Techniques and technologies for the bioanalysis of Sativex®, metabolites and related compounds

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Abstract

Sativex® is an oromucosal spray indicated for the treatment of moderate to severe spasticity in multiple sclerosis and is also an effective analgesic for advanced cancer patients. Sativex contains Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD) in an approximately 1:1 ratio. The increasing prevalence of medicinal cannabis products highlights the importance of reliable bioanalysis and re-evaluation of the interpretation of positive test results for THC, as legal implications may arise in workplace, roadside and sports drug testing situations. This article summarises published research on the bioanalysis of THC and CBD, with particular focus on Sativex. Common screening and confirmatory testing of blood, urine, oral fluid and hair samples are outlined. Correlations between matrices and current analytical pitfalls are also addressed.

Background

Cannabis has been used for its psychoactive effects and medicinal properties since at least the sixth century BC. Research into cannabis expanded after the mid-1960s as recreational use of the drug erupted in the United States (US) and Europe [1]. Following the structural elucidation of Δ^9-tetrahydrocannabinol (THC) in 1964 and its synthesis in 1967 by Mechoulam *et al.* [1], it became widely researched as the only major psychoactive constituent of cannabis. Mechoulam *et al.* also elucidated the structure of cannabidiol (CBD), another major cannabinoid found in cannabis, in 1963. However, it was initially neglected in research due to its lack of psychoactivity [1]. More recently, CBD has been found to have a number of therapeutic pharmacological effects [2].

Cannabinoid content varies considerably between *Cannabis sativa* L. plant varieties. Many strains have been developed to be rich in THC for increased potency. Even within strains there is great variability in cannabinoid ratios. There is little data available on the CBD content in cannabis products, however as THC content has increased over the years, CBD levels have remained low. In the US, THC content in cannabis rose from <3.4% in 1993 to 8.8% in 2008, while CBD content remained low at 0.4% in 2008 [3]. In Australia, an average THC and CBD content in cannabis samples obtained from recreational users were found to be 14.88% and 0.14%, respectively [4].

THC affects the central nervous system (CNS) by slowing down the messages travelling between the brain and the body through its actions at presynaptic receptors, inhibiting the release of neurotransmitters [5]. The psychotropic effects associated with cannabis use have been correlated to the actions of THC as a partial agonist to cannabinoid CB1 receptors in the CNS and peripheral CB2 receptors [6]. Cannabis has both hallucinogenic and depressant

properties resulting in CNS effects such as a sense of relaxation, loss of inhibition, impaired coordination, reduced concentration, hallucinations, anxiety, reduced brain function and paranoia, most of which are attributed to the actions of THC [7]. CBD in particular, has antipsychotic and anxiolytic CNS effects [2]. CBD has a weak affinity for the CB_1 and CB_2 receptors, but has been shown to act as an antagonist of CB_1 and CB_2 agonists, resulting in some observed modulation of the effects of THC [8]. CBD has also been found to have antioxidant, anti-inflammatory and neuroprotective properties [2]. CBD itself has thus been investigated for its potential clinical use in the treatment of psychosis, epilepsy, anxiety and sleep disorders, diabetes, hypertension, cerebral and myocardial ischemia, depression, obesity, and neurodegenerative diseases including amyotrophic lateral sclerosis (ALS), Alzheimer's, Parkinson's and Huntington's diseases [2, 8, 9].

Sativex® (nabiximols) is an oromucosal spray containing THC and CBD in an approximately 1:1 ratio. It is indicated for the treatment of moderate to severe spasticity in multiple sclerosis where patients have not adequately responded to existing treatments [10, 11] and is also effective as an analgesic for advanced cancer patients [6, 12, 13]. Pre-clinical trials of 1:1 formulations of THC and CBD and clinical trials of Sativex have also been carried out to evaluate potential in treating pain caused by rheumatoid arthritis [14], as a neuroprotective agent in ALS [15] and Huntington's disease [16], and in minimising withdrawal symptoms in chronic cannabis smokers [17].

There is a large body of research covering the bioanalysis of THC and its metabolites due to the widespread illicit use of cannabis. Clinical, forensic, and workplace drug testing of cannabis generally target THC and its major metabolites, 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC) and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH) in blood, urine, oral fluid and hair to monitor cannabis use. Bioanalysis of CBD has also been investigated, albeit to a lesser extent. The use of cannabinoids in medicinal cannabis products increases the importance of reliable bioanalysis and re-evaluation of the interpretation of positive test results for THC, as legal implications may arise in workplace, roadside, and sports drug testing situations.

This review summarises the recent research published on the bioanalysis of THC and CBD, with particular focus on Sativex, in blood, urine, oral fluid and hair. Available screening tests as well as confirmatory testing techniques using gas chromatography–mass spectrometry (GC–MS) and liquid chromatography–mass spectrometry (LC–MS) are outlined. Correlations between matrices and analytical pitfalls are also addressed.

Formulation and synthesis of Sativex

Sativex is the lead product of manufacturer GW Pharmaceuticals. The oromucosal spray is available in a 10 mL vial containing 27 mg/mL THC and 25 mg/mL CBD dissolved in ethanol, propylene glycol and peppermint oil [10]. The spray delivers approximately 100 μL per actuation containing 2.7 mg THC, 2.5 mg CBD, and up to 0.04 g ethanol [10]. The process of manufacturing Sativex at GW Pharmaceuticals was reviewed by Potter [18] and is briefly summarised here.

GW Pharmaceuticals grows cannabis plants in pots in a highly regulated glasshouse environment. Lighting and temperature are strictly controlled to minimise variability between crops and to enhance yield. Sativex is produced from two separate cannabis extracts. Homozygous plants that produce mainly either THC or CBD are grown, and the resulting extracts are mixed to provide a standardised medicine containing THC and CBD at an approximately 1:1 ratio. The use of two homozygous plants gives a more reliable ratio of THC: CBD than growing heterozygous plants that produce both THC and CBD, due to a large variability in ratios being dependent on a multitude of environmental factors. Additionally, cuttings of the highest performing plants are taken for propagation as this leads to more uniform plants than if the seedlings of those plants were used.

The majority of cannabinoids are produced in the female flowers of the cannabis plant, therefore GW Pharmaceuticals enhance cannabinoid yields in their crops by growing female plants in the absence of pollen which extends the flowering period. Cannabis naturally begins to flower in autumn, when night length begins to increase. This is simulated in the glasshouse using electric lighting to create 12 hr days and 12 hr nights when appropriate. Sativex (THC genotype) plants are typically harvested after eight weeks in short day length. The dried plant material, including the foliage and flora, is uniformly heated to decarboxylate the precursors THC-A and CBD-A to THC and CBD. The plant material is then immersed in liquid carbon dioxide at a high pressure to dissolve the ingredients, which are then separated and purified to create the extracts. Finally, the extracts from the THC and CBD plants are mixed with the other excipients, ethanol, propylene glycol and peppermint oil.

Disposition and metabolism

When cannabis is smoked, THC is quickly absorbed and can be measured in the plasma seconds after beginning smoking [19]. Plasma protein binding of THC is high (94–99%) [20]. The high lipophilicity of cannabinoids results in rapid redistribution into the fatty tissues where they can be stored for weeks and slowly excreted at low concentrations [21]. CBD has similar pharmacokinetic properties as THC. Both THC and CBD are rapidly converted to

their metabolites with a similar plasma clearance [21]. Median plasma maximum concentrations of THC and CBD were found to be 76 and 2.0 ng/mL, respectively, at 15 min after beginning smoking a single cigarette containing 54 mg of THC and 2.0 mg of CBD [22]. CBD was not detectable after 1 hr whereas THC was detected for at least 2 hr with a 30% detection rate in plasma after 22 hr [22]. Mean plasma levels of CBD were 5.9–11.2 ng/mL during daily oral dosage of 10 mg/kg CBD via capsules [23]. CBD was detectable for a week at 1.5 ng/mL after dosage ceased [23], a much longer window of detection than that observed following smoking. Following smoking of one cannabis cigarette, concentration of THC and its metabolites were found to be higher in frequent smokers and detectable for longer, when compared with occasional smokers, however, CBD levels did not vary greatly [24].

THC and CBD administered via an oromucosal spray, result in significantly lower cannabinoid blood concentrations due to the relatively slower adsorption via this route and the subsequent rapid redistribution into the fatty tissues [25]. Mean peak plasma concentrations were found to be reached an average of 1 hr after administration of Sativex and increased with increasing multiple doses, though there was no evidence of accumulation with multiple doses [25]. Concentrations of THC and CBD have been found to reach a maximum ~2.5 hr later in subjects who had been fed versus those who had fasted [26]. THC bioavailability is low at 6% when administered orally through capsules compared with inhaling (up to 27%) due to extensive first-pass metabolism [27]. The bioavailability of THC from capsules containing cannabis extract with 10 mg THC and 5.4 mg CBD was 3–14% [28]. The bioavailability of THC from administered Sativex was 13.1% and 11.0% for 5 and 15 mg doses, respectively [27]. When CBD was smoked, bioavailability ranged from 11% to 45% with an average of 31% across five subjects [21]. CBD was observed to have a lower bioavailability than THC after administration of Sativex [25].

Phase I metabolism of THC and CBD occurs primarily in the liver with the aid of cytochrome P450 enzymes. THC is hydroxylated at the C11 position to the active metabolite 11-OH-THC (Figure 1a). This is further oxidised to the inactive THC-COOH (Figure 1b) and a number of other minor metabolites [20, 29]. CBD is extensively metabolised and similarly undergoes hepatic first-pass metabolism to 7-OH-CBD which is further oxidised to CBD-7-oic acid (Figure 2a-b) [30]. A number of additional hydroxylated and oxidised minor metabolites of THC [20] and CBD [31] are also formed, however the aforementioned major phase I metabolites are the most important from a bioanalysis point of view. Phase II metabolism of the cannabinoids by UDP-glucuronosyltransferases [32] yields more hydrophilic metabolites including THC-glucuronide (THC-glu) and THC-COOH-glucuronide (THC-COOH-glu) from THC (Figure 1c-d) [24, 33] and CBD-glucuronide (CBD-glu) and 7-OH-CBD-glucuronide (7-OH-CBD-glu) from CBD (Figure 2c-d) [34, 35]. The major excretion route of THC is via the faeces as the conjugated 11-OH-THC and THC-

COOH. THC-COOH and other carboxylic acid metabolites are also excreted in the urine in both free and conjugated forms [20]. Plasma concentrations of THC-COOH increase slowly over the first hour following the commencement of smoking and levels plateau by 2–4 hours [24]. A high percentage of free-CBD is excreted in the faeces [36]. A significant portion of CBD excreted in urine is in its glucuronide form [34, 35].

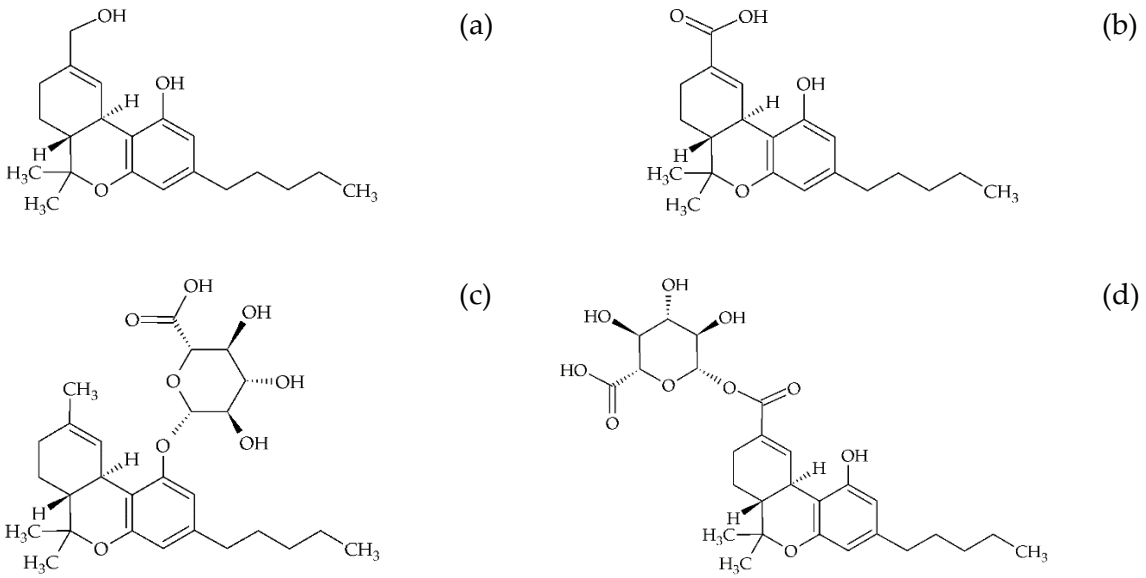

Figure 1: Major metabolites of THC. Phase I metabolites 11-OH-THC (a) and THC-COOH (b); Phase II metabolites THC-glu (c) and THC-COOH-glu (d).

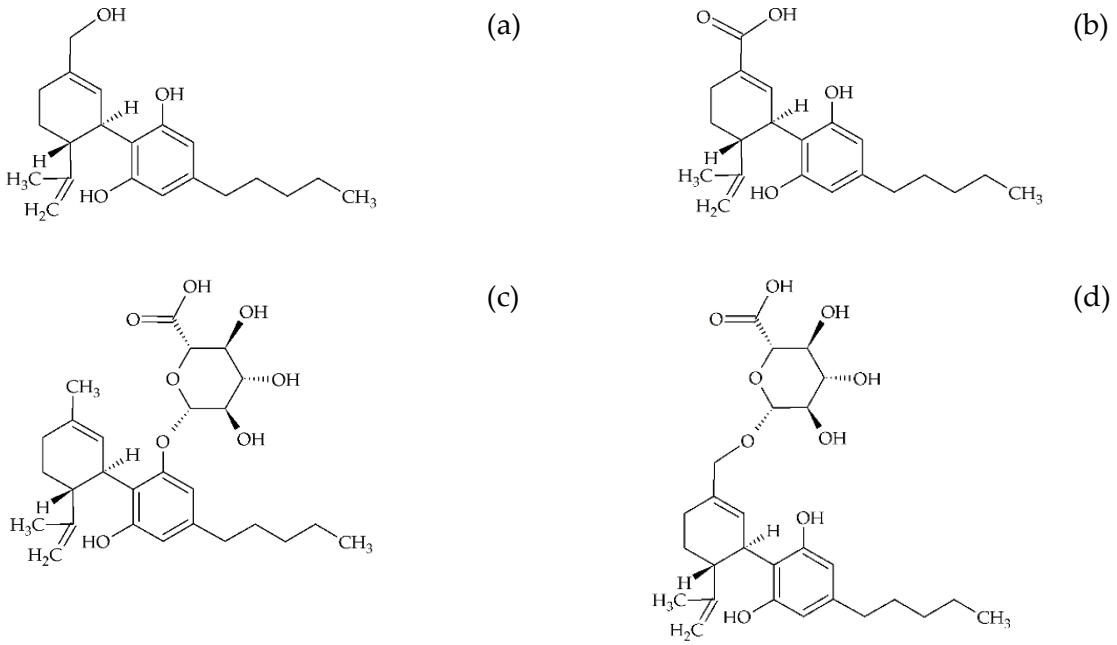

Figure 2: Major metabolites of CBD. Phase I metabolites 7-OH-CBD (a) and CBD-7-oic acid (b); Phase II metabolites CBD-glu (c) and 7-OH-CBD-glu (d).

Nadulski *et al.* [28] found evidence that CBD partially inhibits the CYP 2C catalysed hydrolysis of THC to 11-OH-THC, although the effect was relatively small. The modulating

effects of CBD on THC were determined to be unlikely due to pharmacokinetic interactions at low, therapeutic doses as the bioavailability of THC did not significantly change when CBD was present [27, 28].

Methods of bioanalysis

Most commonly, cannabis testing is performed using urine, plasma or whole blood, or oral fluid. Other matrices such as hair and sweat have also been regarded as useful for drug analysis in some circumstances. Target analytes vary between matrices, but generally include the major cannabinoids, THC, CBD and cannabinol (CBN), and THC metabolites, 11-OH-THC, THC-COOH and THC-COOH-glu and THC-glu. On-site immunoassay screening tests for cannabis are widely available for urine and oral fluid testing, though none target CBD. Urine screening tests mainly target THC-COOH, while oral fluid tests target the parent THC. Laboratory-based immunoassay screening techniques include enzyme-multiplied immunoassay technique (EMIT), enzyme-linked immunosorbent assay (ELISA), cloned enzyme donor immunoassay (CEDIA) and fluorescence polarisation immunoassay (FPIA), which mainly target THC-COOH in urine and blood. Confirmatory laboratory testing focusses heavily on mass spectrometric techniques. GC–MS is the most widespread, although LC–MS/MS is catching up as detection limits have recently matched those of GC–MS and less samples preparation is required due to the lack of a derivatising step necessary for GC–MS. GC–MS has continued to be prominent as it evolves into more sensitive GC–MS/MS and 2D–GC–MS forms. Due to the isobaric relationship and structural similarities between THC and CBD, they produce a similar mass fragmentation pattern and therefore need to be separated chromatographically, or subjected to a derivatisation that yields distinguishable products in the mass spectrum [37, 38].

Blood

Both plasma and whole blood matrices can be analysed to determine drug use. These matrices are most useful for post-accident and post-mortem analyses as samples are collected off-site at a hospital or morgue, where the necessary specialised personnel and facilities are readily available. THC has a plasma half-life of about 2 hours; after which it is converted into its metabolites, therefore the detection of THC rather than THC-COOH in plasma, is a useful indicator of recent use [39]. However, THC is present in lower concentrations, requiring sensitive analysis techniques. Karschner *et al.* [27] found average peak plasma CBD concentrations of 1.6 and 6.7 ng/mL, 3.7 and 4.0 hr after low (5.4 mg THC; 5.0 mg CBD) and high (16.2 mg THC; 15.0 mg CBD) Sativex doses, respectively; THC gave average peak plasma concentrations of 5.1 and 15.3 ng/ml at 3.3 and 4.0 hr after low and high Sativex doses. THC-COOH was present in plasma in much higher concentrations at 108.0 and 126.6 ng/ml, 4.4 and 4.8 hr following dosage [27]. Subjects given oral doses of 10 mg THC and 5.4 mg CBD via capsules had peak plasma concentrations of 4.05 ng/mL THC and 0.95 ng/mL CBD 1 hr following dosage [38].

Elimination of cannabinoids is variable between individuals and metabolites can be detected in the blood of chronic daily smokers during a month of sustained abstinence [40]. There is also some evidence that fat-stored THC can be redistributed into the blood of chronic users following extensive exercise [41]. These effects can have implications for the interpretation of single positive test results.

Screening tests

Laboratory-based blood screening for drugs is well established; the most common methods of screening are immunoassay-type tests such as ELISA, and LC–MS. LC–MS/MS techniques have been utilised to rapidly screen for multiple drugs at once. These generally target the major metabolite of each drug, so THC-COOH is generally the target compound for cannabis. ELISA kits have been used to detect THC-COOH in blood with a cut-off of 20 ng/mL [42]. Neither ELISA nor LC–MS screening methods target CBD.

Confirmatory tests

Nadulski *et al.* [38] quantified cannabinoids and THC metabolites in plasma by GC–MS. Derivatisation was found to be essential for the detection of 11-OH-THC and THC-COOH, and improved chromatography for THC, CBD and CBN. Various derivatising agents were evaluated, and *N*-*O*-bistrifluoroacetamide (BSTFA) was found to be more suitable than methylating and other silylating agents based on derivatising efficiency, stability and availability. Quantification limits of the trimethylsilyl (TMS) derivatives monitored in selected ion monitoring (SIM) mode following auto-SPE were 0.080 ng/mL (THC), 0.95 ng/mL (CBD), 3.9 ng/mL (CBN), 0.5 ng/mL (11-OH-THC) and 0.88 ng/mL (THC-COOH). Stott *et al.* [26] also used GC–MS to detect the TMS derivatives of THC, CBD and 11-OH-THC, and achieved an LOQ of 0.1 ng/mL for all analytes. Karschner *et al.* [43] validated a 2D–GC–MS method to quantify THC, CBD, 11-OH-THC and THC-COOH in plasma. THC, CBD and THC-COOH were quantifiable down to 0.25 ng/mL and 11-OH-THC was quantifiable at 0.125 ng/mL from 1 mL plasma samples extracted by SPE and derivatised with BSTFA.

Jagadeo *et al.* [44] achieved quantification limits of 2 ng/mL for THC and 3 ng/mL for CBD, 11-OH-THC and THC-COOH in 1 mL samples of whole blood using a method involving protein precipitation with acetonitrile and LC–MS/MS with online SPE. Whole blood analysis of cannabinoids and THC metabolites including glucuronides was achieved by Schwope *et al.* [45] using LC–MS/MS following protein precipitation and an SPE method modified to reduce build-up of phospholipids that can cause ion suppression. The LOQ of THC, CBD, CBN, 11-OH-THC and THC-COOH was 1.0 ng/mL, THC-glu had an LOQ of 0.5 ng/mL and THC-COOH-glu had an LOQ of 5.0 ng/mL [45]. THC-glu and THC-COOH-glu were quantified in a real sample at 0.6 ng/mL and 96 ng/mL, respectively [45].

Urine

Routine urine testing involves the detection of cannabis metabolites, THC-COOH and THC-COOH-glu. The other major metabolite, 11-OH-THC, is also of interest in research studies [46]. Due to its relatively large window of detection, urine testing is useful for determining if a person has used drugs within the last few days. Additionally, urine testing can detect the metabolites of THC regardless of the route of consumption. Urine has been thoroughly researched as a matrix for drug testing and testing procedures are well-established. However, urine testing has some significant disadvantages, such as its ease of adulteration either through dilution or substitution, particularly due to privacy issues with collection. The large and varied window of detection observed can also pose problems when interpreting results, as the metabolites of THC may be found in the urine up to 12 days after a single oral dose [20] and even longer if the subject is a regular user. Although drug testing of any kind cannot itself confirm that an individual is impaired, urine testing is widely accepted as a means of identifying the risk of impairment.

Due to the introduction of CBD-containing medicines such as Sativex, it has become of interest to detect CBD in urine. Generally, urine testing for cannabis targets the THC-COOH metabolite as it is present in much higher concentrations than the parent THC. Analysis of CBD in urine is relatively new, and so the parent compound is targeted due to lack of knowledge of the exact mechanism of its metabolism to CBD-7-oic acid. Commercial standards for this compound are not yet available, partly due to the difficulty in its synthesis. A 10-step synthesis has been outlined previously [47]. As need arises for the analysis of the CBD metabolite in urine, more effort will be put into developing a standard.

Screening tests

Immunoassay screening is often performed on urine samples either on-site or in a laboratory. Common types of tests include ELISA and CEDIA with cut-off concentrations typically ranging from 25 to 100 ng/mL. EMIT has also been used [38]. These immunoassays all target the metabolite THC-COOH, though they also have varying cross-reactivities with other cannabinoids. The cross-reactivity with CBD is generally quite low in these tests, and so they are unlikely to give a positive result if CBD is the sole cannabinoid present.

Confirmatory tests

Cannabinoids are mainly present in urine in their glucuronated forms. Alkaline or enzymatic hydrolysis of urine samples is performed to cleave glucosidic bonds, allowing for the free THC, THC-COOH and CBD to be analysed by GC–MS techniques following derivatisation. Alkaline hydrolysis is more effective for de-conjugating THC-COOH-glu [34], while enzyme hydrolysis using *E. coli* beta-glucuronidase is preferred for releasing free

THC [48]. Enzyme hydrolysis using beta-glucuronidase isolated from red abalone resulted in a 250-fold higher concentration of CBD compared with alkaline hydrolysis or no hydrolysis [34]. Tandem alkaline/enzyme hydrolysis has also been performed when analysing THC and THC-COOH simultaneously [49]. This may be useful for future simultaneous analyses of CBD and CBD-7-oic acid in urine. To avoid uncertainties due to hydrolysis variability, glucuronide conjugated metabolites can be analysed directly with the use of LC–MS/MS [50]. Following hydrolysis, sample clean-up procedures utilising either SPE or LLE are performed.

Derivatisation before GC–MS analysis is typically performed using BSTFA with 1% trimethylsilyl chloride (TMCS) giving trimethylsilylated derivatives [34, 49, 51]. A combination of (pentafluoropropionic anhydride) PFPA and (pentafluoropropanol) PFPOH has also been used to produce acylated derivatives [52]. An LOQ of 0.9 ng/mL was achieved for THC-COOH using GC–MS following enzyme hydrolysis and derivatisation. CBD and THC were found to be quantifiable down to 3.4 and 3.9 ng/mL, respectively [51].

An LOQ of 2 ng/mL for THC and THC-COOH was achieved using an LC-MS method after alkaline hydrolysis [46]. Use of LC with tandem MS following enzyme hydrolysis achieved an LOQ of 1 ng/mL for CBD, THC and THC-COOH [53]. Without hydrolysis, an LC–MS/MS method was able to simultaneously quantify THC-glu at 0.5 ng/mL and THC-COOH-glu at 5 ng/mL; free THC-COOH and CBD were also quantified down to 1 ng/mL and THC down to 2 ng/mL [50]. Also using LC–MS/MS without any hydrolysis, Wei *et al.* [54] recently achieved very low LOQ values at 0.008 ng/mL for THC and CBN, 0.012 ng/mL for CBD, 0.018 ng/mL for THC-COOH and 0.028 ng/mL for 11-OH-THC.

Drug metabolite concentrations in urine are heavily dependent on hydration and may be diluted with increased urine output. Therefore, measured concentrations of any drug compound or metabolite are routinely normalised using creatinine concentrations, which are relatively stable in urine. Determining concentrations of THC-COOH as ng/mg creatinine allows for comparisons between samples and can help identify re-use based on THC-COOH/creatinine ratios in sequential samples from an individual [55-57]. These could potentially be applied to CBD or CBD-7-oic acid concentrations found in urine.

Oral fluid

Oral fluid testing is advantageous for determining recent use of cannabis since the target analyte is the parent THC compound. THC is deposited in the oral cavity directly from smoking and is only detectable for a few hours. Following administration of a dose of Sativex containing 5.4 mg THC and 5.0 mg CBD, Lee *et al.* [58] found oral fluid concentrations of THC and CBD both peaked at well over 1000 ng/mL. Both cannabinoids remained at detectable levels (1.0–60.0 ng/mL THC, 0.5–67.8 ng/mL CBD) up to 10.5 hr after

dosage [58]. Following heavy dosage patterns, THC concentrations peaked between 5356 and 15,468 ng/mL and CBD peaked over the range of 3826 to 17,233 ng/mL [37]. The average THC/CBD ratio was 1.10 (%RSD 19.9), consistent with the composition of the Sativex spray. CBN was also detected (2.08–593 ng/mL) in all Sativex positive samples with peak concentrations correlating with peak THC and CBD concentrations. CBN is likely present in the formulation as a result of the extraction process. Although the blood concentrations of THC are much lower in Sativex patients, and perhaps do not result in significant impairment, oral fluid concentrations may still be quite high due to these initial deposits left in the mouth by the spray. This could lead to difficulties interpreting positive results when the purpose of testing is to determine the likelihood of impairment.

Screening tests

There are a vast number of point-of-care screening devices available for the detection of THC in oral fluid. Some of the devices that have been evaluated in peer-reviewed journals within the last few years include the Cozart® DDS, Dräger DrugTest® 5000, RapidSTAT®, DrugWipe® 5+, OraLab® 6, OrAlert™ and OraTect® III (Table 1) [59-63]. All the currently available devices target the parent compound THC, though many have recorded cross-reactivity with 11-OH-THC and THC-COOH. No current screening methods detect CBD. A laboratory based ELISA screening kit from Immunalysis has also been used for the detection of THC in oral fluid [64, 65]. This test kit targets THC, however it has also showed cross-reactivity with other cannabinoids, including CBD at 50% [64].

Table 1: Oral fluid screening tests for cannabis

Test device	Manufacturer	THC cut-off (ng/mL)	Reference(s)
Cozart® DDS	Alere	31	[59, 61]
Dräger DrugTest® 5000	Dräger Safety	5	[59-63]
RapidSTAT®	Mavand	15	[59-62]
DrugWipe® 5+	Securetec	30	[59-62]
OraLab® 6	Varian	50	[59]
OrAlert™	Innovacon	100	[59]
OraTect® III	Branan	40	[59]
ELISA kit	Immunalysis	4	[64, 65]

The DrugWipe® II Twin and Cozart® DDS devices were used to conduct screening tests in participants receiving Sativex treatment as an aid to withdrawal. The Cozart® DDS device successfully detected THC in all the participants who were receiving the Sativex treatment, with confirmed oral fluid concentrations all >52.4 ng/mL [37]. The DrugWipe® device however, had a very low sensitivity to the Sativex spray, as negatively screened samples were found to contain 52–11,624 ng/mL THC [37]. Samples that screened positive were in the range of 166–15,468 ng/mL THC [37].

Confirmatory tests

Due to the viscosity of oral fluid, many collection devices dilute oral fluid at the point of collection so lower limits of detection are necessary to accurately quantify cannabinoids in the neat oral fluid. Additionally, the oral fluid matrix and some commercial collection buffers can cause ion suppression issues in LC analyses; however, this is largely overcome by using a sample clean-up method. A number of methods to simultaneously analyse multiple cannabinoids including both THC and CBD in oral fluid have recently been published. These methods utilise GC–MS [66-68], 2D–GC–MS [69] and LC–MS/MS [37, 68, 70-72]. Solid phase extraction is the most common form of sample preparation, however modified versions including SPME (solid phase micro-extraction) [68] and MEPS (micro-extraction by packed sorbent) [72] have also been used, as well as liquid-liquid extraction [37, 71].

Expectorated oral fluid is sometimes used, however oral fluid is often collected using a collection device such as the Quantisal [69-71] and Cozart DDS [37] collectors. Both collectors dilute the collected oral fluid 1:3 with a stabilising buffer. Volumes of oral fluid or oral fluid/buffer mixes used for analysis range from 125–1000 μL. LOQs of THC and CBD were 0.5–2 and 0.9–2 ng/mL, respectively, when analysed by GC–MS [66-68]. Using LC–MS/MS, LOQs were 0.25–1 ng/mL for THC and 0.3–2 ng/mL for CBD [37, 71-73]. Milman *et al.* achieved a 0.5 ng/mL LOQ for both THC and CBD collected by the Quantisal device and analysed using 2D–GC–MS [69]. Similarly, Concheiro *et al.* achieved the same quantification limits using micro-flow LC–MS/MS [70]. Milman *et al.* also successfully validated their method for 500 μL neat, expectorated oral fluid with an LOQ of 0.25 ng/mL for both THC and CBD [74].

Cannabinoids CBN and tetrahydrocannabinolic acid A (THCA-A) and THC metabolites, 11-OH-THC and THC-COOH are also often targeted in simultaneous cannabinoid quantification methods. It has been suggested that the detection of 11-OH-THC and THC-COOH in oral fluid is indicative of active cannabis smoking, rather than passive exposure, since these metabolites are not present in cannabis smoke [64]. THC-COOH is present in oral fluid at only pictogram per millilitre concentrations, hence methods must be suitably sensitive. It may be worth investigating whether any THC precursor, THCA-A remains in the cannabis extracts used to produce Sativex. Smoking cannabis results in only partial decarboxylation of THCA-A [75]. Fabritius *et al.* [71] found relatively high concentrations of THCA-A in oral fluid after the smoking of cannabis joints. Peak THCA-A concentrations of 44–2031 ng/mL correlated with peak THC concentrations in the oral fluid shortly after commencing smoking indicating that it is also deposited into the oral cavity [71]. If Sativex contains a very small amount of THCA-A or none at all, elevated levels found in the oral fluid could indicate use of other cannabis products.

Hair

Cannabinoids may be incorporated into hair by passive diffusion from the bloodstream during growth of the hair fibre, secretions from sweat and sebaceous glands, or by deposition of external contaminants [76]. This matrix is most useful for estimating the approximate time and duration of past events as the growth rate of the hair and distance of the hair section from the scalp can provide this type of information. Specific time intervals cannot be determined due to the varied growth rates between individuals [77]. Increases and decreases in the concentration of cannabinoids found in different segments of hair can also indicate patterns of use over time. This makes hair analysis useful in the contexts of rehabilitation and child exposure to cannabis, among others [78]. Hair testing has a long window of detection and a user would have to abstain for three months to produce a negative sample for a typical 3 cm hair sample [77]. Though problematic if used alone, hair testing may be complementary to other matrices as it can give an indication as to whether a positive blood, urine or oral fluid test is due to a single use or regular use of cannabis.

Methods for the analysis of cannabinoids in hair often include CBD, and CBN as well as THC. Therefore, these methods could be directly applied to Sativex patients to monitor medication compliance. An issue described with hair analysis is the difficulty in distinguishing between cannabinoids that are within the hair and external contamination [79]. This has been mostly overcome by performing confirmatory analysis of the THC metabolite, THC-COOH. However, the metabolite is incorporated into hair at smaller concentrations compared with the parent THC, so it may not be detected if only small doses were used [77]. According to the Society of Hair Testing, the recommended cut-off for the screening of cannabinoids in hair is 100 pg/mg and the cut-offs for confirmatory analyses are 50 pg/mg for THC and 0.2 pg/mg for THC-COOH [80].

Hair collection is simple and non-invasive. Strands are collected from the back of the head (vertex posterior) either by pulling, or cutting as close to the scalp as possible [76, 81, 82]. Hair segments of 3 cm in length are most commonly used though different sectioning patterns are used for different testing purposes [77]. Hair samples are much more stable than other matrices and can be stored at ambient temperatures until analysis [76, 82]. In order to minimise the detection of external contaminants, hair samples are decontaminated by sequential washes, sometimes first with water [76, 78], followed by two or three washes with a solvent such as methylene chloride [82, 83], dichloromethane [76, 78, 84] or isopropanol [81]. The final wash is typically retained for analysis in order to confirm no external cannabinoids remain in the samples. Strands are then either cut into segments <1–2 mm [78, 81, 84] or pulverised using a ball mill [82, 85]. Samples of 10 to 100 mg are then subjected to alkaline hydrolysis to destroy the hair and release the analytes, most commonly performed using 1 mL of 1 M sodium hydroxide and incubated for 10–30 min at 90–100° C [76, 78, 81-84]. Analytes are extracted after cooling, often by liquid-liquid extraction with a

mixture of n-hexane and ethyl acetate [78, 81-84]. Solid phase extraction has also been used [86]. If THC-COOH is an analyte of interest, acetic acid is added to reduce the pH to allow for the extraction of the acidic metabolite [78, 83, 86].

Screening tests

Cannabinoids are present in hair in much lower concentrations than urine, blood, and oral fluid, so sensitive instrumentation is required for its analysis and GC–MS is commonly used for this purpose. Cirimele *et al.* [82] recommended using GC–MS in SIM mode to rapidly screen for THC, CBD and CBN and then confirm results by analysing for THC-COOH. The limits of detection achieved were 0.1 ng/mg for THC, 0.02 ng/mg for CBD and 0.01 ng/mg for CBN. Screening using the rapid GC–MS method detected cannabinoids in real samples at ranges of 0.1–0.29 ng/mg THC, 0.03–3.00 ng/mg CBD and 0.01–1.07 ng/mg CBN. Huestis *et al.* [86] adapted an ELISA kit designed for use with blood and urine samples to analyse hair samples. The immunoassay achieved an LOD of 2 pg/mg THC, though they ultimately used a cut-off of 5 pg/mg for screening and found cross-reactivity of 340% THC-COOH, 51% CBN but only 0.1% CBD when compared with THC [86].

Confirmatory tests

GC–MS methods have been most common for the analysis of cannabinoids in hair, however, due to the low concentrations of THC-COOH found, more sensitive instrumentations are now preferred. These typically include tandem MS systems coupled to either GC [76, 86] or LC [78, 84].

Samples are generally derivatised before analysis by GC–MS. Derivatisation using PFPA/PFPOH allowed for the detection of THC-COOH in collected hair samples at 0.02–0.39 ng/mg hair by negative chemical ionisation GC–MS [83]. Another method by Kim *et al.* involving derivatisation using *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide/trimethylsilyl chloride/*N*-(trimethylsilyl)imidazole (MSTFA/TMCS/TMSI) at 60 °C for 15 min achieved an LOQ of 0.05 ng/mg hair for THC, CBD and CBN [81]. A method utilising GC–MS/MS with an LOQ of 1 pg/mg for THC and 0.1 pg/mg for THC-COOH enabled Huestis *et al.* [86] to detect THC down to 3.4 pg/mg and THC-COOH at 0.10–7.3 pg/mg in real hair samples. Emidio *et al.* [76] achieved LOQ values of 62 pg/mg THC, 12 pg/mg CBD and 30 pg/mg CBN using headspace SPME–GC–MS/MS. This technique efficiently combines analyte extraction and pre-concentration into a single step. LC–MS/MS methods using LLE have been able to quantify THC-COOH at 0.2 pg/mg [78] and THC, CBD and CBN at 3.9, 18 and 5.3 pg/mg [84]. Montesano *et al.* [87] were able to quantify THC-COOH at 0.1 pg/mg using pressurised liquid extraction followed by SPE before analysis by LC–MS/MS. LOQ values of 1.0 pg/mg for THC and 2.0 pg/mg for CBD and CBN were achieved by this method.

Using GC–MS, Kim *et al.* detected THC, CBD and CBN in samples collected from cannabis users at an average of 0.14, 0.04 and 0.36 ng/mg, respectively [81]. CBN was the most detected cannabinoid, THC the least, possibly due to pyrolitic degradation of THC during

smoking [81]. Similarly, Emidio *et al.* found hair samples more commonly contained CBN than THC with an average CBN concentration of 96 pg/mg compared with an average THC concentration of 56 pg/mg [76]. Analyses of hair samples by LC–MS/MS however, have not shown this trend. Salamone *et al.* [84] found THC, CBD and CBN in hair in ranges of 50–553 pg/mg, 18–1862 pg/mg and 31–205 pg/mg, respectively. Dulaurent *et al.* [78] detected a high amount of THC (1122 pg/mg) and no CBN in a hair sample.

Other matrices

Alternative matrices for the non-invasive detection of cannabis include sweat and exhaled air. Sweat patches that are placed on the body for hours or even days collect excreted sweat that can be analysed for the presence of drugs [88]. This is most suited to patient care and monitoring purposes, due to the timeframe required for sample collection. A faster screening test, the DrugWipe "K" is an immunoassay that can detect a number of illicit drugs including THC after being wiped across the forehead a few times [89]. The parent compounds are the analytes of interest in sweat testing so CBD could also be targeted in confirmatory analyses. However, passive contamination may be an issue and there is a 1–12 hr delay between drug administration and excretion into sweat [90]. Kintz *et al.* [90] found THC in the range of 4 to 152 ng/pad in 16 sweat samples collected using cosmetic pads spiked with water/isopropanol (1:1). Actual concentrations in sweat could not be determined since the volume of sweat collected is unknown. Metabolites 11-OH-THC and THC-COOH were not detected. SAMHSA guidelines include cut-off values of 4 ng/patch and 1 ng/patch for screening and confirmatory analysis, respectively, for the detection of parent THC in sweat patches [91]. Saito *et al.* [92] achieved an LOQ of 0.4 ng/patch for THC in sweat using PharmChek patches and GC–NCI–MS. Huestis *et al.* [93] found positive sweat patch results in daily cannabis users for at least a week after cessation of use, however THC was not detected after oral ingestion of up to 14.8 mg THC.

Skoglund *et al.* [94] found exhaled breath had a narrower window of detection than plasma or urine, making it useful for indicating recent use, however it was less sensitive than plasma analysis. Stephanson *et al.* [95] achieved an LOQ of 6 pg/filter THC in exhaled breath collected using a SensAbues device and analysed by LC–MS/MS. THC was successfully measured using this method in eight real samples at 27–557 pg/filter [95].

The LOQ values for the confirmatory analysis of cannabinoids in the matrices discussed above are summarized in Table 2.

Table 2: Summary of LOQ values for the detection of cannabinoids in biological matrices.

Matrix	Analysis method	LOQ* (analyte)	Ref.
Plasma	GC–MS	0.080 (THC); 0.95 (CBD); 3.9 (CBN); 0.5	[38]

		(11-OH-THC); 0.88 (THC-COOH)	
	GC–MS	0.1 (THC, CBD, 11-OH-THC)	[26]
	2D–GC–MS	0.25 (THC, CBD, THC-COOH); 0.125 (11-OH-THC)	[43]
Whole blood	LC–MS/MS	2 (THC); 3 (CBD, 11-OH-THC, THC-COOH)	[44]
	LC–MS/MS	1.0 (THC, CBD, CBN, 11-OH-THC, THC-COOH); 0.5 (THC-glu); 5.0 (THC-COOH-glu)	[45]
Urine	GC–MS	3.4 (THC); 3.9 (CBD); 0.9 (THC-COOH)	[51]
	LC–MS	2 (THC, THC-COOH)	[46]
	LC–MS/MS	1 (THC, CBD, THC-COOH)	[53]
	LC–MS/MS	0.5 (THC-glu); 5 (THC-COOH-glu); 1 (CBD, THC-COOH); 2 (THC)	[50]
	LC–MS/MS	0.008 (THC, CBN); 0.012 (CBD); 0.018 (THC-COOH); 0.028 (11-OH-THC)	[54]
Oral fluid	GC–MS	0.5 (THC, CBN); 1 (CBD, THCA-A)	[66]
	GC–MS	1.9 (THC); 0.9 (CBD); 4.8 (THC-COOH); 12.7 (11-OH-THC); 5.6 (CBN)	[67]
	SPME–GC–MS	2 (THC, CBD, CBN)	[68]
	LC–MS/MS	1 (THC); 2 (CBD, CBN)	[37]
	LC–MS/MS	0.5 (THC, CBD, CBN, 11-OH-THC, THCA-A); 0.08 (THC-COOH)	[71]
	LC–MS/MS	0.25 (THC); 0.3 (CBD, CBN); 0.02 (THC-COOH); 0.4 (11-OH-THC)	[72]
	LC–MS/MS	1 (THC)	[73]
	2D–GC–MS	0.5 (THC, CBD)	[69]
	Micro-flow LC–MS/MS	0.5 (THC, CBD)	[70]
	LC–MS/MS	0.25 (THC, CBD)	[74]
Hair	GC–MS	50 pg/mg (THC, CBD, CBN)	[81]
	GC–MS/MS	1 pg/mg (THC); 0.1 pg/mg (THC-COOH)	[86]
	Headspace SPME–GC–MS/MS	62 pg/mg (THC); 12 pg/mg (CBD); 30 pg/mg (CBN)	[76]
	LC–MS/MS	0.2 pg/mg (THC-COOH); 50 pg/mg (THC, CBD, CBN)	[78]
	LC–MS/MS	3.9 pg/mg (THC); 18 pg/mg (CBD); 5.3 pg/mg (CBN)	[84]
	LC–MS/MS	0.1 pg/mg (THC-COOH); 1.0 pg/mg (THC); 2.0 pg/mg (CBD, CBN)	[87]
Sweat	GC–NCI–MS	0.4 ng/patch (THC)	[92]

Exhaled breath	LC–MS/MS	6 pg/filter (THC)	[95]

*LOQ measurements given in ng/mL unless otherwise stated.

Correlation between matrices

Urine has a large window of detection since THC-COOH is eliminated slowly and variably over time. Blood analysis generally targets the parent THC to give a shorter window of detection and hence a better indication of recent use as THC levels decline to low, 'baseline' levels within a few hours of use. However, THC may still be detectable in blood at low levels for days or even weeks in chronic heavy users. Odell *et al.* [96] measured an average of 1.9 ng/mL THC in whole blood 148 hr after last use and Bergamaschi *et al.* [40] detected THC (≥0.3 ng/mL) in blood samples up to 30 days after last use. Major metabolite THC-COOH, also remains detectable in the urine of chronic users for prolonged periods. Peak concentrations of THC and CBD in plasma following cannabinoid administration via oral capsules was reached at ~1 hr, while the peak concentration of THC-COOH was reached at ~2 hr [38]. THC-COOH levels in blood were found to peak 1.2–7.5 hr after Sativex administration [27]. These provide some correlation with the detection of urinary THC-COOH, however lower concentrations of the metabolite are observed in blood.

Correlation between oral fluid and plasma varies, depending on route of exposure. Huestis and Cone [97] found that after the initial deposits from smoking had been depleted, the concentration of THC in oral fluid was well correlated to plasma concentrations until 4 hours after use. However, Kauert *et al.* [98] found that the similar elimination rates of THC from oral fluid and plasma are merely coincidental. Toennes *et al.* [99] and Laloup *et al.* [100] obtained data indicating that the detection of THC in oral fluid is a good predictor for THC also being found in plasma and therefore the subject being under the influence of cannabis. Sativex administration has been shown to result in much lower plasma concentrations compared with smoking cannabis, and hence these correlations will not be useful since high oral fluid concentrations will not equate to high plasma concentrations or a high risk of impairment. Conversely, ingested cannabis (brownies or capsules) result in lower oral fluid concentrations relative to plasma, so an individual may be influenced while no THC is detectable in the saliva. Niedbala *et al.* [101] found mean peak concentrations of 23.3 and 25.3 ng/mL of THC in oral fluid collected from the left and right sides of the mouth, respectively, after subjects smoked a single cigarette containing 20–25 mg of THC. Comparatively, the mean peak concentrations detected following the ingestion of brownies containing the same amount of THC was only 3.4 and 4.8 ng/mL in oral fluid collected from the left and right sides of the mouth, respectively. Additionally, Milman *et al.* [74] noted that orally administered capsules of Marinol® (synthetic THC) are unlikely to result in detectable concentrations of THC appearing in the oral fluid.

Skoglund *et al.* [94] found good correlation between exhaled breath and plasma with positive breath tests for THC concurring with positive plasma results and negative breath tests corresponding to negative or low concentrations of THC in plasma. Their results indicated that exhaled breath had a shorter window of detection than plasma.

The suitability of a biological matrix depends on the purpose of testing and the available facilities. Table 3 summarizes the major advantages and disadvantages of using blood, urine, oral fluid and hair matrices for cannabinoid testing.

Table 3: Summary of the benefits and drawbacks of the major testing matrices.

Matrix	Advantages	Disadvantages
Plasma/whole blood [27, 39]	Short window of detection for parent THC is useful for determining recent use and possible impairment. Relatively high concentrations of THC-COOH allow for easier detection.	Specialist equipment and personnel required for sample collection. Highly sensitive methods required to detect parent THC.
Urine [20, 49]	Longer window of detection is useful for long-term drug monitoring. Cannabinoid concentrations are unaffected by route of exposure. Simple to collect.	Longer window of detection can be problematic when interpreting a positive result. Privacy concerns surrounding sample collection. Prone to adulteration practices.
Oral fluid [102]	Shortest window of detection for parent THC. Simple, non-invasive collection methods.	Large variations in cannabinoid concentrations depending on route of exposure.
Hair [77-79]	Longest window of detection is useful for determining historic use or exposure. Simple, non-invasive collection. Helpful as a complementary testing matrix to confirm positive results from other tests.	Difficulties in distinguishing between active use and passive exposure. Varied growth rates prevent determination of specific time intervals. Sensitive detection methods required for the low picogram concentrations present.

When attempting to determine recent use or risk of impairment, the detection of parent THC is most appropriate, either in blood or oral fluid. Due to its short half-life THC is generally only detectable for a few hours in these matrices for occasional users. The window of detection in blood and oral fluid is extended when analysing for the metabolites, as these appear later and persist for longer. However, recent research shows the length of time the various cannabinoids and their metabolites remain is dependent on a number of factors and is highly variable between individuals [102]. Consequently, while a positive blood or oral fluid result may be indicative of recent use, determining a specific time of last use is not currently feasible. Detecting multiple cannabinoids and using oral fluid/plasma ratios are promising ways to indicate recent use, however more research is required before these can be used to determine more accurate windows of detection [103, 104].

Analytical pitfalls

Analysis of cannabinoids in biological matrices has been subject to difficulties due to the instability of the compounds when stored in these matrices. Degradation of cannabinoids in urine and blood is often the result of oxidative processes with pH and temperature playing major roles [56]. Even freezing cannot halt degradation; a 26% decrease in THC concentration was observed in urine stored at –70 °C in silanised glass after five months [53]. Nevertheless, freezing of urine and blood or plasma samples is preferred as higher losses are observed in refrigerated samples and even more so in samples stored at room temperature. Desrosiers *et al.* [105] recommended analysing urine within three months if THC-COOH-glu is of interest since it undergoes deconjugation over time, and thus increasing THC-COOH concentrations while the glucuronide decreases. Similarly, Scheidweiler *et al.* [106] found THC-COOH-glu and 11-OH-THC concentrations in plasma fell whilst THC-COOH concentrations increased over one week at room temperature.

Oral fluid is commonly collected using commercial kits that dilute the sample with a stabilising buffer at the point of collection. This improves cannabinoid stability compared with neat oral fluid samples, though refrigerated storage and analysis within a few weeks is still recommended for samples collected using the Quantisal, StatSure and Oral-Eze devices [107, 108]. THC in expectorated oral fluid diluted with phosphate buffer at pH ~6 was stable for three weeks when refrigerated, while samples diluted with Cozart DDS buffer solution was stable for at least four weeks at room temperature [109].

In addition to losses through degradation, THC is known to be highly lipophilic, commonly resulting in adsorptive losses to precipitants and surfaces during storage or sample preparation [109, 110]. CBD is also lipophilic [110] and is likely subject to similar effects. The lipophilicity of THC coupled with the high polarity of THC-COOH has been problematic for developing extraction procedures capable of extracting both analytes efficiently in a single

sample [44]. These attributes must be taken into consideration when collecting, storing and analysing samples for cannabinoids. Bergamaschi *et al.* [40] processed blood samples in an ice bath to minimise adsorption of cannabinoids onto precipitant materials. Jagadeo *et al.* [44] added acetonitrile to blood samples to desorb any cannabinoids from the container walls during their sample preparation. Addition of a non-ionic surfactant has also been shown to reduce the degree of adsorption of THC to polypropylene surfaces in neat oral fluid samples [109].

THC and CBD give identical fragmentation patterns when analysed by LC-MS/MS with ESI in positive ionisation mode. Broecker *et al.* [111] determined that this phenomenon is due to an acid-catalysed equilibrium between THC and CBD that occurs specifically under positive ESI conditions. Therefore, it should be noted that in any +ESI–LC–MS/MS analysis of THC or CBD (or both), THC and CBD must be chromatographically separated as they cannot be distinguished by MS or MS/MS data alone.

Sativex treatment compliance

Most illicit cannabis contains high amounts of THC and is low in CBD [3, 4]. Observing similar concentrations of THC and CBD in any biological matrix is a good indication of Sativex treatment since the formulation contains an almost equal amount of the two. However, this current distinction may become irrelevant in the future as cannabis plants containing higher CBD content increase in popularity, and with the emergence of e-cigarettes containing high levels of CBD with respect to THC [102, 112]. Sativex doses may also mask concurrent illicit use [37]. Presence of THC-COOH in oral fluid has been used to indicate active use of cannabis rather than passive exposure. A marker for distinguishing Sativex treatment from illicit cannabis use may be the precursor, THCA-A. THCA-A was found in the oral fluid of cannabis smokers indicating it is only partially decarboxylated during smoking [71]. The method of manufacturing Sativex should result in no or very low amounts of THCA-A present in the formulations, so detecting this may indicate illicit use.

Plasma 11-OH-THC/THC ratios were found to be higher after either Sativex administration or oral dosage with THC capsules (mean peak ratios of 1.1–2.1) than following cannabis smoking (0.0–0.5) [27]. Therefore elevated 11-OH-THC levels compared with THC may be an indicator of medicinal cannabis use, though not Sativex specifically.

For long term monitoring of treatment compliance, hair testing may be appropriate. Hair testing is more expensive per sample, but it has the potential to give information over a much larger time frame and replace multiple blood or urine samples, reducing costs in the long term [77].

Future perspective

Increasingly, studies are including the analysis of CBD along with THC and its metabolites in biological matrices. Further research into CBD and its metabolites is necessary, however CBD-7-oic acid is currently lacking in commercial supply. Once this metabolite becomes available, additional pharmacokinetic studies are inevitable and will help identify how best to analyse each matrix for evidence of CBD intake. Accurate detection and quantification of CBD, THC and its metabolites will be useful for identifying Sativex use, however distinguishing this from concurrent use of recreational cannabis continues to be challenging. More research into potential markers for either Sativex or recreational cannabis will hopefully result in a reliable means to determine the source of cannabinoids present in a sample.

Blood and urine remain the principal tools used for monitoring Sativex use. Oral fluid testing continues to be investigated and may be the preferred testing matrix if screening sensitivity and collection device reliability are significantly improved. Hair analysis also has its benefits, however, continued efforts in distinguishing internal exposure from external contamination are essential. Though still in its infancy, exhaled air is a promising matrix for future testing, particularly due to the non-invasiveness of breath testing.

GC–MS is well established as a confirmatory testing tool for the quantification of cannabinoids in biological matrices. Although GC–MS/MS and 2D–GC–MS techniques have improved on the standard GC–MS methods, LC–MS methods, in particular LC–MS/MS, has caught up in terms of sensitivity and is set to overtake GC–MS. LC–MS is also becoming increasingly popular as a screening tool and may in future replace immunoassay-based testing for cannabinoids following Sativex administration.

Executive summary

Background

- Quantitation of CBD and its metabolites in biological matrices is essential to monitoring Sativex administration.

Future Perspective

- Identification of markers that can distinguish between Sativex use and recreational cannabis use will be useful for determining concurrent cannabis use with Sativex administration.
- LC–MS continues to be increasingly popular for both screening and confirmatory analyses for cannabinoids in biological matrices and is an important tool for the monitoring of Sativex use.

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