A review on the recent advances in HPLC, UHPLC and UPLC analyses of naturally occurring cannabinoids (2010-2019)

Lutfun Nahar[1*] | Alev Onder[2] | Satyajit D. Sarker[3]

[1]Laboratory of Growth Regulators, Institute of Experimental Botany ASCR & Palacký University, Šlechtitelů 27, 78371 Olomouc, Czech Republic

[2]Ankara University, Faculty of Pharmacy, Department of Pharmacognosy, 06100 Tandogan, Ankara, Turkey

[3]Centre for Natural Products Discovery, School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, James Parsons Building, Byrom Street, Liverpool L3 3AF, United Kingdom

Correspondence

Lutfun Nahar, Laboratory of Growth Regulators, Institute of Experimental Botany ASCR & Palacký University, Šlechtitelů 27, 78371 Olomouc, Czech Republic

Email: drnahar@live.co.uk

Abstract

Introduction: Organic molecules that bind to cannabinoid receptors are called cannabinoids, and they have similar pharmacological properties like the plant, *Cannabis sativa* L. Hyphenated liquid chromatography (LC), incorporating high performance liquid chromatography (HPLC) and ultra performance liquid chromatography (UPLC, also known as ultra high performance liquid chromatography, UHPLC), usually coupled to a UV, UV-PDA or MS detector, has become a popular analytical tool for the analysis of naturally occurring cannabinoids in various matrices.

Objective: To review literature on the use of various LC-based analytical methods for the analysis of naturally occurring cannabinoids published since 2010.

Methodology: A comprehensive literature search was performed utilizing several databases, like Web of Knowledge, PubMed and Google Scholar, and other relevant published materials including published books. The keywords used, in various combinations, with cannabinoids being present in all combinations, in the search were *Cannabis*, hemp, cannabinoids, *Cannabis sativa*, marijuana, analysis, HPLC, UHPLC, UPLC, quantitative, qualitative and quality control.

Results: Since 2010, several LC methods for the analysis of naturally occurring cannabinoids have been reported. While simple HPLC-UV or HPLC-UV-PDA-based methods were common in cannabinoids analysis, HPLC-MS, HPLC-MS/MS, UPLC (or UHPLC)-UV-PDA, UPLC (or UHPLC)-MS and UPLC (or UHPLC)-MS/MS, were also used frequently. Applications of mathematical and computational models for optimization of different protocols were observed, and pre-analyses included various environmentally friendly extraction protocols.

Conclusions: LC-based analysis of naturally occurring cannabinoids has dominated the cannabinoids analysis during the last ten years, and UPLC and UHPLC methods have been shown to be superior to conventional HPLC methods.

Keywords

Cannabis sativa; cannabinoids; liquid chromatography (LC); HPLC, UPLC, UHPLC, hemp; *Cannabis*; marijuana; LC-MS; LC-PDA; analysis; detection

1 INTRODUCTION

Compounds that bind to the cannabinoid receptors (endocannabinoid system) and possess similar pharmacological properties as produced by the plant, *Cannabis sativa* L. are known as cannabinoids[1-3]. Major naturally occurring cannabinoids are presented in Figure 1. However, naturally occurring cannabinoids include over 113 different organic compounds, of which, Δ^9-tetrahydrocannabinol (**12**, Δ^9-THC or simply, THC) and cannabidiol (**3**, CBD) are the two major cannabinoids (Figure 1), biosynthesized by *C. staiva*[2]. Δ^9-THC (**12**) is the main contributor to the psychoactive property of *C. sativa*, but interestingly, the other major compound, cannabidiol (**3**), possesses antipsychoactive property[1]. Natural cannabinoids, commonly known as phytocannabinoids, are mainly accumulated in a viscous resins produced predominantly in the glandular trichomes of *C. sativa*, and can be structurally grouped into at least eight major structural classes, *i.e.,* cannabichromenes (**1**, CBC), cannabicyclols (**2**, CBL), cannabidiols (**3**, CBD), cannabigerols (**6**, CBG), cannabinols (**8**, CBN), tetrahydrocannabinols (**12**, THC), cannabielsoins, iso-tetrahydrocannabinols and cannabicitrans[1,2]. Cannabinoids are of great interest for their therapeutic value as *Cannabis* is often indicated for the treatment of pain, glaucoma, nausea, depression, and neuralgia[1]. Medicinal *Cannabis* generally has a higher level of CBD (>20%) than THC (~1%), whereas recreational *Cannabis* contains higher amounts of THC (>20%) than CBD (~2%). The medicinal and psychotropic value of phytocannabinoids can vary significantly between cultivars. Apart from *C. sativa*, several other plants including *Acmella oleraceae, Echinacea angustifolia, E. purpurea, Helichrysum umbraculigerum* and *Radula marginata* also produce phytocannabinoids[1,3].

Cannabinol (**8**, CBN) is the first cannabinoid discovered in 1940 by the British Chemist Robert S. Cann, followed by the discovery of cannabidiol (**3**, CBD) and then tetrahydrocannabinol (**12**, THC) and so on. Since the discovery of these major cannabinoids, several analytical tools and methods have been introduced for the detection, identification, quantification and analysis of various naturally occurring cannabinoids, predominantly from the plant *C. sativa*, as well as in various other biological matrices, *e.g.,* human blood, urine, hair and nails, often linking to pharmacokinetic studies and/or forensic analysis[2]. GC-based methods initially dominated the *Cannabis* analysis arena until the discovery of the fact that the hot injection port of a GC results in the incomplete decarboxylation of acidic cannabinoids[1]. GC-based techniques are still in use for the analysis of cannabinoids, but a

derivatization step before injection is required to protect the -COOH functional groups. Because of this extra step, LC-based techniques, especially since the introduction of HPLC and UPLC (or UHPLC)[4] technology, have become popular over the last few decades and are preferred for the determination of cannabinoids in most testing laboratories. While, ultraviolet (UV) detection, sometime UV-PDA, is most frequently used detection tool with LC analysis based on low initial cost, ease of use, and robustness, the use of MS detection has also become quite common nowadays[5-14]. In fact, the analysis of *Cannabis* has gained new importance globally, predominantly for quality control within the legalized recreational and medical *Cannabis* industry, but also for forensic differentiation between drug-type *Cannabis* and legal products such as fibre hemp and CBD (**3**)-rich/THC (**12**)-poor *Cannabis*. Methods based on LC-MS and LC-UV-PDA have been used for the determination of major natural cannabinoids and their metabolites in various matrices, e.g., *Cannabis* plant extracts, hemp, food products, biomass, cannabis oils, whole blood, plasma, oral fluids, hair and so on.

Since 2010, the world has witnessed a remarkable advancement in computational methods and technologies positively impacting analytical methods pertinent to phytochemical analysis including analysis of naturally occurring cannabinoids, and at the same time, significant developments in hyphenated LC and related technologies. A review article published in 2018 tried to capture only the published literature on LC-MS/MS methods and sample preparation techniques for the analysis of endocannabinoids[9], but there is hardly any comprehensive and critical review on the literature covering all detection technologies hyphenated to HPLC and UPLC for the analysis of naturally occurring cannabinoids. Therefore, this present review aims to highlight the developments in HPLC and UPLC (or UHPLC) methods applied to cannabinoids analysis since 2010, and to critically appraise the scientific publications in this topic published during the past decade.

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

High performance liquid chromatography or high pressure liquid chromatography (HPLC), is one of the most popular, modern, powerful and versatile chromatographic separation techniques that have been routinely used to separate, identify and quantify components from complex mixtures, *e.g.,* an herbal extracts or products, and to obtain

chemical profiles or fingerprints of crude mixtures[15,16]. In a standard HPLC operation, a compact column is usually 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 (2-5 μm particle size). HPLC is arguably the most widely used analytical separation technique for qualitative and quantitative determination of compounds in natural products extracts, fractions or in finished products. The most commonly used detector hyphenated with an HPLC system is UV and/or photodiode array (PDA) detector. However, hyphenation between an HPLC and more sophisticated detection techniques, *e.g.,* mass spectrometer (MS) or nuclear magnetic resonance (NMR) spectrometer, has now become quite common, and this hyphenation offers enhanced capability of separating and solving structural problems of complex natural products[15]. Sometimes, multiple detection techniques are employed, e.g., LC-UV-Vis-MS, LC-MS-MS and LC-NMR-MS. In relation to naturally occurring cannabinoids analysis, mass spectrometric detection is the most useful detection technology that provides valuable structural information for the identification of various cannabinoids. The ionization techniques used in HPLC-MS are almost exclusively soft ionization techniques, e.g., electrospray ionization mass spectrometry (ESI-MS), that display mainly the molecular ion species with only a few fragment ions. The use of tandem mass spectrometry (MS/MS), which provides fragments through collision-induced dissociation of the molecular ions, has increased significantly in recent years[14]. However, an HPLC-MS or HPLC-MS/MS system does not always allow a complete and unambiguous on-line identification of any component, unless it is a well-known compound, and complementary on-line spectroscopic information is available in databases for comparison[15,16]. The quality of MS response invariably depends on a number of factors, e.g., nature of the compounds to be analyzed, the solvent and buffer used as the mobile phase, the flow rate, and, of course, the type of interface used, and thus, often creates difficulties in relation to reproducibility of information. NMR, albeit probably the least sensitive of all detection techniques, is also used as a detector for HPLC, as it offers the most useful structural information towards the structure elucidation of natural products[15]. Other detectors, for example, evaporative light scattering detector (ELSD), infrared (IR) detector, electrochemical detector, and fluorescent detector are also in use.

UPLC is an advanced liquid chromatographic technique that offers a significantly short analysis time and small amount of solvent(s) as a mobile phase[17]. It also offers much better separation efficiency and resolution of analyte mixtures. UHPLC and UPLC are essentially the

same techniques, and should not be confused as different techniques. UPLC was launched and trademarked in 2004 by Waters based on sub 2-micron porous particles[4]. When other manufacturers entered the market to offer liquid chromatographic systems with ultra performance as the UPLC, which was introduced by Waters, they had to use a different name from the Waters' registered trademarked name, UPLC, and consequently UHPLC was coined as a way to refer to instruments similar to UPLC. In fact, UPLC and UHPLC are synonyms. The main characteristic feature of UHPLC and UPLC instruments is sub 2-micron particles as opposed to particle size between 2.5-10 microns in conventional HPLC systems. The smaller particles (<2 μm) require a higher pressure to work with, and consequently, UHPLC or UPLC systems must be able to perform above 6,000 psi, which is usually the upper limit of classical HPLCs. The latest UPLC model by Waters, called Acquity UPLC Plus Series, was unveiled last year to introduce new performance benchmark for chromatographic separations in the analytical laboratories (https://www.businesswire.com/news/home/20180410005189/en/).[4] In the remainder of the text (not in Tables) of this manuscript, to avoid unnecessary repetition and confusion, the term UPLC will be used for referring to both UPLC and UHPLC.

In UPLC, because of the small particle size (<2 μm), the diffusion path between the sample analytes and the stationary phase is shorter and the efficiency is higher. Recently introduced solid core particles, which are encapsulated by a surface of small particles, offer even further lesser diffusion path and higher efficiency. UPLC enables phytochemists to address analytical challenges associated with separation, detection and quantification of various classes of secondary metabolites from various matrices more quickly than was previously possible.

In UPLC, the run time can be up to three and nine fold shorter than that of the LC systems using 3 and 5 μm columns, respectively. The column size is usually 50-100 mm with an internal diameter of 2.1 mm. The separation in UPLC is achieved under extremely high pressure (100 MPa or 14.5 K psi). Like any other modern LC systems, UPLC can be hyphenated with various types of detection techniques, UV, PDA or MS. A UPLC system enables the detection of analytes at a very low concentration owing to its improved signal-to-noise ratio, and requires much smaller injection volume without any loss of sensitivity[4]. Because of different obvious advantages over conventional HPLCs, UPLC has now become a routine technique for chemical, biomedical and pharmaceutical analysis as well as for the analysis of

phytochemicals including cannabinoids from various matrices. Despite a series of advantages of using UPLC one of the major disadvantages of UPLC is the higher back pressures 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 narrow use.

Owing to the richness of structural information that can be obtained from MS data, HPLC and UPLC, simply referred to as LC, are routinely coupled to MS detectors, creating HPLC-MS or UPLC-MS, and used for the analysis of various types of natural products, including cannabinoids. Most often tandem MS, also depicted as MS/MS is used with an LC system. In LC-MS, electrospray ionization (ESI), both in positive and negative ion modes, is the most common ionization mode for the analysis of cannabinoids.

In addition to conventional HPLC and UPLC methods, there have been a few other recent liquid chromatographic techniques employed for cannabinoid samples. For example, a fairly new ultra-high performance supercritical fluid chromatography (UHPSFC) coupled to tandem mass spectrometry was employed for the detection and quantification of cannabinoids, e.g., THC (**12**) and its metabolites including monohydroxylated, dihydroxylated and carboxylated derivatives, in waste water[18] at sub nanogram per litre level. Similar UHPSFC methods were previously reported for quantitative determination of cannabinoids, e.g., cannabidiol (CBD, **3**), cannabidiolic acid (CBDA, **4**), cannabigerol (CBG, **6**), cannabigerolic acid (CBGA, **7**), cannabinol (CBN, **8**), Δ^8-THC (**11**), Δ^9-THC (**12**), tetrahydrocannabinolic acid (THCA, **13**) and tetrahydrocannabivarin (THCV, **14**) in *Cannabis* and *Cannabis* products[19,20]. Another example is the use of nanoliquid chromatography coupled with in-tube solid-phase microextraction for the analysis of contact traces of *Cannabis* containing CBD (**3**), CBN (**8**) and THC (**12**) obtained from plastic bags, office papers, aluminium foil, cotton cloths and hand skin, applying UV-DAD detection[21].

In the following sections/subsections various specific LC-based analytical methods for the analysis of cannabinoids in various matrices are discussed.

3 HPLC and UPLC (or UHPLC) ANALYSIS OF NATURALLY OCCURRING CANNABINOIDS

A significant body of published literature has been made available to researchers since the year 2010 on the use of LC-based methods, HPLC and/or UPLC, for the analysis of naturally

occurring cannabinoids in different matrices[5-14], which highlights the importance of these techniques as well as remarkable advancements in sensitivity and versatility associated with them. While the earlier part of the last decade was dominated by the use of conventional HPLC methods, the later part has witnessed a steady increase in the methods using UPLC methods in the analysis of naturally occurring cannabinoids in *Cannabis sativa* L. plant parts, commercially available *Cannabis* products, and in forensic samples of human origins. The published literature also demonstrates that, albeit there are various types of detection technologies, which can be used with an LC system, the UV-PDA and the MS/MS technologies are the two most popular detection methods for LC-based naturally occurring cannabinoids analysis. The introduction and application of different mathematical and computation modelling methods as well as chemometric tools seem to have made the analysis of LC-based data more useful and reliable during the past decade.

Different types of LC columns are available to date, but reversed-phase C_{18} packed columns are still the most popular columns, with the occasional use of C_8 or phenyl columns, for cannabinoids analysis. 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 0.3-1.5 mL/min, depending on the use of HPLC or UPLC. The following subsections deal with the applications of LC-based methods in the analysis of naturally occurring cannabinoids in various matrices.

3.1 HPLC and UPLC analysis of cannabinoids in *Cannabis sativa* L. plant samples and *Cannabis* consumer products

Various HPLC and UPLC methods that have been reported for the analysis of cannabinoids in *Cannabis sativa* L. plant samples[22-38] and *Cannabis* consumer products, e.g., hashish, marijuana and cannabis oils[39-55], since the year 2010, are summarized in Tables 1 and 2, and appraised in the following subsections.

3.1.1 *Cannabis sativa* L. plant samples

Both HPLC and UPLC methods have been applied to separate, identify and quantify various cannabinoids in *Cannabis sativa* L. samples, including whole plants, roots, inflorescences and biomass containing *Cannabis* plant parts[22-38] (Table 1). However, the use of simple HPLC-based methods has been observed more often than UPLC-based methods for

the analysis of *C. sativa* plant samples in the last decade. Gul et al. (2015)[22] analyzed a biomass that had *C. sativa* plant materials and detected the presence of several cannabinoids including, CBC (**1**), CBL (**2**), CBD (**3**), CBDA (**4**), CBG (**6**), CBGA (**7**), CBN (**8**), Δ^8-THC (**11**), Δ^9-THC or THC (**12**), Δ^9-THCA or THCA (**13**) and THCV (**14**), using a simple water-ACN (both containing 0.1% HCOOH) based gradient elution on a standard C_{18} reversed-phase column and UV-PDA detection monitored at 220 nm. Much later, a UPLC-based separation and quantification method for cannabinoids, CBC (**1**), CBD (**3**), CBDA (**4**), CBN (**8**), THC (**12**) and THCA (**13**) (Figure 1), in *C. sativa* plant parts containing biomass, and *Cannabis* resin samples was reported, where a UPLC column with a particle size of 1.6 μm was used, the gradient elution was with water-ACN containing 0.1% HCOOH, and UV-PDA detection was employed[34].

Several HPLC and UPLC-based methods for the analysis of various extracts of *C. sativa* whole plants have been reported[23-33, 35, 36]. While the UV or UV-PDA-based detection is quite common, ESI-MS/MS detection has been increasingly becoming the method of choice for cannabinoids analysis from *C. sativa* plant crude extracts by HPLC or UPLC. In an HPLC-UV based method, a 50 mm long monolithic column of C_{18} packing with an internal diameter of 4.6 mm, and particle size of 5 μm has recently been used with a linear ACN-water gradient (flow rate: 2 mL/min) to determine THC (**12**) (at 210 nm) in the plant extract obtained (yield: >26%) by the supercritical extraction method at different pressures (15–33 MPa), temp (40–80°C) and ethanol (EtOH) as a co-solvent (0-5%)[23]. Δ^9-tetrahydrocannabinol (**12**) was identified based on its retention time. Although, it is somewhat unusual to use a high flow rate like 2 mL/min in an analytical HPLC system, the reported method appears to have worked well for the determination of THC (**12**). Another similar HPLC-UV method (detection at 220 nm) for the separation of cannabinoids, CBD (**3**), CBDA (**4**), CBDV (**5**), CBG (**6**), CBGA (**7**), CBN (**8**), THC (**12**) and THCA (**13**) (Figure 1), in different strains of *C. sativa* including hemp, has been reported using a longer column, 150 mm of length[27]. With the same length of column, but using a UV-PDA detection with an HPLC was utilized for the determination of, CBD (**3**), CBDA (**4**), CBG (**6**), CBGA (**7**), THC (**12**), THCA (**13**), THCV (**14**) and THCVA (**15**)[28] (Figure 1). A much longer C_{18} column (250 mm) was used for the separation and quantification of seven cannabinoids in a *C. sativa* plant extract applying a water-ACN based gradient elution and using a UV-PDA detector set at 214 nm for quantification; the cannabinoids were CBD (**3**), CBDA (**4**), CBG (**6**), CBGA (**7**), CBN (**8**), THC (**12**) and THCA (**13**)[24]. The retention order (in

increasing retention time) of these cannabinoids were CBDA (**4**) < CBGA (**7**) < CBG (**6**) < CBD (**3**) < CBN (**8**) < THC (**12**) < THCA (**13**). A very similar fast-HPLC-PDA method (detection at 211 nm) has recently been published, but instead of a water-ACN gradient, an isocratic elution at 35^{o}C was used for the simultaneous detection of CBD (**3**), THC (**12**) and THCA (**13**) within a run time of only 5 min[26], establishing this method as a low cost alternative to UPLC for routine analyses of cannabinoids. During the past decade, it seems that only one UPLC-UV based method for the detection of several cannabinoids in plant samples was reported[27], where the particle size of C_{18} silica was of typical UPLC (or UHPLC) range, 1.7 μm (Table 1).

The use of both UV-PDA and ESI-MS/MS detection (both positive and negative ion modes) for the analysis of CBD (**3**), CBDA (**4**), CBN (**8**), THC (**12**) and THCA (**13**) in plant extracts has recently been published[25]. A water-ACN (both containing 0.1% HCOOH) based isocratic elution on a Poroshell 120 EX-C_{18} column (150 mm x 2.1 mm; particle size: 2.7 μm) coupled with a Poroshell 120 EX-C_{18} guard column (5 mm x 2.1 mm; particle size: 2.7 μm) and using an ESI-MS/MS detection afforded separation, identification and quantification of CBD (**3**), CBG (**6**), CBN (**8**), THC (**12**), THCA (**13**) and THCV (**14**)[29]. A couple of UPLC-PDA-MS based methods were published for the analysis of cannabinoids, CBC (**1**), CBL (**2**), CBD (**3**), CBDA (**4**), CBDV (**5**), CBG (**6**), CBGA (**7**), CBN (**8**), Δ^8-THC (**11**), THC (**12**), Δ^9-THCA (**13**) and THCV (**14**) in plant samples[35, 36]. In both cases a 100 mm long column with 2.1 mm diameter and particle size 1.6-1.7 μm was used.

Flowers or inflorescences of *C. sativa* are one of the most significant plant parts for cannabinoids contents. A simple UV-detection based HPLC analytical method, using a conventional C_{18} column, has recently been reported for the analysis of cannabinoids in inflorescences, resulting in the detection of cannabinoids **1**, **3**, **4**, **6**-**8** and **11**-**14**[30]. Two convenient HPLC-UV-PDA methods using a water-ACN (both containing 0.1% HCOOH) gradient on a C_{18} column of 150 mm length, internal diameter of 3 mm and the particle size 2.7 μm, using a flow rate of 0.4 mL/min have been utilized successfully for the analysis of *Cannabis sativa* inflorescences to separate and identify cannabinoids **3**, **4**, **6** and **7**[31,32]. The same researchers also reported an ESI-MS/MS method in both positive and negative ion modes using an ion trap LC-MS system for the analysis of same cannabinoids. A heated ESI-MS/MS detection using both positive and negative ion modes on a HPLC system was employed to detect CBD (**3**), CBDA (**4**), CBG (**6**), CBGA (**7**)**,** CBN (**8**), THC (**12**) and THCA (**13**) in

inflorescences and their macerated oils, where the length of the C_{18} column was 150 mm, but the internal diameter and the particle size were 2 mm and 4 μm, respectively, and the flow rate was only 0.3 mL/min[33]. It was demonstrated that this LC-MS method could completely overcame previously reported drawbacks of LTQ-Orbitrap–MS, such as slow switching between the positive and negative modes. It was noted that THCA (**13**) and CBDA (**4**) exhibited a molecular ion at *m/z* 357 and presented the same fragments, but they significantly differed in the intensities of two most characteristic signals, *m/z* 313 and *m/z* 339. The deprotonated *pseudo*molecular ion lost either the carboxylic group producing a fragmentation of *m/z* 313, or a -OH group forming the ion at *m/z* 339. Apparently only one UPLC-PDA-MS based method has been reported since 2010 for the analysis of the flowers of *C. sativa* for the detection and quantification of cannabinoids, **1**-**4**, **6**-**8** and **11**-**14**[36]. In this experiment, the column was shorter (100 mm), the internal diameter was 2.1 mm and the particle size was 1.6 mm, with a flow rate of just 0.25 mL/min in a gradient elution with water-ACN (both containing 0.05% HCOOH).

Cannabinoids **1**, **3**, **4**, **8**, **12** and **13** have been detected qualitatively and quantitatively in medicinal *Cannabis* resins using a simple UV-PDA-based UPLC method emplying a Phenomenex Luna Omega C_{18} column (150 mm × 2.1 mm; particle size: 1.6 μm), and a multi-step gradient of water and ACN, both containing 0.1% HCOOH[34]. Prior to UPLC analysis, the resin extract was obtained by supercritical fluid extraction. It was noted that the use of a multi-step gradient could improve the resolution of all cannabinoid species investigated, negating the need for mass spectrometry peak differentiation, particularly when comparing CBC (**1**) and THCA (**13**) elution.

Cannabis sativa L. roots and seeds were analyzed for the detection and quantification of cannabinoids using UPLC-MS methods, employing a short C_{18} column (50 mm) with a diameter of 2.1 mm and particle size of 1.7 μm[37,38]. While cannabinoids **1**, **3**, **4**, **7**, **8** and **11**-**14**[37] were detected in the roots, the seeds revealed the presence of 11-hydroxy-Δ^9-THC (THC-OH, **9**), 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH, **10**) and THC (**12**)[38].

In fact, LC-based methods are generally useful for chemical fingerprinting and quality assessment of plant extracts containing cannabinoids. Presence of cannabinoids, both qualitatively and quantitatively, depends on the extraction method used to extract cannabinoids from a plant matrix. While the traditional ways of extracting cannabinoids from

plants involve solvent-based extraction methods, nowadays, more environmentally friendly extraction techniques, *e.g.,* supercritical fluid extraction (SFE), have become desirable[1].

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

Various LC-based analytical techniques have become quite popular in the analysis of cannabinoids in *Cannabis* consumer products, which include smoking products, e.g., hashish and marijuana, cannabidiol oil, *Cannabis* olive oil, *Cannabis* tinctures, *Cannabis* medicinal products and *Cannabis* tea (Table 2)[39-55]. In the HPLC and UPLC (or UHPLC) methods, the use of PDA or MS detection technologies have become routine for the analysis of *Cannabis* consumer products. However, the use of HPLC as opposed to UPLC still remains the method of choice, because of more affordability of an HPLC system than a UPLC. Appropriate quality control methods are essential for ensuring the quality of medicinal *Cannabis* and thus, optimizing the therapeutic outcome. At the same time, the quality of other *Cannabis* consumer products also needs to be assessed by various analytical tools.

There are at least eleven different HPLC methods have been published during the last decade for the analysis of commercial *Cannabis* using either a HPLC-UV-PDA or HPLC-MS/MS techniques[39-49]. Cannabidiol (CBD, **3**)-based oil preparations have become one of the most popular consumer products because of a variety of beneficial effects of CBD (**3**), which is not a controlled substance, on human health[39]. The use of *Cannabis* oil rich in CBD (**3**) has been the recent trend in high value cosmetic and cosmeceutical products. In a recent study, 14 different commercially available CBD oils have been analysed for proving quality assessment by chemical profiling by a HPLC-Q-Exactive-Orbitrap-MS method[39]. Cannabidiol (CBD, **3**), together with CBGA (**7**), THC (**12**) and THCA (**13**) were quantified as quality markers.

During the past decade, Italian pharmacists have been given the permission to serve medical prescriptions that require preparation and dispensing of *Cannabis* extracts to patients, prompting the necessity of evaluating the CBD (**3**) and THC (**12**) contents in cannabis extracts prior to sale. *Cannabis* olive oil extracts are one of such prescription products that are prepared from dried female cannabis inflorescences[40]. Very recently, a fast HPLC-UV method, developed by applying an analytical quality by design strategy (AQbD) and response surface methodology, has been reported for the analysis and quantification of CBD (**3**) and THC (**12**), and also CBDA (**4**) in *Cannabis* olive oil extracts[40]. Isocratic elution with a mixture of ACN-water containing 5 mM of K_2HPO_4 adjusted to pH 3.45 (range 3.11-3.50) in 3:1 ratio, at a

flow rate of 0.38 mL/min, was used on an Agilent Poroshell 120 SB-C_{18} column (150 mm, 2.1 mm; particle size: 2.7 μm) at a raised column temperature of 53°C (Table 2). It can be noted that the AQbD strategy offered a good understanding of the parameters that generally affect the quality control process and to control them. This study showed that a high degree of variability in CBD (**3**) and THC (**12**) contents in the tested samples of *Cannabis* olive oil existed, and suggested that this simple HPLC-UV-PDA method could be used for routine analysis of *Cannabis* olive oil extracts. Earlier, a similar isocratic HPLC method, but using ESI-MS/MS in positive ion mode, was reported for the analysis of these two major cannabinoids in *Cannabis* olive oil samples[41]. There have been two UPLC-MS methods reported for the analysis of cannabinoids, **3**, **4**, **8**, **12** and **13**[50,51]. In both cases, water-ACN gradients were employed, and ESI-MS/MS was used for the detection of individual cannabinoids. It can be noted that a much shorter column of only 30 mm length was used in both cases. In the UPLC-MS analysis reported by Carcieri et al., 2018, a significant variability in cannabinoids concentrations was observed in galenic preparations of *Cannabis* olive oil, which is rather alarming because the exact concentration of cannabinoids in galenic preparations is crucial for confirming their quality and accurately administering the prescribed dose[50].

One of the major *Cannabis* consumer products, albeit illegal in many countries, is the *Cannabis* smoking products, often known as hashish and marijuana. There have been quite a few HPLC-based analytical methods, using UV-PDA or ESI-MS/MS detection and quantification, reported in the past decade for the analysis of cannabinoids in hashish and/or marijuana[42-46] (Table 2). An accurate and high throughput method for the quantitative determination of CBD (**3**), CBDA (**4**), CBG (**6**), CBGA (**7**), CBN (**8**), Δ^8-THC (**11**), THC (**12**) and THCA (**13**), in marijuana, using an HPLC-UV-PDA method was reported, where two cultivars, which included ten individual samples, four composite samples, seven calibration standards, and four quality control standards, were analyzed within 24 h[43]. A gradient elution with the mobile phase consisting of 50 mM aqueous solution of ammonium formate, pH 5.19 and MeOH at a flow rate of 0.7 mL/min on an Agilent Poroshell 120 SB-C_{18} column (75 mm x 3.0 mm; particle size: 2.7 μm) was employed. An HPLC-UV-PDA method applying an isocratic elution with 34% ACN containing 0.5% CH_3COOH, and 66% water containing 0.5% CH_3COOH at a flow rate of 1 mL/min for the analysis of cannabinoids in hashish samples was documented by Ciolino et al.[46], which was applied for the analysis of 60 commercial hashish products, including hash oil, hemp seed oil products, etc., representing diverse product types

and a broad range of cannabinoids amounts, detecting cannabidiol (CBD, **3**), CBDA (**4**), CBN (**8**), Δ^9-THC (**12**) and Δ^9-THCA (**13**) (Table 2). This HPLC-PDA method apparently addressed the need for a reliable quantitative procedure for the determination of the cannabinoids in a variety of *Cannabis* consumer products and sample types. It was also demonstrated that this method could easily be applied to foods, candies, beverages, topicals, vapes/e-liquids, oral liquid supplements, pastes, capsules, tablets, cannabis plants, and plant extracts or preparations[46].

An isocratic HPLC-UV-PDA method using the mobile phase consisting of water and ACN, both containing 0.1% HCOOH, has recently been reported for the analysis of *Cannabis* consumer products, where a core-shell C_8 column (100 mm x 2.1 mm; particle size: 2.7 μm) was used[44]. Neutral and acidic cannabinoids, CBD (**3**), CBDA (**4**), CBN (**8**), THC (**12**) and THCA (**13**) could be successfully detected in confiscated *Cannabis* consumer product (hashish) samples. It can be noted that most of the published LC methods for the analysis of cannabinoids employed C_{18} columns of various sizes, but the use of C_8 columns has been rather limited. In addition to using a UV-PDA, Protti et al. (2019) have recently used ES-MS/MS, both in positive and negative ion modes, for the detection and quantification of cannabinoids **3**, **4**, **8**, **12** and **13** in hashish samples[45]. This methods could find its application in the analysis of recreational, drug-type, and fibre-type *Cannabis* samples, offering conclusive cannabinoid profiling for a rational use of this plant, its extracts, and purified compounds in medicinal chemistry and other fields, including the nutraceutical, cosmeceutical and cosmetic products for fibre-type varieties[45]. Another similar HPLC-MS method applying an ESI-MS/MS detection in positive and negative ion modes detecting CBD (**3**), CBDA (**4**), THC (**12**) and THCA (**13**) in 40 different *Cannabis* consumer products has been published, where an isocratic elution with 90% ACN (containing 0.1% HCOOH) in water (containing 0.1% HCOOH) at a flow rate of 0.5 mL/min was utilized[42].

The UPLC-based methods for the analysis of cannabinoid consumers products published during the last decade have utilized both UV-PDA and MS detectors[36, 52-55]. In those UPLC methods, an isocratic as well as a gradient elution with a mobile phase comprising acidified water-ACN or water-MeOH has been used routinely on UPLC columns, mainly of 100 mm length. Cannabinoids **3**, **8** and **12** were detected in confiscated *Cannabis* consumer products using a PDA and/or a MS detector in ESI mode on a Waters Acquity UPLC H-class[55] (Table 2); the binary mobile phase comprised MeOH (containing 0. 1% HCOOH) and water,

and a Waters UPLC BEH C_{18} column (50 mm x 2. 1 mm, particle size: 1.7 μm) with isocratic elution at a flow rate of 0. 2 mL/ min were used. A simple UPLC-UV-PDA method using a gradient elution with 10 mM ammonium formate, pH 3.6 at a flow rate of 0.6 mL/min could quantify cannabinoids **1**, **3**, **4**, **6**, **8** and **12**-**14** in marijuana[54]. An ESI-TWIM (travelling wave ion mobility)-MS in positive ion mode was used in the UPLC analysis of CBN (**8**), THC (**12**) and THCA (**13**), in *Cannabis* consumer products, where a gradient elution with water-MeOH (containing 0.1% HCOOH) at a flow rate of 0.5 mL/min was used; the column temperature was maintained at 55°C[53] (Table 2). A gradient elution with 0.05% HCOOH in both water and ACN at a flow rate of 0.25 mL/min could successfully detect and quantify a series of cannabinoids including CBC (**1**), CBL (**2**), CBD (**3**) CBDA (**4**), CBG (**6**), CBGA (**7**), CBN (**8**), Δ^8-THC (**11**), THC (**12**), THCA (**13**) and THCV (**14**), in cannabinoid consumer products[36]. Most recently, Dos Santos et al. (2019) have reported an ESI-QTOF and ESI-travelling wave ion mobility-MS detection method for the UPLC analysis of cannabinoids using a Waters Acquity UPLC HSS T3 column (100 mm x 2.1 mm; particle size: 1.8 μm) and a multi-step gradient mobile phase consisting of water-MeOH (containing 0.1% HCOOH)[52] (Table 2).

Cannabinoids were analyzed in traditional *Cannabis* tincture samples using a simple HPLC-PDA method as a part of developing a quality control procedure for cannabinoids[47]. A gradient elution with water-ACN (containing 0.1% TFA) was used on a standard ACE 250 mm C_{18} column, to identify CBD (**3**), CBDA (**4**), CBG (**6**), CBGA (**7**), CBN (**8**), THC (**12**) and THCA (**13**), and establish significant variations in traditional *Cannabis* tincture quality. Hemp nut concentrated powder products from Taiwan were assessed by an HPLC method employing ESI-MS/MS on a Triple Quadrupole Tandem Mass Spectrometer, both in positive and negative ion modes, and with the help of chemometric techniques for the rapid evaluation and classification of samples; CBD (**3**), CBN (**8**) and THC (**12**) were quantified[48].

Some medicinal *Cannabis* products were analyzed by HPLC using both UV-PDA and MS detectors to identify cannabinoids **3**, **4**, **8**, **12** and **13**[49]. An isocratic elution with water-ACN (containing 0.1% HCOOH) at a flow rate of 0.5 mL/min was applied. *Cannabis* tea, rather a 'not-so-common' *Cannabis* consumer product, was assessed by a UPLC method using ESI-MS/MS detection; a gradient elution was employed with the mobile phase comprising water-ACN, both containing 0.1% HCOOH on an Acquity UPLC HSS T3 column, (30 mm x 2.1 mm;

particle size: 1.8 μm)[51]; CBD (**3**), CBDA (**4**), CBN (**8**), THC (**12**) and THCA (**13**) were detected as major cannabimoids[51].

3.2 HPLC and UPLC analysis of cannabinoids in biological and forensic samples

Marijuana or hashish, a cocktail of at least 30 different major cannabinoids, generally prepared from crushing the leaves, flowers (inflorescenses) and even stems of *C. sativa*, is one of the oldest recreational and addictive natural products used by the humans for centuries[1]. However, the nonmedical use of *Cannabis* or marijuana is illegal in many countries, which prompts the use of analytical tools, like HPLC and UPLC, to analyze biological and forensic samples like blood, oral liquid, hair and urine, to confirm marijuana usage (Tables 3-7)[56-106]. After consumption of marijuana, THCA (**13**), which is present in the crude marijuana, but converted to THC (**12**) by heat during smoking, is excreted in the urine as its glucuronide conjugate, and can be analysed comfortably by LC methods.

3.2.1 Human blood samples

Among the biological and forensic samples, the blood is probably one of the most popular samples for cannabinoids analysis; it can be whole blood, plasma or serum. In fact, blood, plasma and serum samples are quite extensively used in forensic analysis to detect the consumption of cannabinoids[1]. HPLC and UPLC methods are commonly used for the analysis cannabinoids in blood samples[56-71]. Table 3 summarizes various HPLC and UPLC-based methods for the analysis of cannabinoids in blood samples. It appears that for the HPLC analysis of blood samples for the presence of cannabinoids and their metabolites, in addition to the commonly used reversed-phase silica C_{18} columns, biphenyl columns are also used. In human whole blood samples, various unaltered cannabinoids and their metabolised products, 11-OH-THC (**9**), THC-COOH (**10**), THC-glucuronide and THC-COOH-glucuronide have been detected by various HPLC-based methods using, almost exclusively, an MS detector employing ESI-MS/MS methods. Cannabidiol (CBD, **3**), CBN (**8**), 11-OH-THC (**9**), THC-COOH (**10**), THC (**12**), THC-glucuronide and THC-COOH-glucuronide were quantitatively determined in human whole blood by an HPLC-MS method using a biphenyl column, and a gradient mobile phase composed of 10 mM ammonium acetate in water adjusted to pH 6.15 with HCOOH and 15% MeOH in ACN (Table 3)[56]. Electrospray ionization (ESI) was applied for cannabinoid detection, utilizing both positive [for CBN (**8**) and THC (**12**)] and negative ion modes [CBD (**3**), 11-OH-THC (**9**), THC-COOH (**10**), THC-glucuronide and TH-CCOOH-glucuronide]. This method

was claimed to be the first robust, sensitive and specific LC-MS/MS technique for direct detection and quantification of several cannabinoids and two cannabinoid glucuronides in human whole blood, providing a comprehensive cannabinoids whole blood profile following cannabis intake[56]. Later, another similar HPLC-MS method employing a biphenyl column of a shorter length (50 mm) was reported for the determination of 11-OH-THC (**9**), THC (**12**), and THCA (**13**) in human whole blood[57]. This validated method was also a sensitive, efficient and robust procedure for the quantitation of cannabinoids in whole blood using a small sample volume of 0.5 mL, and was successfully applied to both human performance and post-mortem casework in two different laboratories using different instrumentations. Scheidweiler et al., reported an HPLC-MS method using ESI-MS/MS on a Sciex 5500 QTrap® mass spectrometer with a Turbo VT ion source, a short (50 mm) reversed-phase silica C_{18} column, and a gradient elution with a mobile phase comprising 10 mM ammonium acetate in water and 15% MeOH in ACN, for the simultaneous determination of cannabinoids and their biotransformed products, including CBD (**3**), CBG (**6**), CBN (**8**), 11-OH-THC (**9**), THC-COOH (**10**), THC (**12**), THCV (**14**), THCVA (**15**), THC-glucuronide and THC-COOH-glucuronide[58]. It was suggested that this method could possibly help whole blood cannabinoid results interpretation by monitoring the most comprehensive panel of major and minor cannabinoids and metabolites to date that might improve identification of recent cannabis intake or distinguish licit medicinal and illicit cannabis administration[58].

At least three different UPLC-MS methods have been reported during the past decade to quantitatively determine naturally occurring cannabinoids and their metabolites in human whole blood samples[67-69]. An ESI-MS/MS detection was used in all three methods. Cannabinoids **3**, **8**-**10**, **12** and THC-COOH-glucuronide were quantified in human whole blood using a UPLC-MS method, where an ACE Excel C_{18}-PFP column (50 mm x 2.1 mm; particle size: 2 μm) and a gradient mobile phase comprising 0.2% HCOOH:MeOH (95:5) and 0.2% HCOOH:MeOH (5:95), were used[67]. Solid-phase extraction was used to prepare samples for UPLC analysis. This UPLC-MS method using an automated solid-phase extraction for sample preparation was effective in the simultaneous identification and quantification of naturally occurring cannabinoids and their metabolites in ante-mortem and post-mortem human whole blood samples in forensic settings. This method arguably had a few advantages over previously reported UPLC methods in its automated extraction procedure, a shorter run time (5.5 min, as opposed to 10-15 min run time) and the ability to detect a wider variety of

cannabinoids[67]. In another similar high throughput UPLC-MS method, CBD (**3**), CBN (**8**), 11-OH-THC (**9**), THC-COOH (**10**), and THC (**12**) were successfully determined in only 100 μL of human whole blood samples using ESI-MS/MS, and this method was also validated for the analysis of cannabinoids in post-mortem blood samples in a forensic set up[68]. Much earlier, a simple UPLC-MS method was published for simultaneous qualitative and quantitative determination of cannabinoids **9**, **10** and **12** in 500 μL of human whole blood[69]; the separation was achieved on an Acquity UPLCW HSS T3 (50 mm x 2.1 mm, particle size: 1.8 μm) reversed-phase silica C_{18} column using a MeOH/2 mM ammonium formate (0.1% HCOOH) gradient in a total run time of 9.5 min (Table 3).

Naturally occurring cannabinoids and their biotransformed products were quantitatively determined in human peripheral blood samples by a universal and robust HPLC-MS method, which was validated for its application in forensic toxicology[59]. In this protocol, protein precipitation, integrated solid-phase extraction and on-line enrichment followed HPLC separation and detection with a triple quadrupole mass spectrometer were employed. Cannabinoids were determined in dried spots of human blood by another HPLC-MS method, and cannabinoids **9**, **10** and **12** were successfully quantified[60] (Table 3). In this selective and sensitive method, instead of a reversed-phase silica C_{18} column, a C_8 column (50 mm x 2.1 mm; particle size: 2.6 μm) was used, and this method was the first LC-MS method for the analysis of THC (**12**) and its hydroxylated (**9**) and carboxylated (**10**) metabolites in human dried blood spots. This method, suitable for roadside testing, could allow assessing the time elapsed after the drug intake and distinguishing between acute or former consumption; this is an important information in specific contexts such as “on street” controls by police forces[60].

A rapid, selective and sensitive HPLC-MS method using ESI-MS/MS in positive and negative ion modes for the quantification of major cannabinoids and their metabolites in micro volume of human blood samples following dabsyl derivatization to enhance signal intensity was published[61]. This validated method comprised protein precipitation followed by derivatization with dabsyl chloride and subsequent analysis using LC-MS/MS on a 150 mm x 2.1 mm reversed-phase silica C_{18} analytical column maintained at 65◦C and eluted with a gradient of water and ACN, both containing 0.2% HCOOH; the run time was 8 min[61]. This method was also shown to be applicable for the analysis of CBD (**3**) and CBN (**8**), 11-OH-THC

(**9**), THC-COOH (**10**) and THC (**12**) in human blood plasma, serum and urine samples. Recently, a HPLC-MS method using the ESI-MS/MS technique on an API 4000 QTrap and an API5500 tandem mass spectrometers has been reported for the analysis of the THC metabolite, THC-COOH (**10**) in human post-mortem blood samples[62]. This simple method utilized protein precipitation for a sample volume of 100 μL and used a Luna 5 mm C_{18} (2) 100 A column (150 mm x 2 mm; particle size: 5 μm) eluting with a gradient elution with water:MeOH = 95:5, and B water:MeOH = 3:97, both containing 10 mM ammonium acetate and 0.1% CH_3COOH.

Blood plasma samples are quite often used in forensic analysis for the detection of illegal cannabinoids consumption, and the use of HPLC and UPLC methods, both applying simple UV-PDA or ESI-MS/MS detection technologies is common[8, 63-65, 70,71]. An HPLC method utilizing APCI (atmospheric pressure chemical ionization)-MS/MS in positive ion mode associated with extensive ion suppression was employed to analyze naturally occurring cannabinoids in 352 human plasma samples, providing simultaneous quantification of several cannabinoids and their biotransformed products including CBC (**1**), CBD (**3**), CBDV (**5**), CBN (**8**), CBG (**6**), THC (**12**), 11-OH-THC (**9**), THCV (**14**), 11-nor-9-carboxy-Δ^9-tetrahydrocannbinol (THC-COOH, **10**), Δ^9-tetrahydrocannabivarin-carboxylic acid (THCV-COOH, **15**), and THC-COOH-glucuronide[64]. A Poroshell Eclipse C_{18} column (40 mm x 4.6 mm; particle size: 2.7 μm) was used in this analysis and a gradient elution with the mobile phase was composed of 20% isopropanol, 20% MeOH and 60% ACN, and water containing 0.1% HCOOH was employed. The method was shown to be useful in clinical monitoring of *Cannabis* usage. A similar method using an APCI-MS/MS method on a on a triple quadrupole mass spectrometer was reported for the analysis of the same cannabinoids as stated earlier in human plasma samples, where a standard Phenomenex Kinetex column (150 mm x 3 mm; particle size: 2.6 μm) was used, and the gradient elution was performed using a combination of water and MeOH, both containing 0.1% HCOOH[65] (Table 3). The limits of detection were below 1 ng/mL for all analytes, the accuracy ranged from 84% to 115%, and both within-day and between-day precision were lower than 12%, which made this method applicable to plasma samples from *Cannabis* users. Much earlier, a simple HPLC-MS method employing ESI-MS/MS was reported for the analysis of THC (**12**), and its two major metabolites 11-OH-THC (**9**) and THC-COOH (**10**) in human plasma samples[8]. Most recently, Roslawski et al. have reported a simple HPLC-UV-based analytical method for simultaneous detection and quantification of several cannabinoids and their metabolites in human plasma samples, using a Kinetex EVO C_{18} column

eluting with a gradient elution with MeOH and 0.2% NH_4OH/water at a flow rate of 0.4 mL/min[63].

The only two UPLC methods reported since 2010 for the analysis of cannabinoids in human plasma samples used MS detection technology, applying the ESI-MS/MS technique[70,71]. While Ocque et al.[70] used an isocratic elution with 18:82:0.02 water: MeOH: HCOOH over 8.5 min to identify cannabinoids **3**, **4**, **8**, **9** and **12**-**14**, the other study[71] employed a gradient elution with water-MeOH (containing 0.1% HCOOH) offering the quantification of CBD (**3**), 11-OH-THC (**9**), THC-COOH (**10**) and THC (**12**).

Since 2010, the two methods of analysis of cannabinoids in human serum samples, using HPLC-based methods with ES-MS/MS detection technique, were reported by the same group of researchers[62, 66]. A simple protein precipitation pre-treatment was employed for a human serum sample volume of 100 μL, analysed on a Luna 5 μm C_{18} (2) 100 A analytical column (150 mm x 2 mm) eluting with a mobile phase consisting of water-MeOH 95:5 and water-MeOH 3:97, both with 10 mM ammonium acetate and 0.1% CH_3COOH to quantify THC-COOH (**10**)[62]. This method complied with the recommendations for qualitative screening methods for major cannabinoid in human samples.

3.2.2 Human breath, oral fluid and breast milk samples

While the analysis of human oral fluid samples for cannabinoids by HPLC or UPLC is rather common, the use of these LC techniques for the analysis of human breath and breast milk samples is rather rare[72-83] (Table 4). Nevertheless, it is known that the presence and concentration of cannabinoids in breath samples correlate with recent marijuana use and possibly to impairment. Thus, the search for sensitive analytical tools for the analysis of THC (**12**) in human breath has been a challenge for some time, which has resulted in an effort in exploring the option of using an LC-MS method after pre-treatment or derivatisation of the breath sample[72]. A novel derivatization method based on an azo coupling reaction that significantly increases the ionization efficiency of cannabinoids for LC-MS/MS analysis has recently been reported sample[72]. This reported derivatization method allowed effective detection of CBN (**8**) and THC (**12**) in human breath sample (Table 4).

Human breast milk, a highly complex biological fluid, is not often used for forensic analysis of cannabinoids, but is used to detect cannabinoids to protect breast-fed infants from possible toxicities of cannabinoids[1]. Cannabinoids extraction from this matrix is quite

challenging because of its high lipid (up to 5% by weight) and protein contents. However, it is essential for monitoring cannabinoids in breast milk resulting from passive or nonrecent active maternal exposure. There are at least one UPLC methods, both employing the ESI-MS/MS detection technology, reported during the past decade[83]. Cannabidiol (CBD, **3**), CBN (**8**) and THC (**12**) were successfully quantified from human breast milk samples using a Phenomenex Kinetex C_{18} (100 mm x 2.1 mm; particle size: 2.6 μm) with a gradient elution with the mobile phase comprising 5.0 mM of ammonium formate with 0.05% HCOOH, and ACN[83]. It can be noted that despite the LC system used was mentioned as a UPLC system, the particle size 2.6 μm of the column was a bit larger for a UPLC column, where normally the particle size is less than 2 μm.

Human oral fluid samples are routinely used in forensic analysis to ascertain illegal *Cannabis* consumption, and LC-based methods for quantification of cannabinoids in human oral fluid samples are quite common (Table 4)[6,13,73-82]. Except for one report[73], where a simple PDA detection was used for a HPLC-based analysis of cannabinoids in human oral sample, all other published HPLC or UPLC-based methods utilized MS detection technology (Table 4). An isocratic elution with 89% ACN in water containing 0.1% HCOOH at a flow rate of 0.5 mL/min was employed for the quantification of CBD (**3**), CBN (**8**), THC-COOH (**10**) and THC (**12**) in oral fluids using an HPLC-PDA method[73]. At the same time, the same group[73] developed a new molecularly imprinted solid-phase extraction methodology followed by a gradient LC-MS/MS using cylindrical shaped molecularly imprinted pills for detection of cannabinoids **3**, **8**, **10** and **12** in human oral fluid. A similar isocratic HPLC-MS/MS method was reported earlier, which only quantified THC (**12**) and its major metabolite, THC-COOH (**10**) in a short run time of 5 min[74]. An APCI-ESI-MS/MS on an ABSciex 6500 QTRAPW triple quadrupole/linear ion trap mass spectrometer with an IonDrive™ Turbo V source, in positive ion mode, was utilized in an HPLC quantification of CBD (**3**) and CBG (**6**), 11-OH-THC (**9**), THC-COOH (**10**), THC (**12**) and THCV (**14**) using a United Chemical Technologies Selectra PFPP column (100 mm x 2.1 mm; particle size: 3 μm) at a raised temp of 40^{o}C and a gradient elution with water-ACN (both containing 0.15% HCOOH) at a flow rate of 0.5 mL/min[6]. This HPLC-MS/MS method was found to be sensitive and rapid, and offered specific and simultaneous quantification of six cannabinoids and metabolites in human oral fluid, with limits of quantification of 0.2 μg/L for THC (**12**), 11-OH-THC (**9**), THCV (**14**), CBD (**3**), and CBG (**6**) and 15 ng/L for THC-COOH (**10**). This

method could monitor THC-COOH (**10**) at clinically relevant concentrations for identifying active *Cannabis* smoking and include minor cannabinoids distinguishing recent *Cannabis* consumption.

An ESI-MS^n on a API 6500 Q-trap mass spectrometer, equipped with a Turbo-Ion-Spray (ESI) source was used with a standard HPLC with an Agilent Zorbax XDB-C_{18} analytical column (100 mm x 2.1 mm; core shell particle size: 2.6 μm) and a linear gradient mobile phase comprising water-ACN, both containing 5 mM ammonium acetate to quantify cannabinoids **3**, **8**-**10** and **12** as picolinates[79]. This method demonstrated that formation of picolinic acid esters of hydroxylated drugs or their biotransformation products could be a promising tool to improve their mass spectrometric ionization efficiency, alter their fragmentation behaviour and enhance sensitivity and specificity of their detection. Much earlier, especially during 2012-2013, there were a few similar HPLC-ESI-MS/MS methods reported for the quantification of major cannabinoids and their biotransformed products, e.g., CBD (**3**), CBN (**8**), 11-OH-THC (**9**), THC-COOH (**10**), THC (**12**), THCA (**13**), THC-COOH-glucuronide and THC-glucuronide, in human oral fluid samples[13, 75-78] (Table 4).

Malaca et al. have recently published a UPLC method utilizing an ESI-MS/MS technique on a triple quadruple MS in positive ion mode for the qualitative and quantitative analysis of CBD (**3**), and THC (**12**) in a sample of 500 μL of human oral fluid[80]; an Acquity UPLC BEH C_{18} UPLC column (75 mm x 2.1 mm; particle size: 1.7 μm) was used for the step gradient elution with water-ACN, both comprising 0.1% HCOOH (Table 4). Another HPLC-ESI-MS/MS method has also been recently reported, which has afforded more extensive identification and quantification of several cannabinoids and their biotransformation products, including **3**, **6**, **8**-**10**, **12**-**14**, THC-COOH-glucuronide and THC-glucuronide[82]. Previously a simple UPLC-MS method was described for the detection of only THC (**12**) in human oral fluid[81].

3.2.3 Human hair samples

Human hair samples frequently used for forensic analysis for drugs of abuse or illegal drugs like *Cannabis*. Hair analysis is used to monitor usage of drugs over long periods, and in recent years, solid-phase microextraction has emerged as an important extraction method for this analysis. In fact, hair analysis has become a routine procedure in most forensic laboratories since this alternative matrix offers obvious advantages over classical matrices, particularly, wider time window, non-invasive sampling and good stability of the analytes over

time. However, the advantages come with some difficulties associated with hair analysis of cannabinoids, which particularly include low concentrations of the major metabolite, THC-COOH (**10**). Thus, the use of an effective extraction method is essential prior to any LC or GC-based analysis of human hair samples. In the case of illicit cannabis exposure, THC (**12**), the main active compound of *Cannabis*, one of its metabolites THC-COOH (**10**), and two cannabinoids **3** and **8** are normally analyzed and quantified by LC-based methods. THC-COOH (**10**) is considered to be the only marker that can distinguish between direct cannabinoid consumption and passive exposure.

While there have been several HPLC-based methods reported during the past decade[84-90], the use of UPLC-based analysis for hair samples is rather limited to the publication by Shah et al.[91] (Table 5). Most of the samples analyzed by LC were adult human hair samples with the only exception of the work reported by Moosmann et al.[90], where they used children hair samples. All HPLC and UPLC methods described since 2010 utilized MS detection technologies of different kinds (Table 5).

Since 2010, the only UPLC method published for the determination of cannabinoids, e.g., THC (**12**), 11-OH-THC (**9**) and THC-COOH (**10**) in human hair sample utilized an Agilent Zorbax Eclipse plus C_{18} UPLC column (100 mm x 2.1 mm; particle size: 1.8 μm) and a linear gradient mobile phase comprising 10 mM ammonium formate/0.02 M HCOOH in water, and 0.02 HCOOH in ACN[91]. An ESI-MS/MS using a 6430 triple quadrupole mass spectrometer was applied for detection of cannabinoids. This selective, sensitive and robust UPLC method was validated for the analysis of drugs of forensic and toxicological natural, including cannabinoids, in human hair samples.

One of the most recent HPLC-MS analyses of human hair samples for the qualitative and quantitative determination of cannabinoids employed a Thermo single-stage Orbitrap (Exactive) MS system, interfaced with an HESI source for the detection of THC (**12**)[84]; a Thermo Acclaim RSLC 120 C_{18} analytical column (100 mm x 2.1 mm; particle size: 2.2 μm) was used (Table 5). The other recent HPLC-MS method used APCI-ESI-MS in positive ion mode to quantify CBD (**3**), CBN (**8**) and THC (**12**), employing a similar column packing but with a longer column (250 mm) and larger particle size (5 μm)[86]. In this fully validated HPLC-ESIMS/MS method was shown useful for the analysis of drugs of abuse including cannabinoids in hair samples as well as on the distribution of the drugs deposition in hair collected from different anatomical body sites. The study was quite extensive and involved 481 samples of human

hair, collected during 2010–2015 from 231 drug abusers. Prior to HPLC analysis, cannabinoids were extracted using an ultrasonic-assisted methanolic extraction protocol.

Earlier, Montesano et al.[85] reported an HPLC-ESI-HRMS/MS method using a gradient elution with a mobile phase composed of water-ACN, both containing 0.1% HCOOH, to detect and quantify CBD (**3**), CBN (**8**), THC (**12**) and THC-COOH (**10**). Pressurised liquid extraction method was used to extract cannabinoids and their metabolites from experimental hair samples. It was shown that this method was fast and accurate the determination of those four cannabinoids in human hair samples, suitable for forensic analysis of hair samples for the presence of cannabinoids. This method was claimed to be the first HPLC–HRMS/MS based method that could allow the detection of THC-COOH (**10**) in hair at lower values than the cut-off (0.2 pg/mg). In the same year, an ESI-MS^n on a API 6500 Q-trap mass spectrometer, equipped with a Turbo-Ion-Spray (ESI) source was applied with a standard HPLC method for the analysis of several cannabinoids as picolinates in human hair samples[79] (Table 5).

A few other HPLC-MS/MS methods were reported between 2010 and 2014 for the analysis of cannabinoids in human hair samples[87-89] (Table 5). A fast LC-ESI-time-of-flight (TOF) mass spectrometric method was described for the automated and simultaneous screening, identification and quantitation of 30 representative multiclass drugs including cannabinoids in hair samples[89]. This method utilized a reversed-phase XDB-C_{18} analytical column (50 mm x 4.6 mm; particle size: 1.8 μm) and a gradient mobile phase consisting of water and ACN, both containing 0.1% HCOOH to detect CBD (**8**) and THC (**12**). Although it was described as an HPLC method, in fact, it was a UPLC method as it used a UPLC column with the particle size of 1.8 μm. This study demonstrated the effectiveness of an LC-TOFMS technique for both screening and quantitation purposes in cannabinoids testing in human hair. In another HPLC-MS/MS method[88], cannabinoids **3**, **8**, **12** and **13** were quantified in human hair samples, using a standard Phenomenex Luna C_{18} analytical column (150 mm x 2 mm; particle size: 5 μm) eluting with a water-ACN gradient. This method was found to be effective for analyzing human hair samples from a study dealing with the external contamination of hair by side-stream marijuana smoke. An ESI-MS/MS in negative ion mode on a hybrid API 5500 QTRAP MS was used with HPLC for the quantitative analysis of cannabinoids **3**, **8**, **10** and **12** in hair samples[87] (Table 5). This method provided the limit of quantification for THC-COOH (**10**), the most challenging biotransformation products of

cannabinoids, at 0.2 pg/mg, which was in accordance with the hair testing recommendations for forensic analysis.

There was only one report on HPLC-based analysis of cannabinoids, CBN (**8**), THC (**12**) and THCA (**13**) in children hair as hair analysis for drugs and drugs of abuse is increasingly applied in child protection cases[90]. This selective and sensitive HPLC method employed an ESI-MS/MS technique using a QTRAP 4000 triple quadrupole linear ion trap mass spectrometer coupled with a Turbolon-Spray interface. It can be noted that THCA (**13**) could be considered as a valuable marker facilitating the interpretation of the results not only in child protection cases but also in other issues such as hair samples from alleged cannabis growers or from law enforcement officers handling seized *Cannabis* plant materials.

3.2.4 Human urine samples

Human urine samples are popular in forensic toxicological analysis for the determination of various illegal drugs, including cannabinoids, and their metabolites[1]. Table 6 summarizes the applications of HPLC and UPLC-based analytical methods for naturally occurring cannabinoids and their biotransformation products in human urine samples[64, 65, 92-103]. Detection techniques used in these studies included simple UV, PDA and MS/MS methods.

An APCI-MS/MS in positive ion mode was applied with an HPLC for the detection of several cannabinoids including CBD **1**, **3**, **5**, **6**, **8**-**10**, **12**, **14**, **15** and THC-COOH-glucuronide in human urine samples using rather a short column (40 mm)[64]. A similar method, but using a 150 mm column, was also reported for the quantification of similar cannabinoids and their biotransformation products[65] (Table 6). Sanchez-Gonzalez et al.[92] utilized a simple HPLC-ESI-MS/MS method for the quantification of THC (**12**) and its major biotransformation products, 11-OH-THC (**9**) and THC-COOH (**10**), where the separation was achieved on a reversed-phase silica C_{18} analytical column (100 mm x 4.6 mm; particle size: 5 μm) eluting with a gradient elution with water and ACN, both containing 0.1% HCOOH. A rather much shorter column (20 mm x 2.1 mm; particle size: 2.5 μm) reversed-phase silica C_{18} analytical column achieved successful separation and ESI-MS/MS based quantitative detection of CBD (**3**), CBN (**8**), THC-COOH (**10**) and THC (**12**)[73]. There have been several other ESI-MS/MS based HPLC analytical methods reported for the analysis of cannabinoids and their biotransformation products in human urine samples [62, 74, 94-98] (Table 6). While the length of most of the HPLC columns used in these studies was between 50 mm and 250 mm, a much shorter SunFire C_{18} column (20

mm) was used by Lendoiro et al.[74], where a simple isocratic elution with 0.1% aqueous HCOOH and ACN, with a total run time of 5 min, was applied.

UPLC-based methods for the analysis of human urine samples for the presence and quantity of cannabinoids reported in the literature since the year 2010 almost exclusively utilized ESI-MS/MS detection technology[100-103] (Table 6). Most of the columns used in those studies were of the length of 50-100 mm. Except for one occasion, where a phenyl column was used[101], all other columns contained C_{18} packing (Table 6). Muller and Opdal[100] have recently reported a rapid semi-automated sample preparation with alkaline hydrolysis in a 96-well plate for quantification of THC-COOH (**10**) in human urine samples by UPLC-MS/MS, where an Acquity UHPLC BEH C_{18} column (100 mm x 2.1 mm; particle size: 1.7 μm) was used with the mobile phase comprising 0.1% HCOOH in ammonium formate (10 mM, pH 3.3) and ACN in a gradient elution mode. However, earlier the method reported by Dong et al.[101] could successfully and simultaneously quantify several cannabinoids and their major metabolites including CBD (**3**), CBN (**8**), 11-OH-THC (**9**), THC-COOH (**10**), THC (**12**) and THC-COOH-glucuronide. An ESI-MS/MS on a 8050 Shimadzu triple quadrupole mass spectrometer with electrospray ionization using scheduled multiple reaction monitoring (MRM) coupled with a UPLC system provided effective quantification of all those cannabinoids and their metabolites and additionally, THCV (**14**) and THCVA (**15**)[103]. Both the UPLC methods reported by Wei et al.[102] and Andersson et al.[103] used columns with particle size above 2.6 μm, which are not really true UPLC columns, as the particle size of a UPLC column is usually less than 2 μm.

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 richness of structural information that this detection may provide. However, the use of simple UV or PDA detectors still remains a popular method because of its simplicity and low cost. An isocratic elution with a mobile phase comprising 1% *o*-phosphoric acid in water containing 4 mL *n*-hexyl amine whose pH was adjusted at 6.0 by dropwise addition of 4 M NaOH and/or *o*-phosphoric acid 1 M), and ACN (87:13) at a flow rate of 1 mL/min was applied for the UV-PDA based quantification of CBD (**3**), CBN (**8**) and THC (**12**) in human urine samples; a Waters ODS-3 column (250 mm x 4.0 mm particle size: 5 μm) was used[93]. While a similar LC-PDA method, using a SunFire C_{18} column (150 mm x 3.0 mm; particle size: 3.5 μm), was used for the quantification of CBD (**3**), CBN (**8**), THC-COOH (**10**) and THC (**12**)[73], a simple UV detector was employed for the detection of CBD (**3**), CBN (**8**) and THC

(**12**), extracted from human urine samples by surfactant-aided dispersive liquid-liquid microextraction, using an HPLC coupled with a reversed-phase silica C_8 analytical column (250 mm x 4.6 mm; particle size: 3 μm)[99].

3.2.5 Miscellaneous biological samples from human

Table 7 lists HPLC or UPLC analysis of a few other less common biological samples from humans for the detection of cannabinoids. One of such samples is meconium sample. Toxicological studies using maternal foetal matrices can be a suitable tool to assess drug use or abuse during pregnancy, including cannabinoids[1]. One of such matrices is meconium, which contains the amniotic fluid swallowed by the foetus in the last half of pregnancy and is released as the first stools after birth. Meconium sample is easier to collect than neonatal urine and offers a much longer window of exposure about 20 weeks. Generally a 3 g sample of meconium is needed for maximum sensitivity. An HPLC-ESI-MS/MS method has recently been published for the simultaneous detection and quantification of CBD (**3**), CBN (**8**), 11-OH-THC (**9**), THC-COOH (**10**), THC (**12**), 8β,11-dihydorxy-THC and THC-glucuronide in 19 authentic meconium samples from uncontrolled pregnancies or women suspicious of drug consumption[104] (Table 7). A Phenomenex Kinetex C_{18} analytical column (50 mm x 2.1 mm; particle size: 2.6 μm) was used and gradient elution was performed using ACN in water (both containing 0.1% HCOOH) for a run time of only 10 min. Pretreatment involved homogenization of meconium samples in MeOH and passing it through a cation exchange solid-phase extraction. One of the new aspects of this method was the inclusion of THC-COOH and THC-glucuronides, which did not require any hydrolysis step for the determination of the free analytes, offering simple and much shorter sample analysis time analysis. This method allowed evaluation of the disposition of CBD (**3**) in positive *Cannabis* meconium specimens for the first time.

Several major cannabinoids and their biotransformation products including **3**, **8**-**10**, **12**, THC-glucuronide and THC-COOH-glucuronide were successfully quantified from post-mortem human tissue homogenates by HPLC using ESI-MS/MS in positive ion mode[105]. This method was applied for five post mortem cases to study the distribution of cannabinoids and their metabolites into some less commonly studied matrices. Pretreatment required protein precipitation and liquid-liquid extraction of cannabinoids from tissue homogenates. Most recently, human skeletal tissue samples have been analysed for detecting presence of

cannabinoids **10** and **12** by a UPLC-based method applying ESI-MS/MS, where an Acquity BEH C_{18} column (150 mm x 2.1 mm; particle size: 1.7 μm) was used with a mobile phase comprising water-MeOH (both containing 0.1% HCOOH) and a run time of 17 min[106]. In another experiment, 11-OH-THC (**9**) along with the above two cannabinoids were quantified in human liver samples using a similar UPLC-ESI-MS/MS method[38].

3.3 HPLC analysis of cannabinoids in animal samples

Table 8 presents the summary of all LC-based analytical methods for the quantification of cannabinoids in various animal samples published during 2010-2019[107-111]. All those LC methods were HPLC-based, and there has been no report on the use of UPLC for animal sample during this period. Also, all those methods utilized isocratic elution. An HPLC-ESI-MS/MS method was established for the analysis of marijuana cannabinoids in mouse brain tissue using an Applied Biosystems 3200 Q trap with a turbo V source for TurbolonSpray attached to a Shimadzu SCL HPLC system, offering simultaneous separation and identification of CBC (**1**), CBD (**3**), 11-OH-THC (**9**), THC-COOH (**10**) and THC (**12**) in mouse brain following *Cannabis* inhalation[110]. The brain tissue was chosen for this study as it is believed to be the site of action for many of the pharmacological effects of naturally occurring cannabinoids. This method utilized a well-established, reliable liquid-liquid extraction procedure for cannabinoids from tissue samples and with a simple isocratic reversed-phase HPLC coupled with an MS detector.

Whilst several published works mainly described the methods of detection and quantification of cannabinoids in various matrices, pharmacokinetic or ADME studies of cannabinoids using LC-based methods have been rather limited in the past decade. Zgair et al.[109] used a simple HPLC-UV-PDA based separation and detection of CBD (**3**) and THC (**12**), and most recently, Ravula et al.[108] have applied ESI-MS/MS on the ABSCIEX API 5500 Q-Trap mass spectrometer using the positive ion mode for the quantification of same cannabinoids present in rat plasma samples. The latter method[108] was designed to investigate pharmacokinetics aspects of cannabinoids after passive cannabis smoke inhalation, and offered effective the quantification of CBD (**3**) and THC (**12**) at concentrations up to 0.1 ng/mL, which could provide better understanding of the elimination phase of these compounds. This method required only 50 μL for processing, which could be considered useful for repeated sampling regimens in small animals such as rodents, making this method cost effective, and

suitable for the detect the analytes over extended periods. Whole blood samples from rat administered with a single dose (50 mg/kg) of CBD (**3**) was analyzed by HPLC-ESIMS/MS for the quantification of CBD (**3**), THC (**12**) and its metabolites[107]. This method was shown to be highly sensitive and selective for simultaneous determination of cannabinoids **3**, **9**, **10**, **12** and THC-COOH-glucuronide in rat whole blood. Earlier, an HPLC- ESIMS/MS method in negative ion mode on an IT-TOF MS was employed for the simultaneous detection and quantification of CBD (**3**), CBDV (**5**), CBG (**6**) and THCV (**14**) in mouse peripheral tissue samples[111]. This method was rapid, precise and accurate, and could be used as a fundamental tool for pharmacokinetic and pharmacodynamic studies on phytocannabinoids in tissues from different animal models.

3.4 HPLC and UPLC analysis of cannabinoids in dietary supplements, food and beverages

Cannabinoids may be present in food and beverages, either as contaminants or as food additives[11, 38, 112, 113] (Table 9). Fibre-type *Cannabis sativa* L. (hemp) is a valuable source for non-psychoactive cannabinoids, CBD (**3**) being the most important one among them, usually biosynthesized in both female and male inflorescences, and these cannabinoids are used medicinally in various food, beverages and pharmaceutical preparations, as well as several cosmetics and cosmeceutical products. Therefore, the qualitative and quantitative analysis of cannabinoids in food and food supplements is pivotal for quality assurance and the dietary intake control of cannabinoids-containing food items. It is particularly important when it comes to apiary products, which are widely consumed and the bees produce them from different floral sources. A new HPLC-based method has recently been developed and validated for the analysis of cannabinoids in honey, using both UV and MS detection methods, providing simultaneous detections and quantification of several cannabinoids, CBD (**3**), CBDA (**4**), CBG (**6**), CBGA (**7**), THC (**12**) and THCA (**13**)[112] (Table 9). This was the first HPLC-based analytical method for detection of non-psychoactive cannabinoids in honey. It was suggested that this method could potentially be applied for the analysis of honey for quality and safety assurance purposes, especially in the context of a European legislation on the amount of cannabinoids allowed in food products[112]. Earlier, a rapid HPLC-ESI-MS/MS method for the determination of a group of cannabinoids **3**, **4**, **6**-**8** and **11**-**14** in *Cannabis sativa* L. based beverages and food was reported[11]. In this study, an Ascentis Express RP-Amide stainless steel column (50 mm × 4.6 mm; particle size: 2.7 µm) was eluted with a linear gradient using the

mobile phase comprising water-ACN 0.1% HCOOH. This method was applied and found to be useful for the analysis of hemp seeds, oil and flour, as well as the food and beverages that contain them.

During the past decade, there seems to be only two UPLC methods reported for the analysis of cannabinoids in dietary supplements, food and beverages[38, 113] (Table 9). Heo et al.[113] reported fully validated UPLC-PDA and UPLC-ESIMS/MS methods for the quantification of THC (**12**) in dietary supplements, using a Waters Acquity UPLC HSS C_{18} column (150 mm x 2.1 mm; particle size: 1.8 μm) and a gradient mobile phase comprising 25 mM sodium phosphate and 0.01% sodium hexane sulfonate in deionized water adjusted to pH 3 with phosphoric acid, and ACN. Forty five samples including those from dietary supplement tablets, capsules, powders, liquids, cookies and candy, collected from Korean markets, were analyzed in this study, and the method was indicated to be useful for adulterant inspection and sample analysis providing targeted screening of cannabinoids in dietary supplement and foods. A simple UPLC-ESIMS/MS method has recently been published for the analysis of 11-OH-THC (**9**), THC-COOH (**10**) and THC (**12**) in milk samples, aiming at ensuring food safety[38]. A total of 13 milk samples (whole, semi-skimmed and skimmed), five junior formula milk products were analysed by this simple method using a Phenomenex Kinetex C_{18} UPLC column (50 mm x 2.1 mm; particle size: 1.7 μm), and a gradient elution with water-MeOH (both containing 0.1% HCOOH). It was demonstrated that this method was superior to previously published methods in terms of recoveries percentages obtained, and time needed to determine the analytes (only required 12 min, 24 min with the post-execution).

3.5 HPLC and UPLC analysis of cannabinoids in waste water and sewerage

In addition to the analyses of cannabinoids in *Cannabis sativa* plant, *Cannabis* products, biological and forensic samples, LC-based methods are also applied for the detection of cannabinoids present in various other matrices, *e.g.,* water and waste water samples. Table 10 presents a list of HPLC and UPLC (or UHPLC) analytical methods for naturally occurring cannabinoids in waste water and sewerage[114-116]. Cannabidiol (CBD, **3**), CBN (**8**), 11-OH-THC (**9**) and THC (**12**) were detected and quantified in sewage sludge by a HPLC-ESI-MS/MS method, and provided, for the very first time, the evidence on the occurrence of these cannabinoids in sewage sludge[114]. While Andres-Costa et al.[115] established a method to determine THC-COOH (**10**) and THC (**12**) in water samples using a

UPLC method applying QqTOF-MS/MS in positive ion mode, an ESI-MS/MS detection method was used to detect and quantify THC-COOH (**10**), THC (**12**) and THC-COOH-glucuronide in waste water samples utilising a UPLC separation on a Phenomenex Kinetex C_{18} column (100 mm x 2.1 mm; particle size: 1.7 μm)[116]. It can be noted that waste water analysis is considered to be one of the most useful methods for the determination of various drugs used in the geographical areas that wastewater treatment plants service, and simply by monitoring human biomarkers in sewage water, the consumption of various drugs, including cannabinoids, can be determined.

4. CONCLUSIONS

During the past decade, LC [HPLC and UPLC (or UHPLC)]-based analytical methods, especially LC-MS/MS techniques, have continued to be one of the most popular and effective methods for the detection and quantification of naturally occurring cannabinoids. However, the use of UPLC (or UHPLC) has become more popular than the conventional HPLC methods because of more precision, shorter run time, less use of solvent (mobile phase), and increased affordability. While water and ACN, both containing 0.05-0.1% HCOOH or CH_3COOH, have appeared as the most widely used mobile phase combination, either as a gradient or an isocratic elution, the use of MeOH instead of ACN has also been observed. A number of new MS interfaces, and mathematical models for method optimization, e.g., analysis quality by design (AQbD), have been introduced to make the LC-based methods even more effective for cannabinoids determination and quantification in different matrices.

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ORCID

Lutfun Nahar https://orcid.org/0000-0002-1157-2405

Alev Onder https://orcid.org/0000-0002-9088-1045

Satyajit D. Sarker http://orcid.org/0000-0003-4038-0514

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.* 2019; in press.
2. Lafaye G, Karila L, Blecha L, et al. Cannabis, cannabinoids, and health. *Dialogues Clin Neurosci.* 2017; 19(3): 309-316.
3. Pertwee RG. Cannabinoids. Springer-Verlag, Berlin, 2005.
4. Nahar L, Sarker SD. Guest Editorial: UPLC in phytochemical analysis. *Trends in Phytochem Res.* 2019; 3: 1-2.
5. Aizpurua-Olaizola O, Omar J, Navarro P, Olivares M, Etxebarria N, Usobiaga A. Identification and quantification of cannabinoids in *Cannabis sativa* L. plants by high performance liquid chromatography mass spectrometry. *Anal Bioanal Chem.* 2014; 406(29): 7549-7560.
6. Desrosiers NA, Scheideiler KB, Huestis MA. Quantification of six cannabinoids and metabolites in oral fluid by liquid chromatography-tandem mass spectrometry. *Drug Testing Anal.* 2015; 7(8): 684-694.
7. Dulaurent S, Gaulier JM, Imbert L, Morla A, Lachatre G. Simultaneous determination of Delta(9)-tetrahydrocannabinol, cannabidiol, cannabinol and 11-nor-Delta(9)-tetrahydrocannabinol-9-carboxylic acid in hair using liquid chromatography-tandem mass spectrometry. *Forensic Sci Int.* 2014; 236: 151-156.
8. Ferreiros N, Labocha S, Walter S, Lotsch J, Geisslinge G. Simultaneous and sensitive LC-MS/MS determination of tetrahydrocannabinol and metabolites in human plasma. *Anal Bioanal Chem.* 2013; 405(4): 1399-1406.
9. Marchioni C, de Souza ID, Acquaro VR, Crippa JAD, Tumas V, Queiroz MEC. Recent advances in LC-MS/MS methods to detmine endocannabinoids in biological samples: Application in neurodegenerative diseases. *Anal Chim Acta.* 2018; 1044: 12-28.
10. Míguez-Framil M, Cocho JA, Tabernero MJ, Bermejo AM, Moreda-Piñeiro A, Bermejo-Barrera P. An improved method for the determination of Δ^9-tetrahydrocannabinol, cannabinol and cannabidiol in hair by liquid chromatography-tandem mass spectrometry. *Microchem J*. 2014; 117: 7-17.

11. De Marco Pisciottano I, Guadagnuolo G, Soprano V, De Crescenzo M, Gallo P. A rapid method to determine nine natural cannabinoids in beverages and food derived from Cannabis sativa by liquid chromatography coupled to tandem mass spectrometry on a QTRAP 4000. *Rapid Commun Mass Spec.* 2018; 32(19): 1728-1736.
12. Roth N, Moosmann B, Auwarter V. Development and validation of an LC-MS/MS method for quantification of Delta 9-tetrahydrocannabinolic acid A (THCA-A), THC, CBN and CBD in hair. *J Mass Spectrometry.* 2013; 48(2): 227-233.
13. Sergi M, Montesano C, Odoardi S, et al. Micro extraction by packed sorbent coupled to liquid chromatography tandem mass spectrometry for the rapid and sensitive determination of cannabinoids in oral fluids. *J Chromatogr A*. 2013; 1301: 139-146.
14. 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.
15. Sarker SD, Nahar L. Natural Products Isolation, 3rd Edition, Humana Press –Springer-Verlag, USA. 2012.
16. 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.
17. Nováková L, Matysová L, Solich P. Advantages of application of UPLC in pharmaceutical analysis. *Talanta*. 2006; 68: 908-918.
18. Gonzalez-Marino I, Homas KV, Reid MJ. Determination of cannabinoid and synthetic cannabinoid metabolites in wastewater by liquid-liquid extraction and ultra-high-performance supercritical fluid chromatography-tandem mass spectrometry. *Drug Testing Anal.* 2018; 10(1): 222-228.
19. Wang M, Wang YH, Avula B, Radwan MM, Wanas AS, Amira S, van Antwerp J, Parcher JF, El-Sohly MA, Khan IA. Decarboxylation study of acidic cannabinoids: A novel approach using ultra-high-performance supercritical fluid chromatography/photodiode array-mass spectrometry. *Cannabis and Cannabinoid Res.* 2016; 1(1): 262-271.
20. Wang M, Wang YH, Avula B, Radwan MM, Wanas AS, Mehmedic Z, van Antwerp J, El-Sohly MA, Khan IA. Quantitative determination of cannabinoids in cannabis and cannabis products using ultra-high-performance supercritical fluid chromatography and diode array/mass spectrometric detection. *J Forensic Scs.* 2017; 62(3): 602-611.

21. Jornet-Martinez N, Ortega-Sierra A, Verdu-Andres J, Herraez-Hernandez R, Campins-Falco P. Analysis of contact traces of *Cannabis* by in-tube solid-phase microextraction coupled to nanoliquid chromatography. *Molecules.* 2018; 23(9): Article no. 2359.
22. Gul W, Gul SW, Radwan MM, Wana AS, Mehmedic Z, Khan II, Sharaf MHM, El-Sohly MA. Determination of 11 cannabinoids in biomass and extracts of different varieties of cannabis using high-performance liquid chromatography. *J AOAC Int.* 2015; 98(6): 1523-1528.
23. Gallo-Molina AC, Castro-Vargas HI, Garzon-Mendez WF, Ranfrez JAM, Monroy ZJR, King JW, Parada-Alfonso F. Extraction, isolation and purification of tetrahydrocannabinol from the *Cannabis sativa* L. plant using supercritical fluid extraction and solid phase extraction. *The J Supercritical Fluids*. 2019; 146: 208-216.
24. Peschel W, Politi M. H-1 NMR and HPLC/DAD for *Cannabis sativa* L. chemotype distinction, extract profiling and specification. *Talanta.* 2015; 140: 150-165.
25. Citti C, Battisti UM, Braghiroli D, Ciccarella G, Schmid M, Vandelli MA, Cannazza G. A Metabolomic approach applied to a liquid chromatography coupled to high-resolution tandem mass spectrometry method (HPLC-ESI-HRMS/MS): Towards the comprehensive evaluation of the chemical composition of cannabis medicinal extracts. *Phytochem Anal.* 2018; 29(2): 144-155.
26. Burnier C, Esseiva P, Roussel C. Quantification of THC in cannabis plants by fast-HPLC-DAD: A promising method for routine analyses. *Talanta.* 2019; 192: 135-141.
27. Zivovinovic S, Alder R, Allenspach MD, Steuer C. Determination of cannabinoids in *Cannabis sativa* L. samples for recreational, medical, and forensic purposes by reversed-phase liquid chromatography-ultraviolet detection. *J Anal Sci Technol.* 2018; 9: article no. 27 (10 pages).
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(6): 1503-1522.
29. Azipurua-Olaizola O, Omar J, Navarro P, Olivares M, Etxenarria N, Usobiaga A. Identification and quantification of cannabinoids in *Cannabis sativa* L. plants by high performance liquid chromatography-mass spectrometry. *Anal Bianal Chem.* 2014; 406(29): 7549-7560.

30. Mandrioli M, Tura M, Scotti S, Toschi TG. Fast detection of 10 cannabinoids by RP-HPLC-UV method in Cannabis sativa L. *Molecules*. 2019; 24: article no. 2113 (1-12 page).
31. Pellati F, Brighenti V, sperlea J, Marchetii L, Bertelli D, Benvenuti S. New methods for the comprehensive analysis of bioactive compounds in *Cannabis sativa* L. (hemp). *Molecules*. 2018; 23(10): article no. 2639.
32. Brighenti V, Pellati F, Steinbach M, Maran D, Benvenuti S. Development of a new extraction technique and HPLC method for the analysis of non-psychoactive cannabinoids in fibre-type *Cannabis sativa* (hemp). *J Pharm Biomed Anal.* 2017; 143: 228-236.
33. Calvi L, Pentimalli D, Panseri S, Giupponi L, Gelmini F, Beretta G, Vitali D, Bruno M, Zilio E, Pavlovic R. Comprehensive quality evaluation of medical *Cannabis sativa* L. inflorescence and macerated oils based on HS-SPME coupled to GC-MS and LC-HRMS (q-exactive orbitrap (R)) approach. *J Pharm Biomed Anal.* 2018; 150: 208-219.
34. Elkins AC, Deseo MA, Rochfort S, Exernieks V, Spangenberg G. Development of a validated method for the qualitative and quantitative analysis of cannabinoids in plant biomass and medicinal cannabis resin extracts obtained by supercritical fluid extraction. *J Chromatog B – Anal Technol Biomed Life Scs.* 2019; 1109: 76-83.
35. Fekete S, Sadat-Noorbakhsh V, Schelling C, Molnar I, Guillarme D, Rudaz S, Veuthey JL. Implementation of a generic liquid chromatographic method development workflow: Application to the analysis of phytocannabinoids and *Cannabis sativa* extracts. *J Pharm Biomed Anal.* 2018; 155: 116-124.
36. Wang YH, Avula B, El-Sohly MA, Radwan MM, Wang M, Wanas AS, Mehmedic Z, Khan IA. Quantitative determination of (9)-THC, CBG, CBD, their acid precursors and five other neutral cannabinoids by UHPLC-UV-MS. *Planta Medica.* 2018; 84(4): 260-266.
37. Gul W, Gul SW, Chandra S, Lata H, Ibrahim EA, El-Sohly MA. Detection and quantification of cannabinoids in extracts of *Cannabis sativa* roots using LC-MS/MS. *Planta Medica.* 2018; 84(4): 267-271.
38. Escriva U, Andres-Costa MJ, Andreu V, Pico Y. Analysis of cannabinoids by liquid chromatography-mass spectrometry in milk, liver and hemp seeds. *Food Chem.* 2017; 228: 177-185.
39. Pavlovic R, Nenna G, Calvi L, Panseri S, Borgonovo G, giupponi L, Cannazza G, Giorgi A. Quality traits of “Cannabidiol Oils”: Cannabinoids content, terpene fingerprint and

oxidation stability of European commercially available preparations. *Molecules.* 2018; 23(5): Article n. 1230.

40. Deidda R, Avohou HT, Baronti R, Davolio PL, Pasquini B, Del Bubba M, Hubert C, Hubert P, Orlandini S, Furlanetto S. Analytical quality by design: development and control strategy for a LC method to evaluate the cannabinoids content in cannabis olive oil extract. *J Pharm Biomed Anal.* 2019; 166: 326-35.
41. Morini L, Porro G, Liso M, Groppi A. Therapeutic use of delta 9-THC and cannabidiol: Evaluation of a new extraction procedure for the preparation of cannabis based olive oil. *Curr Pharm Biotechnol.* 2017; 18(10): 828-833.
42. Meng Q, Buchanan B, ZuccoloJ, Poulin Z-M, Gabriele J, Baranowski DC. A reliable and validated LC-MS/MS method for the simultaneous quantification of 4 cannabinoids in 40 consumer products. *PLOS One.* 2018; 13(5): e0196396.
43. Patel B, Wene D, Fan ZH. Qualitative and quantitative measurement of cannabinoids in cannabis using modified HPLC/DAD method. *J Pharm Biomed Anal.* 2017; 146: 15-23.
44. Hädener M, König S, Weinmann W. Quantitative determination of CBD and THC and their acid precursors in confiscated cannabis samples by HPLC-DAD. *Forensic Sci Int.* 2019; 299: 142-150.
45. Protti M, Brighenti V, Battaglia MR, Anceschi L, Pellati F, Mercolini L. Cannabinoids from *Cannabis sativa* L.: A new tool based on HPLC DAD-MS/MS for a rational use in medicinal chemistry. *ACS Med Chem Letts.* 2019; 10(4): 539-544.
46. Ciolino LA, Ranieri TL, Taylor Am. Commercial cannabis consumer products part 2: HPLC-DAD quantitative analysis of cannabis cannabinoids. *Forensic Sci Int.* 2018; 289: 438-447.
47. Peschel W. Quality control of traditional cannabis tinctures: Pattern, markers and stability. *Scientia Pharmaceutica.* 2016; 84(3): 567-584.
48. Chang CW, Tung CW, Tsai CC, Wu YT, Hsu MC. Determination of cannabinoids in hemp nut products in Taiwan by HPLC-MS/MS coupled with chemometric analysis: quality evaluation and pilot human study. *Drug Testing Anal.* 2017; 9(6): 888-897.
49. Citti C, Ciccarella G, Braghiroli D, Parenti C, Vandelli MA, Cannazza G. Medicinal cannabis: Principal cannabinoids concentration and their stability evaluated by a high performance liquid chromatography coupled to diode array and quadrupole time of flight mass spectrometry method. *J Pharm Biomed Anal.* 2016; 128: 201-209.

50. Carcieri C, Tomasello C, Simiele M, De Nicolo A, Avataneo V, Canzoneri L, Cusato J, Di Perri G, D'Avolio A. Cannabinoids concentration variability in cannabis oive oil galenic preparations. *J Pharm Pharmacol.* 2018; 70(1): 143-149.
51. Pacifici R, Marchei E, Salvatore F, Guandalini L, Busardo FP, Pichini S. Evaluation of cannabinoids concentration and stability n standardized preparations of cannabis tea and cannabis oil by ultra-high performance liquid chromatography tandem mass spectrometry. *Clin Chem Lab Med.* 2017; 55(10): 1555-1563.
52. Dos Santos NA, Tose LV, da Silva SRC, Murgu M, Kuster RM, Ortiz RS, Camargo FAO, Vaz BG, Lacerda Jr V, Ramao W. Analysis of isomeric cannabinoid standards and *Cannabis* products by UPLC-ESI-TWIM-MS: a comparison with GC-MS and GC x GC-QMS. *J Braz Chem Soc.* 2019; 30(1): 60-70.
53. Tose LV, Santos NA, Rodrigues RRT, Murgu M, Gomes AF, Vasconcelos GA, Souza PCT, Vaz BG, Romao W. Isomeric separation of cannabinoids by UPLC combined with ionic mobility mass spectrometry (TWIM-MS) Part 1. *Int J Mass Spec.* 2017; 418: 112-121.
54. Mudge EM, Murch SJ, Brown PN. Leaner and greener analysis of cannabinoids. *Anal Bioanal Chem.* 2017; 409(12): 3153-3163.
55. Sun WL, Zheng XY, Zhao YB, Zeng LH, Gao LS, Zheng H, Liu Y. Determination of three kinds of cannabinoids in cannabis using ultra high performance liquid chromatography-tandem mass spectrometry and analysis of phenotype of cannabis. *Chinese J Anal Chem.* 2017; 45(7): 1052-1058.
56. Schwope DM, Scheidweiler KB, Huestis MA. Direct quantification of cannabinoids and cannabinoid glucuronides in whole blood by liquid chromatography-tandem mass spectrometry. *Anal Bioanal Chem.* 2011; 401(4): 1273-1283.
57. Tiscione NB, Miller R, Shan XQ, 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.
58. Scheidweiler KB, Newmeyer MN, Barnes AJ, Huestis MA. Quantification of cannabinoids and their free and glucuronide metabolites in whole blood by disposable pipette extraction and liquid chromatography-tandem mass spectrometry. *J Chromatog A.* 2016; 1453 34-42.
59. König S, Aebi B, Lanz S, Gasser M, Weimann W. On-line SPE LC-MS/MS for the quantification of Delta 9-tetrahydrocannabinol (THC) and its two major metabolites in

human peripheral blood by liquid chromatography tandem mass spectrometry. *Anal Bioanal Chem.* 2011; 400(1): 1-16.

60. Mercolini L, Mandrioli R, Sorella V, Somaini L, Giocondi D, Serpelloni G, Raggi MA. Dried blood spots: Liquid chromatography-mass spectrometry analysis of Delta(9)-tetrahydrocannabinol and its main metabolites. *J Chromatog A.* 2013; 1271: 33-40.
61. Lacroix C, Saussereau E. Fast liquid chromatography/tandem mass spectrometry determination of cannabinoids in micro volume blood samples after dabsyl derivatization. *J Chromatog B – Anal Technol Biomed Life Scs.* 2012; 905: 85-95.
62. Dziadosz M, Teske J, Henning K, Klintschar M, Nordmeier F. LC-MS/MS screening strategy for cannabinoids and methadone in human serum, urine, and post-mortem blood as an effective alternative to immunoassay based methods applied in forensic toxicology for preliminary examination. *Forensic Chem.* 2018; 7: 33-37.
63. Roslawski MJ, Remmel RP, Karanam A, Leppik IE, Marino SE, Birnbaum AK. Simultaneous quantification of 13 cannabinoids and metabolites in human plasma by liquid chromatography tandem mass spectrometry in adult epilepsy patients. *Ther Drug Monit.* 2019; 41(3): 357-370.
64. Klawitter J, Sempio C, Morlein S, De Bloois E, Klepacki J, Henthorn T Leehy MA, Hoffenberg EJ, Knupp K Wang GS. An atmospheric pressure chemical ionization MS/MS assay using online extraction for the analysis of 11 cannabinoids and metabolites in human plasma and urine. *Ther Drug Monitoring.* 2017; 39(5): 556-564.
65. Aizpurua-Olaizola O, Zarandona I, Ortiz L, Navarro P, Etxebarria N, Usobiaga A. Simultaneous quantification of major cannabinoids and metabolites in human urine and plasma by HPLC-MS/MS and enzyme-alkaline hydrolysis. *Drug Testing Anal.* 2017; 9(4): 26-633.
66. Dziadosz M, Klitschar M, Teske J. Simple protein precipitation-based analysis of delta(9)-tetrahydrocannabinol and its metabolites in human serum by liquid chromatography-tandem mass spectrometry. *Forensic Toxicol.* 2017; 35(1): 190-194.
67. House J, Lyttle C, Blanchard C. An ultra-high-pressure chromatography tandem mass spectrometry (UPLC-MS/MS) method for the detection of cannabinoids in whole blood using solid phase extraction. *Can Soc Forensic Sci J.* 2017; 50(3): 103-113.

68. Sørensen LK & Hasselstrøm JB. Sensitive determination of cannabinoids in whole blood by LC-MS-MS after rapid removal of phospholipids by filtration. *J Anal Toxicol.* 2017; 41(5): 382-391.

69. Simões SS, Ajenjo AC, Dias MJ. Qualitative and quantitative analysis of THC, 11-hydroxy-THC and 11-nor-9-carboxy-THC in whole blood by ultra-performance liquid chromatography/tandem mass spectrometry. *Rapid Comm Mass Spectrometry.* 2012; 25(18): 2603-2610.

70. Ocque AJ, Hagler CE, DiFrancesco R, Lombardo J, Morse GD. Development and validation of an assay to measure cannabidiol and delta 9-tetrahydrocannabinol in human EDTA plasma by UHPLC-MS/MS. *J Chromatog B – Anal Technol Biomed Life Scs.* 2019; 1112: 56-60.

71. Jamwal R, Topletz AR, Ramratnam B, Akhlaghi F. Ultra-high performance liquid chromatography tandem mass-spectrometry for simple and simultaneous quantification of cannabinoids. *J Chromatog B – Anal Technol Biomed Life Scs.* 2017; 1048: 10-18.

72. Luo YR, Cassandra Y, Lynch KL. Quantitation of cannabinoids in breath samples using a novel derivatization LC-MS/MS assay with ultra-high sensitivity. *J Anal Toxicol.* 2019; 43(5): 331-229.

73. Cela-Perez MC, bates F, Jimenez-morigosa C, Lendoiro E, de Castro A, Cruz A, Lopez-Rivadulla M, Lopez-Vilarino M, Gonzalez-Rodriguez MV. Water-compatible imprinted ills for sensitive determination of cannabinoids in urine and oral fluid. *J Chromatog A.* 2016; 1429: 53-64.

74. Lendoiro E, de Castro A, Fernandez-Vega H, Cela-Perez MC, Lopez-Vilarino JM, Gonzalez-Rodriguez M, Cruz A, Lopez-Rivadulla M. Molecularly imprinted polymer for selective determination of Delta(9)-tetrahydrocannabinol and 11-nor-Delta(9)-tetrahydrocannabinol carboxylic acid using LC-MS/MS in urine and oral fluid. *Anal Bioanal Chem.* 2014; 406(15): 3589-3597.

75. Concheiro M, Lee D, Lendoiro E, Huestis MA. Simultaneous quantification of Delta(9)-tetrahydrocannabinol, 11-nor-9-carboxy-tetrahydrocannabinol, cannabidiol and cannabinol in oral fluid by microflow-liquid chromatography-high resolution mass spectrometry. *J Chromatog A.* 2013; 1297: 123-130.

76. Scheidweiler KB, Himes SK, Chen XH, Liu HF, Huestis MA. 11-Nor-9-carboxy-Delta 9-tetrahydrocannabinol quantification in human oral fluid by liquid chromatography-tandem mass spectrometry. *Anal Bioanal Chem.* 2013; 405(18): 6019-6027.
77. Fabritius M, Staub C, Mangin P, Giroud C. Analysis of cannabinoids in oral fluid by liquid chromatography-tandem mass spectrometry. *Forensic Toxicol.* 2013; 31(1): 151-163.
78. Zancanaro I, Limberger RP, Bohel PO, dos Santos MK, De Boni RB, Pechansky F, Caldas ED. Prescription and illicit psychoactive drugs in oral fluid-LC-MS/MS method development and analysis of samples from Brazilian drivers. *Forensic Sci Int.* 2012; 223(1-3): 208-216.
79. Thieme D, Sachs U, Sachs H, Moore C. Significant enhancement of 11-hydroxy-THC detection by formation of picolinic acid esters and application of liquid chromatography/multi stage mass spectrometry (LC-MS3): Application to hair and oral fluid analysis. *Drug Testing Anal.* 2015; 7(7): 577-585.
80. Malaca S, Busardo FP, Gottardi M, Pichini S. Dilute and shoot ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) analysis of psychoactive drugs in oral fluid. *J Pharm Biomed Anal.* 2019; 170: 63-67.
81. Di Rago M, Chu M, Rodda LN, Jenkins E, Kotsos A, Gerostamoulos D. Ultra-rapid targeted analysis of 40 drugs of abuse in oral fluid by LC-MS/MS using carbon-13 isotopes of methamphetamine and MDMA to reduce detector saturation. *Anal Bioanal Chem.* 2016; 408(14): 3737-3749.
82. Sobolesky PM, Smith BE, Hubbard JA, Stone J, Marcotte TD, Grelotti DJ, Grant I, Fitzgerald RI. Validation of a liquid chromatography-tandem mass spectrometry method for analysing cannabinoids in oral fluid. *Clinica Chimica Acta.* 2019; 491: 30-38.
83. Wei BN, McGuffey JE, Blount BC, Wang LQ. Sensitive quantification of cannabinoids in milk by alkaline saponification-solid phase extraction combined with isotope dilution UPLC-MS/MS *ACS Omega.* 2016; 1(6): 1307-1313.
84. Odoardi S, Valentini V, de Giovanni N, Pascali VL, Strano-Rossi S. High-throughput screening for drugs of abuse and pharmaceutical drugs in hair by liquid-chromatography-high resolution mass spectrometry (LC-HRMS). *Microchem J.* 2017; 133: 302-310.
85. Montesano C, Simeoni MC, Vannutelli G, Gregori A, Ripani L, Sergi M, Compagnone D, Curini R. Pressurized liquid extraction for the determination of cannabinoids and

metabolites in hair: Detection of cut-off values by high performance liquid chromatography-high resolution tandem mass spectrometry. *J Chromatog A.* 2015; 1406: 192-200.

86. Tzatzarakis MN, Alegakis AK, Kavvalakis MP, Vakonaki E, Stivaktakis PD, Barbounis EG, Tsatsakis AM. Comparative evaluation of drug deposition in hair samples collected from different anatomical body sites. *J Anal Toxicol.* 2017 41(3): 214-223.
87. Dulaurent S, Gaulier JM, Imbert L, Morla A, Lachâtre G. Simultaneous determination of Δ9-tetrahydrocannabinol, cannabidiol, cannabinol and 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid in hair using liquid chromatography-tandem mass spectrometry. *Forensic Sci Int*. 2014; 236: 151-156.
88. Roth N, Moosmann B, Auwärter V. Development and validation of an LC-MS/MS method for quantification of Δ9-tetrahydrocannabinolic acid A (THCA-A), THC, CBN and CBD in hair. *J Mass Spectrom*. 2013; 48(2): 227-233.
89. Dominguez-Romero JC, Garcia-Reyes JF, Molina-Diaz A. Screening and quantitation of multiclass drugs of abuse and pharmaceuticals in hair by fast liquid chromatography electrospray time-of-flight mass spectrometry. *J Chromatog B – Anal Technol Biomed Life Scs.* 2011; 879(22): 2034-2042.
90. Moosmann B, Roth N, Hastedt M, Jacobsen-Bauer A, Pragst F, Auwarter V. Cannabinoid findings in children hair - what do they really tell us? An assessment in the light of three different analytical methods with focus on interpretation of 9-tetrahydrocannabinolic acid A concentrations. *Drug Testing Anal.* 2015; 7(5): 349-357.
91. Shah I, Petroczi A, Uvacsek M, Ranky M, Naughton DP. Hair-based rapid analyses for multiple drugs in forensics and doping: application of dynamic multiple reaction monitoring with LC-MS/MS. *Chem Central J.* 2015; 8: Article number: 73.
92. Sanchez-Gonzalez J, Salgueiro-Fernandez R, Cabarcos P, Bermejo AM, Bermejo-Barrera P, Moreda-Pineiro A. Cannabinoids assessment in plasma and urine by high performance liquid chromatography-tandem mass spectrometry after molecularly imprinted polymer microsolid-phase extraction. *Anal Bioanal Chem.* 2017; 409(5): 1207-1220.
93. Feizbakhsh R, Ebrahimi M, Davoodnia A. Preconcentration and analysis of cannabinoid compounds (THC-9, CBN, CBD) in urine samples by IL-ISFME/D-mu-SPE/HPLC-DAD. *Int J Med Res Health Scs.* 2016; 5(1): 235-244.

94. Scheidweiler KB, Desrosiers NA, Huestis MA. Simultaneous quantification of free and glucuronidated cannabinoids in human urine by liquid chromatography tandem mass spectrometry. *Clin Chim Acta.* 2012; 413(23-24): 1839-1847.
95. Zazoğlu S, Anilanmert B, Aydin M. Cengiz S. Fast confirmation for marijuana metabolite: THC-COOH, ultra-fast LC-MS/MS run time, and application to routine samples. *Acta Chromatographica/* 2017; 29(2): 253-265.
96. Hädener M, Weinmann W, van Staveren DR, Konig S. Rapid quantification of free and glucuronidated THCCOOH in urine using coated well plates and LC-MS/MS analysis. *Bioanal.* 2017; 9(5): 485-496.
97. Montesano C, Sergi M, Odoardi S, Simeoni MC, Compagnone D, Curini R. A micro-SPE procedure for the determination of cannabinoids and their metabolites in urine by LC-MS/MS. *J Pharm Biomed Anal.* 2014; 91: 169-175.
98. Li XW, Shen BH, Jiang Z, Huang Y. Rapid screening of drugs of abuse in human urine by high-performance liquid chromatography coupled with high resolution and high mass accuracy hybrid linear ion trap-Orbitrap mass spectrometry. *J Chromatog A.* 2013; 1302: 95-104.
99. Moradi M, Yamini Y, Baheri T. Analysis of abuse drugs in urine using surfactant-assited dispersive liquid-liquid microextraction. *J Separation Sci.* 2011; 34(14): 1722-1729.
100. Muller LD, Opdal MS. Developing a rapid semi-automated sample preparation with alkaline hydrolysis in a 96-well plate for quantification of 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid in urine samples by UHPLC-MS/MS. *J Pharm Biomed Anal.* 2018; 161: 296-304.
101. Dong XR, Li LL, Ye YH, Zheng LX, Jiang Y. Simultaneous determination of major phytocannabinoids, their main metabolites, and common synthetic cannabinoids in urine samples by LC-MS/MS. *J Chromatog B – Anal Technol Biomed Life Sce.* 2016; 1033: 55-64.
102. Wei BN, Wang LQ, Blount BC. Analysis of cannabinoids and their metabolites in human urine. *Anal Chem.* 2015; 87(20): 10183-10187.
103. Andersson M, Scheidweiler KB, Sempio C, Barnes AJ, Huestix MA. Simultaneous quantification of 11 cannabinoids and metabolites in human urine by liquid chromatography tandem mass spectrometry using WAX-S tips. *Anal Bioanal Chem.* 2016; 408(23): 6461-6471.

104. Prego-Meleiro P, Lendoiro E, Concheiro M, Cruz A, Lopez-Rivadulla M, da castro A. Development and validation of a liquid chromatography tandem mass spectrometry method for the determination of cannabinoids and phase I and II metabolites in meconium. *J Chromatog A.* 2017; 1497: 118-126.
105. Gronewold A, Skopp G. A preliminary investigation on the distribution of cannabinoids in man. *Forensic Sci Int.* 2011; 210(1-3): E7-E11.
106. Orfanidis A, Gika H, Mastrogianni O, Krokos A, Theodoridis G, Zaggelidou E, Raikos N. Determination of drugs of abuse and pharmaceuticals in skeletal tissue by UHPLC-MS/MS. *Forensic Sci Int.* 2018; 290: 137-145.
107. Palazzoli F, Citti C, Licata M, Vilella A, Manca L, Zoli M, Vandelli MA, Forni F, Cannazza G. Development of a simple and sensitive liquid chromatography triple quadrupole mass spectrometry (LC-MS/MS) method for the determination of cannabidiol (CBD), Delta(9)-tetrahydrocannabinol (THC) and its metabolites in rat whole blood after oral administration of a single high dose of CBD. *J Pharm Biomed Anal.* 2018; 150: 25-32.
108. Ravula A, Chandasana H, Setlow B, Febo M, Bruijnzeel AW, Derendorf H. Simultaneous quantification of cannabinoids tetrahydrocannabinol, vannabidiol and CB1 receptor antagonist in rat plasma: An application to characterize pharmacokinetics after passive cannabis smoke inhalation and co-administration of rimonabant. *J Pharm Biomed Anal.* 2018; 160: 119-125.
109. Zgair A, Wong JCM, Sabri A, Fischer PM, Barrett DA, Constantinescu CS, Gershkovich P. Development of a simple and sensitive HPLC-UV method for the simultaneous determination of cannabidiol and delta(9)-tetrahydrocannabinol in rat plasma. *J Pharm Biomed Anal.* 2015; 114: 145-151.
110. Poklis JL, Thompson CC, Long KA, Litchman AH, Poklis A. Disposition of cannabichromene, cannabidiol, and delta(9)-tetrahydrocannabinol and its metabolites in mouse brain following marijuana inhalation determined by high-performance liquid chromatography-tandem mass spectrometry. *J Anal Toxicol.* 2010; 34(8): 516-520.
111. Piscitelli F, Pagano E, Lauritano A, Izzo AA, Di Marzo V. Development of a rapid LC-MS/MS method for the quantification of cannabidiol, cannabidivarin, delta(9)-tetrahydrocannabivarin, and cannabigerol in mouse peripheral tissues. *Anal Chem.* 2017; 89(8): 4749-4755.

112. Brighenti V, Licata M, Pedrazzi T, Maran D, Bertelli D, Pellati F, Benvenuti S. Development of a new method for the analysis of cannabinoids in honey by means of high-performance liquid chromatography coupled with electrospray isonisation-tandem mass spectrometry section. *J Chromatog A.* 2019; 1597: 179-186.
113. Heo S, Yoo GJ, Choi JY, Park HJ, Do JA, Cho S, Baek SY, Park SK. Simultaneous analysis of cannabinoid and synthetic cannabinoids in dietary supplements using UPLC with UV and UPLC-MS/MS. *J Anal Toxicol.* 2016; 40(5); 350-359.
114. Mastroianni N, Posyigo C, de Alda ML, Barcelo D. Illicit and abused drugs in sewage sludge: Method optimization and occurrence. *J Chromatog A.* 2013; 1322: 29-37.
115. Andres-Costa MJ, Andreu V, Pico Y. Analysis of psychoactive substances in water by information dependent acquisition on a hybrid quadrupole time-of-flight mass spectrometer. *J Chromatog A.* 2016; 1461: 98-106.
116. Jacox A, Wetzel J, Cheng S-Y, Concheiro M. Quantitative analysis of opioids and cannabinoids in wastewater sample. *Forensic Scs Res.* 2017; 2(1): 18-25.

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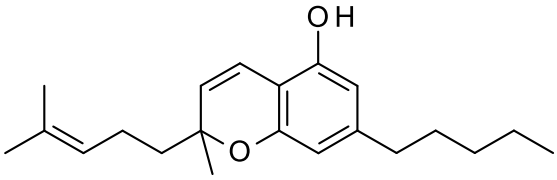
<tr><td colspan="2"> 
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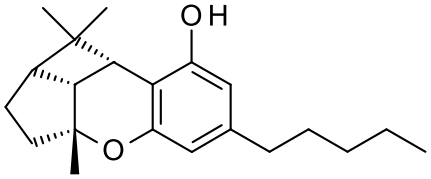
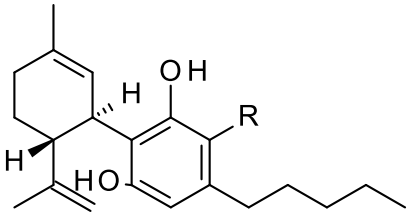
<tr><td> 
Cannabicyclol (2, CBCL)</td><td> 
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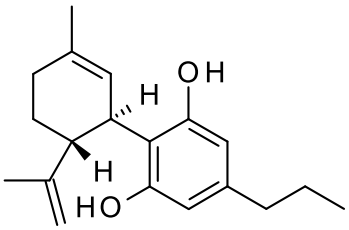
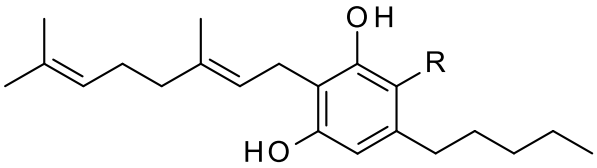
<tr><td> 
Cannabidivarin (CBDV, 5)</td><td> 
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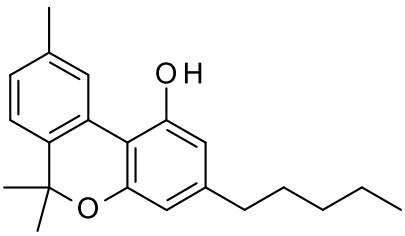
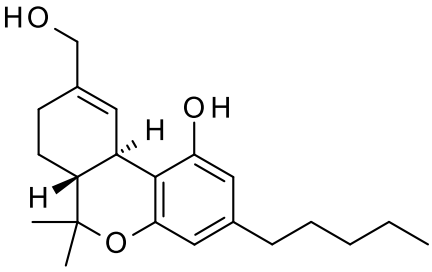
<tr><td> 
Cannabinol (8, CBN)</td><td> 
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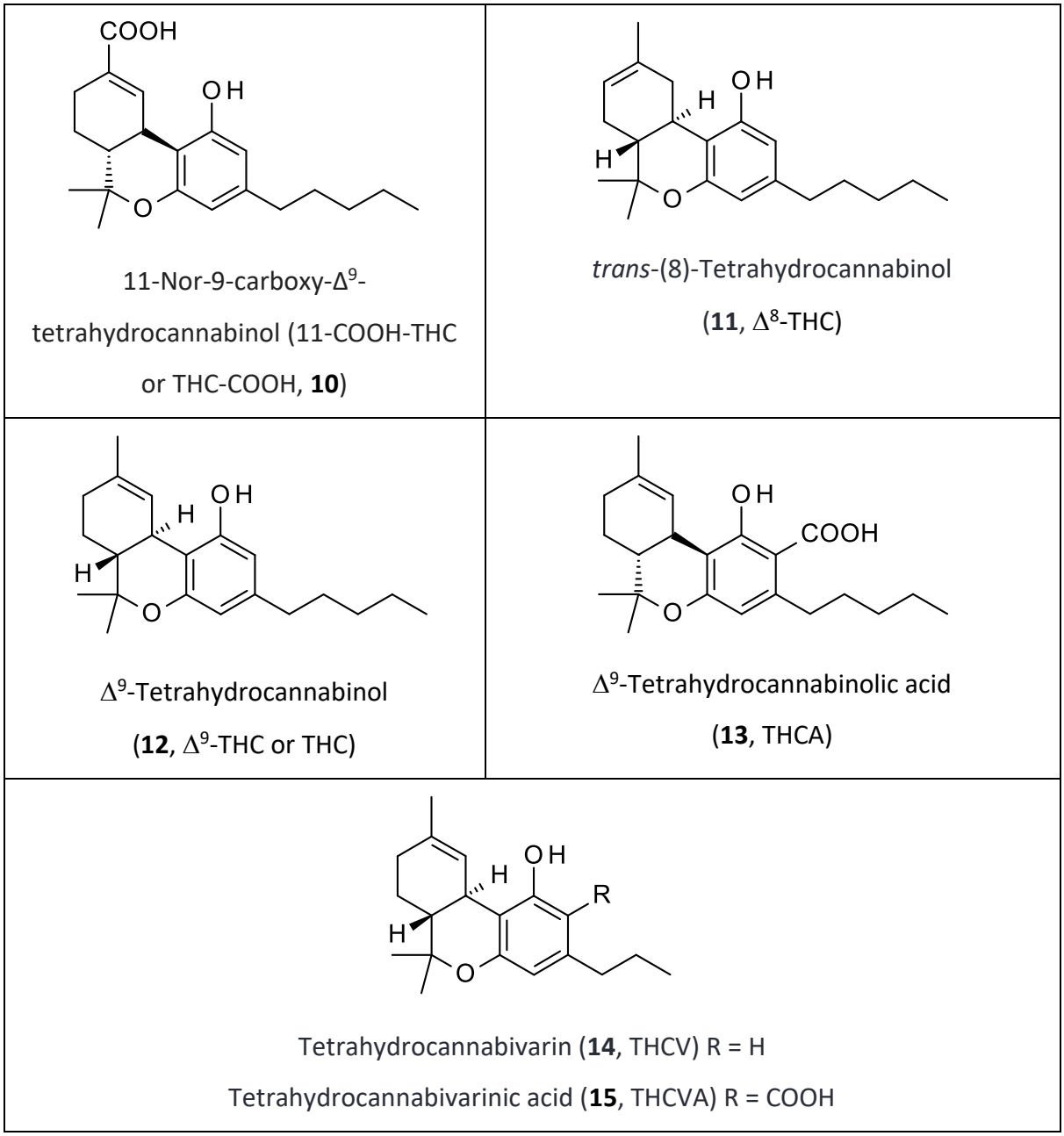

FIGURE 1 Cannabinoids in different matrices analyzed by HPLC and UPLC (or UHPLC)

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

Instrumentation	Column	Mobile phase	Detection	Plant parts	Cannabinoids analyzed/detected
HPLC methods					
Shimadzu Ultra Fast LC Prominence System	Phenomenex Luna C_{18} column (150 mm x 4.6 mm; particle size: 3 μm) linked to a Phenomenex C_{18} guard column cartridge.	0.1% HCOOH in both water (A) and ACN (B). Gradient elution: 30-70% B in A in 0-6 min, 70-77% B in A in 6-12 min, kept at 77% B in A for 10 min, and restored to initial condition in 0.2 min. Column temp: 28°C; flow rate: 1.2 mL/min; injection volume: 10 μL.	UV-DAD scanned for 210-400 nm, and set at 220 nm for quantification	A	CBC (**1**), CBL (**2**), CBD (**3**), CBDA (**4**), CBG (**6**), CBGA (**7**), CBN (**8**), Δ^8-THC (**11**), Δ^9-THC or THC (**12**), Δ^9-THCA or THCA (**13**) and THCV (**14**)[22]
Agilent 1260 HPLC	A monolithic Chromolith C_{18} column (50 x 4.6 mm; particle size: 5 μm; Merck, Darmstadt, Germany).	A linear gradient with 5-100% ACN in water in 17 min, and a flow rate of 2 mL/min.	UV detector set at 210 nm	B	Δ^9-THC or THC (**12**)[23]
Waters 900 HPLC System	Agilent Zorbax RX-C_{18} column (250 mm x 4.6 mm; particle size: 5 μm)	Column temp: 25°C, flow rate: 0.9 mL/min; injection volume: 10 μL. Solvent A contained water-ACN mixture (65:35) with 0.1% TFA, and solvent C was ACN. Gradient elution: solvent A:	UV-PDA set at 214 nm for quantification, and scanned or 210-400 nm		CBD (**3**), CBDA (**4**), CBG (**6**), CBGA (**7**), CBN (**8**), THC (**12**), and THCA (**13**)[24]

		0 min 70%, 30 min 35%, 43 min 5% and 48 min 70%			
Agilent 1200 HPLC	A Poroshell column (Poroshell 120 EC-C_{18}, 50 mm x 3.0 mm; particle size: 2.7 µm, Agilent, Milan, Italy)	0.1% HCOOH in both water (A) and ACN (B). A linear gradient from 5% to 95% B over 45 min, held at 95% B for 10 min and then brought back to the initial composition (5% B) over 5 min and the column equilibrated for another 5 min. The flow rate: 0.3 mL/min; injection volume: 5 µL; column temperature: 25°C; the total run time: 65 min.	UV-DAD, and ESI-MS/MS on a 6540 quadrupole time-of-flight (QTOF) mass analyser using both positive and negative ion modes		CBD (**3**), CBDA (**4**), CBN (**8**), Δ^9-THC or THC, (**12**)and Δ^9-THCA or THCA (**13**)[25]
Waters 1515 HPLC-DAD	Nucleodur® C_{18} Gravity column (250 mm x 4.6 mm; particle size: 5 µm)	0.1% HCOOH in both water (A) and ACN (B). Isocratic elution with 80% B in A. Flow rate: 1-3 mL/min; column temperature: 35°C.	UV-DAD set at 211 nm		CBD (**3**), CBN (**8**), Δ^9-THC or THC, (**12**) and Δ^9-THCA or THCA (**13**)[26]
LaChrom Elite System (Hitachi, Ltd., Tokio, Japan) HPLC system	Phenomenex Kinetex XB-C_{18} column (150 mm ×4.6 mm; particle size: 2.6 µm)	0.1% HCOOH in both water (A) and ACN (B). Gradient elution with A and B. Injection volume: 15 µL; column temperature: 50°C; flow rate: 0.8 mL/min.	UV-Vis detector set at 220 nm		CBD (**3**), CBDA (**4**), CBDV (**5**), CBG (**6**), CBGA (**7**), CBN (**8**), Δ^9-THC or THC, (**12**), and Δ^9-THCA or THCA (**13**)[27]

Agilent 1290 HPLC System	Poroshell 120 EX-C_{18} column (150 mm x 2.1 mm; particle size: 2.7 μm) coupled with a Poroshell 120 EX-C_{18} guard column (5 mm x 2.1 mm; particle size: 2.7 μm)	0.1% HCOOH in both water (A) and ACN (B). 0-8 min isocratic elution with 66% B, 8-12 min: linear gradient 66-95% B, held for 1 min at 95% B, followed by re-equilibration at 66% for 4 min. Injection volume: 15 μL; flow rate: 0.5 mL/min.	UV-DAD scanned at 200-400 nm, set at 214 nm for quantification		CBD (**3**), CBDA (**4**), CBG (**6**), CBGA (**7**), Δ^9-THC or THC (**12**), Δ^9-THCA or THCA (**13**), THCV (**14**) and THCVA (**15**)[28]
	Reversed-phase C_{18} analytical column	Gradient elution with ACN in water	ESI-MS/MS on a quadrupole-time-of-flight (Q-ToF) detector		CBD (**3**), CBG (**6**), CBN (**8**), THC (**12**), THCA (**13**) and THCV (**14**)[29]
Potency Prominence-i LC-2030C	Nex-Leaf CBX Potency C_{18} column (150 mm x 4.6 mm, particle size: 2.7 μm; with a guard column Nex-Leaf CBX 5 x 4.6 mm, 2.7 μm; Shimadzu, Japan)	Water containing 0.085% phosphoric acid (A) and CAN containing 0.085% phosphoric acid (B). Gradient elution: 0-3 min 0-70% B, 3-7 min 85% B, 7-8 min from 95% B, 8-10 min 70% B; flow rate: 1.6 mL/min; column temp. 35°C; injection volume: 5 μL.	UV detector set at 220 nm	C	CBC (**1**), CBD (**3**), CBDA (**4**), CBG, (**6**), CBGA (**7**), CBN (**8**), Δ^8-THC (**11**), Δ^9-THC or THC (**12**), Δ^9-THCA (**13**) and THCV (**14**)[30]
Agilent 1110		0.1% HCOOH in both water (A) and ACN (B). Gradient elution: 0-13 min 60% B, 13-	UV-DAD set at 220 nm for quantification, and		CBD (**3**), CBDA (**4**), CBG (**6**) and CBGA, **7**)[31,32]

	Ascentis Express C_{18} column (150 mm x 3.0 mm particle size: 2.7 μm; Supleco, USA)	17 min from 60% to 80% B, 17-22 min from 80% to 90% B; post-running time 15 min; flow rate: 0.4 mL/min; column temp. 30°C; injection volume: 3 μL.	scanned for 190-600 nm		
Agilent 1200			ESI-MS/MS in both positive and negative ion modes using an Ion Trap LC-MS		
Agilent 1100			UV-PDA at 210 and 220 nm (scanned for 190-600 nm), and ESI-MS/MS		
Thermo Fisher HPLC system	Synergi Hydro RP column (150 mm x 2 mm; particle size: 4 μm; Phenomenex) coupled with a guard column (4 mm x 3 mm; particle size: 4 μm)	0.1% HCOOH in water (A) and ACN (B). Gradient elution: 60-95% B in A in 10 min; held at 95% B for 4 min before 6 min equilibration at 60% B. Injection volume: 15 μL; column temperature: 30°C; flow rate: 0.3 mL/min.	Heated electrospray ionization (HESI-MS/MS using both positive and negative ionisation modes, on a Thermo Q-Exactive Plus Mass Spectrometer.		CBD (**3**), CBDA (**4**), CBG (**6**), CBGA (**7**), CBN (**8**), Δ^9-THC or THC (**12**), and Δ^9-THCA or THCA (**13**)[33]
UPLC (or UHPLC) methods					
Agilent 1290 UHPLC	Phenomenex Luna Omega C_{18} Column (150 mm × 2.1 mm; particle size: 1.6 μm)	0.1% HCOOH in both water (A) and ACN (B). Multi-step gradient elution: 0-2 min with 15% B, 2-3 min 15-75% B, 3-10 min from 75-90% B, 10-11 min 90-100% B and 11-15 min 100% B, followed	UV-PDA set at 280 nm, but scanned for 190-640 nm	A, D	CBC (**1**), CBD (**3**), CBDA (**4**), CBN (**8**), THC (**12**) and THCA (**13**)[34]

		by equilibration to initial condition for 5 min; flow rate: 0.3 mL/min; column temperature 30°C; injection volume: 3 μL.			
HITACHI ChromasterUltra UHPLC system	A Phenomenex Kinetex XB-C_{18} column (150 mm × 2.1 mm; particle size: 1.7 μm)	0.1% HCOOH in both water (A) and ACN (B). Gradient elution with A and B. Injection volume: 5 μL; column temperature: 50°C; flow rate: 0.8 mL/min.	UV-Vis detector set at 220 nm	B	CBD (**3**), CBDA (**4**), CBDV (**5**), CBG (**6**), CBGA (**7**), CBN (**8**), Δ^9-THC or THC (**12**) and Δ^9-THCA or THCA (**13**)[27]
Waters Acquity UPLC H-class	Acquity UPLC BEH C_{18} (50 mm x 2.1 mm; particle size: 1.7 μm), Acquity UPLC BEH Shield C_{18} (50 mm x 2.1 mm, and 100 mm x 21 mm; particle size: 1.7 μm), Acquity UPLC BEH Phenyl (50 mm x 2.1 mm; particle size: 1.7 μm), Acquity CSH Fluoro-Phenyl (50 mm x 2.1 mm; particle size: 1.7 μm), and a Acquity UPLC BEH Shield RP18 guard pre-column (5 mm x 2.1 mm; particle size: 1.7 μm) from Waters (Milford, USA).	A generic gradient from 45% to 100% ACN (B) in ethanol (A) in 5 min at 0.35 mL/min flow rate. Column temperature: 45°C	UV-PDA-MS		CBC (**1**), CBDA (**4**), CBDV (**5**), CBG (**6**), CBGA (**7**), CBN (**8**), Δ^8-THC (**11**), THC, (**12**), Δ^9-THCA or THCA (**13**) and THCV (**14**)[35]

Waters Acquity UPLC-I Class	Waters Cortec UPLC C_{18} column (100 mm x 2.1 mm; particle size: 1.6 μm)	A gradient elution with 0.05% HCOOH in both water (A) and ACN (B). Flow rate: 0.25 mL/min.	UV-PDA-MS using a single quadrupole MS analyser	B, C	CBC (**1**), CBL (**2**), CBD (**3**), CBDA (**4**), CBG (**6**), CBGA (**7**), CBN (**8**), Δ^8-THC (**11**), Δ^9-THC or THC (**12**), Δ^9-THCA or THCA (**13**) and THCV (**14**)[36]
Waters Acquity UPLC-I Class	Waters Cortec UPLC C_{18} column (100 mm x 2.1 mm; particle size: 1.6 μm)	A gradient elution with 0.05% HCOOH in both water (A) and ACN (B). Flow rate: 0.25 mL/min.	ESI-MS/MS	E	CBC (**1**), CBD (**3**), CBDA (**4**), CBGA (**7**), CBN (**8**), Δ^8-THC (**11**), Δ^9-THC or THC (**12**), Δ^9-THCA or THCA (**13**) and (THCV (**14**)[37]
Agilent 1260 UHPLC	Phenomenex Kinetex C_{18} (50 mm x 2.1 mm; particle size: 1.7 μm)	Gradient elution with water (A) and MeOH (B), both containing 0.1% of HCOOH; flow rate of 0.2 mL/min; starting at 70% of B increased linearly in 5 min to 95% B and held for 7 min, and returned at 70% with an equilibration time of 12 min before the next injection; injection volume: 5 μL.	ESI-MS/MS in positive ion mode using an Agilent 6410 Triple quadrupole mass spectrometer	F	THC-OH (**9**), THC-COOH (**10**) and THC (**12**)[38]

A = Biomass containing *Cannabis sativa* L. plant parts; B = *Cannabis sativa* L. whole plant parts; C = Inflorescences; D = *Cannabis* resins; E = *Cannabis sativa* L. roots; F = *C. sativa* L. seeds

TABLE 2 HPLC and UPLC (or UHPLC) methods for the analysis of cannabinoids in *Cannabis* consumer products

Instrumentation	Column	Mobile phase	Detection	Source	Cannabinoids analyzed/detected
HPLC methods					
Thermo Fisher HPLC Surveyor system	Phenomenex Synergy Hydro RP column (150 mm x 2 mm; particle size: 4 μm) with a C_{18} guard column (4 mm x 3 mm)	Mobile phase consisted of solvent A (0.1% HCOOH in water) and B (ACN). The gradient was initiated with 60% eluent A with a linear decrease up to 95% in 10 min. This condition was maintained for 4 min. The mobile phase was returned to initial conditions at 14 min, followed by a 6-min re-equilibration period (total run time: 20 min). Flow rate: 0.3 mL/min; injection volume: 2 μL; column temp. 30°C	ESI-MS/MS on a Thermo Q-Exactive Plus MS	A	CBD (**3**), (CBGA (**7**), Δ^9-THC or THC (**12**) and Δ^9-THCA or THCA (**13**)[39]

Thermo Fisher Surveyor Plus™ HPLC	Agilent Poroshell 120 SB-C_{18} column (150 mm, 2.1 mm; particle size: 2.7 μm; Agilent Technologies, USA) coupled with a Agilent Poroshell SB-C_{18} guard column (5 mm, 2.1 mm; particle size: 2.7 μm)	Isocratic elution with a mixture of ACN/water containing 5 mM of K_2HPO_4 adjusted to pH 3.45 (range 3.11–3.50) in 75:25 ratio. Column temperature: 53 ^oC; flow rate: 0.38 mL/min; injection volume: 10 mL	UV-DAD set at 220 nm for detection	B	CBD (**3**), CBDA (**4**) and THC (**12**)[40]
Agilent 1260 HPLC	Agilent Poroshell 120 SB-C18 column (75 mm x 3.0 mm; particle size: 2.7 μm)	Isocratic elution with ACN in water	ESI-MS/MS in positive ion mode		CBD (**3**) and THC (**12**)[41]
Agilent 1260 HPLC	Agilent Eclipse Plus 95Å C_{18} column (100 mm x 4.6 mm; particle size: 3.5 μm) coupled with a guard column.	Isocratic elution with 90% ACN (containing 0.1% HCOOH) in water (containing 0.1% HCOOH); flow rate: 0.5 mL/min; run time: 11 min; column temperature: 40^oC; injection volume: 20 μL.	ESI-MS/MS using both positive and negative ion modes	C	CBD (**3**), CBDA (**4**), Δ^9-THC or THC (**12**) and Δ^9-THCA (**13**)[42]

	Agilent Poroshell 120 SB-C18 column (75 mm x 3.0 mm; particle size: 2.7 μm)	Gradient elution with a 50 mM aqueous solution of ammonium formate, pH 5.19 (mobile phase A) and MeOH (mobile phase B): 0-8.2 min 68.0-85.0% mobile phase B, 8.2-9.0 min 85.0-95.0% mobile phase B, 9.0-10.0 min: 95.0-68.0% mobile phase B. total run time: 10 min; flow rate: 0.7 mL/min; injection volume: 10 μL; column temp: 30°C..	UV-DAD set at 220 nm for detection and quantification		CBD (**3**), CBDA (**4**), CBG (**6**), CBGA (**7**), CBN (**8**), Δ^8-THC (**11**), THC (**12**) and THCA (**13**)[43]
Dionex UltiMate 3000 HPLC	Kinetex Core-shell C_8 column (100 mm x 2.1 mm; particle size: 2.7 μm)	0.1% HCOOH in both water (A) and ACN (B). Isocratic elution: 0-2 min 50% B, gradient elution: 2-9 min 50-65% B, isocratic elution: 9-10 min 65% B, gradient elution: 10-10.1 min 65-50% B; post run 3 min at 50% B; flow rate: 0.6 mL/min; column temperature 25°C; injection volume: 5 μL.	UV/DAD detector set at 210 nm for quantification (scanned 200-800 nm)		CBD (**3**), CBDA (**4**), CBN (**8**), THC (**12**) and Δ^9-THCA (**13**)[44]
Waters Alliance e2695	Waters Cortecs C_{18}+ column (100 mm x 2.1 mm; particle size: 2.7 μm)	0.1% HCOOH in both water (A) and ACN (B). Gradient elution with 50% B, linearly ramping up to 95% of solvent B over 5 min,	UV-DAD and ESI-MS/MS on a Waters Micromass Quattro Micro Triple Quadrupole in multiple		CBD (**3**), CBDA (**4**), CBN (**8**), Δ^9-THC or THC (**12**) and Δ^9-THCA (**13**)[45]

		and held at 95% B for 5 min, The gradient was then changed to the starting conditions over 1 min and kept constant for 2 min to re-equilibrate the system. Flow rate: 0.3 mL/min; column temperature 25°C; injection volume: 10 μL.	reaction monitoring mode, using both positive and negative ion mode with ionization polarity switching		
Agilent 1100, Agilent 1200 and Agilent 1260	MacMod ACE 5 C18-AR (250 mm x 4.6 mm; particle size: 5 μm)	Isocratic elution with 34% ACN containing 0.5% CH_3COOH., and 66% water containing 0.5% CH_3COOH. Flow rate: 1 mL/min; column temp. 25°C; injection volume: 25 μL; run time 50 min	UV-DAD set at 220, 240, 270 and 307 nm, and scanned for 190-400 nm.		CBD (**3**), CBDA (**4**), CBN (**8**), Δ^9-THC or THC (**12**) and Δ^9-THCA (**13**)[46]
Waters 900 HPLC system	Ace 5 Phenyl column (250 mm x 4.6 mm; particle size: 5 μm) and Agilent Zorbax RX-C_{18} column (250 mm x 4.6 mm; particle size: 5 μm) connected to a Nova-Pak® C_8 Guard Column 3.9 mm x 20 mm	Gradient elution with water with 0.1% TFA (solvent A), and water-ACN mixture (65:35) containing 0.1% TFA (solvent B), and ACN (solvent C) were used for the fingerprint (80 min including pre and washing phase). Solvent B and C were used for the cannabinoid profile (55 min including pre and washing phase).	UV-PDA set at 214 nm for detection and quantification	D	CBD (**3**), CBDA (**4**), CBG (**6**), CBGA (**7**), CBN (**8**), THC (**12**) and THCA (**13**)[47]
Agilent 1200	Supelco Ascentis C_{18} column (100 mm x 2.1	Mobile phase: water (A) and ACN (B) both containing 0.1%	ESI-MS/MS using a Triple Quadrupole	E	CBD (**3**), CBN (**8**) and THC (**12**)[48]

	mm; particle size: 3 μm) coupled with a Supelco Discovery HS C_{18} guard column (20 mm x 2.1 mm; particle size: 3 μm)	HCOOH. Gradient elution with 0-8 min: 40-90% B in A, 8-12 min: 90% B, 12-13 min: 90-40% B, 13-20 min: 40% B. Flow rate: 0.3 mL/min; injection volume: 20 μL	Tandem Mass Spectrometer (Agilent, USA), both in positive and negative ion modes.		
Agilent 1200 HPLC	Poroshell 120 SB-C_{18} column (100 mm x 2.1 mm; particle size: 2.7 μm, Agilent, Milano, Italy)	Isocratic elution with water (A) and ACN (B), both containing 0.1% HCOOH at a flow rate of 0.5 mL/min. Total run time: 10 min; column temp: 25°C; injection volume: 5 μL.	UV-DAD set at 228 nm, and scanned for 190-500 nm, and ESI-MS/MS in positive ion mode on a 6540 quadrupole time of flight (QTOF) mass analyzer with an electrospray ionization (ESI) source	F	CBD (**3**), CBDA (**4**), CBN (**8**), THC (**12**) and THCA (**13**)[49]
UPLC (or UHPLC) methods					
Shimadzu Nexera X2 LC system	Acquity UPLC HSS T3 column, (30 mm x 2.1 mm; particle size: 1.8 μm; Waters, Milan, Italy)	Gradient elution mobile phases A (ACN : water 75:25 + 0.05% formic acid) and B (isopropanol: ACN 80:20 + 0.05%); flow rate: at 0.4 mL/min; column temp. 30°C; injection volume 5 μL. The initial condition 0% B, increased to 100% over 1.5 min, held for 1 min, re-	ESI-MS/MS on a LCMS-8050 tandem mass spectrometer	B	CBD (**3**), CBDA (**4**), CBN (**8**), THC (**12**) and THCA (**13**)[50]

		equilibrated to initial conditions for 1 min (total run time 3.5 min).			
Agilent 1290 UPLC	Acquity UPLC HSS T3 column, (30 mm x 2.1 mm; particle size: 1.8 μm; Waters, Milan, Italy)	A gradient elution with water and ACN, both containing 0.1% HCOOH	ESI-MS/MS		CBD (**3**), CBDA (**4**), CBN (**8**), THC (**12**) and THCA (**13**)[51]
Waters Acquity UPLC-I Class	Waters Acquity UPLC HSS T3 column (100 mm x 2.1 mm; particle size: 1.8 μm)	0.1% HCOOH in both water (A) and MeOH (B). Multi-step gradient elution: 0-8 min 10-60% B, 8-10 min 60-95% B, 10-12 min 95% B, followed by equilibration to initial condition for 2 min; flow rate: 0.5 mL/min; column temp: 55°C.	ESI-QTOF and ESI-travelling wave ion mobility (TWIM)-MS in positive and negative ion modes	C	Seven constitutional isomers of Δ^9-THC (**12**) and four isomers of Δ^9-THCA (**13**)[52]
			ESI-TWIM (travelling wave ion mobility)-MS in positive ion mode		CBN (**8**), THC (**12**) and THCA (**13**)[53]
Agilent 1200 RRLC	Phenomenex Kinetex C_{18} column (100 mm x 3.0 mm; particle size: 1.7 μm)	Mobile phase A: 10 mM ammonium formate, pH 3.6, and mobile phase B: ACN. Gradient elution with 0-8 min, 52-66% B; 8-8.5 min, 66-70% B; 8.5-13 min, 70-80% B; 13-15 min, 80% B, and column equilibration time: 7 min. Flow rate 0.6 mL/min; injection volume: 5 μL.	UV-PDA set at 220 nm for detection		CBC (**1**), CBD (**3**), CBDA (**4**), CBG (**6**), CBN (**8**), THC (**12**), THCA (**13**), and THCV (**14**)[54]

Waters Acquity UPLC-I Class	Waters Cortec UPLC C_{18} column (100 mm x 2.1 mm; particle size: 1.6 μm)	A gradient elution with 0.05% HCOOH in both water (A) and ACN (B). Flow rate: 0.25 mL/min.	UV-PDA-MS using a single quadrupole MS analyser		CBC (1), CBL (**2**), CBD (**3**), CBDA (**4**), CBG (**6**), CBGA (**7**), CBN (**8**), Δ^8-THC or THC (**12**), Δ^9-THCA or THCA (**13**) and THCV (**14**)[36]
Waters Acquity UPLC H-class	Waters UPLC BEH C_{18} column (50 mm x 2.1 mm; particle size: 1.7 μm)	1% HCOOH both in water and MeOH. Isocratic elution at a flow rate of 0.2 mL/min.	UV-PDA set at 220 nm for detection, and ESI-MS		CBD (**3**), CBN (**8**) and THC (**12**)[55]
Agilent 1290 UPLC	Acquity UPLC HSS T3 column, (30 mm x 2.1 mm; particle size: 1.8 μm; Waters, Milan, Italy)	A gradient elution with water and ACN, both containing 0.1% HCOOH	ESI-MS/MS	G	CBD (**3**), CBDA (**4**), CBN (**8**), THC (**12**) and THCA (**13**)[51]

A = Cannabidiol oils; B = *Cannabis* olive oil; C = *Cannabis* consumers products, *e.g.,* hashish and marijuana; D = *Cannabis* tinctures; E = Hemp nut products; F = Medicinal *Cannabis* products; G = *Cannabis* tea

TABLE 3 HPLC and UPLC (or UHPLC) methods for the analysis of naturally occurring cannabinoids in human blood samples

Instrumentation	Column	Mobile phase	Detection	Matrices/source	Cannabinoids analyzed/detected
HPLC methods					
Shimadzu UFLCxr System	Ultra Biphenyl column (100 mm x 2.1 mm; particle size: 5 μm) fitted with an Ultra II Biphenyl guard cartridge (10 mm x 2.0 mm)	Gradient elution: solvent A (10 mM ammonium acetate in water adjusted to pH 6.15 with HCOOH and solvent B (15% MeOH in ACN). The initial gradient conditions were 30% B, held for 30 sec, then increased to 90% B at 6.0 min, which (90% B) was maintained for 7.5 min, at which time the column was re-equilibrated to 30% B over 0.75 min and held for 1.75 min. Column temp: 40°C; injection volume: 25 μL; flow rate: 0.4 mL/min.	ESI-MS/MS on an AB Sciex 3200 Qtrap triple quadrupole mass spectrometer with a TurboV ESI source	A	CBD (**3**), CBN (**8**), 11-OH-THC (**9**), THC-COOH (**10**), THC (**12**), THC-glucuronide and THC-COOH-glucuronide[56]
Shimadzu Prominance XR LC System	Raptor Biphenyl column (50 mm x 2.1 mm; particle size: 2.7 μm)	Mobile phase comprised water (A) and MeOH (B), both containing 0.1% HCOOH.	ESI-MS/MS using a Sciex 3200QTrap tandem MS		11-OH-THC (**9**), THC (**12**) and THCA[57]

		Gradient elution: 60% B increased to 100% B at 6.5 min and held at 100% until 8 min. Re-equilibration back to the starting conditions of 60% B at 8.01 min and held at 60% until 9 min. Column temp: 40°C; injection volume 20 µL			
Shimadzu LC-20AD XR HPLC System	Phenomenex Kinetex C_{18} column (50 mm x 2.1 mm; particle size: 2.6 m) combined with a SecurityGuard C_{18} guard column (4 mm x 2 mm)	Gradient elution with 10 mM ammonium acetate in water (A) and 15% MeOH in ACN (B). Mobile phase B concentration was initially 30% for 0.5 min, increased to 50% over 0.5 min, to 70.7% over 7.33 min, and to 100% over 0.67 min, then held for 4.5 min before returning to 30% B over 0.1 min and held for 2.4 min (total run time 16 min). Flow rate: 0.5 mL/min until 9.00 min, increased to 0.75 mL/min over 0.10 min and held for 4.1 min, and 0.5	ESI-MS/MS on a Sciex 5500 QTrap® mass spectrometer with a Turbo VT ion source (Framingham, MA, USA).		CBD (**3**), CBG (**6**), CBN (**8**), 11-OH-THC (**9**), THC (**12**), THCA (**13**), THCV (**14**), THCVA (**15**), THC-glucuronide and THCA-glucuronide[58]

		mL/min over 0.1 min and held for 2.7 min. Column temp: 40◦C; injection volume 20 μL			
Dionex HPLC system	Phenomenex Luna C_8 column (100 mm x 2.0 mm; particle size: 3 μm)	A step gradient elution with water and ACN, both containing 0.1% HCOOH.	ESI-MS/MS in positive ion mode on a triple-stage quadrupole mass spectrometer with linear ion trap capability	B	11-OH-THC (**9**), THC-COOH (**10**) and THC (**12**)[59]
Waters Alliance e2695 System	Phenomenex Kinetex C_8 column (50 mm x 2.1 mm; particle size: 2.6 μm)	Mobile phase: 0.1% HCOOH in MeOH (B) and 0.1% HCOOH in water (A) The gradient programme: started with 50% B in A then ramped up linearly to 95% of B over 5 min; this ratio was maintained for 5 min, then ramped down linearly to 50% B over 1 min and held for 2 min. Injection volume: 10 μL; flow rate: 0.5 mL/min	ESI-MS/MS on a Waters Micromass Quatro Micro Triple Quadrupole MS	C	11-OH-THC (**9**), THC-COOH (**10**) and THC (**12**)[60]

Waters Alliance 2795 HPLC	Waters Atlantis C_{18} column (150 mm x 2.1 mm; particle size: 3 μm)	Gradient elution with water (A) and ACN (B), both with 0.2% of HCOOH. The flow rate was 0.3 mL/min and the gradient was as follows: 0.0–1.0 min: linear from 70 to 100% B; 1.0–6.0 min: 100% B; 6.0–6.1 min: linear from 100 to 70% B; 6.1–8 min: 70% B. For on-line dabsylation, exactly the same gradient was used but the last part at 70% B was prolonged until 13 min.	ESI-MS/MS in both positive and negative ion modes	D	CBD (**3**), CBN (**8**), 11-OH-THC (**9**), THC-COOH (**10**) and THC (**12**)[61]
Shimadzu (LC-20AD) HPLC	Phenomenex Luna C_{18} column (150 mm x 2 mm; particle size: 5 μm)	The mobile phase A: 10 mM ammonium acetate buffer with 0.1% acetic acid in 95% aqueous MeOH, and mobile phase B: 10 mM ammonium acetate buffer with 0.1% acetic acid in 97% aqueous MeOH. Step gradient: starting with 20% B, ramping to 100% B from 0.0 to 8.0 min, holding 100% B from 8.0 to 9.0 min,	ESI-MS/MS using an API 4000 QTrap and an API5500 tandem mass spectrometers	E	THC-COOH (**10**)[62]

		reducing to 20% B from 9.0 to 9.5 min, holding 20% B from 9.5 to 15.0 min, using a flow rate of 0.2 mL/min, injection volume of 20 μL, and column temperature at 25°C.			
Agilent 1100	Kinetex EVO C_{18} column	A 65%-95% gradient of MeOH and 0.2% NH_4OH/water at a flow rate of 0.4 mL/min	UV detector set at 220 nm	F	CBC (**1**), CBD (**3**), CBDA (**4**), CBV (**5**), CBG (**6**), CBGA (**7**), CBN (**8**), 11-OH-THC (**9**), THC-COOH (**10**), THC (**12**), THCA (**13**), THCV (**14**), and 11-nor-9-carboxy-Δ-tetrahydrocannabinol glucuronide (THC-COOH-glu)[63]
Agilent 1200 HOLC	Reversed-phase C_{18} column	Water-ACN gradient	ESI-MS/MS		11-OH-THC (**9**), THC-COOH (**10**) and THC (**12**)[8]
Agilent 1200	Poroshell Eclipse C_{18} column (40 mm x 4.6 mm; particle size: 2.7 μm; Agilent Technologies)	Mobile phase B: 20% isopropanol, 20% MeOH, and 60% ACN, and mobile phase A: water containing 0.1% HCOOH. Gradient elution with a flow rate of 0.75 mL/minute and 60% of solvent B for the first	APCI (atmospheric pressure chemical ionization)-MS/MS in positive ion mode		CBC (**1**), CBD (**3**), CBDV (**5**), CBG (**6**), CBN (**8**), 11-OH-THC (**9**), THC-COOH (**10**), THC (**12**), THCV (**14**), THCV-COOH, **15**) and THC-C-glucuronide[64]

		minute. Within the following 3 min, the flow rate was increased to 1 mL/min and 95% solvent B. From 4 to 6 min, the B was increased to 100%. At minutes 6.2, the system returned to starting conditions for 1.8 min to equilibrate before the following injection. Column temp: 60^{o}C			
Agilent 1260 HPLC	Phenomenex Kinetex column (150 mm x 3 mm; particle size: 2.6 μm) coupled with a Phenomenex guard column (0.5 μm x 0.1 mm)	Both solvents A (water) and B (MeOH) contained 0.1% HCOOH. Gradient elution: 60-80% B at 0-1 min, held at 80% B for 7 min, increased to 95% B over the next 2 min, held at 95% B for 10 min, decreased to 60% B over the next 1 min and held at 60% B for 7 prior to the next injection. Flow rate: 0.3 mL/min; column temp. 30°C; injection volume: 10 μL.	APCI-MS/MS on a triple quadrupole mass spectrometer (Agilent 6430) in positive ion mode		CBD (**3**), CBG (**6**), CBN (**8**), 11-OH-THC (**9**), THC-COOH (**10**), THC (**12**) and THCV (**14**)[65]

Shimadzu (LC-20AD) HPLC	Phenomenex Luna C_{18} column (150 mm x 2 mm; particle size: 5 μm)	The mobile phase A: 10 mM ammonium acetate buffer with 0.1% acetic acid in 95% aqueous MeOH, and mobile phase B: 10 mM ammonium acetate buffer with 0.1% acetic acid in 97% aqueous MeOH. Step gradient: starting with 20% B, ramping to 100% B from 0.0 to 8.0 min, holding 100% B from 8.0 to 9.0 min, reducing to 20% B from 9.0 to 9.5 min, holding 20% B from 9.5 to 15.0 min, using a flow rate of 0.2 mL/min, injection volume of 20 μL, and column temp. 25°C.	ESI-MS/MS using a API 4000 QTrap and a API5500 tandem mass spectrometers	G	THC-COOH (**10**)[62]
Agilent 1260 HPLC	Phenomenex Luna C_{18} column (150 mm x 2.0 mm; particle size: 5 μm)	Mobile phase comprised A (H_2O/MeOH, 95:5) and B (H_2O/MeOH, 3:97), both with 10 mM ammonium acetate and 0.1 % CH_3COOH.	ESI-MS/MS		11-OH-THC (**9**), THC-COOH (**10**) and THC (**12**)[66]

UPLC (or UHPLC) methods					
Waters Acquity UPLC	ACE Excel C_{18}-PFP column (50 mm x 2.1 mm; particle size: 2 μm)	Mobile phase (A) was 0.2% HCOOH:MeOH (95:5) and (B) was 0.2% HCOOH:MeOH (5:95). Flow rate: 0.5 mL/min. Gradient elution: initial 40% A held for 1.0 min, followed by a gradient change to 15% A for 0.5 min and held for 2 min. A gradient change to 100% B for 6 sec, and held for 1 min, followed by a final gradient change to 40% A for 0.6 sec, held for 53.4 sec, with a final run time of 5.5 min	ESI-MS/MS in positive ion mode	A	CBD (**3**), CBN (**8**), THC (**12**), 11-OH-THC (**9**), THC-COOH, **10**) and THC-COOH-glucuronide[67]
Waters Acquity UPLC system	Acquity UPLC HSS C_{18} column (100 mm x 2.1 mm; particle size: 1.8 μm)	The mobile phases A and B consisted of 0.05% HCOOH in water, and 0.05% HCOOH in MeOH/ACN (1:1). Gradient elution: 68-100% B in A over 0-5 min, held for 1 min, returned to 68% B in 0.1 min, and equilibrated for 2.9 min. Injection volume: 10 μL; flow rate:	ESI-MS/MS on a Waters Xevo TQ-Stripple-quadrupole instrument with an ESI ion source		CBD (**3**), CBN (**8**), 11-OH-THC (**9**), THC-COOH (**10**) and THC (**12**)[68]

		0.4 mL/min; column temp: 45°C			
Waters Acquity UPLC System	Waters Acquity UPLC HSS T3 C_{18} column (50 mm x 2.1 mm; particle size: 1.8 μm)	Gradient elution with MeOH/2 mM ammonium formate (formic acid 0.1%) (95:5, (A) and 2 mM ammonium formate (formic acid 0.1%)/MeOH (95:5) (B). The gradient programme: 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 to the initial conditions in 0.05 min and equilibration time for 1.95 min. Column temp: 45°C; injection volume 10 μL; flow rate: 0.4 mL/min	ESI-MS/MS in positive ion mode on a TQD detector (triple quadrupole mass spectrometer, Waters, Milford, MA, USA) equipped with an electrospray ionization source		11-OH-THC (**9**) THC-COOH (**10**) and THC (**12**)[69]
Waters Acquity UPLC	Waters Acquity HSS T3 column (100 mm x 2.1 mm; particle size: 1.8 μm) with a VanGuard T3 (2.1 mm x 5 mm;	Isocratic elution with 18:82:0.02 water: MeOH: HCOOH over 8.5 min. Column temperature: 40°C and injection volume: 20 μL.	ESI-MS/MS	F	CBD (**3**), CBDA (**4**), CBN (**8**), 11-OH-THC (**9**), THC (**12**), THCA (**13**) and THCV (**14**)[70]

	particle size 1.8 μm) pre-column.				
Waters Acquity UHPLC	Acquity BEH C_{18} column (50 mm x 2.1 mm; particle size: 1.7 μm) linked to an Acquity UHPLC BEH C_{18} VanGuard pre-column (2.1 mm × 5 mm)	Both A (water) and B (MeOH) contained 0.1% HCOOH. A linear gradient elution using 0-3.5 min: 75-95% B, 3.5-4.5 min: held at 95% B 4.5-5.5 min: 95-75% B and maintained at 75% for 0.5 min before the next injection. Total run time 6 min; flow rate: 0.4 mL/min; column temp: 45°C; injection volume: 10 μL.	ESI-MS/MS using a Xevo TQ MS detector (Waters)		CBD (**3**), 11-OH-THC (**9**), THC-COOH (**10**) and THC (**12**)[71]

A = Whole blood; B = Peripheral blood; C = Dried spots of blood; D = Micro volume blood samples; E = Post-mortem blood; F = Plasma; G = Serum

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

Instrumentation	Column	Mobile phase	Detection	Sources	Cannabinoids analyzed/detected
HPLC methods					
Agilent 1200	Reversed-phase C_{18} column	Water-ACN gradient	ESI-MS/MS in positive ion mode	A	CBN (**8**) and THC (**12**)[72]
Waters 2695 HPLC	SunFire C_{18} column (150 mm x 3.0 mm; particle size: 3.5 μm)	Isocratic elution with 89% ACN in water containing 0.1% HCOOH. Flow rate: 0.5 mL/min; injection volume: 20 μL; column temp: 30°C	PDA set at 235 nm (scanned 200-400 nm)	B	CBD (**3**), CBN (**8**), THC-COOH (**10**) and THC (**12**)[73]
Waters Alliance 2795 HPLC	SunFire C_{18} column (20 mm x 2.1 mm; particle size: 2.5 μm)	Formic acid 0.1% (A) and ACN (B) were used as mobile phase at a flow rate of 0.5 mL/min using a gradient: 40% B at 0 min, increased to 65% over 0.8 min, increased to 100% B over 2 min, and returned to initial conditions at 2.1 min, and equilibrated until 5 min. Column temp: 26°C	ESI-MS/MS on a Quattro Micro API triple quadrupole MS detector in positive ion mode		
	SunFire C18 column (20 mm x 2.1 mm; particle size: 2.5 μm)	Isocratic elution with 0.1% aqueous HCOOH and ACN, with a total run time of 5 min	ESI-MS/MS		THC-COOH (**10**) and THC (**12**)[74]

Shimadzu HPLC	United Chemical Technologies Selectra PFPP column (100 mm x 2.1 mm; particle size: 3 μm) combined with a guard column (10 mm × 2.1 mm, 3 μm particle size)	Mobile phase A (water) and B (ACN) both had 0.15% HCOOH. Gradient elution: 0-8.5 min – 70-78.5% B, 8.5-8.7 min – 78.5-98% B, held at 98% B for 3 min, re-equilibrated to 70% B in 0.2 min and held there for 2.1 min. Flow rate: 0.5 mL/min; column temp: 40°C	APCI-ESI-MS/MS on an ABSciex 6500 QTRAPW triple quadrupole/linear ion trap mass spectrometer with an IonDrive™ Turbo V source, using a positive ion mode		CBD (**3**) and CBG (**6**), 11-OH-THC (**9**), THC-COOH (**10**), THC (**12**) and THCV, (**14**)[6]
Perkin Elmer Series 200 Micro-LC System	Phenomenex Kinetex C_{18}-XB column (100 mm x 2.1 mm; particle size: 2.6 μm) coupled with a Phenomenex security Guard Ultra Cartridge (packed with C_{18} particles)	Mobile phase contained water (A) and MeOH (B) both having 1.25 mM ammonium acetate. Gradient elution was as follows: phase B increased from 65 to 80% in 2 min, then up to 85% in 2.5 min and in the following 0.3 min brought to 100%, and held for 3.2 min before switching back to the initial 65% in 2 min. Flow rate: 0.35 mL/min	ESI-MS/MS in positive ion mode on an API 4000 Qtrap® from PE-Sciex		CBD (**3**), CBN (**8**), 11-OH-THC (**9**), THC-COOH (**10**) and THC (**12**)[13]
Thermo Scientific Dionex 3000 RSLCnano System	Hypersil Gold C_{18} analytical column (50 mm x 0.5 mm; particle size: 3 μm)	Gradient elution with mobile phase A (10 mM ammonium acetate adjusted to pH 6 with 1% HCOOH) and B (15%	ESI-MS/MS in positive ion mode on a Thermo Scientific Q Exactive Mass spectrometer		CBD (**3**), CBN (**8**), THC-COOH (**10**) and THC (**12**)[75]

		MeOH in ACN) at a 30 μL/min flow rate at 35 ◦C. The initial composition (30% B) was maintained for 0.5 min, B was increased from 30% to 90% over 5.5 min, held at 90% for 1.5 min, and returned to initial conditions over 0.5 min. A 2 min equilibration followed, yielding a total run time of 10 min.			
Shimadzu LC-20AD System	Phenomenex Kinetex C_{18} column (50 mm x 2.1 mm; particle size: 2.6 μm)	Gradient elution: solvent A 0.01 % CH_3COOH in water and solvent B 0.01 % CH_3COOH in MeOH at a flow rate of 0.5 mL/min. The initial gradient conditions were 20 % B, held for 1 min, then increased to 60 % B at 1.5 min and increased to 98 % B over 2 min, held there for 3.5 min, at which time the column was re-equilibrated to 20 % B over 0.1 min and held for 1.9 min (total	ESI-MS/MS in negative ion mode on an an ABSciex 5500 QTrap® triple quadrupole/ linear ion trap mass spectrometer with a TurboIonSpray source		THC-COOH (**10**)[76]

		runtime, 9 min). Flow rate increased to 1.0 mL/min at 3.7 to 7.2 min to increase column rinsing efficiency.			
Dionex UltiMate 3000 Rapid Separation LC System	Pheneomenex Kinetex C_{18} column (150 mm x 2.1 mm; particle size: 2.6 μm)	Gradient elution was performed with solvent A (ammonium formate buffer 5 mM, pH 6.8) and solvent B (ACN) at a constant flow rate of 0.4 mL/min. The initial gradient conditions were 30 % B, held for 30 s, and then linearly increased to 90 % B over 7 min. The final B concentration was held for 2 min. Solvent B was reduced from 90 to 30 % over 30 s and held for 1.5 min. Column temp. 40°C; injection volume: 10 μL	ESI-MS/MS on an AB Sciex API 5000 triple quadrupole MS interfaced with a Turbo VTM source with a TurboIonSpray_ probe (ESI).		CBD (**3**), CBN (**8**), 11-OH-THC (**9**), THC-COOH (**10**), THC (**12**), THCA (**13**), THC-COOH-glucuronide and THC-glucuronide[77]
Shimadzu LV-20AD System	Phenomenex Luna C_{18} column (150 mm x 2.0 mm particle size: 5 μm)	Gradient elution with water with 5 mmol/L ammonium formate (solvent A), and MeOH with 20% of ACN and 5 mmol/L ammonium formate (solvent B).	ESI-MS/MS on a mass spectrometer Applied Biosystems/MDS Sciex 4000 QTRAP MS system		CBN (**8**) and THC (**12**)[78]

		Gradient programme: 32.5% B during 2 min linearly increased to 75% in 6.5 min, 80% in 7.4 min, 95% in 13-13.2 min, 100% in 17 min, decreased to original conditions for 5 min. Column temp: 40°C; injection volume: 10 μL; flow rate: 0.2 mL/min			
Agilent 1290 HPLC system	Agilent Zorbax XDB-C_{18} analytical column (100 mm x 2.1 mm; core shell particle size: 2.6 μm)	Mobile phase: A contained 10% ACN in water, and B 90% ACN in water, both had 5 mM ammonium acetate. A linear gradient was set to an initial composition of 20 % B at a flow rate of 0.4 mL/min. The composition was increased to 70% (t = 5 min) before a final concentration of 100 % B was reached at 0/8 mL/min (t = 8 min). Column temp: 60°C; injection volume: 20 μL.	ESI-MS^n on a API 6500 Q-trap mass spectrometer (AB Sciex, Framingham, MA, USA), equipped with a Turbo-Ion-Spray (ESI) source.		CBD (**3**), CBN (**8**), 11-OH-THC (**9**), THC-COOH (**10**) and THC (**12**) as picolinates[79]
UPLC (or UHPLC) methods					
Waters Acquity UPLC	Acquity UPLC BEH C_{18} column (75 mm x 2.1 mm; particle size: 1.7 μm)	0.1% HCOOH in both water (A) and ACN (B). Isocratic elution: 0-0.25 min with 10% B, gradient elution: 0.25-2.30	ESI-MS/MS on a triple quadruple MS in positive ion mode	B	CBD (**3**) and THC (**12**)[80]

		min 10-30% B, 2.30-2.60 min from 30-93% B, isocratic elution: 2.60-3.50 min 93% B, gradient elution: 3.50-3.60 min 93-10% B, isocratic elution: 3.60-6.00 min 10% B; flow rate: 0.35 mL/min; column temperature 50°C; injection volume: 2 μL.			
Sciex (R) API 4500 Q-Trap LC-MS/MS system	C_{18} analytical column	Water-ACN gradient	ESI-MS/MS		THC (**12**)[81]
Waters Acquity I-class UPLC	Waters Aquity UPLC BEH C_{18} column (50 mm x 2.1 mm; particle size: 1.7 μm; Waters Corporation, USA) coupled with a Waters Acquity UPLC BEH C_{18} VanGuard pre-column (5 mm x 2.1 mm; particle size: 1.7 μm)	0.1% HCOOH in both water (A) and ACN (B). flow rate: 0.4 mL/min; column temperature 40°C; injection volume: 10 μL. Isocratic elution: 0-30 sec 50% B, gradient elution: 30 sec-3.5 min 50-90%% B. The final mobile phase B concentration was maintained for 15s, before returning to initial conditions and holding for 45s.	ESI-MS/MS on a Waters TQ-S-micro quadrupole MS in negative ion mode		CBD (**3**), CBG (**6**), CBN (**8**), 11-OH-THC (**9**), THC-COOH (**10**), THC-COOH-glucuronide, THC-glucuronide, THC (**12**), THCA (**13**) and THCV (**14**)[82]

Shimadzu UPLC	Phenomenex Kinetex C_{18} (100 mm x 2.1 mm; particle size: 2.6 μm)	Gradient elution: 5.0 mM of ammonium formate with 0.05% formic acid (solvent A), and 100% ACN (solvent B). Flow rate at 0.4 mL/min and column temp: 40 °C.	ESI-MS/MS using both positive and negative ion modes, on a Sciex triple quadrupole 6500 with a TurboIonSpray source	C	CBD (**3**), CBN (**8**) and THC (**12**)[83]

A = Human breath sample; B = Human oral fluid; C = Human breast milk

TABLE 5 HPLC and UPLC (or UHPLC) methods for the analysis of naturally occurring cannabinoids in human hair samples

Instrumentation	Column	Mobile phase	Detection	Sources	Cannabinoids analyzed/detected
HPLC methods					
Thermo/Dionex UltiMate 3000 HPLC	Thermo Acclaim RSLC 120 C_{18} column (100 mm x 2.1 mm; particle size: 2.2 μm)	Mobile phase comprised water (A) with 5 mM ammonium formate and 0.1% HCOOH, mobile phase B was MeOH/ACN 1:1 with 0.1% of HCOOH. 100% A for 1 min, from 0% to 10% B in 0.1 min, linear gradient to 15% B in 4 min, linear gradient to 50% B in 1.8 min, to 70% B in 1.7, to 80% B in 1.1 min, to 100% in 1 min held for 3.5 min. Column temp. 40°C; injection volume: 10 μL; flow rate: 0.4 mL/min; total rune time: 14.5 min.	Thermo single-stage Orbitrap (Exactive) MS system, interfaced with an HESI source	A	THC (**12**)[84]
	Phenomenex Kinetex C_{18} column (100 mm x 2.1 mm; particle size: 2.6 μm) protected by a Phenomenex security Guard C_{18} Ultra Cartridge	Mobile phase A (water) and B (ACN) both contained 0.1% HCOOH. Gradient: 60-75% phase B in 2.4 min, 75-90% B in 2.4-3.6 min and in 3.6-4.0 min 90-100% B, which was held for 1.2 min and then switched back to the initial. Flow rate: 0.5 mL/min	ESI-HRMS/MS		CBD (**3**), CBN (**8**), THC-COOH (**10**) and THC (**12**)[85]
Shimadzu LC 20AB system	Discovery HS C_{18} column (250 mm x 4.6	Mobile phase: 0.1% HCOOH both in water (A) and ACN (B). Starting at 5%	APCI-ESI-MS in positive ion mode		CBD (**3**), CBN (**8**) and THC (**12**)[86]

	mm; particle size: 5 μm)	of solvent B (0.0–2.0 min), 70% of B (2.1-30.0 min) and finally 5% of B (30.1–36.0 min). Column temp. 30°C; flow rate: 0.6 mL/min; injection volume: 10 μL			
Shimadzu LC 20AD system	Waters Atlantis T3 column (150 mm x 2.1 mm; particle size: 3 μm)	Gradient elution with solvent A (0.1% HCOOH in water), and solvent B (a mixture of MeOH with 5% A), using the programme: 0-1 min, 30% B; 1-7 min, increase from 30 to 90% B; 7-14 min, 90% B; 14-14.3 min decreased from 90 to 30% B; 14.3-17 min, column equilibration with 30% B. Flow rate: 0.3 mL/min; column temp. 40°C; injection volume: 20 μL.	ESI-MS/MS in negative ion mode on a hybrid API 5500 QTRAP (Quadrupole/ Quadrupole/Ion Trap) mass spectrometer (AB Sciex, Courtaboeuf, France)		CBD (**3**), CBN (**8**), THC-COOH (**10**) and THC (**12**)[87]
Shimadzu Prominence Series 20A HPLC	Phenomenex Luna C_{18} column (150 mm x 2 mm; particle size: 5 μm) equipped with a Phenomenex Luna C_{18} guard column (4 mm x 2.0 mm)	Gradient elution with 0.1% HCOOH in water (A) and 0.1% HCOOH in ACN (B) using the programme 0-1 min 20% B, 1-8 min 20-95% B, held there for 4 min, 12-13 min 95-30% B, held there at 30% B for 2.5 min for re-equilibration. The flow rate was increased after 9.5 min from 0.6 mL to 0.8 mL per min to speed up the run. Column temp. 50 °C. Overall run time was 15.5 min.	ESI-MS/MS in positive ion mode on a QTrap 4000 triple quadrupole linear ion trap MS from AB Sciex		CBD (**3**), CBN (**8**), THC (**12**) and THCA (**13**)[88]

Agilent 1200 HPLC	Reversed-phase XDB-C_{18} analytical column (50 mmx 4.6 mm; particle size: 1.8 μm)	Mobile phase comprised water and ACN, both containing 0.1% HCOOH. Gradient elution: 0-3 min isocratic 10% B, 3-15 min 10-100% B, held there for 5 min. Injection volume: 20 μL; flow rate: 0.5 mL/min.	ESI-MS/MS in positive ion mode		CBD (**3**) and THC (**12**)[89]
Agilent 1290 HPLC system	Agilent Zorbax XDB-C_{18} analytical column (100 mm x 2.1 mm; core shell particle size: 2.6 μm)	Mobile phase: A contained 10% ACN in water, and B 90% ACN in water, both had 5 mM ammonium acetate. A linear gradient was set to an initial composition of 20 % B at a flow rate of 0.4 mL/min. The composition was increased to 70% (t = 5 min) before a final concentration of 100 % B was reached at 0/8 mL/min (t = 8 min). Column temp: 60°C; injection volume: 20 μL.	ESI-MS^n on a API 6500 Q-trap mass spectrometer (AB Sciex, Framingham, MA, USA), equipped with a Turbo-Ion-Spray (ESI) source.		CBD (**3**), CBN (**8**), 11-OH-THC (**9**), THC-COOH (**10**) and THC (**12**) as picolinates[79]
Shimadzu Prominence HPLC system	Phenomenex Luna C_{18} column (150 mm x 2 mm; particle size: 5 μm) with a Phenomenex guard column (4 mm x 2 mm)	Mobile phase A was 0.1% HCOOH in water and mobile phase B 0.1% HCOOH in ACN. Gradient elution with 20% B for 1 min, increased to 95% B in 7 min and held at 95% for 4 min. Starting conditions were restored within 1 min and the system was left to re-equilibrate at 20% B for 2.5 min prior to the next	ESI-MS/MS on a QTRAP 4000 triple quadrupole linear ion trap mass spectrometer fitted with a TurboIon-Spray interface	B	CBN (**8**), THC (**12**) and THCA (**13**)[90]

		injection. Flow rate: 0.6 mL/min for the first 9.5 min, increased to 0.8 mL/min for 3.5 min and afterwards reduced to 0.6 mL/min for the rest of the run. The column temp: 50 °C; injection volume: 20 µL.			
UPLC (or UHPLC) methods					
Agilent 1260 UPLC System	Agilent Zorbax Eclipse plus C_{18} column (100 mm x 2.1 mm; particle size: 1.8 µm)	Mobile phase comprised solvent A (10 mM ammonium formate/0.02 M HCOOH in water) and solvent B (0.02 HCOOH in ACN). Linear gradient elution: 90% solvent A for 0.5 min and decreasing to 50% solvent A in 3 m, decreasing further to 5% solvent A and 95% solvent B, and held from 4 to 6 min and then returned to 90% of solvent A until 7 min, and stabilised until 8 min before next injection. Column temp. 65^{o}C.	ESI-MS/MS on a 6430 triple quadrupole mass spectrometer	A	11-OH-THC (**9**) and THC-COOH (**10**), THC (**12**)[91]

A = Adult human hair; B = Human children hair

TABLE 6 HPLC and UPLC (or UHPLC) methods for the analysis of naturally occurring cannabinoids in human urine samples

Instrumentation	Column	Mobile phase	Detection	Cannabinoids analyzed/detected
HPLC methods				
Agilent 1200	Poroshell Eclipse C_{18} column (40 mm x 4.6 mm; particle size: 2.7 μm; Agilent Technologies)	Mobile phase B: 20% isopropanol, 20% MeOH, and 60% ACN, and mobile phase A: water containing 0.1% HCOOH. Gradient elution with a flow rate of 0.75 mL/minute and 60% of solvent B for the first minute. Within the following 3 min, the flow rate was increased to 1 mL/min and 95% solvent B. From 4 to 6 min, the B was increased to 100%. At minutes 6.2, the system returned to starting conditions for 1.8 min to equilibrate before the following injection. Column temp: 60°C	APCI (atmospheric pressure chemical ionization)-MS/MS in positive ion mode	CBC (**1**), CBD (**3**), CBDV (**5**), CBG (**6**), CBN (**8**), 11-OH-THC (**9**), THC-COOH (**10**), THC (**12**), THCV (**14**), THCV-COOH (**15**), and THC-C-glucuronide[64]
Agilent 1260 HPLC	Phenomenex Kinetex column (150 mm x 3 mm; particle size: 2.6 μm) coupled with a Phenomenex guard column (0.5 μm x 0.1 mm)	Both solvents A (water) and B (MeOH) contained 0.1% HCOOH. Gradient elution: 60-80% B at 0-1 min, held at 80% B for 7 min, increased to 95% B over the next 2 min, held at 95% B for 10 min, decreased to 60% B over the next	APCI-MS/MS on a triple quadrupole mass spectrometer (Agilent 6430) in positive ion mode	CBD (**3**), CBG (**6**), CBN (**8**), 11-OH-THC (**9**), THC-COOH (**10**), THC (**12**) and THCV (**14**)[65]

		1 min and held at 60% B for 7 prior to the next injection. Flow rate: 0.3 mL/min; column temp. 30°C; injection volume: 10 μL.		
	Reversed-phase silica C_{18} column (100 mm x 4.6 mm; particle size: 5 μm)	Gradient elution with water and ACN, both containing 0.1% HCOOH.	ESI-MS/MS	11-OH-THC (**9**), THC-COOH (**10**) and THC (**12**)[92]
Waters HPLC	Waters ODS-3 column (250 mm × 4.0 mm; particle size: 5 μm)	Isocratic elution with eluent A (1% *o*-phosphoric acid in water containing 4 mL *n*-hexyl amine whose pH was adjusted at 6.0 by dropwise addition of 4 M NaOH and/or *o*-phosphoric acid 1 M) and eluent B (ACN): 87:13 at a flow rate of 1 mL/min; column temp: 27°C; injection volume 10 μL; total run time: 15 min.	UV-PDA at 220 nm	CBD (**3**), CBN (**8**) and THC (**12**)[93]
Shimadzu 20AD LC system	Ultra Biphenyl column (100 mm x 2.1 mm; particle size: 5 μm) couples with a guard column (10 mm x 2.1 mm) of same packing material	Gradient elution with solvent A (10 mM ammonium acetate adjusted to pH 6.15 with formic acid) and solvent B (15% MeOH in ACN). The initial gradient conditions were 30% B, held for 30 sec, then increased to 90% B at 6.0 min, and held at 90% B for 7.5 min, at which	ESI-MS/MS in positive and negative ion modes	CBD (**3**), CBN (**8**), 11-OH-THC (**9**), THC-COOH (**10**), THC (**12**), THC-glucuronide and THC-COOH-glucuronide[94]

		time the column was re-equilibrated to 30% B over 0.75 min and held for 1.75 min. Column temp. 40°C; flow rate: 0.4 mL/min; injection volume: 10 μL.		
Shimadzu (LC-20AD) HPLC	Phenomenex Luna C_{18} column (150 mm x 2 mm; particle size: 5 μm)	The mobile phase A: 10 mM ammonium acetate buffer with 0.1% acetic acid in 95% aqueous MeOH, and mobile phase B: 10 mM ammonium acetate buffer with 0.1% acetic acid in 97% aqueous MeOH. Step gradient: starting with 20% B, ramping to 100% B from 0.0 to 8.0 min, holding 100% B from 8.0 to 9.0 min, reducing to 20% B from 9.0 to 9.5 min, holding 20% B from 9.5 to 15.0 min, using a flow rate of 0.2 mL/min, injection volume of 20 μL, and column temp. 25°C.	ESI-MS/MS using a API 4000 QTrap and a API5500 tandem mass spectrometers	THC-COOH (**10**)[62]
Zivak Tandem Gold LC-MS/MS System	Phenomenex Luna C_{18} column (50 mm x 3.0 mm; particle size: 3 μm) coupled with a Phenomenex guard column (4.0 mm x 2.0 mm; particle size: 3 μm)	Column temp: 45°C; flow rate 0.3 mL/min; injection volume: 10 □m. Isocratic elution with 80% ACN in water, both containing 0.1% HCOOH.	ESI-MS/MS in negative ion mode	THC-COOH (**10**)[95]

Agilent 1260 HPLC	Reversed-phase silica C_{18} analytical column (250 mm x 4.6 mm; particle size: 5 μm)	Gradient elution with water and ACN, both containing 0.1% HCOOH	ESI-MS/MS	THC-COOH (**10**) and THC-COOH-glucuronide[96]
Perkin Elmer HPLC	Phenomenex Kinetex C_{18}-XB column (50 mm x 2.1 mm; particle size: 2.6 μm) with a Phenomenex Security Guard Ultra Cartridge	The mobile phases were: (A) MeOH and (B) water both containing 1.25 mM ammonium acetate. Gradient elution: phase A increased from the initial 65% to 80% in 1 min, then up to 85% in 1.5 min and in the following 0.3 min brought to 100%. The latter was maintained for 1 min and then switched back to the initial 65% in 2 min. Flow rate: 0.75 mL/min	ES-MS/MS	CBD (**3**), CBN (**8**), 11-OH-THC (**9**)THC-COOH (**10**) and THC (**12**)[97]
Thermo Fisher Surveyor HPLC System	Pentafluorophenyl (Hypersil Gold PFP) analytical column (50 mm × 2.1 mm; particle size: 3 μm)	Gradient elution with solvent B (ACN) and solvent A (0.1% HCOOH, 1% ACN/10 mM ammonium acetate), using the programme: 0 min 10% B, 0-3 min 10% B, 3-10 min 10-90% B, 10-15 min 90% B, 15-15.1 min 90-10% B, and 16.1-20 min 10% B. Re-equilibration time was 5 min. Flow rate: 0.3 mL/min	ESI-MS/MS in positive ion mode on a LTQ-Orbitrap-MS	CBD (**3**), CBN (**8**) and THC (**12**)[98]

Varian 9012 HPLC	Zorbax C_8 column (250 mm x 4.6 mm; particle size: 3 μm)	Isocratic elution with water and ACN (13:87) at a flow rate 1.0 mL/min	UV set at 220 nm	CBD (**3**), CBN (**8**) and THC (**12**)[99]
Waters 2695 HPLC	SunFire C_{18} column (150 mm x 3.0 mm; particle size: 3.5 μm)	Isocratic elution with 89% ACN in water containing 0.1% HCOOH. Flow rate: 0.5 mL/min; injection volume: 20 μL; column temp: 30°C	UV-PDA set at 235 nm (scanned 200-400 nm)	CBD (**3**), CBN (**8**), THC-COOH (**10**) and THC (**12**)[73]
Waters Alliance 2795 HPLC	SunFire C_{18} column (20 mm x 2.1 mm; particle size: 2.5 μm)	Formic acid 0.1% (A) and ACN (B) were used as mobile phase at a flow rate of 0.5 mL/min using a gradient: 40% B at 0 min, increased to 65% over 0.8 min, increased to 100% B over 2 min, and returned to initial conditions at 2.1 min, and equilibrated until 5 min. Column temp: 26°C	ESI-MS/MS on a Quattro Micro API triple quadrupole MS detector in positive ion mode	
	SunFire C_{18} column (20 mm x 2.1 mm; particle size: 2.5 μm)	Isocratic elution with 0.1% aqueous HCOOH and ACN, with a total run time of 5 min	ESI-MS/MS	THC-COOH (**10**) and THC (**12**)[74]
UPLC (or UHPLC) methods				
Agilent 1290 Infinity UHPLC	Acquity UHPLC BEH C_{18} column (100 mm x 2.1 mm; particle size: 1.7 μm) coupled with a pre-column, Acquity UHPLC BEH C_{18}	Column temperature: 60°C; injection volume: 10 μm; flow rate: 0.6 mL. The mobile phase consisted of solvent A (0.1% HCOOH in ammonium formate (10 mM, pH 3.3) and solvent B (ACN). The	ESI-MS/MS	THC-COOH (**10**)[100]

	VanGuard (2.1 mm × 5 mm).	separation of the compounds was achieved by a linear gradient. The gradient conditions were as followed: 60% B; 0-0.2 min., 60% B–70%B; 0.2–1.7 min., 70% B–100% B; 1.7–1.9 min., 100% B; 1.9–3.4 min. (washing step) and 100% B–60% B; 3.4–3.5 min., 60% B; 3.5–4.9 min. (re-equilibrating).		
Dionex UltiMate 3000 UHPLC	Acquity UPLC BEH Phenyl column (100 mm x 2.1 mm; particle size: 1.7 μm) couple with a VanGuard pre-column (2.1 mm x 5 mm)	Both water (A) and ACN (B) contained 0.1% HCOOH. Gradient elution: B increased from 5% for the initial 0.6 min to 70% at 1.5 min, and to 95% at 5 min, and held at 95% B until 6.5 min. Thereafter, the column was re-equilibrated to 5% B for additional 2.5 min, resulting in a total run time of 9 min. Flow rate: 0.3 mL/min; column temp. 25°C.	ESI-MS/MS on a TSQ Quantiva with triple-stage quadrupole mass spectrometer	CBD (**3**), CBN (**8**), 11-OH-THC (**9**), THC-COOH (**10**), THC (**12**) and THC-COOH-glucuronide[101]
Shimadzu UHPLC System	Phenomenex Kinetex C_{18} column (100 mm x 2.1 mm; particle size: 2.6 μm)	The gradient programme contained 5.0 mM of ammonium formate with 0.05% HCOOH (solvent A) and ACN (solvent B). Flow rate: 0.4 mL/min; column temp. 40°C.	ESI-MS/MS in both positive and negative ionisation modes	11-OH-THC (**9**), THC-COOH (**10**) and THC (**12**)[102]

Shimadzu Nexera LC-30 UHPLC System	Phenomenex Kinetex C_{18} column (50 mmx 2.1 mm; particle size: 2.6 μm) with a guard column (2 mm x 2.1 mm).	Gradient elution with mobile phase A (10 mM ammonium acetate in water and B (15 % MeOH in ACN at a flow rate of 0.5 mL/min. The gradient programme was 30% B for 0.50 min, to 50% B at 1.0 min, 70.7% B at 8.33 min, 98% B at 9.0 min holding for 3.0 min, re-equilibration to 30% B over 0.10 min and held for 1.80 min.	ESI-MS/MS on a 8050 Shimadzu triple quadrupole mass spectrometer with electrospray ionization using scheduled multiple reaction monitoring (MRM)	CBD (**3**), CBG (**6**), CBN (**8**), 11-OH-THC (**9**), THC-COOH (**10**), THC (**12**), THCV (**14**), THCVA (**15**), THC-COOH-glucuronide and THC-glucuronide[103]

TABLE 7 HPLC and UPLC (or UHPLC) methods for the analysis of naturally occurring cannabinoids in miscellaneous biological samples from human

Instrumentation	Column	Mobile phase	Detection	Matrices/source	Cannabinoids analyzed/detected
HPLC methods					
Alliance 2795	Phenomenex Kinetex C_{18} column (50 mm x 2.1 mm; particle size: 2.6 μm)	Gradient elution with ACN in water (both containing 0.1% HCOOH); 40% B from 0 -0.2 min, linearly increased to 40-100% in 0.2-6 min, returned to initial conditions in 6-6.8 min. Column temp: 35°C; flow rate: 0.3 mL/min.	ESI-MS/MS in positive ion mode using a Quattro Micro API ESCI triple quadrupole (Waters).	Meconium sample	CBD (**3**), CBN (**8**), THC-OH (**9**), THC-COOH (**10**), THC (**12**), 8β,11-dihydroxy-THC (diOHTHC), and THC-COOH-glucuronides[104]
Agilent 1100 Series HPLC	Phenomenex Luna C_{18} column (150 mm x 2.0 mm; particle size: 5 μm)	ACN/MeOH/0.4 mM ammonium acetate solution pH 3.2 as the mobile phase (16:4:5) at a flow rate of 0.28 mL/min. Injection volume: 20 μL	ESI-MS/MS in positive ion mode	Post-mortem human tissue homogenates	CBD (**3**), CBN (**8**), THC-OH (**9**), THC-COOH (**10**), THC (**12**), 8β,11-dihydroxy-THC (diOHTHC), and THC-COOH-glucuronides[105]
UPLC (or UHPLC) methods					
Acquity UPLC H-Class	Acquity BEH C_{18} column (150 mm x 2.1 mm; particle size: 1.7 μm; Waters) couples with an Acquity BEH	0.1% HCOOH in both water (A) and MeOH (B). Flow rate: 0.4 mL/min; column temperature 40°C; injection volume: 10 μL. Gradient elution: 0–2.00 min: 80% solvent A, 2.00–2.13 min:	ESI-MS/MS	Human skeletal tissue	THC-COOH (**10**) and THC (**12**)[106]

	C_{18} VanGuard pre-column (5 mm x 2.1 mm; 1.7 μm; Waters)	linear change from 80.0 to 68.1% solvent A, 2.13–6.80 min: from 68.1 to 66.9% solvent A, 6.80–6.81 min: from 66.9 to 30% solvent A, 6.81– 10.00 min: from 30 to 1% solvent A, 10.00–13.00 min: 1% solvent A, 13.00–17.00 min: 80% solvent A, where the column was re- equilibrated.			
Agilent 1260 UHPLC	Phenomenex Kinetex C_{18} (50 mm x 2.1 mm; particle size: 1.7 μm)	Gradient elution with water (A) and MeOH (B), both containing 0.1% of HCOOH at a flow rate of 0.2 mL/min; starting at 70% of B increased linearly in 5 min to 95% B and held there for 7 min, and returned at 70% with an equilibration time of 12 min before the next injection; injection volume: 5 μL.	ESI-MS/MS in positive ion mode using an Agilent 6410 Triple quadrupole mass spectrometer	Human liver	11-OH-THC (**9**), THC-COOH (**10**) and THC (**12**)[38]

TABLE 8 HPLC methods for the analysis of naturally occurring cannabinoids in animal samples

Instrumentation	Column	Mobile phase	Detection	Matrices/source	Cannabinoids analyzed/detected
Agilent 1200 HPLC	Kinetex EVO C_{18} column (100 mm x 2.1 mm; particle size: 5 μm; Phenomenex, Italy)	The mobile phase was composed of (A) 2.0 mM aqueous ammonium acetate and (B) ACN using the gradient elution: 0.0–10.0 min, linear gradient from 30 to 90% (B); 10.0–15.0 min, isocratic at 90% (B), 15.0–18.0 min, linear gradient from 90 to 30% (B). A pre-equilibration period of 2.0 min was applied between each run. Flow rate: 0.35 mL/min; column temp: 40◦C; injection volume was 25 μL	ESI-MS/MS using a using a SCIEX API4000 QTRAP mass analyser	Rat whole blood	CBD (**3**), 11-OH-THC (**9**), THC-COOH (**10**), THC (**12**) and THC-COOH-glucuronide[107]
Shimadzu UFLC-Nexera X2 HPLC system	Waters Symmetry C_{18} column (150 mm x 4.6 mm; particle size: 5 μm)	The mobile phase A: was 10 mM ammonium formate buffer with 0.1% formic acid, and mobile phase B: MeOH. Isocratic elution with 90% B in A, with a flow rate of 1 mL/min injection volume of 20 μL, and total run time of 6 min.	ESI-MS/MS on the ABSCIEX API 5500 Q-Trap mass spectrometer using the positive ion mode	Rat plasma	CBD (**3**) and THC (**12**)[108]

Waters Alliance 2695 HPLC	ACE C18-PFP column (150 mm x 4.6 mm; particle size: 3 mm) coupled with an ACE C18-PFP 3 μm guard column	Isocratic elution with 62% ACN in water at a flow rate of 1 mL/min for 20 min. Column temp. 55°C.	UV-PDA detector set at 220 nm		CBD (**3**) and THC (**12**)[109]
Shimadzu SCL HPLC system	Zorbax Eclipse XDBC18 column (75 mm x 4.6 mm; particle size: 3.5 μm)	Isocratic elution with 90% MeOH in water with 0.1 mM ammonium formate at a flow rate of 0.5 mL/min. Total run time: 8 min	ESI-MS/MS in positive ion mode on an Applied Bio systems 3200 Q trap with a turbo V source for TurbolonSpray	Mouse brain	CBC (**1**), CBD (**3**), 11-OH-THC (**9**), THC-COOH (**10**) and THC (**12**)[110]
LC20AB HPLC	Phenomenex Kinetex C_{18} column (100 mm x 2.1 mm; particle size: 5 μm)	Isocratic elution with 75% MeOH in water containing 0.1% ammonium acetate, at a flow rate of 0.15 mL/min, with a column temp. 30°C	ESI-MS/MS in negative ion mode on an IT-TOF MS (Shimadzu, Japan)	Mouse peripheral tissue	CBD (**3**), CBDV (**5**), CBG (**6**) and THCV (**14**)[111]

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

Instrumentation	Column	Mobile phase	Detection	Matrices/source	Cannabinoids analyzed/detected
HPLC methods					
Agilent 1100	Ascentis Express C_{18} column (150 mm x 3 mm; particle size: 2.7 μm; Suppelco, USA)	0.1% HCOOH in both water (A) and 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; post-running time 10 min; flow rate: 0.4 mL/min; column temperature 30°C; injection volume: 3 μL.	UV-DAD	Honey	CBD (**3**), CBDA (**4**), CBG (**6**), CBGA (**7**), THC (**12**) and THCA (**13**)[112]
Agilent 1200	Phenomenex Kinetex EVO C_{18} column (100 mm x 2.1 mm; particle size: 5 μm; Phenomenex, Italy)	2 mM aqueous CH_3COONH_4 (A) and ACN (B). Linear gradient elution: 0-10 min 30-90% B, 10-15 min isocratic elution with 90% B, 15-18 min from 90% to 30% B; post-running time 2 min; flow rate: 0.35 mL/min; column temperature 40°C; injection volume: 25 μL.	ESI-MS/MS in negative ion mode on a linear ion trap quadrupole (QTRAP) mass analyser		
Agilent 1200	Ascentis Express RP-Amide stainless steel column (50 mm × 4.6 mm;	0.1% HCOOH in both water (A) and ACN (B). The analysis	ESI-MS/MS on a 4000 QTRAP spectrometer	Beverages and food	CBD (**3**), CBDA (**4**), CBG (**6**), CBGA (**7**), CBN (**8**), Δ^8-THC (**11**), THC (**12**),

	particle size: 2.7 μm, Supelco, Bellefonte, PA, USA)	started from 40% B for 1.0 min, followed by a linear gradient 40-95% B in 9.0 min, and held at 95% B for 3 min; mobile phase B was then decreased to 40% in 2 min and the column equilibrated for further 7.0 min. Complete run time: 22 min; flow rate: 0.8 mL/min; column temperature: 25°C; injection volume: 10 μL			THCA (**13**) and THCV (**14**)[11]
UPLC (or UHPLC) methods					
Waters Acquity UPLC-I Class	Waters Acquity UPLC HSS C_{18} column (150 mm x 2.1 mm; particle size: 1.8 μm)	Mobile phase: 25 mM sodium phosphate and 0.01% sodium hexane sulfonate in deionized water adjusted to pH 3 with phosphoric acid (solvent A) and ACN (solvent B). Gradient elution: 0 min, 60% B; 4 min, 80% B; 9 min, 100% B; 11 min, 100% B; 11.1 min, 60% B and 15 min, 60% B. Column temp. 30°C; injection volume: 10 mL	UV-PDA set at 210 nm, and ESI-MS/MS	Dietary supplement	THC (**12**)[113]
Agilent 1260 UHPLC	Phenomenex Kinetex C_{18} UPLC column (50 mm x	Gradient elution with water (A) and MeOH (B), both containing 0.1% of HCOOH at a flow rate of 0.2 mL/min; starting at 70% of B	ESI-MS/MS in positive ion mode using an Agilent 6410	Milk	11-OH-THC (**9**), THC-COOH (**10**) and THC (**12**)[38]

	2.1 mm; particle size: 1.7 μm)	increased linearly in 5 min to 95% B and held there for 7 min, and returned at 70% with an equilibration time of 12 min before the next injection; injection volume: 5 μL.	Triple quadrupole mass spectrometer		

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

Instrumentation	Column	Mobile phase	Detection	Matrices/source	Cannabinoids analyzed/detected	References
HPLC methods						
HPLC Symbiosis Pico System	Purospher Start RP-18 end-capped column (125 mm x 2.0 mm; particle size: 5 mm) connected to a guard column of same materials.	Mobile phase contained ACN (B) and water (A) both having 20 mM of HCOOH/ammoniu m formate buffer (pH 3.8). Gradient elution: 0 min 5% B, 0-12 min 40% B, 12-18 min 70% B, 18-19 min 80% B, 19-26 min 100% B, held for 2 min, 28-30 min 5% B, and held for 10 min for re-equilibration. Flow rate: 0.3 mL/min.	ESI-MS/MS in positive ion mode on a 4000QTRAP hybrid triple quadrupole-linear ion trap mass spectrometer	Sewage sludge	CBD (**3**), CBN (**8**)11-OH-THC (**9**) and THC (**12**)[114]	

UPLC (or UHPLC) methods						
Agilent 1260 Infinity	Phenomenex Kinetex C_{18} column (50 mm x 2.1 mm; particle size: 1.7 μm)	Mobile phase comprised water (A) and MeOH (B), both containing 0.1% HCOOH. The gradient elution started at 10% B for 5 min, then increased linearly to 95% B until 12 min and continued at 95% B up to 25 min. Re-equilibration time: 15 min. Column temp: 30°C; flow rate: 0.2 mL/min; injection volume: 5 μL.	QqTOF-MS/MS in positive ion mode	Water samples	THC-COOH (**10**) and THC (**12**)[115]	
Shimadzu Nexera UHPLC	Phenomenex Kinetex C_{18} column (100 mm x 2.1 mm; particle size: 1.7 μm) with a	Mobile phases: A (0.1% HCOOH in water) and B (0.1% HCOOH in ACN). Gradient elution:	ESI-MS/MS on a triple quadrupole LCMS 8030 from Shimadzu	Waste water samples	THC-COOH (**10**), THC (**12**) and THC-COOH-glucuronide[116]	

	Security Guard ULTRA cartridge C_{18} (2 mm x 2.1 mm, Phenomenex)	40-95% B in 0-4 min, held for 1 min, decreased to 40% B in 0.5 min and held at 40% B for 1.5 min. The total run time was 7 min and the mobile phase flow rate was 0.5 mL/min. Column temp. 40^{o}C; injection volume: 50 μL.				