), CBGA (**9**), CBNA (**11**), Δ^9-THC (**14**) and THCV (**16**).[81]

			increased to 60% B in A in 50 min and then raised again up to 90% B in A, maintained at 90% B for other 2 min and then came back to the initial conditions. Flow rate = 0.2 mL/min; injection volume = 10 μL; column temperature = 50°C.		
	Dionex Ultimate 3000 Thermo Fisher UHPLC coupled with a PDA detector	Titan C_{18} column (100 mm × 3 mm, 1.9 μm)	Mobile phase = water (A) and ACN (B), both containing 0.1% TFA. Gradient elution: 0–14 min from 65 to 100% B in A, 15–16 min isocratic elution with 100% B, 17–22 min from 100 to 65% B in A. Run time = 22 min; flow	PDA set at 214 nm	CBC (**1**), CBD (**5**), CBDA (**6**), CBDV (**18**), CBG (**8**), CBN (**10**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[82]

			rate = 0.5 mL/min column temperature = 35°C; injection volume = 10 μL.		
	Shimadzu NEXERA UHPLC system coupled with TOF-MS and TOF-MS/MS	Luna Omega C_{18} column (50 mm × 2.1 mm, 1.6 μm)	Mobile phase = water (A) and ACN (B), both containing 0.1% TFA. Gradient elution: 0→1 min, 25–55% B in A; 1→8.5 min, 55–95% B in A; 8.5→9.5 min, 95% B in A. Then the starting conditions were restored and kept for 2 min. Run time = 11.5 min; flow rate = 0.5 mL/min; injection volume = 2 μL.	TOF-MS/MS	Butyl-cannabidiolic acid (CBDA-C4), CBC (**1**), CBCA (**2**), CBD (**5**), CBDA (**6**), CBDV (**18**), CBDVA (**19**), cannabidicannabidiorcolic acid (CBDOA), cannabifuranic acid (CBFA), CBN (**10**), tetrahydrocannabinolic acid B (THCA-B), Δ^9-THC (**14**), Δ^9-THCA (**15**) and Δ^9-THCVA (**17**).[83]
	Shimadzu NEXERA UHPLC system-PDA	Titan C_{18} column (100 mm × 3 mm, 1.9 μm)	Mobile phase = water (A) and ACN (B), both containing 0.1% TFA. Gradient	PDA set at 214 nm	CBC (**1**), CBD (**5**), CBDV (**18**), CBG (**8**), CBN (**10**) and Δ^9-THC (**14**).[84]

			elution: 50% B (0 min), 50% B (1 min), 100% B (16 min), 100% B (20 min), 50% B (21 min) and 50% B (30 min). Flow rate = 0.5 mL/min; column temperature = 30 ◦C; injection volume = 1 μL.		
Leaves	Agilent 1290 infinity UHPLC system coupled with a 6530 QTOF system with a Dual AJS ESI source operating in positive ionization mode	Zorbax Eclipse Plus C_{18} column (100 mm x 2.1 mm, 1.8 μm)	Mobile phase: 0.1% HCOOH in water (A) and ACN (B). Gradient elution: 0–11 min, 5–35% B in A; 11–12 min, 35–65% B in A; 12–15 min, 65–70% B in A; 15–21 min, 70–80% B in A; 21–25 min, 80–100% B; 25–27 min, 100% B; and post time, 3 min. Column temperature =	ESI-MS in positive ion mode	CBD (**5**) and Δ^9-THC (**14**).[91]

			30oC; flow rate = 0.4 mL/min; injection volume = 1.5 μL.		
Leaves and flowers	Agilent 1290 UHPLC	Hypersil Gold RP column (150 mm × 2.1 mm × 1.9 μm)	Isocratic elution using a mixture of 0.1% formic acid in water and ACN (49:51). Flow rate = 0.45 mL/min; temp. 35^{o}C; run time: 25 min.	PDA set at 280 nm	CBD (**5**) and CBN (**10**).[92]
Hemp microgreens	Dionex Ultimate 3000 Thermo Fisher UHPLC coupled with an Orbitrap Q-Exactive mass spectrometer	Luna Omega column (50 mm x 2.1 mm, 1.6 μm)	The eluent phase consisted of phase A: 0.1 % HCOOH in water and phase B: 0.1 % HCOOH in ACN. Injection volume = 1 μL, flow rate = 0.5 mL/min and column temperature = 30^{o}C.	ESI-MS positive ion mode, and MS/MS	CBDA (**6**), CBG (**8**), CBGA (**9**), CBN (**10**), CBNA (**11**), Δ^{9}-THC (**14**) and Δ^{9}-THCA (**15**).[93]

TABLE 2 HPLC and UPLC (or UHPLC) methods for the analysis of phytocannabinoids in cannabis consumer products

Consumer products	Instrument	Column	Mobile phase	Detection	Cannabinoids analysed
HPLC methods					
Algerian seized hashish	Thermo Finnigan Surveyor HPLC system with DAD	Phenomenex C_{18} analytical column (150 mm x 4.6 mm, 5 µm)	Mobile phase: A = ACN, B = MeOH and C = 0.1% *o*-phosphoric acid. Gradient elution: started with 38% A, 33% B and 29% C for 1 min; increased to 53% A, 47% B and 0% C over 20 min; column was set to initial condition in 0.5 min and re-equilibrated for 5 min. Flow rate = 1.2 mL/min; injection volume = 10 mL; column temperature = 30°C.	DAD set at 220 nm	CBD (**5**), CBDA (**6**), CBN (**10**), CBNA (**11**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[95]
			The mobile phase consisted of ACN (A), MeOH (B), and 0.1% *o*-phosphoric acid (C). The initial setting was 38% A, 33% B, and 29% C for 1 min, which linearly increased to 53% A, 47% B, and 0% C over 20 min. Then, the column was set to initial conditions in 0.5 min and re-equilibrated under these conditions for 5 min. Flow rate = 1.2 mL/min, injection volume = 10 µL and column temperature = 30°C.		CBD (**5**) and Δ^9-THC (**14**).[96]

Aqueous tinctures (CBD containing)	Agilent 1260 HPLC	Raptor ARC-18 LC reversed-phase column (150 mm × 4.6 mm, 2.7 µm)	Mobile phase A = aqueous ammonium formate (5mM)/HCOOH (0.1%) and B = ACN/HCOOH (0.1%). Isocratic elution with 75% B in A. Column temperature = 30^{o}C; flow rate = 1.5 mL/min; run time = 10 min; injection volume = 5 µL.	UV detection at 228 nm	CBD (**5**) and CBDA (**6**).[97]
Cannabis-based products like edible extract, infusion and liquid	Agilent Infinity 1260 HPLC	Agilent Core-shell C_{18} column (150 mm × 2.1 mm, 2.7 µm)	Isocratic elution. 75% ACN in water (containing 0.1% HCOOH).	PDA detection at 220 nm	CBD (**5**) and Δ^9-THC (**14**).[98]
		Reversed-phase Luna C_{18}(2) column			CBC (**1**), CBCA (**2**), CBCL (**3**), CBD (**5**), CBDA (**6**), CBDV (**18**), CBDVA (**19**), CBG (**8**), CBGA (**9**), CBN (**10**), Δ^8-THC (**13**), Δ^9-THC (**14**), Δ^9-THCA (**15**), THCV (**16**) and THCVA (**17**).[30]
Cannabis doping samples	Agilent 1290 HPLC coupled with a QTRap 6500 MS	Eclipse XDB-C_8 column (100 mm x 2.1 mm, 3.5 µm), and Eclipse XDB-C_{18} column (100 mm x 2.1 mm, 3.5 µm)	Mobile phase: A = 5% ACN in water and 2 mmol/L ammonium acetate and B = 95% ACN in water and 2 mmol/L ammonium acetate. Gradient elution: 0 min 40% B, 0.5 min 40% B, 3 min 100% B and 6 min 100%. Flow rate = 0.25 mL/min; injection	ESI-MS/MS in negative MRM mode	Δ^8-THC (**13**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[99]

			volume = 3 mL. Also, isocratic elution with 58% B in A.		
Cannabis light preparation (products containing dried female inflorescences of *Cannabis sativa* of Chemotype III)	Shimadzu Prominence-I HPLC-UV	Shimadzu NexLeaf CBX for Potency C_{18} column (150 mm x 4.6 mm, 2.7 μm)	The mobile phase comprised water (A) and ACN (B), both containing 0.085% phosphoric acid. Gradient elution: 0-7 min (70–85% B in A), 7.0–7.1 min (85–95% B in A), 7.1–8.0 min (95% B in A), 8.0–8.1 min (95-70% B in A) and 8.1-10 min (70 % B in A). Flow rate = 1.6 mL/min; column temperature = 35°C.	UV detection at 220 nm	CBD (**5**), CBDA (**6**), CBN (**10**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[100]
Cannabis seized products	Agilent 1260 Infinity Series	Kinetex C_{18} (100 mm × 4.6 mm, 2.6 μm)	Isocratic elution with MeOH and 10 mM ammonium acetate at pH 5.2 (80:20). Fow rate = 0.5 mL/min and column temperature = 30°C.	PDA set at 228 nm	CBD (**5**), CBDA (**6**), CBG (**8**), CBGA (**9**), CBN (**10**), Δ^8-THC (**13**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[101]
Cannabis oil	Agilent 1220 Infinity II LC coupled with a variable wavelength UV detector	Poroshell 120 EC-C_{18} column (50 mm x 3 mm, 2.7 μm)	Mobile phase: A = 0.1% HCOOH in water and B = 0.05% HCOOH in MeOH. Gradient elution started with 60% B in A for 0-1 min, increased to 77% B in A in 1-7 min, again increased to 95% B in A in 7.0-8.2 min, came back to 60% B in 8.2-9.5 min, and finally column equilibration until 11 min. Column temperature = 50°C,	UV detection at 230 nm	CBD (**5**), CBDA (**6**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[102]

			injection volume = 5 μL; run time = 11 min.		
	Flexar HPLC system - Perkin Elmer	Purospher STAR RP-18 column (120 mm X 4.6 mm, 5 μm)	Isocratic elution with 80% ACN in water with HCOOH (0.1%) at a flow rate of 1 mL/min. Column temperature = 25°C and injection volume = 10 μL.	UV-PDA detection at 208 and 280 nm	CBD (**5**) and Δ^9-THC (**14**).[103]
	Agilent 1200 series HPLC coupled with an AB SCIEX API 4000 QTRAP triple quadrupole mass analyzer equipped with an ESI source operating in the negative ion mode	Kinetex EVO C_{18} column (100 mm × 2.1 mm, 5 μm)	Mobile phase: A = 20 mM ammonium acetate and B = ACN. Gradient elution: 30 to 90% B in A in 10 min, kept at 90% B for 5 min, and decreased to 30% of B in 3 min. The post-running time was set at 2 min. Flow rate = 0.35 mL/min; column temperature = 40°C; injection volume = 25 mL.	ESI-MS/MS in negative ion mode	CBD (**5**), CBDA (**6**), CBG (**8**), CBGA (**9**), CBN (**10**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[104]
Cannabis (hemp) oil	LaChrom D-7000 LC system	Spheri-5 C_{18} column (250 mm × 4.6 mm, 5 μm)	Mobile phase: 0.5% CH_3COOH in water (A) and ACN (B). Isocratic elution with 34% B in A. Flow rate = 1 mL/min; column temperature 25°C.	PDA detection at 220 and 240 nm	CBD (**5**) and CBDA (**6**).[105]
	LaChrom D-7000 LC system	Spheri-5 C_{18} column (250 mm × 4.6 mm, 5 μm)	Mobile phase: 0.5% CH_3COOH in water (A) and ACN (B) The gradient started from 70% B in A, then 80% B in A for 5 min, and	PDA detection	CBD (**5**), CBDA (**6**), CBN (**10**) and Δ^9-THC (**14**).[106]

			finally the gradient returned to the starting conditions for 5 min. Flow rate = 1 mL/min; run time = 20 min; column temperature 25°C; injection volume = 20 μL.	at 220 and 240 nm	
	PerkinElmer Flexar FX-15 UHPLC system coupled with a PDA detector	PerkinElmer Brownlee SPP C_{18} column (150 mm x 3 mm, 2.7 μm)	The mobile phase comprised water (A) and ACN (B) both containing 0.1% HCOOH. Gradient elution: started with 67% B in A, increasing to 95% B in A in 5.5 min and maintaining at 95% B for 2 min. Equilibration time was 4.5 min at 67% B in A before each injection. Injection volume = 10 μL; flow rate = 1 mL/min; column temperature = 40°C.	PDA set at 228 nm	CBC (**1**), CBD (**5**), CBDA (**6**), CBDV (**17**), CBG (**8**), CBGA (**9**), CBN (**10**), Δ^8-THC (**13**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[107]
Cannabis oil infused products	Jasco Extrema HPLC system coupled with a UV-Vis detector	Solas C_{18} column (150 mm × 4.6 mm, 5 μm)	Isocratic elution with 75% ACN in water at a 1.5 mL/min flow rate. Column temperature = 25°C; injection volume = 10 μL; run time = 20 min.	UV detection at 214 nm	CBD (**5**) and Δ^9-THC (**14**).[108]
Cannabis oil and oil containing capsules	Sciex ExionLC coupled with a Sciex Triple QuadTM 5500+ triple-quadrupole MS	Kinetex C_{18} column (100 mm × 3 mm, 2.6 μm)	Mobile phase comprised water (A) and MeOH (B), both containing 0.1% HCOOH and 2 mM ammonium formate. Gradient elution: started with 60% B in A, increased to 90% B in A in 7 min, kept for 2 min at 90% B in A, and dropped back to initial condition and re-equilibrated for 3.5 min. Column temperature = 35°C; injection	ESI-MS in positive ion mode	CBC (**1**), CBD (**5**), CBDA (**6**), CBDVA (**18**), CBG (**8**), CBGA (**9**), cannabigerovarinic acid (CGBVA), CBN (**10**), cannabivarin

			volume = 20 µL; flow rate = 0.5 mL/min; run time = 13 min.		(CBV), Δ^9-THC (**14**), Δ^9-THCA (**15**), THCV (**16**) and THCVA (**17**).[109]
Cannabis essential oil from inflorescences	Shimadzu Prominence LC	Poroshell 120 EC-C_{18} column (150 mm x 4.6 mm, 2.7 µm)	A binary gradient of 0.1% HCOOH in water (A) and 0.1% HCOOH in ACN (B) at a flow rate of 0.40 mL/min. The gradient started at 70% of B for 5 min, and then increased linearly to 77% of B in 7 min and at 90% of B in 0.10 min. This composition was kept constant for up to 30 min before a linear decrease to the initial conditions (70% of B) in 0.10 min and held up to 60 min for the equilibration of the column.	UV-PDA at 220 nm	CBD (**5**), CBDA (**6**), CBN (**10**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[110]
Cannabidiol oil (CBD oil)	Agilent 1100 series LC chromatographic system	Kinetex C_{18} (150 mm x 2.1 mm, 2.6 µm) column equipped with a guard column C_{18}, 2.1 mm	Isocratic elution: 75% ACN in water with 0.1% HCOOH. Flow rate = 0.5 mL/min, run time = 30 min, column temperature = 20 °C and injection volume = 10 µL.	UV-PDA detection at 230 nm	CBD (**5**), CBDA (**6**), CBN (**10**), Δ^8-THC (**13**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[44]
	Agilent 1260 HPLC	Raptor ARC-18 LC reversed-phase column (150 mm × 4.6 mm, 2.7 µm)	Mobile phase A = aqueous ammonium formate (5 mM)/HCOOH (0.1%) and B = ACN/formic acid (0.1%). Isocratic elution with 75% B in A. Column temperature = 30°C; flow rate = 1.5 mL/min; run time = 10 min; injection volume = 5 µL.	UV detection at 228 nm	CBD (**5**) and CBDA (**6**).[97]

Cannabidiol full spectrum oil	Agilent 1260 HPLC system coupled with a UV-vis	MultoHigh 100 RP C_{18} column (125 mm x 4.6 m, 3 μL)	Isocratic elution with 87% MeOH in water at a 0.2 mL/min flow rate. Column temperature = 35°C.	UV detection at 212 nm	CBD (**5**), CBDA (**6**), CBDV (**18**), CBDVA (**19**), CBGVA, CBG (**8**), CBGA (**9**), CBN (**10**) and CBT (**22**).[111]
	Dionex Ultimate 3000 UHPLC coupled with a UV/vis detector and a TSQ Quantum Access MAX triple quadrupole (TQD) mass spectrometer detector			ESI-MS in positive ion mode	
Cannabidiol-containing powder and sunflower oil	Dionex Ultimate 3000 HPLC coupled with a DAD and a 3200 Q TRAP MS system	Eclipse C_{18} Plus column (50 mm x 2.1 mm, 5 μm) coupled with a guard column (5 mm x 2.1 mm, 5 μm)	Mobile phase = 0.1% HCOOH both in water (A) and ACN (B). Gradient elution: 0.0–10.0 min linearly from 5% to 100% B; 100% B was maintained from 10.0 to 12.0 min; then from 12.0 to 12.5 min 100% to 5% B. Finally, 5% B was maintained at 20.0 min. Column temperature = 25°C; flow rate = 0.2 mL/min; injection volume = 5 μL.	DAD and ESI-MS in positive ion mode	CBD (**5**).[112]
Cannabidiol salt	HPLC-UV	Reversed-phase C_{18} column (250 mm x 4.6 mm x 5 μm)	Isocratic elution with ACN in water.	UV detection at 225 nm	Cannabidiol phenolate salts.[113]

Commercial cannabinoids	Agilent UHPLC Infinity 1290	Nine polysaccharide-based columns (250 mm × 4.6 mm), namely Chiralpak IA, IB-N, IC, ID, IE, IF, IG, IH and IJ	Mobile phase = ACN and orthophosphoric acid (pH = 2.2). The amount of ACN varied in the range of 55–80%. Flow rate = 1 mL/min, injection volume 5 μL and column temperature = 25°C.	UV detection at 228 and 306 nm	CBC (**1**), CBD (**5**), CBDA (6), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[114]
	Acquity UPLC H-class system coupled with a PDA detector	Poroshell 120 EC-C_{18} column (150 mm × 2.1 mm, 2.7 μm)	Mobile phase: A = 0.1% formic acid in water and B = 0.1% formic acid in ACN. Gradient elution: 0-2.8 min 68% B in A, increased to 73% B in A in 0.5 min, held for 3.7 min, then increased to 95% B in A in 5.0 min, and held for 1.0 min, back to 68% B in A in 0.5 min and maintained for 4.5 min to re-equilibrate the system. Flow rate = 0.5 mL/min; injection volume = 1 μL.	PDA set at 220 nm. Design by Experiment (DoE)	CBC (**1**), CBD (**5**), CBDA (**6**), CBG (**8**), CBGA (**9**), CBN (**10**), Δ^8-THC (**13**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[115]
Commercial industrial hemp products	HPLC-DAD	Reversed-phase C_{18} column	Isocratic elution with 80% ACN in water over 8 min. Flow rate = 1 mL/min	UV detection at 220 and 240 nm	CBD (**5**) and Δ^9-THC (**14**).[116]
e-Liquid (CBD containing)	Agilent 1260 HPLC	Raptor ARC-18 LC reversed-phase column (150 mm × 4.6 mm, 2.7 μm)	Mobile phase A = aqueous ammonium formate (5mM)/HCOOH (0.1%) and B = ACN/HCOOH (0.1%). Isocratic elution with 75% B in A. Column temperature = 30°C; flow rate = 1.5 mL/min; run time = 10 min; injection volume = 5 μL.	UV detection at 228 nm	CBD (**5**) and CBDA (**6**).[97]

Hemp cosmetics	iChrom5100 analytical domestic LC	Supersil-ODS2 column (250 mm x 4.6 mm, 5 μm)	Mobile phase: 0.1% HCOOH acid in water (A) and ACN (B). Gradient 1: 80 – 85% B for 0 – 10 min; 85 – 90% B for 10 – 15 min; 90% B for 15 – 18 min; 90 – 80% B for 18 – 22 min; 80% B for 22 – 25 min. Gradient 2: 70 – 90% B for 0 – 20 min; 90%B for 20 – 22 min; 90 – 70% B for 22 – 24 min; 70% B for 24 – 30 min. Flow rate = 1 mL/min.	DAD set at 220 nm	CBD (**5**), CBDA (**6**), CBN (**10**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[117]
Hemp products	Thermo Fisher Scientific Vanquish Core HPLC coupled with a DAD and Orbitrap Exploris 120 MS	Poroshell 120 EC-C_{18} column (100 mm × 3 mm, 2.7 μm) coupled with a guard column (50 mm × 3 mm, 2.7 μm)	Mobile phase: 0.1% HCOOH in water (A) and ACN (B). Isocratic elution with 75% B for 6 min, washing with 98% B for 6-8 min, column re-equilibration at 75% B for 8-13 min. Flow rate = 0.5 mL/min.	DAD at 210 nm and HRMS	CBG (**8**), cannabigerobutol (CBGB) and cannabigerovarin (CBGV).[118]
Infused consumer products containing cannabis (cream, lip balm, lotion, shampoo)	Shimadzu LC-20A Prominence HPLC	Harmony Secure RP C_{18} column (150 mm x 4.6 mm, 3.5 μm)	Isocratic elution with 80% ACN with 0.1% formic acid and 20% water with 5 mM ammonium formate with 0.1% formic acid at a flow rate of 1.00 mL/min over 15 min. Column temperature = 40°C; injection volume = 50 μL	UV detection at 210 and 228 nm	CBD (**5**), CBDA (**6**), CBN (**10**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[119]
	Agilent 1200 HPLC paired to Agilent 6460 triplequadrupole MS	Raptor TM C_{18} column (150 mm x 4.6 mm, 3.5 μm)	Elution with 0.1% HCOOH in water (A) and 0.1% HCOOH in ACN (B) over 16 min gradient with a flow rate of 1 mL/min. The gradient started at 35% B in A and increased to 55% B in A in 2 min and held	MS in multiple reaction monitorin	

			for 1 min, increased to 80% B in A in 7 min, increased again to 95% B in A in 8 min and 100% B in A at 10 min, held for 3 min before decreasing to 35% B in A at 13.5 min and equilibrating until 16 min. Column temperature = 40°C; injection volume = 50 μL	g (MRM) mode	
Lotions	Reversed-phase HPLC	C_{18} column with a column guard	Isocratic elution with, 80% ACN with 0.1% HCOOH and 20% water with 5 mM ammonium formate and 0.1% formic acid. Run time 10 min; column temperature = 40°C	UV detection at 210 and 228 nm	CBD (**5**), CBN (**10**) and Δ^9-THC (**14**).[120]
Medical cannabis oil	HPLC–MS-MS TSQ Fortis II	Poroshell 120 C-18 (150 mm x 4.6 mm, 2.7 μm)	A gradient elution with 0.1% HCOOH in water (A) and MeOH (B), starting at 10% B in A, rising to 90% B in A in 2 min, 2-5 min at the same composition, then coming back to 10% A in 01.1 min and equilibration until 7 min	MS/MS	CBD (**5**) and Δ^9-THC (**14**).[121]
	Agilent 1260 HPLC	ACE 3 C_{18}-PFP column (150 mm × 3.0 mm, 3 μm)	Isocratic elution. 75% ACN and 0.1% formic acid in water.	UV detection at 228 nm	CBC (**1**), CBD (**5**), CBDA (**6**), CBG (**8**), CBN (**10**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[57]
	Shimadzu Prominence-i	Luna C_{18}(2) column (150 mm × 4.6 mm × 5 μm) coupled who a Security C_{18}	Mobile phase: A = water buffered with 20 mM ammonium formate and 0.1% HCOOH, B = ACN and C = MeOH buffered	PDA detection at 232 nm	CBC (**1**), CBD (**5**), CBDA (**6**), CBDV (**18**), CBG (**8**),

	LC-2030C 3D Plus HPLC-PDA	guard column (20 mm × 4.6 mm × 5 μm)	with 10 mM ammonium formate and 0.05% HCOOH. Gradient elution: started with 50% B in A, increased to 53% B in A in 17 min, 17-30 min 25% A, 30% B and 45%C, 30.01 min 10% A, 45% B and 45%C, held until 32.00 min, 32.01 min 50% B in A, and held until 34 min. Column temperature = 40°C; flow rate = 2.5 mL/min; injection volume 10 μL.		CBGA (**9**), CBN (**10**), Δ^8-THC (**13**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[69]
Medicinal cannabis products	Shimadzu HPLC system	ACE C_{18} column (150 mm x 4.6 mm, 5 μm)	The mobile phase was composed of water (A) and ACN (B), both containing 0.1% HCOOH. Gradient elution: 0-16 min 5-95% B in A, and then coming back to 5% B in 16-17 min. Flow rate = 0.6 mL/min; column temperature = 25°C; run time = 18 min.	DAD set at 220 nm	CBD (**5**), CBDA (**6**), CBN (**10**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[122]
Medicinal cannabis products including flos and oils	Shimadzu HPLC system	Restek Raptor ARC-18 column (150 mm x 4.6 mm, 2.7 mm)	Isocratic elution with 75% ACN containing 0.1% HCOOH in water containing 5 mM ammonium formate and 0.1 % HCOOH at a flow rate of 0.5 mL/min. Run time = 11 min	UV detection at 228 nm	CBC (**1**), CBCA (**2**), CBCL (**3**), CBCLA (**4**), CBD (**5**), CBDA (**6**), CBDV (**18**), CBDVA (**19**), CBG (**8**), CBGA (**9**), CBN (**10**), CBNA (**11**), Δ^8-THC (**13**), Δ^9-THC (**14**), Δ^9-THCA (**15**), THCV

					(**16**) and THCVA (**17**).[123]
Nonprescription commercial CBD products	Dionex Ultimate 3000 HPLC system	Waters XTerra MS C_{18} column (250 mm x 2.1 mm, 5 μm)	A gradient elution with the mobile phases consisted of a 50 mM aqueous solution of ammonium formate, pH 5.19 (phase A) and MeOH (phase B).	DAD detection at 235 nm	CBD (**5**), CBN (**10**) and Δ^9-THC (**14**).[124,125]
Nonpsychotropic cannabis oil	Dionex Ultimate 3000 coupled with a UV-Vis detector	ZORBAX Eclipse XDB C_{18} column (250 mm x 3 mm, 5 μm)	The mobile phase was composed of A = 0.1% formic acid in water, and B = ACN. Isocratic elution with 80% B in A over 25 minutes. Flow rate = 0.5 mL/min; column temperature 30°C.	UV detection at 220 nm	CBD (**5**), CBDA (**6**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[126]
Resins	Jasco reversed-phase HPLC coupled with a UV-Vis detector	C_{18} Agilent column (150 mm x 46 mm, 5 μm)	The mobile phase comprised water (A) and ACN (B). A linear gradient elution started with 10% B in A and increased to 90% B in A in 20 min. Injection volume = 10 μL; column temperature = 35°C	UV set at 220 nm	CBD (**5**), CBN (**10**) and Δ^9-THC (**14**).[127]
Resins and cannabis oil	Shimadzu LC-20A coupled with PDA	Hypersil BDS C_{18} column (150 mm x 4.6 mm, 5 μm)	The mobile phase comprised A = MeOH and B = 25 mM ammonium acetate solution. Gradient elution: 75% A: 1 min, 75% to 95% A in 15 min, 95% A: 2 min, 95% to 75% A in 2 min, and 75% A: 5 min. Total run time was 25 min. Flow rate = 1mL/min; run time = 25 min	UV set at 205 nm	CBD (**5**), CBDA (**6**), CBN (**10**), Δ^9-THC (**14**), and Δ^9-THCA (**15**).[38]
Resins and medicinal	Thermo-Fisher HPLC with PDA detector	ACE C_{18} column (250 mm x 4.6 mm, 5 μm).	Mobile phase: A = 50 mM ammonium formate, and B = MeOH. Step-gradient elution. Column temperature = 30°C; run	PDA detection at 240 nm.	CBD (**5**), CBN (**10**) and Δ^9-THC (**14**).[128]

cannabis extract			time = 30 min; flow rate = 1 mL/min; injection volume = 10 μL.		
UPLC (or UHPLC) methods					
Cannabis extract, tincture and cream	Acquity Classic UPLC coupled with a Xevo TQ MS detector	Acquity UPLC BEH C_{18} column (100 mm x 2.1 mm, 1.7 mm) connected to an Acquity UPLC BEH C_{18} VanGuard precolumn (5 mm x 2.1 mm)	The mobile phase comprised water (A) and ACN (B), both containing 0.1% HCOOH. Gradient elution: 50% B in A for 1 min; 100% B for 8 min; 50% B in A for 3 min; and equilibration of the column for 1 min. Injection volume = 5 μL; flow rate = 0.5 mL/min; column temperature = 45°C.	ESI-MS/MS in positive and negative ion modes	CBC (**1**), CBD (**5**), CBDA (**6**), CBDV (**18**), CBG (**8**), CBGA (**9**), CBN (**10**), Δ^8 -THC (**13**), Δ^9 -THC (**14**), Δ^9 -THCA (**15**) and THCV (**16**).[45]
Cannabis oil	Acquity UPLC H-Class coupled with a tunable UV detector (TUV)	Cortecs C_{18} UPLC column (150 mm x 2.1 mm, 1.6 μm)	Mobile phase: A = 0.1% acetic acid in water and B = ACN. Gradient elution: 0–16 min, 53% B in A; 17–31 min, 62% B in A; and 31–33 min, 95% B in A, the conditions were reset at 53% B (3 -35 min). Column temperature = 40°C; flow rate = 0.4 mL/min; injection volume = 2 μL	UV set at 230 nm	CBD (**5**) and Δ^9-THC (**14**).[129]
	Agilent 1200 RRLC system coupled to a diode array detector (DAD)	Kinetex C_{18} column (100 mm × 3.0 mm, 1.7 μm)	Mobile phase: A = 10 mM ammonium formate and B = ACN. Gradient elution: 0–8 min, 52–66% B in A; 8–8.5 min, 66–70% B in A; 8.5–13 min, 70–80% B in A; 13–15 min, 80% B in A; a 7-min column equilibration after each run. Flow rate = 0.6 mL/min; injection volume = 5 μL.	DAD set at 220 nm	CBC (**1**), CBD (**5**), CBDA (**6**), CBG (**8**), CBGA (**9**), CBN (**10**), Δ^9-THC (**14**), Δ^9-THCA (**15**), THCV (**16**) and THCVA (**17**).[48]

	Shimadzu Nexera X2 LC system coupled with a LCMS-8050 tandem mass spectrometer	Acquity UPLC HSS T3 column (30 mm x 2.1 mm, 1.8 μm)	Mobile phase A (ACN:water 75:25 + 0.05% HCOOH) and B (isopropanol:ACN 80:20 + 0.05%). Gradient elution started with 0% solution B, which was linearly increased to 100% over 1.5 min, this condition was maintained for 1 min, and then the column was re-equilibrated to initial conditions for 1 min Injection volume = 5 μL; flow rate = 0.4 mL/min; run time = 3.5 min; column temperature = 30°C.	ESI-MS in positive ion mode	CBD (**5**), CBDA (**6**), CBN (**10**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[130]
Cannabis oil galenic preparations	Acquity UPLC coupled with a triple quadrupole detector (TQD, MS/MS)	Acquity UPLC HSS-T3 column (30 mm x 2.1 mm, 1.8 μm)	Mobile phase A consisted of 0.05% HCOOH acid in water:ACN (30:70), and mobile phase B of 0.05% HCOOH in isopropanol:ACN (80:20). Gradient elution: started with 100% A for 4.6 min, reached 100% B phase and kept for 1.5 min, then the column was reconditioned at 100% B. Run time = 7 min; flow rate = 0.4 mL/min; injection volume = 4 μL.	ESI-MS in positive ion mode	CBD (**5**), CBDA (**6**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[131]
	Agilent 1200 series HPLC coupled to a 6430 Triple Quad MS	Zorbax Eclipse Plus C_{18} column (50 mm x 2.1 mm, 1.8 μm)	Mobile phase: 0.1% HCOOH in water (A) and 0.1% HCOOH in ACN (B). Gradient elution: started with 50% B in A for 0.2 min, increased to 100% B in 2.8 min, kept for 2 min, and came back to 50% B for column reconditioning. Run time = 8.5		

			min; flow rate = 0.3 mL/min; injection volume = 5 μL.		
Cannabidiol oil	Shimadzu Nexera UHPLC system	Phenomenex XB-C_{18} column (50 mm × 2.1 mm, 1.7 μm)	A gradient elution with 0.1% HCOOH in water (A) and 0.1% HCOOH in ACN (B), starting with 5% B, the gradient was increased to 100% B over 18 min. Injection volume 1-10 μL and flow rate = 0.3 mL/min	DAD detection at 201 and 273 nm	CBC (**1**), CBD (**5**), CBDV (**18**), CBG (**8**), CBN (**10**) and *cis*- and *trans*-Δ^9-THC (**14**).[132]
Commercial cannabinoids	Agilent 1260 Infinity II LC coupled with an Agilent 6545 quadrupole time-of-flight (Q-TOF) mass spectrometer, equipped with a Dual AJS (Agilent Jet Stream) ESI source operating in positive-ion mode	Luna Omega Polar C_{18} column (150 mm × 2.1 mm, 1.6 μm)	Isocratic elution with 73% B (ACN) in A (0.028% HCOOH in water at pH 3.6). Column temperature = 30°C, run time = 15 min, injection volume 3 μL and flow rate = 0.3 mL/min	PDA at 223, 230, 251, 269 and 285 nm, and ESI-TOF (time of flight) in positive ion mode	CBC (**1**), CBCA (**2**), CBD (**5**), CBDA (**6**), CBDV (**18**), CBDVA (**19**), CBG (**8**), CBGA (**9**), CBCLA (**4**), CBN (**10**), CBT (**22**), Δ^8-THC (**13**), Δ^9-THC (**14**), Δ^9-THCA (**15**), THCV (**16**) and THCVA (**17**).[134]
	Agilent 1290 Infinity II UHPLC	Daicel's immobilized sub-2 μm chiral column series:	Both normal and reversed-phase solvents. Normal phase: *n*-	DAD set at 220 nm	CBC (**1**), CBD (**5**), CBDA (**6**), CGB (**8**),

	equipped with a DAD	Chiralpak IA-U [amylose tris (3,5- dimethyl-phenylcarbamate)], IB-U [cellulose tris (3,5-dimethylphenylcarbamate)], IC-U [cellulose tris (3,5-dichloro-phenylcarbamate)], ID-U [amylose tris (3-chloro-phenylcarbamate)], IG-U [amylose tris (3-chloro-5-methyl-phenylcarbamate)], and IH-U [amylose tris (S)-α-methyl-benzylcarbamate. All columns were 100 mm x 3 mm.	hexane/isopropanol/ethanol/trifluoroacetic acid (TFA) (96: 3: 1: 0.1), isocratic elution at a flow rate of 0.21 mL/min at 25°C with an injection volume of 0.5 μL. Reversed-phase: water/ACN/TFA (45: 55: 0.1), isocratic elution at a flow rate of 0.8 mL/min at 25°C with an injection volume of 0.5 μL.		CBGA (**9**), CBN (**10**), Δ^8-THC (**13**), Δ^9-THC (**14**), Δ^9-THCA (**15**) and THCV (**16**).[135]
Decoction and oil	Acquity UPLC I-Class coupled with a Xevo TQ-S micro mass spectrometer	Acquity UPLC BEH C_{18} column (50 mm x 2.1 mm, 1.7 μm)	Mobile phase = ammonium formate 50 mM pH 3 (A) and MeOH (B). Gradient elution: started with 40% B in A, held for 0.5 min, increased to 100% B within 7.0 min, decreased to 40% B within 8.0 min, held for 2.0 min. Run time = 10 min; injection volume = 10 μL; flow rate = 0.4 mL/min; column temperature = 50°C.	ESI positive ion mode	CBD (**5**), CBDA (**6**), Δ^9-THC (**14**), Δ^9-THCA (**15**) and Δ^9-THC conjugates.[133]

Ground cannabis inforescence packaged in tamper-proof cartridges (Syqe cartridges)	Acquity UPLC H-Class	Acquity UPLC C_{18} column (150 mm x 2.1 mm, 1.7-µm)	Mobile phase: water (A) and ACN (B), both containing 0.1% HCOOH. Gradient elution: 70% to 100% B 10.5 min, held for 0.5 min, and returned to the initial conditions. Column temperature = 30°C; flow rate = 0.4 mL/min; run time = 14 min	PDA detection at 228 nm	CBD (**5**), CBDA (**6**), CBGA (**9**), CBN (**10**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[136]
e-Liquid	Agilent 1290 HPLC coupled to a TIMS-TOF Pro MS detector	Poroshell EC-C_{18} column (150 mm x 3 mm, 1.9 µm)	Mobile phase A = 0.1% formic acid in water and B = 0.1% formic acid in ACN. Isocratic elution with 75% B in A for 24 min. Injection volume = 5 µL; flow rate = 0.5 mL/min; column temperature = 30°C.	ESI-MS in positive and negative ion mode	CBD (**5**), 7-OH-CBD, 6α-OH-CBD, CBDV (**18**) and CBE (**20**).[137]
Resins and cannabis oil	Dionex Ultimate 3000 UHPLC system coupled with a Q-Exactive Plus orbital trap mass spectrometer	Acquity UPLC BEH C_{18} column (100 mm × 2.1 mm, 1.7 µm)	Mobile phase: A = 5% MeOH in water and B = isopropanol-MeOH-water (65:30:5); both A and B with 5 mM ammonium formate and 0.1% formic acid. Gradient elution: started with 5% B, increasing to 60% B within the first minute, followed by a gradual change to 70% B in 10 min, rising sharply to 100% B in 0.5 min, washing with 100% B for 5 min and reconditioning to initial conditions for 2.5 min. Run time = 19 min; flow rate = 0.3 and 0.4 mL/min; injection volume 3 µL	HRMS/MS; ESI-MS in positive ion mode	CBD (**5**), CBG (**8**), CBN (**10**) and Δ^9-THC (**14**).[26]

TABLE 3 HPLC and UPLC (or UHPLC) methods for the analysis of phytocannabinoids and their conjugates in human blood samples

Matrices/sources	Instrumentation	Column	Mobile phase	Detection	Cannabinoids analysed/detected
HPLC methods					
Plasma	Acquity H-Class PLUS coupled with a XEVO TQ-S micro MS detector with an ESI interface	Kinetex C_{18} column (100 mm x 2.1 mm, 2.6 μm)	Mobile phase: water (A) and ACN (B), both containing 0.1% formic acid. Gradient elution: 0-0.6 min 18% B in A, 0.6-5.5 min 72% B in A, 5.5-6.5 min 72% B in A, 5.5-7.3 min 79% B in A, 7.0-7.8 min 79% B in A, 7.8-8.8 min 95% B in A, 8.8-9.2 95% B in A, 9.2-10 min 82% B in A. Column temperature = 40°C; flow rate = 0.5 mL/min; run time = 10 min.	ESI-MS/MS in positive and negative ion modes	CBD (**5**), CBDA (**6**), CBN (**10**), 11-OH–Δ^9-THC (**12**), Δ^8-THC (**13**), Δ^9-THC (**14**), Δ^9-THCA (**15**), 11-nor-9-carboxy-Δ^9-THC and Δ^9-THCA-glucuronide.[138]
	Agilent 1250 HPLC coupled with an AB Sciex API5500 triple quadrupole mass spectrometer with a Turbo V Ion Spray source	Ascentis Express RP-Amide analytical column (150 mm x 3 mm, 2.7 μm)	Mobile phase A = 0.04% formic acid in water and B = ACN:MeOH:isopropanol (3:1:1). Gradient elution: 60% B for 1.1 min, to 100% B at 5.0 min, holding for 3.5 min, re-equilibrated to 60% B over 0.1 min and held for 2.4 min. Run time = 10 min; column temperature = 60°C; flow rate = 0.55 mL/min	Atmospheric pressure chemical ionization (APCI) in combination with multiple reaction monitoring in positive ion mode	CBC (**1**), CBD (**5**) and its metabolites including CBD-glucuronide (CBD-glu), CBDA (**6**), 6α-hydroxy-CBD (CBD-6α-OH), 7-hydroxy-CBD (CBD-7-OH), CBN (**10**), Δ^9-THC (**14**) and its metabolites including Δ^9-THCA-glucuronide, 11-OH-Δ^9-

					THC (**12**) and THCVA (**17**).[139]
	Dionex Ultimate 3000 UHPLC coupled with a linear trap quadrupole-Orbitrap mass spectrometer (LTQ-Orbitrap Velos)	Gemini C_{18} column (100 mm x 4.6 mm, 3 µm)	Mobile phase: A = 25 mM formic acid in water and B = 25 mM formic acid in ACN. Isocratic elution with 40% B in A. Flow rate = 0.5 mL/min; run time = 15 min.	ESI-MS/MS in positive ion mode	CBD (**5**), CBG (**8**), CBN (**10**) and Δ^9-THC (**14**).[141]
UPLC (or UHPLC) methods					
Blood	Acquity UPLC H-Class System coupled with an MS detector	Acquity BEH C_{18} column (150 mm × 2.1 mm, 1.7 µm) connected to an Acquity BEH C_{18} VanGuard pre-column (5 mm × 2.1 mm, 1.7 µm)	Mobile phase: 0.1% formic acid in water (A) and 0.1% formic acid in MeOH. Multiple gradient elution started with 10% B in A. Flow rate 0.3 mL/min; runtime = 17 min; injection volume = 5 µL.	ESI-MS/MS in positive and negative ion modes	CBD (**5**), CBN (**10**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[142]
	Acquity UPLC I-Class System coupled with a Xevo® TQ-S micro MS equipped with an ESI source operating in both negative and positive-ion modes	Acquitytm Premier UPLC BEH C_{18} column (100 mm x 2.1 mm, 1.7 µm)	The mobile phase comprised A = water with ammonium formate 5 mM pH 7.5 and B = ACN. Gradient elution: from 5% B in A for 0.25 min to 30% B in A after 1 min, 80% B in A after 11.5 min and held for 0.5 min, 100% B	ESI-MS/MS in positive and negative ion modes	CBD (**5**), 7-COOH-CBD (**7**), 7-OH-CBD, 6α-OH-CBD, 6β-OH-CBD, Δ^9-THC (**14**), 11-OH-Δ^9-THC and (11-COOH-THC (or THC-COOH, **29**).[143]

			after 11.55 min till 13.5 min, and then back to 5% B in A after 13.55 min and held for the remaining 17:00 min. Run time = 17 min; flow rate = 0.4 mL/min; column temperature = 50°C		
	Sciex Exion LC system coupled with a QTRAP® 6500+ triple quadrupole linear ion trap MS equipped with a TurboIonSpray interface	Acquity UPLC HSS T3 C_{18} column (100 mm x 2.1 mm, 1.8 μm)	The mobile phase comprised A = MeOH/water containing 2 mM ammonium formate with formic acid 0.1% (95/5) and B = MeOH/water containing 2 mM ammonium formate with formic acid 0.1% (5/95). Gradient elution: 45% A at 0 min, linearly increased to 60% A in 5 min and to 95% A in 1 min, isocratic for 1.5 min, followed by a decrease in the initial conditions in 0.05 min and equilibration time for 1.95 min. Run time = 10 min; flow rate = 0.4 mL/min; column temperature = 45°C.	MS detection in two modes, MRM and MS^3	Δ^9-THC (**14**), Δ^9-THC-OH and Δ^9-THC-COOH (**29**).[144]
Capillary blood	Dionex Ultimate 3000 UHPLC coupled to a	Acquity UPLC BEH C_{18} column	Mobile phase = 5 mM ammonium acetate (A) and 0.1% formic acid in ACN (B). Gradient elution that	Atmospheric pressure chemical	CBD (**5**) and Δ^9-THC (**14**).[145]

	TSQ Quantiva™ Triple Quadrupole	(50 mm x 2.1 mm, 1.7 μm)	started with 60% B in A. Column temperature = 40°C; flow rate = 0.3 mL/min.	ionization (APCI) in the positive ion mode	
Plasma	Acquity UPLC H-Class system coupled to the Xevo® TQ-D tandem quadrupole MS equipped with a Z-spray source	Coreshell Kinetex C_{18} column (100 mm x 2.1 mm, 1.7 μm)	The mobile phase comprised A (5 mM ammonium acetate) and B (ACN with 0.1% formic acid). Isocratic elution with 60% B in A. Column temperature = 40°C; flow rate = 0.3 mL/min.	ESI-MS/MS in positive ion mode	CBD (**5**) and Δ^9-THC (**14**).[146]
	Acquity-Xevo-TQD UHPLC-MS/MS system	Kinetex C_{18} column (100 mm x 2.1 mm, 1.7 μm)	Mobile phase: A = 5 mmol/L ammonium formate solution with 0.1% formic acid and B = ACN. Gradient elution: 60% B in A (0–1.2 min), 60% to 75% B in A (1.2–2.2 min), 75% B in A (2.2–2.8 min), 100% B (2.8–3.5 min), 100% B (3.5–7.0 min), 60% B in A (7.01–9.0 min). Flow rate = 0.3 mL/min; column temperature = 40°C; injection volume = 10 μL.	ESI-MS/MS in in the multiple reaction monitoring (MRM) mode	CBD (**5**) and Δ^9-THC (**14**).[147]
	Dionex Ultimate 3000 UHPLC system coupled with a TSQ Quantiva™ Triple	Acquity UPLC BEH C_{18} column (50 mm x 2.1 mm, 1.7 μm)	Mobile phase = 5 mM ammonium acetate (A) and 0.1% formic acid in ACN (B). Gradient elution that started with 60% B in A. Column	ESI-MS/MS in positive ion mode	CBD (**5**) and Δ^9-THC (**14**).[148]

	Quadrupole MS detector		temperature = 40°C; flow rate = 0.3 mL/min.		
	Shimadzu Nexera X2 UHPLC system coupled with a tandem MS detector	Acquity UPLC® BEH C_{18} column (50 mm x 2.1 mm, 1.7 μm)	ACN/water 70-90% gradient in 2 min.	ESI-MS/MS in negative ion mode	7-COOH-CBD and Δ^9-THC metabolites.[149]
Serum	Acquity UPLC I-Class coupled with a Waters Xevo TQ-S micro mass spectrometer (triple quadrupole) equipped with an electrospray ionization source operating in negative-ion mode (ESI-)	Acquity UPLC BEH C_{18} column (100 mm x 2.1 mm, 1.7 μm)	A gradient mobile phase composed of ammonium formate 5 mM at pH 7.5 (A) and ACN (B). Flow rate = 0.4 mL/min; column temperature = 50°C; run time = 8 min.	ESI-MS/MS in negative ion mode	CBD (**5**), CBDA (**6**), 7-hydroxycannabidiol (CBD-7-OH) and 6α-hydroxycannabidiol (CBD-6α-OH).[150]
	Acquity UPLC I-Class coupled with a Xevo TQ-S micro mass spectrometer	Acquity UPLC® BEH C_{18} column (50 mm x 2.1 mm, 1.7 μm)	Mobile phase = ammonium formate 50 mM pH 3 (A) and MeOH (B). Gradient elution: started with 40% B in A, held for 0.5 min, increased to 100% B within 7.0 min, decreased to 40% B within 8.0 min, held for 2.0 min. Run time = 10 min; injection	ESI-MS/MS in positive ion mode	CBD (**5**), CBDA (**6**), Δ^9-THC (**14**), Δ^9-THCA (**15**) and Δ^9-THC conjugates.[133]

			volume = 10 μL; flow rate = 0.4 mL/min; column temperature = 50°C.		
	Acquity UPLC I-Class LC coupled with a Waters® Xevo® TQ-S micro mass spectrometer (triple quadrupole) prepared with an electrospray ionization source operating in negative-ion mode (ESI−)	Acquity UPLC BEH C_{18} column (100 mm x 2.1 mm, 1.7 μm)	The mobile phase comprised ammonium formate 5 mM at pH 7.5 (A) and ACN (B). Gradient elution: initial conditions were 5% B in A, held for 0.25 min, increased gradually to 100% B within 5.3 min, decreased to 5% B in A within 5.4 min, held for 2.6 min. Run time = 8 min; flow rate = 0.4 mL/min; column temperature = 50°C	ESI-MS/MS in negative ion mode	CBD (**5**), CBDA (**6**), 7-COOH-CBD (**7**), 6α-OH-CBD, 6β-OH-CBD and 7-OH-CBD.[151]
	HPLC ExionLC 100 integrated system coupled with a 3200 QTRAP® triplequadrupole linear ion trap mass spectrometer fitted with a TurboIonSpray interface	Phenomenex Onyx monolithic C_{18} column (100 mm x 3 mm, 1.5 μm)	Mobile phase: A = 2% MeOH in water containing 10 mM ammonium formate and 0.1% formic acid, and B = MeOH/CAN/isopropanol (80:10:10) containing 8 mM ammonium formate and 0.08% formic acid. Gradient elution: started at 80% Bin A, ramped to 98% B in A over 3 min and then maintained for 2 min, an	ESI-MS/MS in positive ion mode	CBD (**5**) and 7-OH-CBD.[152]

			additional cleaning step of 3 min with 0.6 mL/min of a mixture 1% B: 99% MeOH before valve activation for re-equilibration of the column at 0.6 mL/min for 3 min (1% B in A). Column temperature = 25°C; run time = 12 min; injection volume = 40 µL; flow rate = 0.5 mL/min.		

TABLE 4 HPLC and UPLC (or UHPLC) methods for the analysis of cannabinoids in sweat, oral fluid and breast milk samples

Matrice/sources	Instrumentation	Column	Mobile phase	Detection	Cannabinoids analysed/detected
HPLC methods					
Breast milk	Agilent 1200 series LC system coupled with a Sciex API5000 tandem mass spectrometer	Poroshell Eclipse C_{18} column (50 mm x 4.6 mm, 2.7 μm)	The mobile phase comprised B = 20% isopropanol, 20% MeOH and 60% ACN, and A = water supplemented with 0.1% formic acid. Gradient elution: began with a flow rate of 0.75 mL/min and 60% of solvent B in A for the first minute, in the following 3 min, the flow rate and the organic solvent content increased to 1 mL/min and 95% solvent B in A, respectively. From 4 to 6 min, B increased to 100%. At minute 6.2, the system returned to starting conditions for 1.8 min to equilibrate for the following injection. Column temperature = 60°C.	ESI-MS/MS positive and negative ion mode	CBD (**5**), CBG (**8**), 11-OH-Δ^9- THC (**12**), Δ^9-THC (**14**), Δ^9-THCA (**15**) and Δ^9-THC-glucuronide.[153]
Oral fluid	Agilent 1100 series equipped with a Sciex API4000 TurboIonSpray interface for pneumatically assisted electrospray	Agilent Pursuit XRs Ultra 2.8 C_{18} column (100 mm × 2.0, 2.8 μm)	Mobile phase: A = 10 mM formic acid in water and B = 5% ACN in MeOH with 10 mM formic acid. Gradient elution: began with 15% B in A, held for 2 min; from 15 to 80% organic B in A in 1.5 min, held for 1 min; from 80 to 100% B in A in 1 min; 100% B,	ESI-MS/MS in positive ion mode	CBD (**5**), CBN (10), Δ^9-THC (**14**), and Δ^9-THC-COOH (**29**).[154]

			held for 5.5 min; back to the starting condition in 0.5 min and re-equilibration for 13.5 min. Flow rate = 0.2 mL/min; injection volume = 2 mL; run time = 25 min.		
UPLC (or UHPLC) methods					
Oral fluid	Acquity UPLC coupled with Xevo TQ-S instrument	Cortecs C_{18} column (50 mm x 2.1 mm, 1.6 μm)	Mobile phase A = 0.1% formic acid in water/acetonitrile (95:5) and B = 0.1% formic acid in ACN. Injection volume was 6.0 μL. Gradient elution: began with 55% B in A for 7.5 min, increased to 100% B in A over 0.8 min and then held at 100% B for 0.3 min, re-equilibration to 55% B in A and then held at 55% Bin A for 0.4 min. Column temperature = 26°C; run time = 9 min; flow rate = 0.5 mL/min.	ESI-MS/MS in positive ion mode	CBC (**1**), CBD (**5**), CBDA (**6**), CBG (**8**), CBN (**10**), Δ^8-THC (**13**), Δ^9-THC (**14**), 11-OH-Δ^9-THC (**12**), Δ^9-THCA (**15**), THCV (**16**), CBDV (**18**) and cannabidiorcol (CBD-C1).[155]
	Agilent Infinity 1290 UHPLC system coupled with a 4500 QTRAP hybrid triple quadrupole/linear ion trap MS with a Turbo V ion source	Ace Excel Super C_{18} column (75 mm × 2.1 mm, 2 μm) linked to a C_{18} guard column	Mobile phase: water/formic acid 5 mM (A) and ACN (B). Gradient elution: started with 50% B in A for 0.3 min, increased to 100% B in 3 min, and continued at 100% B for 0.5 min. Flow rate = 0.6 mL/min; column temperature = 45°C; run time 5 min.	ESI-MS in negative ion mode, and MS/MS (MS^2) selected reaction monitoring	CBD (**5**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[156]

				(SRM) acquisition	
Oral fluid and sweat	Acquity UPLC I-Class coupled with a Xevo TQ-S micro mass spectrometer	Acquity UPLC BEH C_{18} column (50 mm x 2.1 mm, 1.7 μm)	Mobile phase = ammonium formate 50 mM pH 3 (A) and MeOH (B). Gradient elution: started with 40% B in A, held for 0.5 min, increased to 100% B within 7.0 min, decreased to 40% B within 8.0 min, held for 2.0 min. Run time = 10 min; injection volume = 10 μL; flow rate = 0.4 mL/min; column temperature = 50°C.	ESI-MS/MS in positive ion mode	CBD (**5**), CBDA (**6**), Δ^9-THC (**14**), Δ^9-THCA (**15**) and Δ^9-THC conjugates.[133]
Saliva	HPLC ExionLC 100 integrated system coupled with a 3200 QTRAP® triplequadrupole linear ion trap mass spectrometer fitted with a TurboIonSpray interface	Onyx monolithic C_{18} column (100 mm x 3 mm, 1.5 μm)	Mobile phase: A = 2% MeOH in water containing 10 mM ammonium formate and 0.1% formic acid, and B = MeOH/ACN/isopropanol (80:10:10) containing 8 mM ammonium formate and 0.08% formic acid. Gradient elution: started at 80% Bin A, ramped to 98% B in A over 3 min and then maintained for 2 min, an additional cleaning step of 3 min with 0.6 mL/min of a mixture 1% B: 99% MeOH before valve activation for re-equilibration of the column at 0.6	ESI-MS/MS in positive ion mode	CBD (**5**) and 7-OH-CBD.[152]

			mL/min for 3 min (1% B in A). Column temperature = 25°C; run time = 12 min; injection volume = 40 μL; flow rate = 0.5 mL/min.		

TABLE 5 HPLC and UPLC (or UHPLC) methods for the analysis of phytocannabinoids in human hair and nail samples

Matrices/source	Instrumentation	Column	Mobile phase	Detection	Cannabinoids analysed/detected
HPLC methods					
Hair	Agilent UHPLC 1290 Infinity II coupled with a 6470 A Triple Quadrupole LC-MS equipped with an ESI source operating in positive and negative-ion modes	Kinetex column (100 mm x 2.1 mm, 2.6 μm)	The mobile phase comprised water and ammonium formate 2 mM at pH 8.5 (A) and ACN (B). Gradient elution: started with 20% B in A, held for 0.25 min, increased to 63% B in A within 3.1 min, to 65% B within 5.00 min, decreased to 20% B in A within 8.10 min, held for 1.9 min. Run time = 10 min; flow rate = 0.4 mL/min; injection volume = 1 μL.	ESI-MS positive and negative ion modes	CBD (**5**) and its metabolites, 11-OH-THC (**12**) 7-COOH-CBD (**7**), 7-OH-CBD, 6α-OH-CBD and 6β-OH-CBD), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[157]
Hair and nail	Acquity I Class coupled with a Waters XEVO TQ-S Micro tandem quadrupole mass spectrometer	Oasis HLB column (20 mm x 4.6 mm, 5 μm)	Mobile phase = 12.5 mM ammonium formate pH 9.5 (A) and ACN (B). Gradient elution: started with 25% B in A, and increased to 100% B in 10 min. Column temperature = 50^{o}C; run time = 10 min; flow rate = 1 mL/min.	ESI-MS in positive ion mode	Δ^9-THC (**14**).[158]
UPLC (or UHPLC) methods					
Hair	Agilent Infinity 1290 UHPLC system coupled	Ace Excel Super C_{18}	Mobile phase: water/formic acid 5 mM (A) and ACN (B). Gradient	ESI-MS in negative ion	CBD (**5**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[156]

	with a 4500 QTRAP hybrid triple quadrupole/linear ion trap MS with a Turbo V ion source	column (75 mm × 2.1 mm, 2 μm) linked to a C_{18} guard column.	elution: started with 50% B in A for 0.3 min, increased to 100% B in 3 min, and continued at 100% B for 0.5 min. Flow rate = 0.6 mL/min; column temperature = 45°C; run time 5 min.	mode, and MS/MS (MS^2) selected reaction monitoring (SRM) acquisition	
Nail	Acquity UPLC coupled to a Waters Xevo TQ-XS triple quadrupole spectrometer (QQQ-MS/MS) with an ESI source	Acquity UPLC HSS column (150 mm x 2.1 mm, 1.8 μm)	Mobile phase: A = 5 mM ammonium acetate and water with 0.075% formic acid, and B = ACN with 0.1% formic acid. Gradient elution: 15% B in A for 0.5 min, increased to 50% B in A from 0.5 min to 10 min, further increased to 95% B in A in 0.75 min, held until 12.25 min, dropped back to 15% B in A in 0.50 min, held until 15 min. Flow rat e= 0.4 mL/min.	ESI-MS in positive ion operating in multiple reaction monitoring (MRM) mode	Δ^9-THC (**14**).[159]

TABLE 6 HPLC and UPLC (or UHPLC) methods for the analysis of phytocannabinoids and their conjugates in human urine samples

Instrumentation	Column	Mobile phase	Detection	Cannabinoids analysed/detected
HPLC methods				
Agilent Infinity 1290 II LC system coupled with an e6470 Triple Quadrupole MS (UHPLC-QQQ-MS)	ACE Excel 3 C_{18}-PFP column (150 mm × 4.6 mm, 3 μm)	Mobile phase: A = 10 mmol/L ammonium formate solution with 0.05% formic acid, and B = MeOH. Gradient elution: %B in A was increased from 5% to 60% in 6 min, reached 95% in 8 min, held at 95% for 3 min, and then reduced to 5% in 2 min. Re-equilibration at the initial gradient conditions at 5% for 4 min was followed. Flow rate = 0.4 mL/min; column temperature = 40°C; run time = 17 min; injection volume = 10 μL.	Jet Stream Technology Ion Source (AJS) ESI-MS/MS in positive and negative ion modes	Δ^9-THCA (**15**).[160]
UPLC (or UHPLC) methods				
Acquity UPLC I-Class coupled with a Xevo TQ-S micro mass spectrometer	Acquity UPLC BEH C_{18} column (50 mm x 2.1 mm, 1.7 μm)	Mobile phase = ammonium formate 50 mM (A) and MeOH (B). Gradient elution: started with 40% B in A, held for 0.5 min, increased to 100% B within 7.0 min, decreased to 40% B within 8.0 min, held for 2.0 min. Run time = 10 min; injection volume	ESI-MS/MS positive ion mode	CBD (**5**), CBDA (**6**), Δ^9-THC (**14**), Δ^9-THCA (**15**), 11-OH-THC (**12**), THC-COOH (**29**) and Δ^9-THC conjugates.[133]

		= 10 μL; flow rate = 0.4 mL/min; column temperature = 50°C.		
Acquity UPLC I-Class coupled with a Waters Xevo TQ-S micro mass spectrometer (triple quadrupole) equipped with an electrospray ionization source operating in negative-ion mode (ESI-)	Acquity UPLC BEH C_{18} column (100 mm x 2.1 mm, 1.7 μm)	A gradient mobile phase composed of ammonium formate 5 mM at pH 7.5 (A) and ACN (B). Flow rate = 0.4 mL/min; column temperature = 50°C; run time = 8 min.	ESI-MS/MS in negative ion mode	CBD (**5**), CBDA (**6**), 7-OH-CBD and 6α-OH-CBD.[150]
Acquity UPLC I-Class coupled with a Xevo TQD tandem MS	Cortecs UPLC C_{18} Plus analytical column (50 mm x 2.1 mm, 1.6 μm)	Mobile phase: A = 0.1% formic acid in water, and B = ACN. Gradient elution: 55% B in A (0–1.9 min), 67% B in A (1.9–4.4 min), 95% B in A (4.4–4.6 min) and 55% B in A (4.6–5.5 min). Column temperature = 30°C; flow rate = 0.4 mL/min; injection volume = 10 μL; run time = 5.5 min.	ESI-MS/MS in positive and negative ion modes	CBD (**5**), Δ^8-THC (**13**), Δ^9-THC (**14**), Δ^{10}-THC, Δ^8-THC-COOH, Δ^9-THC-COOH (**29**).[161]
Agilent Infinity 1290 UHPLC system coupled with a 4500 QTRAP hybrid triple quadrupole/linear ion trap MS with a Turbo V ion source	Ace Excel Super C_{18} column (75 mm × 2.1 mm, 2 μm) linked to a C_{18} guard column.	Mobile phase: water/formic acid 5 mM (A) and ACN (B). Gradient elution: started with 50% B in A for 0.3 min, increased to 100% B in 3 min, and continued at 100% B for 0.5 min. Flow rate = 0.6 mL/min; column temperature = 45°C; run time 5 min.	ESI-MS/MS in negative ion mode and MS/MS (MS^2) selected reaction monitoring (SRM) acquisition	CBD (**5**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[156]

Agilent 1260 Infinity LC system equipped with an AB Sciex QTRAP 4500 MS system	Acquity UPLC HSS T3 (100 mm x 2.1 mm, 1.8 μm)	Mobile phase: water containing 0.05% acetic acid (A) and MeOH (B). Gradient elution: started with 60% B in A and kept for 2 min, increased to 80%B in A from 2 to 6 min and maintained until 12 min, increased to 95% B in A from 12 to 16 min and maintained until 19 min. Finally, mobile phase B was decreased to 60% B in A from 19 to 19.1 min and maintained until 25 min to stabilize the LC system. Flow rate = 0.2 mL/min; column temperature = 35°C.	ESI-MS/MS in negative ion mode using multiple reaction monitoring (MRM) transitions	CBD (**5**), 6-OH-CBD, 7-OH-CBD, 7-COOH-CBD (**7**), Δ^8-THC (**13**), Δ^9-THC (**14**), Δ^9-11-OH-THC, Δ^8-THC-COOH Δ^9-THC-COOH (11-COOH-THC, **29**) and Δ^9-THC-COOH-glucuronide.[162]
Dionex Ultimate 3000 UHPLC coupled with Q Exactive Plus Hybrid Quadrupole-Orbitrap MS (UHPLC-QE Plus-HRMS)	Hypersil GOLD column (100 mm × 2.1 mm, 1.9 μm)	Mobile phase: A = 110 mmol/L ammonium formate solution with 0.05% formic acid was adjusted to a pH value of around 3.6, and B = MeOH. Gradient elution: %B in A was increased from 5% to 90% in 11 min, to 100% in 14 min, and then went into post-run equilibrium time for 4 min to the initial conditions. Column temperature = 30°C; run time = 18 min; flow rate = 0.25 mL/min; injection volume = 10 μL.	ESI-MS/MS in positive and negative ion modes	Δ^9-THCA (**15**).[160]

TABLE 7 HPLC and UPLC (or UHPLC) methods for the analysis of phytocannabinoids in miscellaneous samples from human

Matrices/source	Instrumentation	Column	Mobile phase	Detection	Cannabinoids analysed/detected
HPLC methods					
Dry bone	Thermo-Fisher HPLC system coupled with a Q-Exactive Orbitrap–mass spectrometer	Synergi Hydro-RP column (150 mm x 2 mm, 4 μm) coupled to a C_{18} guard column (4 mm x 3 mm, 4 μm)	Mobile phase: A = 20 mM ammonium formate in water and B = 0.1% HCOOH in MeOH. Gradient elution: started with 90% B in A, increased to 95% B in A in 13 min, kept at 95% B in A until 20 min, decreased to 10% B in A by 22 min and stayed there until 28 min. Flow rate = 0.7 mL/min.	Heated ESI (HESI)	11-COOH-THC (or THC-COOH) (**29**).[163]
S9 liver fraction	Dionex Ultimate 3000 UHPLC coupled with a Q Exactive Focus Quadrupole Orbitrap MS	Acclaim 120 C_{18} column (100 mm x 2.1 mm, 3 μm)	Mobile phase: A = 2 mM ammonium acetate buffer containing 0.1% HCOOH and B = ACN with 0.1% HCOOH. Gradient elution: started with 50% B in A, kept for 0.5 min, raised to 95% until 9 min, kept for 2 min at 95% B in A, decreased to 50% B in A within 0.1 min and kept until 14 min for equilibration. Flow rate = 0.3 mL/min; injection volume = 2 μL; Column temperature = 40°C.	Heated ESI (HESI) in positive and negative modes	11-OH-THC (**12**) and its glucuronide, 11-COOH-THC (**29**) and its glucuronide, and Δ^9-THC-glucuronic acid.[164]

UPLC (or UHPLC) methods					
Cell-free matrices	Agilent 1290 Infinity II LC coupled with an Agilent 6470 Triple Quadrupole Mass Spectrometer, equipped with ESI interface	Acquity UPLC BEH C_{18} column (100 mm x 2.1 mm, 1.7 μm) linked to an Acquity UPLC BEH C_{18} Guard column (5 m x 2.1 mm, 1.7 μm)	Mobile phase: A = 0.1% HCOOH in water, and B = 0.1% HCOOH in ACN. Gradient elution: 0 min, 60% B in A; 5 min, 60% B in A; 7 min, 95% B in A; 8.5 min, 95% B in A; 9.5 min, 60% B in A; 14 min, 60% B in A. Column temperature = 40°C; injection volume = 2 μL; flow rate = 0.6 mL/min	ESI-MS/MS	CBC (**1**), CBD (**5**), CBDV (**18**), CBG (**8**) and CBN (**10**).[165]
Cerumen (earwax)	Acquity I-Class UPLC coupled with a Xevo TQ-XS triple quadrupole MS	Acquity UPLC BEH C_{18} column (50 mm x 2.1 mm, 1.7 μm)	The mobile phase comprised water (A) and ACN (B), both containing 0.1% formic acid. Gradient elution: started with 55% B in A and increased to 95% B in A in 6 min and dropped back to 55% B in A in 1 min. Flow rate = 0.35 mL/min; injection volume = 10 μL; column temperature = 35°C.	ESI-MS/MS in positive ion mode	CBD (**5**), CBN (**10**), Δ^9-THC (**14**), 11-OH-THC (**12**) and 11-COOH-THC (**29**).[166]
Cerumen (earwax)	Acquity I-Class UPLC coupled with a Xevo TQ-XS triple quadrupole MS	Acquity UPLC BEH C_{18} column (50 mm x 2.1 mm, 1.7 μm)	The mobile phase comprised water (A) and ACN (B), both containing 0.1% formic acid. Gradient elution: 0–0.5 min, 10% B in A; 0.5–1.5 min, 10% B in A → 30% B in A; 1.5–5 min, 30% B in A;	ESI-MS/MS in positive ion mode with multiple reaction	CBC (**1**), CBD (**5**), CBG (**8**), CBN (**10**), Δ^9-THC (**14**), and 11-COOH-THC (**29**).[167]

			5–5.5 min, 30% → 90% B in A; 5.5–7.5 min, 90% B in A; 7.5–8.5 min, 90% B in A → 10% B in A; and from 8 to 10 min, 10% B in A. Flow rate = 0.3 mL/min; injection volume = 10 μL; column temperature = 35°C.	monitoring (MRM)	

TABLE 8 UHPLC (or UPLC) methods for the analysis of naturally occurring cannabinoids in animal samples

Animal	Instrumentation	Column	Mobile phase	Detection	Matrices/source	Cannabinoids analysed/detected
UHPLC (or UPLC) methods						
Cattle	Acquity H UPLC and a TQ-S triple quadrupole MS	Eclipse Plus C_{18} column (100 mm, 2.1 mm, 1.8 μm)	The mobile phase comprised water (A) and ACN (B), both containing 0.1% HCOOH. Gradient elution: 0 min: 60% Bin A, 6.50 min: 86% B in A, 7.50 min-9 min: 100%B, 9.01 – 12 min: 60% B in A. Column temperature = 55°C; flow rate = 0.5 mL/min; injection volume = 5 μL; run time = 12 min.	ES-MS in positive and negative ion modes	Plasma	CBC (**1**), CBCA (**2**), CBD (**5**), CBDV (**18**), CBDVA (**19**), CBG (**8**), CBN (**10**), 11-OH-Δ^9-THC (**12**), Δ^8-THC (**13**) Δ^9-THC (**14**), Δ^9-THC-COOH (**29**), Δ^9-THC-glucuronic acid and, Δ^9-THCV (**16**) and Δ^9-THCA (**15**).[168]
Dogs	Acquity UPLC coupled with a Xevo TQ-XS triple quadrupole mass spectrometer	Acquity UPLC HSS-T3 column (100 mm x 2.1 mm, 1.8 μm)	Mobile phases A and B were 0.1% HCOOH in water and 0.1% HCOOH in MeOH, respectively. Gradient elution: 0 min (60% A, 40% B in A), 0–2 min (linear gradient to 100% B), 2–4 min (100% B), 4–4.1 min (linear gradient to 60% A, 40% B) and 4.1–8 min (60% A, 40% B). Flow rate = 0.4 mL/min	ESI-MS/MS in positive ion mode	Blood sample after intranasal, intrarectal and oral administration of cannabidiol	CBD (**5**).[169]

	Acquity UPLC coupled with a Xevo TQ-S triple quadrupole mass spectrometer	Acquity UPLC HSS-T3 column (50 mm x 2.1 mm, 1.8 μm)	Water-ACN (both containing 0.1% HCOOH) linear gradient. Gradient programme: from 0 to 3.5 min B (75–95%), at 3. 5 min 95% B (5%), held for 0.5 min, and at 4.2 min 75% A for 0.8 min. Column temperature = 40°C; flow rate = 0.4 mL/min	ES-MS/MS in positive ion mode	Serum and urine	11-OH-Δ^9-THC (**12**), Δ^9-THC (**14**), Δ^9-THCA (**15**), and Δ^9-THC-glucuronide in serum. In the urine samples: CBD (**5**), 11-OH-Δ^9-THC (**12**), Δ^9-THC (**14**), Δ^9-THCA (**15**), Δ^9-THC-glucuronide and Δ^9-THCA-glucuronide.[170]
Donkeys	Acquity UPLC H-class coupled with a triple quadrupole mass spectrometer (TQ-S) using both positive and negative ionization modes	Acquity UPLC HSS-T3 column (100 mm x 2.1 mm, 1.8 μm)	Water-ACN (both containing 0.1% HCOOH) linear gradient.	ES-MS/MS in positive and negative ion modes	Plasma	11-OH-Δ^9-THC (**12**), Δ^9-THC (**14**) and Δ^9-THCA (**15**).[171]
Mice	Acquity UPLC Class I coupled with an ESI-MS detector	Acquity BEH C_{18} column (50 mm × 2.1 mm, 1.7 μm)	The mobile phase comprised 0.1% HCOOH in water (A) and 0.1% HCOOH in ACN (B). Gradient elution: 50% B in A for 1 min, increased linearly to 95% B in 3 min, kept constant till 3.5 min and the column was set back to initial conditions at 3.6 min and saturated with the mobile phase for the next injection for	ESI-MS/MS in positive ion mode	Brain and peripheral tissues	Δ^9-THC (**14**) and Δ^9-THCA (**15**).[172]

			24 s. Flow rate = 0.6 mL/min; run time = 4 min			
	Acquity UPLC Class I coupled with a Xevo TQ-S Micro Triple Quadrupole MS detector	Acquity BEH C_{18} column (100 mm x 2.1 mm, 1.7 μm)	Mobile phase: A = 0.1% HCOOH in water, and B = MeOH. Gradient elution: started with 75% B in A until 0.5 min; increased to 95% B until 4.5 min; re-equilibrated to 75% B in A in f min. Flow rate = 0.35 mL/min; run time = 5 min.	ESI-MS/MS in positive ion mode	Brain and plasma from mice after being exposed to cannabis smoke	Δ^8-THC (**14**), Δ^9-THC (**14**), 11-OH-Δ^9-THC (**12**) and Δ^9-THC-COOH (**29**).[173]
	Vanquish UHPLC Q-Exactive system coupled with a Hybrid Quadrupole-Orbitrap MS	HSS T3-C_{18} column (100 mm x 2.1 mm, 1.8 μm)	Mobile phase: A = 0.1% HCOOH in water, and B = ACN. Gradient elution: 1% B in A at 0–1 min, 1–70% B in A at 1–3 min, 70–100% B in A at 3–15 min, and 100–1% B in A at 15–18 min. Injection volume = 5 μL; column temperature = 25°C; flow rate = 0.3 mL/min	ESI-MS in positive and negative ion modes	Primary hepatocytes	Δ^9-THC (**14**), Δ^9-THCA (**15**) and Δ^9-THCV (**16**) and their metabolites.[174]

TABLE 9 HPLC and UPLC (or UHPLC) analysis of naturally occurring cannabinoids in dietary supplements, food and beverages

Matrices/source	Instrumentation	Column	Mobile phase	Detection	Cannabinoids analyzed/detected
HPLC methods					
Cannabinoids-infused ice cream	Flexar HPLC system - Perkin Elmer	Purospher STAR RP-18 column (120 mm x 4.6 mm, 5 μm)	Isocratic elution with 80% ACN in water with HCOOH (0.1%) at a 1 mL/min flow rate. Column temperature = 25°C and injection volume = 10 μL.	UV-PDA detection at 208 and 280 nm	CBD (**5**) and Δ^9-THC (**14**).[103]
Chocolates	Agilent 1260 Infinity II coupled with a DAD	Restek Raptor ARC-18 column (150 mm × 4.6 mm, 2.7 μm)	The mobile phase comprised water (A) and ACN (B), both containing 0.1% HCOOH. Gradient elution: 75% B in A for 0−3 min and then 75−100% B in A over 3−7 min. Column temperature = 40°C; flow rate = 1.5 mL//min; injection volume = 2 μm.	UV-DAD at 218 nm	CBD (**5**), CBG (**8**), CBN (**10**) and Δ^9-THC (**14**).[177]
Food supplements containing CBD	HPLC-DAD	C18 ODS column (250 mm x 4.6 mm, 5 μm)	Isocratic elution with 80% ACN in water with a 1 mL/min flow rate.	DAD set at 220 nm	CBD (**5**).[178]
Hemp-seed-based food products	Shimadzu UHPLC system LC-2040 3D Nexera-i PDA/ESIMS	Ascentis Express C18 column (150	The mobile phase was composed of A = water and B = ACN, both containing 0.1% HCOOH. Gradient elution: : 0–4.5 min, 67%	UV-PDA at 228 nm, and ESI-MS in positive ion mode	CBD (**5**), CBDA (**6**), CBDV (**18**), CBDVA (**19**), CBGA (**9**), CBN (**10**), Δ^9-THC (**14**), Δ^9-THCA (**15**), Δ^9-THCV

		mm × 3.0 mm, 2.7 μm)	B in A; 4.5–6 min, 67–95% B in A, 6–8 min, 95% B in A. Flow rate = 1 mL/min; injection volume = 5 μL; column temperature = 40°C.		(**16**) and Δ^9-THCVA (**17**).[179]
UPLC (or UHPLC) methods					
Cannabinoid containing food including chocolate, fondant, biscuits, beverage, cookies and baijiu	Acquity UPLC coupled with MS detector	Acquity UPLC BEH Shield RP18 column (100 mm x 3 mm, 1.7 μm)	Mobile phase: 0.1% HCOOH in water (A) and 0.1% HCOOH in ACN. Gradient elution: 0-9 min 65-100% B in A, 9-9.1 min 100-65% B in A, and 9.1-12 min 65% B in A. Injection volume = 5 μL; column temperature = 30°C; flow rate = 0.3 mL/min.	ESI-MS/MS in negative ion mode using multiple reaction monitoring (MRM)	CBD (**5**), CBDA (**6**), CBDV (**18**), CBG (8), CBN (**10**), Δ^9-THC (**14**), Δ^9-THCA (**15**), THCV (**16**), and Δ^9-THC-COOH (**29**).[180]
Coffee blends enriched with shredded inflorescences of different cultivars of industrial hemp	Shimadzu Nexera UHPLC system	Omega Luna C_{18} column (50 mm x 2.1 mm, 1.6 μm)	Mobile phase: 0.1% HCOOH in water (A) and 0.1% HCOOH in ACN. A linear gradient: 0–5 min, 5→15% B; 5–10 min, 15% B; 10–12 min, 15→17.5% B; 12–15 min, 17.5→45% B; 15–16 min, 45→55% B; 16–21 min, 55→75% B; 21–22 min, 75→95% B; 22–23 min, 95% B. Then, the system was allowed to re-equilibrate before the next injection. Injection volume = 2 mL; flow rate = 0.4 mL/min.	HRMS/MS and APCI in positive and negative ion modes	CBD (**5**), CBDA (**6**), and Δ^9-THCA (**15**).[181]

TABLE 10 HPLC and UPLC (or UHPLC) analysis of naturally occurring cannabinoids in wastewater and sewerage

Matrices/Source	LC system	Column	Mobile phase	Detection method	Detected cannabinoids
HPLC methods					
Sewage water	AB Sciex API 8060 instrument, fitted with an ESI-MS detector	Eclipse plus C_{18} column (100 mm × 2.1 mm, 5 µm)	Mobile phase: A = 0.1% HCOOH in water and B = ACN. Gradient elution: 0–5 min 95% B in A. Flow rate = 1 mL/min; injection volume = 5 µL; column temperature = 40^0C.	ESI-MS/MS in positive ion mode	Δ^9-THC (**14**).[182]
Wastewater samples from a manhole in a city in eastern Massachusetts	Horizon Vanquish UHPLC coupled to a TSQ Altis triple-stage quadrupole MS/MS system comprising a heated electrospray ionization (HESI) source	Reversed-phase Accucore biphenyl column (100 mm × 2.1 mm, 2.6 µm)	The mobile phase comprised water (A) and MeOH (B), both containing 0.1% HCOOH. Gradient elution: 0.5 min equilibration at 0% B, held 0% B for 0.2 min, increase to 99% B over 2.3 min, held constant for 1 min, decreased to 0% B and held for 0.5 min. Flow rate of 0.5 mL/min; run time = 7.5 min; column temperature = 40°C.	ESI-MS/MS in positive and negative ion modes	Δ^9-THC (**14**), Δ^9-THCA-glucuronide and THC-COOH-glucuronide.[183]
UPLC (or UHPLC) methods					
Wastewater collected from the wastewater treatment plant, located along the	Acquity UPLC coupled with a triple-quadrupole mass detector	Acquity UPLC BECH C_{18} column (100	Mobile phase: MeOH (A) and water (B), both containing 0.1% HCOOH. Gradient elution: 20% A in B at 0 min, linearly increased to	ESI-MS/MS multiple reaction	CBD (**5**), CBDA (**6**), CBDV (**18**), CBG (**8**), CBN (**10**), Δ^9-THC (**14**), Δ^9-THCA (**15**),

Febros River shoreline in Vila Nova de Gaia, Porto.		mm x 2.1 mm, 1.7 μm)	90% A in B in 6 min and 98% A in B in 2 min, followed by a decrease to the initial conditions in 0.1 min and equilibration time for 1 min. Column temperature = 25°C; flow rate = 0.3 mL/min; injection volume = 10 μL; run time = 9.1 min.	monitoring (MRM) mode	THC-COOH (**29**) and THCV (**16**).[184]
Wastewater from urban wastewater treatment plants in China	Vanquish UHPLC coupled with a triple quadrupole MS detector	Acquity UHPLC BEH C_{18} (100 mm x 2.1 mm, 1.7 μm)	Mobile phase: A = 0.1% HCOOH in water and B = ACN. Gradient elution: (A:B): 0.0 min (25:75) – 1.0 min (25:75) – 1.5 min (0:100) – 3.0 min (0:100) – 3.1 min (25:75) – 6.0 min (25:75). Column temperature = 40°C; flow rate = 0.4 mL/min; injection volume = 10 μL.	ESI-MS/MS	Δ^9-THC-COOH (**29**).[185]