Gas chromatographic analysis of naturally occurring cannabinoids: A review of literature published during the past decade

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Abstract

Introduction:

Cannabinoids are organic compounds, natural or synthetic, that bind to the cannabinoid receptors and have similar pharmacological properties as produced by the cannabis plant, *Cannabis sativa.* Gas chromatography (GC), *e.g.,* GC-MS, is a popular analytical tool that has been used extensively to analyze cannabinoids in various matrices.

Objective:

To review published literature on the use of various GC-based analytical methods for the analysis of naturally occurring cannabinoids published during the past decade.

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 cannabinoids, *Cannabis sativa*, marijuana, analysis, GC, quantitative, qualitative and quality control.

Results:

During the past decade, several GC-based methods for the analysis of cannabinoids have been reported. While simple 1D GC-MS and GC-FID methods were found to be quite common in cannabinoids analysis, 2D GC-MS as well as GC-MS/MS also were popular because of their ability to provide more useful data for identification and quantification of cannabinoids in various matrices. Some degree of automation in sample preparation, and applications of mathematical and computational models for optimization of different protocols were observed, and pre-analyses included various derivatisation techniques, and environmentally friendly extraction protocols.

Conclusions:

GC-based analysis of naturally occurring cannabinoids, especially using GC-MS, has dominated the cannabinoids analysis in the last decade; new derivatisation methods, new ionization methods, and mathematical models for method optimization have been introduced.

Keywords

Cannabis sativa; cannabinoids; gas chromatography (GC); cannabis; marijuana; GC-MS; GC-FID; analysis; detection

1 INTRODUCTION

Cannabinoids are organic compounds, natural or synthetic, that bind to the cannabinoid receptors (endocannabinoid system) and have similar pharmacological properties as produced by the cannabis plant, *Cannabis sativa*[1]. However, often cannabinoids are referred to over 100 different compounds, Δ^9-tetrahydrocannabinol (**10**, Δ^9-THC or simply, THC) and cannabidiol (**3**, CBD) being the two major ones (Figure 1), biosynthesized by *C. staiva*[2]. The psychoactive property of *C. sativa* is fundamentally contributed by Δ^9-THC (**10**), whilst the other major compound, cannabidiol (**3**), is believed to possess antipsychoactive property. Natural cannabinoids, also known as phytocannabinoids, are concentrated in a viscous resin produced in the glandular trichomes of *C. sativa*, and can be structurally classified into at least eight different classes, *i.e.,* cannabigerols (**5**, CBG), cannabichromenes (**1**, CBC), cannabidiols (**3**, CBD), tetrahydrocannabinols (**10**, THC) and cannabinols (**7**, CBN), cannabielsoins, iso-tetrahydrocannabinols, cannabicyclols (**2**, CBCL), and cannabicitrans[2]. Medicinal cannabis generally has a higher level of cannabidiol CBD (>20%) than THC (~1%), whereas recreational cannabis contains higher amounts of THC (>20%) than CBD (~2%). Medicinal applications of phytocannabinoids, as supported by certain level of clinical evidence, include their uses for the treatment of chronic pain, some treatment resistant epilepsies, and chemotherapy induced nausea and vomiting. In addition to *C. sativa*, phytocannabinoids are also produced by several other plants including *Acmella oleraceae, Echinacea angustifolia, E. purpurea, Helichrysum umbraculigerum* and *Radula marginata*[3].

Since the discovery of the very first individual cannabinoid, cannabinol (**7**, CBN), in 1940, by the British Chemist Robert S. Cann, and subsequent discoveries of cannabidiol (**3**, CBD) and then tetrahydrocannabinol (**10**, THC), several analytical tools and methods have been applied for the detection, identification, quantification and analysis of various naturally occurring cannabinoids, predominantly from the plant *C. sativa*, as well as in various biological matrices, *e.g.,* the blood, urine, hair and nails, often linking to pharmacokinetic studies and/or forensic analysis[2]. Gas chromatography (GC)[4] coupled with various detection technologies, *e.g.,* FID and MS, is one of those analytical tools that has been used extensively to analyze cannabinoids[5-8]. Over the past decade, with the advancement of computational tools, MS databases, and various advanced detection technologies, GC has become one of the major tools in forensic, pharmacokinetic and phytochemical analysis of naturally occurring

cannabinoids. This review aims to capture the developments in GC methods applied to cannabinoids analysis since 2009, and to appraise the scientific publications in this topic published in the past decade.

2 GAS CHROMATOGRAPHY (GC)

Gas chromatography (GC) is a chromatographic technique that is used for the separation and analysis of compounds, which can be vaporized without decomposition, from various matrices[4]. In this chromatography, the mobile phase is an inert gas, *e.g.,* helium, whilst the stationary phase is a microscopic layer of liquid or polymer on an inert support, often a glass or metal tubing, called GC column (Figure 2). The most commonly used detectors with a GC are flame ionization detector (FID) and thermal conductivity detector (TCD), but the use of a mass spectrometer (MS) with different ionization modes has recently become routine in most of the analytical labs. Other types of detectors used infrequently with a GC may include alkali flame detector (AFD), atomic emission detector (AED), dry electrolytic conductivity detector (DELCD), electron capture detector (ECD), flame photometric detector (FPD), helium ionization detector (HID), nitrogen-phosphorus detector (NPD), photo ionization detector (PID), pulsed discharge ionization detector (PDD) and thermionic ionization detector (TID). The most recent developments in detection technology for GC have enabled multiple hyphenation with MS and NMR together to offer GC-MS-NMR, and vacuum ultraviolet detector to give the new hyphenated technique GC-VUV; the use of an infrared detector with GC is also now available (GC-IRD).

Because of the quality and extent of structural information that can be obtained from MS data, GC-MS, and GC-MS/MS, also known as tandem MS gas chromatography, have now been used routinely for the analysis of various types of natural products, including cannabinoids. In GC-MS, electron impact (EI) ionization mode is the most popular mode for the analysis of cannabinoids as this mode produces MS spectra that contain characteristic fragment ion peaks essential for the identification of compounds. However, in this interface, in the most cases, the molecular ion is absent because of the extent of fragmentation of any molecule. Softer ionization techniques, *e.g.,* chemical ionization (CI), that offers information on molecular ions, is also frequently used with GC nowadays for the analysis of cannabinoids. It can be mentioned that GC-MS analysis of cannabinoids is not necessarily restricted to the

use of these two ionization techniques, rather, other ionization techniques, *e.g.,* photo ionization (PI) technique[6], are also in use.

One of the major components of *C. sativa* is thermolabile tetrahydrocannabinolic acid (**11**, THCA) (Figure 1), which is the precursor of THC (**10**), formed through *in situ* heat-mediated decarboxylation of THCA (**11**) during smoking, cooking, or at the hot injection port of any GC. Therefore, in a GC operation, THCA (**11**) cannot be detected, but its decarboxylated product, THC (**10**), can be. A GC method is typically simpler and faster than an HPLC method for the detection of cannabinoids, which make a GC method preferable to an HPLC method. In most of the routine analysis of cannabinoids, a GC-FID is the method of choice because of its simplicity and reliability in routine identification and quantification of cannabinoids in plant materials and/or other matrices. However, for correct identification of individual cannabinoids, a GC-MS is more appropriate. The use of a GC method for the determination of the potency of cannabis is based on the concentration of THC (**10**) and CBD (**3**).

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

3 GC ANALYSIS OF NATURALLY OCCURRING CANNABINOIDS

Several publications published during the past decade on the use of GC-based methods for the analysis of cannabinoids demonstrate that GC remains to be one of the most popular methods of choice when it comes to cannabinoids analysis. The published literature also shows that there are various types of detection technologies can now be used for GC, and different mathematical and computation modelling as well as chemometric tools can make the analysis of GC data more useful and reliable.

Different types of GC columns are available to date, and the correct choice of a GC column, along with the application of the correct detection technology, is crucial to success of cannabinoids analysis by GC. Generally, most columns used in cannabinoid analysis have small diameter and are thin-filmed nonpolar stationary phase (*e.g.,* 100% dimethyl silicone) columns. However, nonpolar columns like 100% dimethyl silicone or 5% diphenyl in dimethyl silicone do not have adequate level of polarity to separate and distinguish cannabidiol (CBD, **3**) from cannabichromene (**1**, CBC), which is absolutely crucial in accurate determination of THC/CBD ratio. In such a situation, the use of an intermediate polarity column, *e.g.,* 35%

diphenyl in dimethyl silicone column can be useful in achieving good separation of CBD (**3**) and CBC (**1**). Two typical GC starting conditions, one using a FID and the other employing an MS detector, for the analysis of cannabinoids are summarised in Table 1.

3.1 GC analysis of cannabinoids in plant samples

Various GC-based analytical techniques have become quite popular in the analysis of cannabinoids in plant samples (Table 2), particularly in cannabis extracts. In fact, these methods are generally useful for chemical fingerprinting of plant extracts containing cannabinoids. Presence of cannabinoids, both qualitative and quantitative, 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.

Omar *et al.*[9] reported a SFE method using supercritical CO_2 and ethanol as a co-solvent to extract cannabinoids from marijuana samples, and used retention time locking GC-MS for the identification and quantification (in the range of 0.96-324 mg/g) of three major cannabinoids, THC (**10**), CBD (**2**) and CBN (**7**). The SFE method could successfully remove terpenes and volatile components, making GC-MS analysis of cannabinoids mixture less complicated. The parameters for the SFE were studied by means of a central composite design (CCD) to get the optimum extraction conditions, and principal component analysis (PCA)[10] was used to analyze data. Cannabinoids determination and quantification in the seeds of *C. sativa* were achieved by ultrasonic extraction using *n*-hexane followed by a simple GC-MS technique[11]. In this piece of work, chemometric tools[10] were used to analyze data.

Derivatization plays a pivotal role in GC-MS analysis of phytocannabinoids, as most of these cannabinoids are not volatile and only derivatization can make them volatile for GC analysis. Fodor and Molnar-Perl[12] studied the role of derivatisation techniques in the analysis of phytocannabinoids, like THC (**10**), CBD (**3**), tetrahydrocannabivarin (**13**, THCV), tetrahydrocannabidivarin (**12**, CBDV), CBC (**1**), cannabicyclol (**2**, CBCL), cannabigerol (**5**, CBG), CBN (**7**), 11-OH-THC (**8**) and THCA (**11**), by GC-MS. The same group reported alkylsilyl speciation by trialkylsilylation and direct sample preparation (solvent free) of cannabinoids, THC (**10**), CBD (**2**), THCA (**11**), CBN (**7**), 11-OH-THC (**8**), CBN (**1**) and CBG (**5**), of plant origins for subsequent GC-MS analysis[13]. A GC-MS and GC-FID based quantification of cannabinoids

present in cannabis olive oil preparation, which is the first choice as a concentrated extract of cannabinoids for medicinal use, has recently been reported[14]. In this study, the effect of temperature and duration of extraction on the amounts of cannabinoids as well as the effect of temperature on decarboxylation of THCA (**11**) was evaluated. While a similar method was also reported for the analysis of cannabinoids in *C. sativa* inflorescence[15], a fast GC-MS method has recently been developed and validated[16] for the determination of cannabinoids in *C. sativa* inflorescence, where different derivatization methods have been tested aiming at avoidance of decarboxylation of carboxyl group of cannabinoids. Fast GC methods are based on six major principles: short columns, fast oven temperature ramp rates, high carrier gas linear velocities, narrow I.D. columns, hydrogen carrier gas and low film thickness. A fast GC offers 3-10 times faster analysis time than conventional GC, resulting in overall lower cost.

As *C. sativa* contains over 550 compounds, including 113 cannabinoids and 120 terpenes, the quality control of medicinal cannabis for medical use has always been a challenging issue[17]. The profiles and contents of cannabinoids and terpenes vary quite considerably among various chemotypes of *C. sativa*. Therefore, appropriate quality control methods are essential for ensuring the quality of medicinal cannabis and thus optimizing the therapeutic outcome. In order to address the quality control aspects of medicinal *C. sativa*, Calvi *et al.*[17] have validated a headspace-solid-phase-extraction method coupled with GC-MS as well as LC-HRMS, providing in depth chemical profiling and fingerprinting of cannabinoids and terpenes in authorized medical grade *C. sativa* inflorescences and macerated cannabis oils. A simple GC-FID method for the determination and quantification of a series of acidic and neutral cannabinoids, tetrahydrocannabivarian (**13**, THCV), CBD (**3**), CBC (**1**), *trans*-(8)-tetrahydrocannabinol (**9**, Δ^8-THC), THC (**10**), CBG (**5**), CBN (**7**), cannabidiolic acid (**4**, CBDA), cannabigerolic acid (**6**, CBGA) and THCA (**11**), has been published recently[18]. This method has also been used for cannabinoids analysis in different parts, *e.g.,* buds, leaves, roots and stems, of the micropropagated *C. sativa*. 1D GC-MS and 2D GC-MS (GC x GC-Quadrupole MS) methods have been shown to be useful for the simultaneous detection of seven cannabinoids: THC (**10**), CBD (**3**), THCA (**11**), CBN (**7**), CBG (**5**), CBDA (**4**) and CBC (**1**)[19].

3.2 GC analysis of cannabinoids in biological and forensic samples

Marijuana, a cocktail of at least 30 different cannabinoids, generally prepared from crushing the leaves, flowers and even stems of *C. sativa*, is one of the oldest recreational and

addictive natural products used by the humans for centuries. However, the use of cannabis or marijuana is illegal in many countries, which necessitates the use of analytical tools to analyze biological samples like blood, bronchoalveolar liquid, and urine to detect marijuana usage (Table 3). After consumption of marijuana, THCA (**11**), which is the major metabolite of THC (**10**), is excreted in the urine as its glucuronide conjugate. It can be mentioned again that THCA (**11**) is also present in the crude marijuana, but is converted to THC (**10**) by heat during smoking.

3.2.1 Bile samples

Among the biological samples, perhaps the bile is the least studied forensic sample for the analysis of cannabinoids in humans. A simple GC-MS method, with the limit of detection of 0.30 ng/mL and the limit of quantification of 1 ng/mL, for the determination of THC (**10**) and THCA (**11**) in the bile samples from 21 forensic cases, was reported[20]. Solid-phase extraction (SPE) was employed and the cannabinoids were derivatized by silylation using *N,O*-bis(trimethylsilyl)-trifluoroacetamide with 1% trimethylchlorosilane. The method also included protein precipitation with acetonitrile after enzymatic hydrolysis.

3.2.2 Blood, plasma and serum samples

Blood, plasma and serum samples are quite extensively used in forensic analysis to detect the consumption of cannabinoids (Table 3). Frazee *et al.*[21] described a GC-MS protocol that involved the addition of deuterated internal standard of Δ^9-THCA (**11**) into the blood or urine sample followed by hydrolysis of THCA-glucuronide by alkali. They used liquid-liquid extraction to extract THCA (**11**) from urine or blood samples, and derivatized THCA (**11**) to its trimethylsilyl adduct for GC-MS analysis. The amount of THCA (**11**) was quantified by comparison of the responses of the unknown sample to that of the calibrators using selected ion monitoring (SIM), which is an MS scanning mode in which a limited mass-to-charge ratio range is detected by the instrument, instead of the full spectrum range, and thus increases sensitivity. A simple GC-MS method was reported by DeLong *et al.*[22] for the determination of CBC (**1**), which is the second most abundant cannabinoid in marijuana, and THC (**10**) in mouse blood and brain. Cannabinoids were extracted from homogenized blood and brain with ice-cold acetonitrile, and trimethylsilyl derivatives were produced for the GC-MS analysis. A 2D-GC-MS method for the analysis of cannabinoids in post mortem blood samples, extracted by a liquid-liquid extraction protocol (efficiency >75%), was found to be effective in the

determination and quantification of THC (**10**), CBD (**2**), CBN (**7**), THCA (**11**) and 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC, **8**)[23]. Simple GC-MS methods have also been used extensively to determine cannabinoids in plasma samples using various extraction and sample preparation techniques. For example, Camargo *et al.*[24] developed an efficient liquid-liquid extraction and GC-MS method for the determination of CBD (**2**) at a concentration range of 2.5-250 ng/mL of plasma.

An automated liquid-liquid extraction coupled with a GC-MS method was reported for the analysis of THC (**10**), THCA (**11**) and 11-OH-THC (**8**) from human blood serum samples after derivatization by *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA)[25]. As this method could detect and quantify THC (**10**) as low as 0.3 mu g/L of concentration, when extracting only 0.5 mL of blood serum, this method was deemed suitable for the limit of quantification for THC (**10**) of 1 mu g/L in driving under the influence of cannabis cases in Germany and other countries. A similar liquid-liquid extraction coupled with a GC-MS/MS method has recently been reported for the detection and quantification of major cannabinoids, THC (**10**), 11-OH-THC (**8**), CBD (**2**) and THCA (**11**), from human blood plasma samples[26]. Cannabinoids were derivatized by MSTFA before GC-MS/MS analysis.

Solid phase extraction (SPE) is the most commonly used sample preparation technique to extract THC (**10**) and its main metabolites from different biological specimens, *e.g.,* blood plasma. The use of SPE employing anion exchange sorbent was found to be an effective method of extraction for THC (**10**), THCA (**11**) and 11-OH-THC (**8**) in blood plasma and serum samples, prior to GC-MS analysis[27]. The benefit of this anion exchange sorbent was that it allowed differential elution of cannabinoids; neutral cannabinoids THC (**10**) and 11-OH-THC (**8**) were eluted first, followed by elution of clean acidic cannabinoid, THCA (**11**). An improved GC-MS/MS-based method for quantification of THC (**10**), 11-OH-THC (**8**) and THCA (**11**) in whole blood samples from cannabis users, employing SPE, and multiple reaction monitoring (MRM) mode, also known as selective reaction monitoring (SRM) has been reported recently[28], and this method has been demonstrated to be specific, precise, linear, and sufficiently sensitive for its application post-mortem samples. In this method, a combination of bis(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) (99:1) was used for derivatisation at 65^{o}C for 30 min.

Microextraction by packed sorbent (MEPS) is practically a mini-SPE technique, in which the sample and solvent volumes are significantly reduced, and the sorbent is packed

into a syringe, which can be used repeatedly. MEPS was successfully used to extract cannabinoids, THC (**10**), THCA (**11**) and 11-OH-THC (**8**), from human blood plasma, and GC-MS/MS technique was used for the detection and quantification (in the range of 0.1-30 ng/mL) of these cannabinoids[29].

Gottardo *et al.*[30] have reported, for the first time, the use of atmospheric pressure chemical ionization gas chromatography tandem mass spectrometry (APGC-MS/MS) for the determination of cannabinoids, THC (**10**), 11-OH-THC (**8**), THCA (**11**), CBD (**3**), cannabidiol acid (**4**, CBDA) and CBG (**5**) in human serum samples, with the limit of quantifications in the range of 0.2-3 ng/mL. APGC interface displayed advantages over the conventional electron ionization (EI) or chemical ionization (CI) sources, which are most often used in the GC-MS analysis of naturally occurring cannabinoids.

3.2.3 Breast milk

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. Cannabinoids extraction from this matrix is quite challenging because of its high lipid and protein contents. Silveira *et al.*[31] published a method combining headspace solid-phase microextraction and GC-MS for the quantification of THC (**10**), CBD (**3**) and CBN (**7**) in human breast milk samples. The method was quite sensitive as evident from its limits of detection and quantification being 10 and 20 ng/mL, respectively, for all analytes, and could determine THC (**10**), CBD (**3**) and CBN (**7**) in human breast milk in a very low volume sample (0.05 mL).

3.2.4 Bronchoalveolar lavages

Rotolo *et al.*[32] have developed a simple and robust GC-MS method for the identification and quantification of CBN (**7**), CBD (**3**), THC (**10**), 11-hydroxy-THC (11-OH-THC, **8**) and THCA (**11**) in bronchoalveolar lavages from hospitalized former or current tobacco smoker patients with lung disease and a long history of cannabis consumption and limited current tobacco use. This is the very first report on the analysis of cannabinoids in bronchoalveolar lavage samples, which has more medical implications than forensic values. Bronchoalveolar lavages, also known as bronchoalveolar washings, is a medical diagnostic procedure, where a bronchoscope is passed through the mouth or nose into the lungs and fluid is squirted into a small part of the lung and then collected for examination.

3.2.5 Hair samples

Hair samples are one of the most frequently used forensic samples for the analysis of drugs of abuse or illegal drugs like cannabis. Hair analysis is used to monitor usage of drugs over long periods, and in recent years, headspace solid-phase microextraction has emerged as an important extraction method for this analysis. Emidio *et al.*[33] described a GC-MS/MS method for the detection and quantification of THC (**10**), CBD (**3**) and CBN (**7**) from human hair samples using a combination of headspace solid-phase microextraction and GC linked to tandem MS. It can be noted that enhanced selectivity and sensitivity can be accomplished using the GC–MS/MS procedure, where the molecular ion or a fragment of the ion having greatest intensity is separated and exposed to a second fragmentation. This secondary fragmentation can then be separated and detected. The hair samples from cannabis users, were first decontaminated using petroleum ether, deionized water and dichloromethane under sonication, followed by digestion in NaOH solution, before GC-MS/MS analysis. An improved GC-MS/MS method for the determination of THC carboxylic acid (THC-COOH, **11**) in hair samples was reported, where a GC-negative ion chemical ionization tandem MS (GC-NCI-MS/MS) technique was used[34]. Instead of SPE, a bead-assisted liquid-liquid extraction was employed using an *n*-hexane/EtOAc solvent mixture. This method was found to be useful in the identification of THCA (**11**) in hair specimens from suspected cannabis abusers.

A forensic standard procedure combining enzyme-linked immunoassay for screening and GC-MS for the detection of cannabinoids, *e.g.,* THC (**10**), CBD (**3**) and CBN (**7**), in human hair samples, which were previously used for the detection of other drugs of abuse, was reported[7]. A simple and rapid single-step tandem GC-MS method using electron impact (EI) ionization mode and liquid-liquid extraction for the analysis of cannabinoids, THC (**10**), CBD (**3**), CBN (**7**) and 11-OH-THC (**8**), in hair samples has recently been reported[35]. They used multiple reaction monitoring (MRM) transitions approach for the detection of all cannabinoids. This method showed excellent limit of detection with 0.03 pg/mg for 11-OH-THC (**8**), and the range 0.3-1.4 pg/mg for the other three cannabinoids. Similarly, the analytical recovery was also good and was within the range of 68-97%. A simple SPE coupled with GC-MS/MS method for the determination of THC (**10**), CBD (**3**) and CBN (**7**) in hair samples from consumers of CBD rich cannabis extracts has been reported by Rodrigues *et al.*[36]. Another similar GC-MS/MS method using EI mode of ionization for simultaneous quantification of THC (**10**), CBD (**3**), CBN (**7**), THCA (**11**), 11-OH-THC (**8**) in human hair samples

has also been described, where SPE was chosen for sample clean-up, applying a mixed-mode anion exchange sorbent[37], and the limit of detection (LOD) was 0.2 pg/mg for THCA (**11**) and 11-OH-THC (**8**), and 2 pg/mg for THC (**10**), CBD (**3**) and CBN (**7**), respectively, which fulfils the Society of Hair Testing recommendations. It can be noted that although GC-MS/MS with NCI is still considered as the gold standard for achieving the highest sensitivity for the analysis of THCA (**11**) in human hair samples, it suffers from lack of flexibility in the chemical ionization mode, when compared to electron impact ionization mode. Paul *et al.*[38] have published a GC-MS/MS method for the identification and quantification of THC (**10**), CBD (**3**), CBN (**7**), THCA (**11**) and 11-OH-THC (**8**) in human hair samples from 10 volunteers after cosmetic application of cannabis oil. Hair samples were prepared by washing with methanol followed by cleaning up with liquid-liquid and SPE methods.

3.2.6 Meconium samples

Toxicological studies using maternal foetal matrices can be a suitable tool to assess drug use or abuse during pregnancy, including cannabinoids. Mantovani *et al.*[39] have recently reported the analysis of cannabinoids in meconium matrix through identification and quantification of THCA (**11**). It is known that meconium is easier to collect than neonatal urine and provides a much longer window of exposure of up to 20 weeks. In this study, accelerated solvent extraction (ASE)[4] was used for simultaneous extraction and hydrolysis of conjugated THCA (**11**) from meconium, followed by an SPE procedure. The ASE method was optimized by a multivariate statistic technique, the response of surface methodology (RSM)[10]. The software Design-Expert® (version 8) was used to design the Box-Behnken experiment and to evaluate the statistical analysis of the surface response results. The extracted cannabinoids were analyzed by a standard GC-MS method. Six meconium samples from babies whose mothers were drug users were used in this experiment, and it demonstrated satisfactory performance to confirm foetal cannabis exposure.

3.2.7 Oral fluid samples

Two dimensional gas chromatography-mass spectrometry (2D-GC-MS)[40], is a powerful technique where two different GC columns are used to facilitate simultaneous separation of multiple compounds in a complex mixture. As cannabis samples contain multiple compounds that belong to cannabinoid class, 2D-GC-MS could be quite useful in the identification and quantification of multiple cannabinoids present in a biological sample, which would otherwise

be difficult to achieve by conventional 1D GC-MS because of different physicochemical characteristics and concentrations of cannabinoid components present in a sample. A 2D-GC-MS method was reported for the simultaneous identification and quantification of Δ^9-THC (**10**), CBD (**3**), CBN (**7**), 11-hydroxy-THC (**8**) and THCA (**11**) extracted by SPE from an oral fluid sample[41]. In this experiment, both electron ionization and negative chemical ionization techniques were used. This SPE-2D-GC-MS method demonstrated efficient quantification of these five components in a single oral fluid specimen collected with the Quantisal™ device. A GC-MS/MS was developed for the quantification of THCA (**11**) in human oral fluid samples, collected from individuals participating in controlled cannabis studies, with the Quantisal and Oral-Eze oral fluid collection devices, and derivatizing by hexafluoroisopropanol and trifluoro acetic anhydride[42]. This method reduced analysis time by 9 min per sample compared to 2D-GC-MS and extended the capability of quantification.

3.2.8 Urine samples

Human urine samples are popular in forensic toxicological analysis for the determination of various illegal drugs, including cannabinoids, and their metabolites. A 2D-GC-MS method was validated for the identification and quantification of cannabinoids present in a urine sample, and was shown to be potentially useful in the monitoring of cannabinoid pharmacotherapy and illicit cannabis use[43]. A GC-MS method was also reported for the determination of CBG (**5**), which in its acid form is one of the major intermediates in the biosynthesis of cannabinoids in *C. sativa*, in urine samples of cannabis users[44]. The study demonstrated that, like other cannabinoids, CBG (**5**) also enters the body during smoking cannabis and is excreted in the urine in its conjugated glucuronide form. De Barbanter *et al.*[45] published a rapid and sensitive GC-based method for the determination of THCA (**11**) in urine samples. It can be noted that the detection of cannabis abuse is generally dependant on the quantification of the most important metabolite THCA (**11**) in urine. This reported method comprised a quick (4 min) GC-MS/MS method with a fast sample preparation involving microwave-assisted derivatisation. The total process only needed 30 min and required only 1 mL of urine sample. The extraction and derivatisation processes were rather simple, and involved the spiking of 1 mL of urine with 50 mL of the internal standard (0.5 mg/mL of THCA), hydrolysis by incubation for 7 min at 56-58^{o}C after addition of 100 mL of 6M NaOH solution, adding 1.5 mL of acetic acid and 10% EtOAc in *n*-hexane, vortexing for 1 min, transferring of

the organic phase, evaporation under nitrogen, and finally, derivatization of the dried sample using 20 mL of acetonitrile, 50 mL MSTFA and 50 mL of MSTFA/ethanethiol/NH_4I (500:4:2) in a microwave reactor at 750 W during 1.5 min. A similar GC-MS method was developed for simultaneous determination of amphetamines, opiates and THCA (**11**) in human urine samples, where similar sample preparation protocol, and two different spin columns packed with C_{18} and mixed-mode C_{18} strong anion exchange bonded monolithic silica were used prior to GC-MS analysis[46]. Derivatization was accomplished by *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA)-trimethylchlorosilane (TMCS) and *N*-methyl-bis(trifluoroacetamide) (MBTFA). This GC-MS method was used for forensic and clinical overdose or poisoning cases and could be suitable for routine analysis of drugs in biological matrices. Kim *et al.*[47] also reported simultaneous determination of amphetamines and cannabinoids in urine samples using GC-MS, using trifluoroacetic anhydride and pentafluoropropanol for derivatisation.

While the GC-MS methods used for cannabinoids analysis are fundamentally same or similar, various types of extraction methods, especially microextractions, have recently been employed to extract different cannabinoids from urine samples. For example, a hollow fibre-liquid phase microextraction (LPME) technique was employed to extract THCA (**11**) from urine samples prior to GC-MS analysis[48]. LPME is a microscale sample preparation technique, where target analytes are extracted from an aqueous sample through a supported liquid membrane that is immobilized in the pores of a porous polymeric material, *e.g.,* a hollow fibre, and into a volume of acceptor solution (typically, 10-30 µL).

3.2.9 Forensic samples of recreational drugs

In addition to cannabinoids or cannabis being used on its own as recreational substance, nontraditional cannabinoids are also added to and hidden in various freely available recreational products, *e.g.,* 'Spice' (an herbal blend, which was available freely in the UK until the end of 2009, and classified as Class B drug). A GC-MS method, using solid probe mass spectroscopy, where no liquid-liquid extraction is needed, was reported for the detection of illegal components like cannabinoids in 'Spice' and other similar herbal products[49]. In fact, cannabinoids are often added to various psychoactive substances like 'legal highs' or 'herbal highs', and simple GC-MS analysis can be used to detect various cannabinoids in those substances[50].

3.3 GC analysis of cannabinoids in pharmacokinetic and ADME studies

Whilst several published works mainly described the methods of detection and quantification of cannabinoids in various matrices, pharmacokinetic or ADME studies of cannabinoids using GC have been rather scarce in the past decade. Only a GC-MS based pharmacokinetic study of THC (**10**) and CBD (**3**) was carried out in dogs under cannabinoids therapy[51], albeit studies on the clinical use of cannabinoids in veterinary medicine are rather limited, and appropriate analytical methodologies for the determination of cannabinoids in animals, especially in dogs.

3.4 GC analysis of cannabinoids from miscellaneous samples

In addition to analyses of cannabinoids in plants, biological and forensic samples, GC methods are also applied for the detection of cannabinoids present in various other matrices, *e.g.,* waster water[52]. Two major cannabinoids that are found in waste water are THC (**10**) and THCA or THC-COOH (**11**). A GC-MS based method for the determination of THC (**10**) and THCA (**11**) in waste water was reported by Racamonde *et al.*[52]. Solid-phase microextraction technique was used to extract cannabinoids from water waste samples, and derivatized using *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) and *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) before GC-MS analysis revealing the presence of considerable amounts of cannabinoids in waste water. It is interesting to note that, to optimize the derivatisation step and parameters, a Box–Behnken response surface design[10] was adopted, and the design was composed of a total of sixteen experiments, including four central points, covering derivatization times between 10 and 30 min, temperatures between 30 and 70 ◦C and volumes of MSTFA between 10 and 60 μL, selected according to the literature for other analytes determined by on-fibre SPME silylation.

Presence of cannabinoids in various food samples was studied by GC-FID (flame ionization detector). Flame-ionization detection (FID) is a good general detector for organic compounds in GC that detects the amount of carbon in a sample. Lalge *et al.*[53] described a GC-FID method for the determination of THC (**10**), CBD (**3**) and CBN (**7**) in hemp seed powder, hemp oil and hemp protein, which are used in the preparation of various food items. THC (**10**) was found in low concentrations in the food samples analyzed in this study.

4. CONCLUSIONS

During the past decade, GC-based analytical methods, especially GC-MS techniques, have remained as one of the most popular methods for the analysis of naturally occurring cannabinoids. A number of new derivatisation methods, new ionization techniques, and mathematical models for method optimization have been introduced to make the GC-based methods even more suitable for cannabinoids determination and quantification in different matrices.

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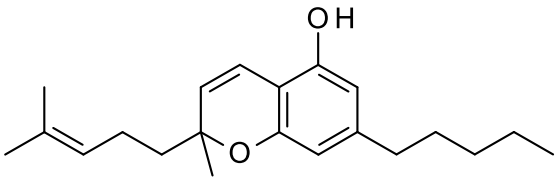
<tr><td colspan="2"> 
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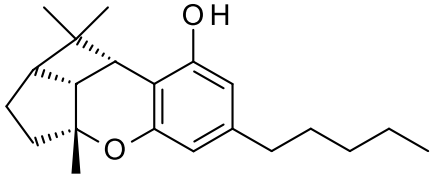
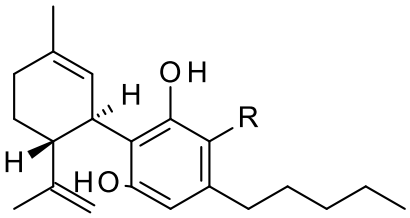
<tr><td> 
Cannabicyclol (2, CBCL)</td><td> 
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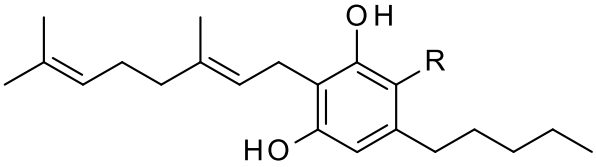
<tr><td> 
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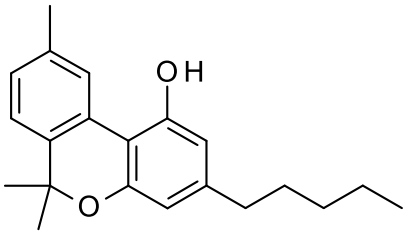
Cannabigerolic acid (6, CBGA) R = COOH</td><td> 
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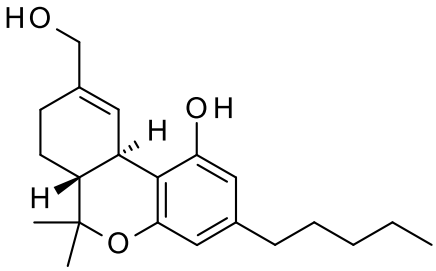
<tr><td> 
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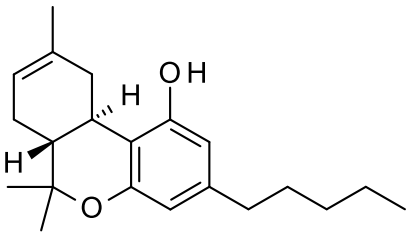
(8, 11-OH-THC)</td><td> 
trans-(8)-Tetrahydrocannabinol
(9, Δ^8-THC)</td></tr>
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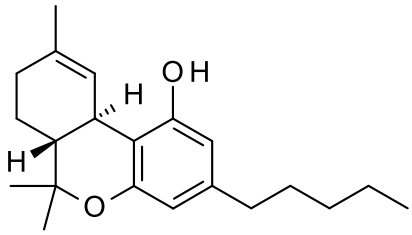
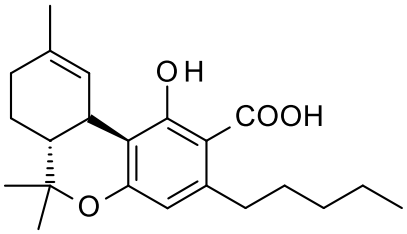
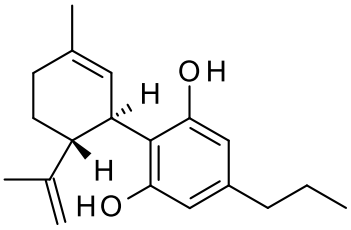
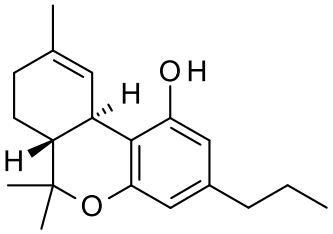
 Δ^9-Tetrahydrocannabinol (**10**, Δ^9-THC or THC)	 Δ^9-Tetrahydrocannabinolic acid (**11**, THCA or THC-COOH)
 Tetrahydrocannabidivarin (**12**, CBDV)	 Tetrahydrocannabivarin (**13**, THCV)

FIGURE 1 Cannabinoids in different matrices analyzed by GC

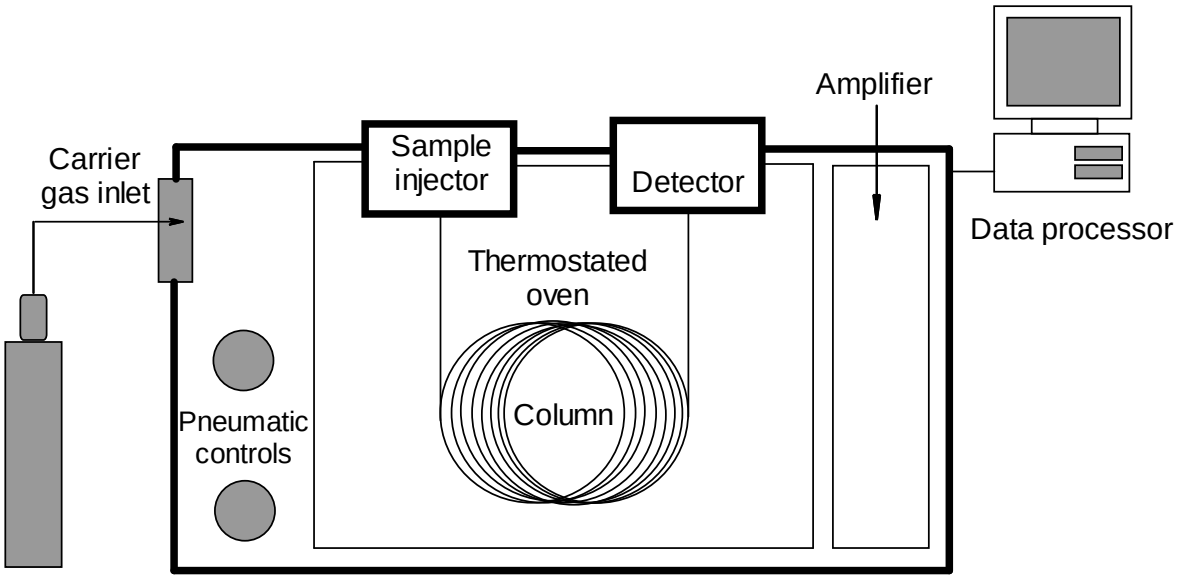


FIGURE 2 A diagram of a typical gas chromatography system (adopted from Sarker and Nahar, 2012[4])

TABLE 1 Two typical starting GC conditions for the analysis of cannabinoids

GC method	Run time	Column	Carrier gas	Injector	Detector
GC-FID	9 min	15 m x 0.53 mm x 0.5 μm	4 mL/min hydrogen	Capillary split injector, split liner with glass wool, 275 °C	FID
GC-MS	30 min	30 m x 0.25 mm x 0.25 μm	1.3 mL helium	Capillary split injector, split liner with glass wool, 275 °C	MS detector. Mass range 50-400 da

TABLE 2 GC analysis of cannabinoids in plants

Sample	Extraction method	Instrumentation	Derivatization	Detected cannabinoids
Marijuana (Cannabis)	Supercritical CO_2 fluid extraction	GC-MS	N/A	THC (**10**), CBD (**3**) and CBN (**7**)[9]
	Extraction with methanol	GC-MS and GC-quadrupole MS (GC x GC-QMS or 2D GC-MS)	N/A	THC (**10**), CBD (**2**), THCA (**11**), CBN, CBG (**5**), CBDA (**4**) and CBC[19]
Cannabis sativa seeds	Ultrasonic extraction with *n*-hexane	GC-MS	N/A	THC (**10**), CBD (**3**), CBG (**5**), CBC (**1**) and CBN (**7**)[11]
Cannabis sativa	Ultrasonic extraction with *n*-hexane	GC-FID	Trimethylsilyl	THCV (**13**), CBD (**3**), CBC (**1**), *trans*-(8)-tetrahydrocannabinol (**9**, Δ^8-THC), THC (**10**), CBG (**5**), CBN (**7**), cannabidiolic acid (**4**, CBDA), cannabigerolic acid (**6**, CBGA) and THCA (**11**)[18]
	Supercritical CO_2 fluid extraction	GC-MS	Trimethylsilyl	THC (**10**)[54]
Cannabis indica and *C. sativa*	N/A	GC-MS	Trialkylsilylation	THC (**10**), CBD (**3**), THCA (**11**), CBN (**7**), 11-OH-THC (**8**), CBC (**1**) and CBG (**5**)[13]
Cannabis sativa inflorescence	Headspace solid-phase microextraction	GC-MS and GC-FID	Silylation	CBD (**3**) and CBDA (**4**)[15]
	Extraction with 90% MeOH in chloroform under sonication	Fast GC-MS	Silylation and esterification	CBD (**3**), CBDA (**4**), CBGA (**6**) and Δ^9-tetrahydrocannabivarine (**13**, THCV)[16]
Cannabis sativa inflorescence and macerated oils	Headspace solid-phase microextraction	GC-MS and LC-HRMS	N/A	Several cannabinoids[17]
Cannabis sativa flowers and leaves	Extraction with methanol	GC-MS and GC-quadrupole MS (GC x GC-QMS or 2D GC-MS)	N/A	THC (**10**), CBD (**3**), THCA (**11**), CBN (**7**), CBG (**5**), CBDA (**4**) and CBC (**1**)[19]

TABLE 3 GC analysis of cannabinoids in biological and forensic samples

Sample	Extraction from sample	Instrumentation	Derivatization	Detected cannabinoids
Bile	Solid-phase extraction	GC-MS	Silylation using *N,O*-bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane	THC (**10**) and THCA (**11**)[20]
Blood	Liquid-liquid extraction	GC-MS	Trimethylsilyl	THCA (**3**)[21]
	Liquid extraction with acetonitrile	GC-MS	Trimethylsilyl	CBC (**1**) and THC (**10**)[22]
	Liquid-liquid extraction	2D-GC-MS	*N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide	THC (**10**), CBD (**3**), CBN (**7**), THCA (**11**) and 11-OH-THC (**8**)[23]
	Solid-phase extraction	GC-MS/MS	Bis(trimethylsilyl)trifluoroacetamide and trimethylchlorosilane (99:1)	THC (**10**), 11-OH-THC (**8**) and THCA (**11**)[28]
Brain	Liquid extraction with acetonitrile	GC-MS	Trimethylsilyl	CBC (**1**) and THC (**10**)[22]
Breast milk	Headspace solid-phase microextraction	GC-MS	Trimethylsilyl	THC (**10**), CBD (**3**) and CBN (**7**)[31]
Bronchoalveolar lavage	Extracted with 10% EtOAc in n-hexane	GC-MS	Trimethylsilyl	THC (**10**), CBD (**3**), THCA (**11**), 11-OH-THC (**8**) and CBN (**7**)[32]
Hair	Headspace solid-phase micro-extraction	GC-MS/MS (ion-trap tandem MS)	N/A	THC (**10**), CBD (**3**) and CBN (**7**)[33]
	Bead-assisted liquid-liquid extraction	GC-NCI-MS/MS	N/A	THC-COOH (**11**)[34]

	Solid-phase extraction (SPE)	GC-MS	Trimethylsilyl	THC (**10**), CBD (**3**) and CBN[7]
	Liquid-liquid extraction	A single-step GC-MS/MS	*N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA)	THC (**10**), CBD (**3**) , 11-OH-THC (**8**) and CBN (**7**)[35]
	Solid-phase extraction (SPE)	GC-MS/MS	*N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA)	THC (**10**), CBN (**7**) and CBD (**3**)[36]
	Solid-phase extraction (SPE)	GC-MS/MS	Trimethylsilyl	THC (**10**), CBD (**3**), CBN, THCA (**11**) and 11-OH-THC (**8**)[37]
	Liquid-liquid and solid-phase extraction	GC-MS/MS	*N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA)	THC (**10**), CBD (**3**), CBN (**7**), THCA (**11**) and 11-OH-THC (**8**)[38]
Meconium samples	Accelerated solvent extraction (ASE) followed by solid-phase extraction (SPE)	GC-MS	N/A	THCA (**11**)[39]
Oral fluid	Solid-phase extraction (SPE)	2D-GC-MS	*N,O*-bis(trimethylsilyl)-trifluoroacetamide, trifluoroacetic anhydride and hexafluoroisopropanol	THC (**10**), CBD (**3**), CBN (**7**), and metabolites, 11-hydroxy-THC (**8**) and THCA (**11**)[41]
	N/A	GC-MS/MS	Hexafluoroisopropanol and trifluoro acetic anhydride	THCA (**11**)[42]
Plasma	Solid-phase extraction (SPE)	2D-GC-MS	*N,O*-bis(trimethylsilyl) trifluoroacetamide with 1% trimethylchlorosilane	THC (**10**), CBD (**3**), 11-OH-THC (**8**) and THCA (**11**)[43]
	Liquid-liquid extraction	GC-MS	Trimethylsilyl	CBD (**3**)[24]
	Liquid-liquid extraction	GC-MS/MS	*N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA)	CBD (**3**), THC (**10**), THCA (**11**), 11-OH-THC (**8**)[26]
	Solid-phase extraction using	GC-MS	Trimethylsilyl	THC (**10**), THCA (**11**), 11-OH-THC (**8**)[27]

	anion exchange sorbent			
	Microextraction by packed sorbent	GC-MS/MS	*N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA)	THC (**10**), 11-OH-THC (**8**) and THCA (**11**)[29]
Serum	Automated liquid-liquid extraction	GC-MS	*N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA)	THC (**10**), 11-OH-THC (**8**), and THCA (**11**)[25]
	Solid-phase extraction using anion exchange sorbent	GC-MS	Trimethylsilyl	THC (**10**), THCA (**11**), 11-OH-THC (**8**)[27]
	Extraction with 10% EtOAc in *n*-hexane	APGC-MS/MS	*N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA)	THC (**10**), 11-OH-THC (**8**), THCA (**11**), CBD (**3**), CBDA (**4**) and CBG (**5**)[30]
Urine	Liquid-liquid extraction	GC-MS	Trimethylsilyl	THCA (**11**)[21]
		GC-MS	*N*-Methyl-*N*-(trimethylsilyl)trifluoroacetamide	CBG (**5**)[44]
		GC-MS/MS	Microwave-accelerated derivatisation. *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA), and MSTFA/ethanethiol/NH_4I	THCA (**11**)[45]
		GC-MS	Tri-fluoroacetic anhydride and pentafluoropropanol	THCA (**11**)[47]
	Silica column-based extraction	GC-MS	*N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA)-trimethylchlorosilane (TMCS) and *N*-methyl-bis(trifluoroacetamide) (MBTFA)	THCA (**11**)[46]
	Hollow fibre-liquid phase microextraction	GC-MS	Trimethylsilyl	THCA (**11**)[48]
Herbal products like 'Spice'	Precludes liquid-liquid extraction	GC-MS with solid probe	N/A	Nontraditional cannabinoids[49]

‘Legal high’ or ‘herbal high’	Ultrasonic extraction with ethanol	GC-MS	N/A	Cannabicyclohexanol[50]