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Differentiation of marijuana headspace volatiles from other plants and hemp products using capillary microextraction of volatiles (CMV) coupled to gas-chromatography–mass spectrometry (GC–MS)

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ABSTRACT

The ability to rapidly detect illicit drugs, such as marijuana, is critical to policing legislation across the country. However, it is often difficult to distinguish or identify small quantities of drugs in large spaces without the aid of trained canines. A new device, the capillary microextractor of volatiles (CMV), has the potential to provide rapid detection due to its ability to collect and preconcentrate volatile organic compounds (VOCs) directly from air within minutes. Analysis of the captured compounds can then be performed using a gas chromatography–mass spectrometer (GC–MS). This study focuses on the detection of marijuana volatiles using the CMV as a sampling and preconcentration device given the hypothesis that marijuana will have a distinct chemical profile, or collection of VOCs, that distinguishes it from related plants and other products that could emit similar compounds. Volatile compounds from the headspace of marijuana, related plants, and hemp products were extracted using the CMV and analyzed with GC–MS. The compounds identified and the chemical profiles of each sample were then compared to the volatiles found in the headspace of authentic marijuana samples. The findings presented here suggest that marijuana plants emit volatiles that are readily distinguished from the other samples tested in this study. The distinguishing compounds included α-santalene, valencene, and β-bisabolene. In some cases, THC and cannabinol were also present in the headspace of marijuana. Although these findings support the hypothesis that marijuana has a distinct chemical VOC signature, further work to create a larger database of potential plants and materials is recommended prior to routine use of the CMV coupled to a GC–MS in forensic casework.

1. Introduction

Marijuana legalization has been an intense and dynamic issue of debate, specifically within the United States. Marijuana, the dried leaves, flowers, stems, and seeds from the hemp plant, *Cannabis sativa*, contain high amounts of tetrahydrocannabinol (THC), which result in hallucinogenic effects when ingested [1]. Because of this, marijuana is considered an illicit drug and is classified as a Schedule I drug under the Controlled Substance Act [2]. In the U.S.A, it was the most commonly used illicit drug in 2013, being used by 80.6% of all illicit drug users [3]. The United Nations Office on Drugs and Crime (UNODC) reports that in 2014, there were 189.5 M users worldwide [4]. Despite the federal laws in the US, several states have been moving towards legalizing marijuana for its medical benefits and for recreational use. Since 1996, Colorado, Washington, Oregon, Alaska and the District of Columbia have legalized recreational marijuana use and a further 20 states have implemented medicinal marijuana legislation [5]. Although marijuana legalization is becoming more commonly accepted, it is still illegal to possess marijuana for any purpose in most states. Growing plants for illicit distribution is also illegal in all states, and the distribution and sale of marijuana is still a crime under federal law. An efficient and rapid method for the detection of marijuana plants in illegal growing operations or hidden within cargo, would assist law enforcement efforts.

Currently, the primary method of illicit drug detection is by use of trained canines, but there are limits to the utility of canines including the fact that canine performance is strongly influenced by the quality of the training and the handler can also influence the false positive detection of illicit material [6]. Further, dog breed and the environment in which they search can also affect the

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reliability of drug alert dogs [7]. Recent work by a number of researchers have attempted to identify the odor signature compounds for the purposes of improving canine training aids [8,9]. Other researchers have reported the composition of smoke from marijuana cigarettes [10] including an attempt to differentiate geographic origin of marijuana and hashish samples by measuring the headspace VOC composition [11].

A new device, capillary microextractor of volatiles (CMV) [12], is proposed as a complement or viable alternative to canine detection due to its high sensitivity, low sampling time, and ability for on-site sampling of volatile compounds.

Capillary microextraction of volatiles (CMV) is a headspace sorbent sampling and pre-concentration technique. It is governed by similar principles as current volatile sampling technologies such as solid phase microextraction (SPME) and sorbent tubes such as Tenax®. SPME is an absorbent or adsorbent fiber that is coated in different sorbents facilitating direct, headspace, and membrane protected sampling [13]. SPME extraction is an equilibrium process whereby volatiles in the headspace that have undergone partitioning between a non-volatile liquid or solid phase and the vapor phase above the liquid or solid, can be absorbed or adsorbed onto the sorbent through an equilibrium process [14]. The volatiles are then analyzed, typically by gas chromatography mass spectrometry (GC–MS), by injecting the fiber directly into the injection port [15]. However, SPME is a passive sampling technique and requires a sampling time of up to several hours [16]. Further, the single fiber geometry of the tool results in a low surface area and reduced sensitivity compared to alternate techniques [14]. It is therefore not a viable in field tool for drug detection.

Sorbent tubes such as Tenax® may offer improved sensitivity over SPME fibers. Tenax® is a porous polymer that shows high efficiency for adsorptivity and desorptivity, as it can sustain relatively high temperatures of up to 375 °C [17]. Tenax also allows for dynamic sampling, facilitating faster sampling times and greater sensitivity than static techniques [18]. Despite the improved sensitivity, Tenax® is a costly sampling device requiring an additional thermal desorption unit to be attached to the standard GC–MS set up. This high cost, and additional instrumentation required for the analysis of sorbent tubes may not be feasible in forensic and law enforcement laboratories. As such an alternate, portable, and inexpensive tool is required.

The CMV was developed based on planar solid phase microextraction (PSPME) [19] and functions using the same equilibrium principles governing solid phase microextraction (SPME). CMVs allow rapid and accurate sampling and detection of compounds in a variety of matrices including explosives, organic gunshot residues and drugs [20–22]. The design of the device is simple, consisting of a 2 cm long by 2 mm wide glass capillary that is packed with sorbent coated glass microfibers [12,20–22]. Polydimethylsiloxane (PDMS) is the absorbent of choice due to its continued use in SPME studies, the wide range of volatile chemicals it can collect and its ability to withstand high injector temperatures [23]. The PDMS is coated on glass fiber filter disks that can be cut, packed and retained within the housing of the CMV. Due to the open nature of the capillary tube, the headspace above a sample can be forced through the tube by a vacuum pump, significantly lowering sampling time. Depending on the target analyte and sample matrix, CMVs can reduce the sampling time from 30 min to as low as 30 s, making for a much more efficient sampling method in the field. Furthermore, the surface area in CMV is 5000 times greater than SPME, increasing adsorption capacity, retention and making it a more sensitive technique [20], thus offering greater capability to detect trace levels of volatiles in the air, such as those required for drug detection. Because it can be coupled to the GC–MS with direct insertion into the injection port using a commercially available thermal separation probe [20], it is also more economical and feasible in current crime labs than sorbent tubes.

The purpose of this study was to characterize the chemical profile of marijuana headspace VOCs through the use of sampling and preconcentration using CMV followed by analysis by GC–MS. Further, the aim of the project was to distinguish marijuana plants from other plants and hemp products through the VOC profile and evaluate the analytical figures of merit for the detection of VOCs in marijuana. The results will help inform the potential application of CMV to differentiate marijuana for field analysis where other plants or products may mask the signal of the volatiles emitted by marijuana. The hypothesis that marijuana plants emit a distinct chemical profile that can be differentiated from other plants and products has been tested through this study. This "characteristic" profile is likely to consist of several compounds that, in isolation, are not identifiable as marijuana, but in combination can be associated with the presence of the drug. The headspace of marijuana has been previously classified into four categories (i.e., fractions) [24] on the basis of the physical properties of the volatile compounds in each fraction. The different fractions are defined as follows: (I) volatiles (bp 20–80 °C; MW < 100 g/mol), (II) intermediate volatiles (bp 150–198 °C; MW > 100 g/mol), (III) less volatiles (bp > 198 °C; MW > 200 g/mol), and (IV) non volatiles (bp > 200 °C; MW > 300 g/mol) [24]. The identified fractions of a particular marijuana sample are dependent upon the operational conditions of the detection (or analytical) technique as well as the sampling procedure.

2. Methods

2.1. Materials

Whole leaf hops samples were purchased from Adventures in Homebrewing (MI, USA) and consisted of Cascade, German Hallertau, Citra, Chinook, Kent Golding, Columbus, Williamette, and US Northern Brewer hops varieties. Hemp paper, fibers, seeds and rope were purchased from Hemp Traders (CA, USA). With the exception of grass (St Augustine grass) which was sampled on the grounds of Florida International University (Modesto campus, Miami, FL, USA), all fresh plant samples were collected from the Fairchild Gardens (Coral Gables, FL, USA). Plant samples consisted of fresh leaves and if present flowers. Authenticated marijuana samples were collected at a local government law enforcement laboratory in Miami, FL, USA. Voucher numbers for the plants, sample sources, and sample weights are described in Table 1.

Marijuana samples were of recent seizures dating to a maximum of several years since the seizure. The physical condition and appearance (freshness) of samples varied. Marijuana sample 1 was the largest and freshest of the samples. Marijuana samples 3–6 had been in storage for over 3 years, and were visibly aged and very dry. Marijuana sample 7 was a grinder with only traces of plant material present. Marijuana sample 8 consisted of 43 small zip lock 'dime bags' of marijuana containing approximately 0.5–1 gram of plant material in each. Marijuana sample 9 was a brown paper bag with tobacco and 16 sealed zip lock 'dime bags' of marijuana. This sample was used to detect interference from other smoking substances. Marijuana sample 10 consisted of 25 zip lock 'dime bags', sealed in a large zip lock bag. Marijuana sample 11 consisted of 175 blue zip lock sealed 'nickel bags', each containing less than 0.5 grams of plant material. Marijuana sample 12 was a sample of 5 plastic snap top vials of visibly aged and dried marijuana sealed in a small evidence bag that was not opened for sampling. Marijuana sample 13 consisted of 11 small zip lock bags that were sealed in larger sandwich zip lock bags. Marijuana sample 14 consisted of 17 'nickel bags', tied in a plastic bag and sealed in a small evidence bag that was not opened for sampling. Marijuana sample 15 was a sample of two zip lock 'dime bags' sealed in a heat sealed bag.

2.2. Sample collection and preparation

All samples, excluding hops and marijuana, were placed in pre-weighed 1 quart metals cans and sealed with metal lids. Cans and lids were purchased from All American Containers (Miami, FL, USA). In order to remove potential artifacts from the can, the cans were baked out prior to use at 250 °C for three days. Holes were made in the lids to provide access for sampling, and were sealed using a rubber septum (Capitol Scientific Inc. Austin, TX). All samples were sealed in the can for at least 24 h to allow volatiles to reach a state of equilibrium between the sample and the headspace in the can. Hops, marijuana, and a cigar were sampled *in situ* directly from the bag they were received in.

Table 1
Sample source, weight and headspace extraction parameters for plant and hemp samples investigated using CMV–GC–MS. Plant specimens were vouchered at the Fairchild Tropical Garden Research Centre.

Sample name	Source/Voucher No.	Sample weight (g)	Extraction time
Cascade hops	Adventures in Homebrewing	28.40 g	30 s
Hallertau hops	Adventures in Homebrewing	28.40 g	30 s
Citra hops	Adventures in Homebrewing	28.40 g	30 s
Chinook hops	Adventures in Homebrewing	28.40 g	30 s
Golding hops	Adventures in Homebrewing	28.40 g	30 s
Columbus hops	Adventures in Homebrewing	28.40 g	30 s
Williamette hops	Adventures in Homebrewing	28.40 g	30 s
US northern brewer Hops	Adventures in Homebrewing	28.40 g	30 s
Whole sterilized hemp seed	Hemp traders	25.17 g	5 min
Hemp paper	Hemp traders	6.12 g	5 min
Hemp rope	Hemp traders	19.90 g	5 min
100% Raw hemp bark fiber	Hemp traders	0.54 g	5 min
100% raw hemp long fiber	Hemp traders	0.98 g	5 min
100% degummed hemp fiber	Hemp traders	0.63 g	5 min
100% Combed hemp fiber	Hemp traders	1.00 g	5 min
100% Short hemp fiber	Hemp traders	1.32 g	5 min
La Historia Cigar	E.P. Carillo	11.40 g	1 min
Salvia coccinea	Jestrow 2015-FTG-11 (FTG)	6.84 g	1 min
Salvia miniata	Jestrow 2015-FTG-12 (FTG)	18.22 g	1 min
Myriocarpa longpipes	Abbott 24125 (FTG)	13.11 g	1 min
Trema micrantha	Jestrow 2015-FTG-15 (FTG)	11.42 g	1 min
Rhaphiolepis umbellata	P. Fantz 3281 (FTG)	12.21 g	1 min
Varronia Bullata	Jestrow 2015-FTG-14 (FTG)	5.70 g	1 min
Pilea nummularifolia	Jestrow & Valdes s.n. (FTG)	9.46 g	1 min
Ficus montana	Jestrow 2015-FTG-09 (FTG)	9.21 g	1 min
Ficus perforata	P. Fantz 3231 (FTG)	14.37 g	1 min
Ficus aurea	Jestrow 2015-FTG-10 (FTG)	11.14 g	1 min
Lantana involucrata	Jestrow 2015-FTG-13 (FTG)	10.28 g	1 min
St Augustine Grass	Florida International University	13.33 g	1 min

Table 2
Sample weight and headspace extraction parameters for authenticated marijuana samples from a local government drug analytical laboratory investigated using CMV–GC–MS. Sampling method is indicated by an X.

Sample Name	Weight of whole sample (g)*	Weight of grab sample (g)	Extraction time	Sampled through evidence bag	Sampled directly above marijuana
Marijuana sample 1	265.5	–	30 s		X
Marijuana sample 2	3.3	–	1 min	X	
Marijuana sample 3	~28	–	1 min		X
Marijuana sample 4	~28	–	1 min		X
Marijuana sample 5	~28	–	1 min		X
Marijuana sample 6	~28	–	1 min		X
Marijuana sample 7	No plant material, traces only	–	1 min	X	
Marijuana sample 8	95.9	4.0	1 min	X	X
Marijuana sample 9	85.0	–	1 min	X	
Marijuana sample 10	204.2	5.4	1 min	X	X
Marijuana sample 11	100.6	0.7	1 min	X	X
Marijuana sample 12	17.6	3.3	1 min	X	X
Marijuana sample 13	80 g	6.4	1 min	X	X
Marijuana sample 14	26.2	1.6	1 min	X	X
Marijuana sample 15	10.7	3.1	1 min	X	X

* Whole sample weights include the weight of its evidence bag packaging.

To account for background on the CMV, all CMVs were first analyzed as blanks using the same desorption and instrumental parameters as described for the samples. Headspace extraction of the volatiles for each specimen was conducted by placing a CMV at the tip of a vacuum pump (Escort Elf Sampling Pump, Ocala, FL) and pumping air through the CMV at a constant flow rate of 1 L/min. Hops varieties were sampled for 30 s due to high concentrations of volatiles in the headspace. Hemp products were sampled for 5 min, due to low concentrations of volatiles. All other plants were sampled for 1 min. Marijuana sampling and extraction time varied depending on the size of the sample and the container it was kept in. Marijuana sample headspace were either sampled within the evidence bag without opening any internal storage bags or sampled directly above the marijuana in a grab sample of any of the internal bags. All sampling procedures, sample sizes and extraction times for marijuana samples are given in Table 2. After sample collection, the CMV was introduced into the injection port of the (GC) for thermal desorption using an Agilent Thermal Separation Probe (Santa Clara, CA, USA).

2.3. Instrumental analysis

Analysis of the headspace for all samples was performed on an Agilent 7890A Gas Chromatograph (GC) and an Agilent 5975C Inert XL MSD mass spectrometer (Santa Clara, CA). Chromatographic separation occurred on a HP-5 ms capillary column (29.17 m × 0.25 mm × 0.25 μm). The oven temperature was programmed for 2 min at 40 °C, then 25 °C/min to 260 °C, and finally at 260 °C for 10 min. The injector was operated in splitless mode at 270 °C and the transfer line was set at 280 °C with a source temperature of 230 °C. The constant GC column flow of helium was set to 1.2 mL/min. The mass spectrometer simultaneously collected total ion (TIC) and selected ion (SIM) data. The selected ion monitoring for THC ions were 299, 271, 231, 314 m/z over the acquisition range 40–340 m/z. Analysis of the headspace of other plant samples, when highly concentrated, used a 5:1 split to protect the detector from saturation. The instrumental conditions were derived from previous work with the CMV and reported by Fan and Almirall [20] and Tarifa and Almirall [21].

2.4. Data analysis

All data was blank corrected to account for artifacts on the CMV. Over corrections were set to an intensity of 0. A visual comparison of the observed sample total ion chromatograms was then conducted based on the relative intensities of each peak to the highest peak in the sample. This accounted for differential absolute intensities observed due to variations in sample size and container volume. A heat map of the relative intensities versus retention time of the total ion chromatogram (TIC) were produced using the ggplots.2 package and heatmap.2 tool in R 3.2 (R Studio, Boston, MA, USA). Samples were grouped based on similarities using the Wards Hierarchical clustering method with a Manhattan distance measure using .JMP software version 10.

Peak identification was conducted using Agilent Technologies MSD Chemstation E.02.01.1177 (Santa Clara, CA, USA) and accompanying 2008 version of National Institute of Standards and Technology (NIST) spectral library. Compounds were identified by comparing retention time and mass spectra of peaks with compounds contained within the library. Where standards were available, identification was confirmed by analysis of a standard solution and comparing both the retention time and mass spectra of specific compounds.

3. Results

3.1. Identification of volatile compounds

The sampling of the headspace directly above the marijuana for samples 8 and 10–15 where used to characterize the chemical profile of marijuana plants. Forty-four compounds were identified with seventeen confirmed by a standard solution (see Table 3). An example total ion chromatogram of Marijuana sample 1 is given in Fig. 2. Of the volatile compounds identified in the headspace of marijuana, 16 were found to be important either due to their abundance or due to their absence in non-marijuana samples. This collection of compounds consisted of α-pinene, β-pinene, β-myrcene, limonene, β-linalool, β-carophyllene, α-carophyllene, α-selinene, α-bergamotene, seline-3,7(11)-diene, α-terpineol, α-santalene, valencene, β-bisabolene, THC and cannabinol. Spectra for all 16 compounds are given in the Supplementary Figs. 1–16. The compounds that were only found in the headspace of marijuana samples were α-santalene, valencene, β-bisabolene, THC and Cannabinol (see Fig. 3).

Of the 16 compounds identified in the headspace of marijuana, several were also detected in the plant and hops samples analyzed (Table 4). Lower molecular mass compounds including α-pinene, β-pinene, β-myrcene, and limonene, were present in most samples. β-caryophyllene was present in all freshly sampled plant material, except grass, and also in dried hops and whole sterilized hemp seed. Hops was the most similar plant to marijuana with the Czech Saaz variety containing 9 of the compounds identified in the headspace of marijuana. Hops headspace contained volatile compounds including β-pinene, β-myrcene, limonene, β-caryophyllene, and α-caryophyllene.

3.2. Relative intensity and peak comparisons

Hierarchical clustering and visual inspection of the heat map identified no clear grouping of marijuana samples based on their total volatile profiles (Fig. 1). Three major divisions were identified. The first group consisted of Marijuana samples 1, 7, 8, 11, 13, 14, the hemp paper and the whole sterilized hemp seed. The profile of Group 1 samples was characterized by a large number of VOC's at similar but low relative intensities. The second grouping was dominated by the plant samples and remaining marijuana samples. It consisted of several sub divisions. Group 2 were the least volatile samples analyzed and were characterized by a low number of different VOCs at low intensities. The first subdivision of Group 2 consisted of Marijuana sample 9 (containing the tobacco), Marijuana sample 10, the cigar, *Trema micrantha*, *Ficus perforata*, all hemp fiber samples and the hemp hurd sample. These were closely associated with Marijuana samples 12 and 15, which grouped together. The next subdivision consisted of the older Marijuana samples 3–6. They were closely related with *Rhaphiolepis umbellata* and *Pilea numalarifolia*. The final subdivision of group two consisted of the remaining plant samples with the *Salvia* spp. grouping closely together on their own. Group 3 was characterized by volatile profiles with a few highly dominant intense peaks. This group consisted of all the hops varieties and also Marijuana sample 2.

Table 3
Qualitative analysis of compounds identified to be emitted directly from the headspace of grab samples of marijuana samples 8, 10–15 captured by CMV after 1 min dynamic sampling at 1 L/min extraction flow.

No.	Compound	Confirmation
1	**α-Pinene**	S
2	**Benzaldehyde**	S
3	**β-Myrcene**	L
4	**β-Pinene**	S
5	**3-Carene**	L
6	**2-Ethylhexanol**	L
7	**Limonene**	S
8	**Benzyl Alcohol**	S
9	**β-Ocimene**	L
10	**γ-Terpinene**	L
11	**α-Terpinolene**	L
12	**β-Linalool**	S
13	**Nonanal**‡	S
14	Allo-Ocimene	L
15	**Exo-Fenchol**	L
16	**Borneol**	L
17	**Dodecane**	S
18	**Tridecane**	S
19	**Ylangene**	L
20	Tetradecane	S
21	Surfynol	L
22	**α-Zingiberene**	L
23	**α-Bergamotene**	L
24	**α-Santalene**	L
25	**β-Caryophyllene**	S
26	**Cyclododecane**	L
27	**α-Caryophyllene**	S
28	**4,11-Selinadiene**	L
29	**Seychellene**	L
30	**α-Guiaene**	L
31	**β-Guaiene**	L
32	**α-Gurjunene**	L
33	**Valencene**	L
34	**3,7,(11)-Selinadiene**	L
35	**β-Maaliene**	L
36	**Guaiol**	L
37	α-Campholene aldehyde	L
38	**α-Bisabolol**	L
39	**Octadecane**	S
40	Eicosane	S
41	Heneicosane	S
42	Tricosane	S
43	**THC**¥	S
44	**CBN**	L

Bolded compounds indicate compounds consistent with compound identification in previously reported studies.
‡ Compound identification confirmed by retention time of standard solutions (S) or mass spectrum in library (L).
¥ THC was confirmed by selected ions 299, 231, 271, 314 *m/z*.

3.3. Quantitation of THC

Fig. 2 illustrates chromatograms of THC identified in the grab sample of marijuana headspace sample #15 and a 1 ppm THC liquid standard solution spiked onto a CMV. An example confirmation of THC by mass spectra is shown in Supplementary Fig. 16. A calibration curve of liquid THC solutions spiked onto CMVs showed linearity ($R^2 = 0.99$) over the concentration range of 0.5–20 ppm. The average of three replicates over the 5–20 ppm concentration range showed relative standard deviations of ⩽12%, while 0.5–2.5 ppm

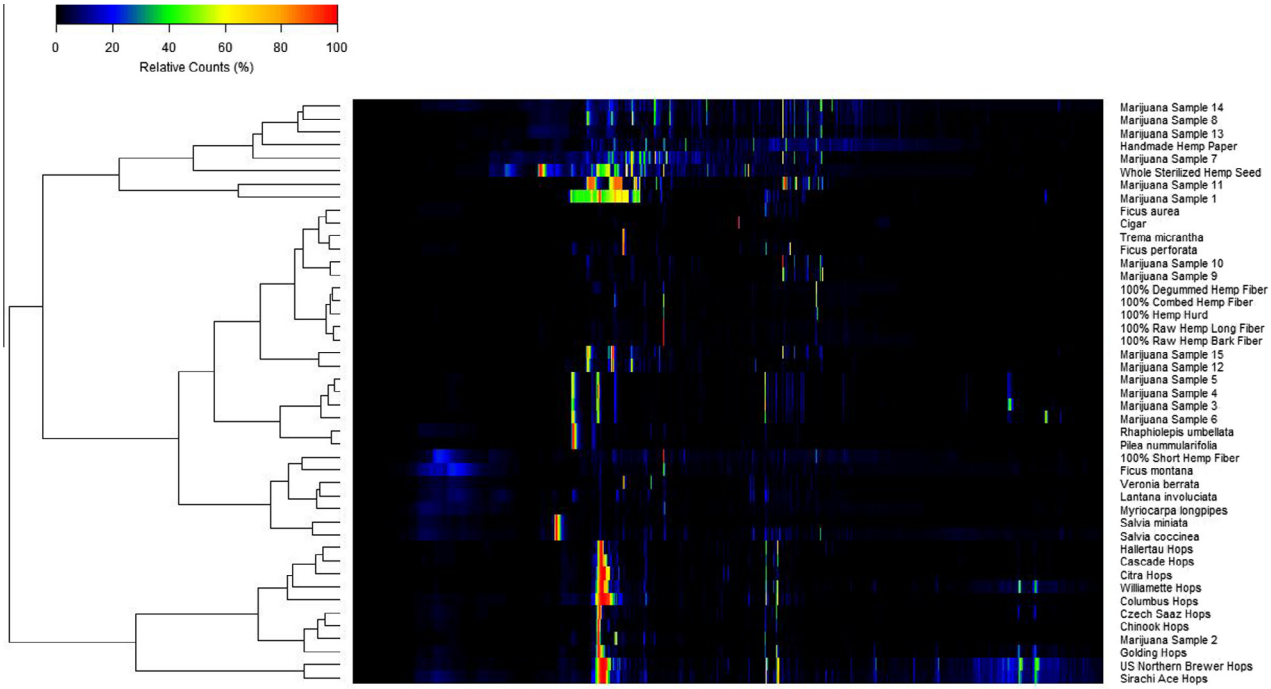

Fig. 1. Heat map and Wards Hierarchical Clustering of the relative abundance of VOCs in the headspace of marijuana and related plants and products.

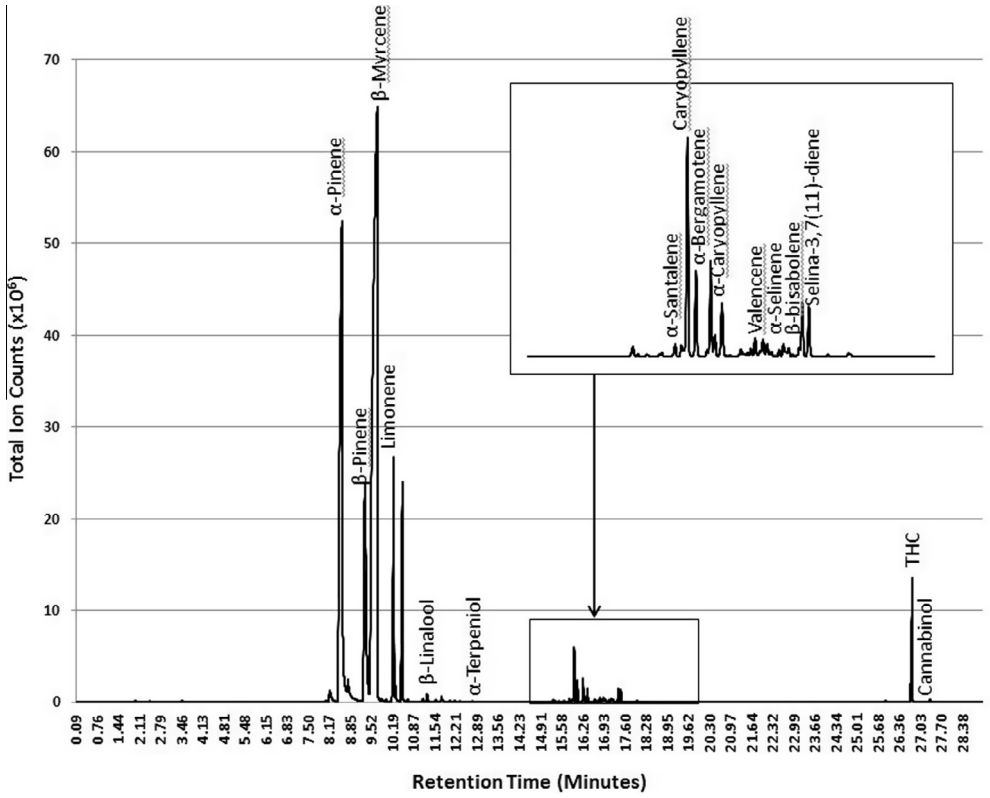

Fig. 2. An example total ion chromatogram of marijuana sample 1.

concentration range showed relative standard deviations of ⩽26%. The limit of detection of THC on CMV is 1.0 ng. An approximation of the mass of THC collected on the CMV from marijuana sample 15, was determined to be 1.8 ng after integration of the THC peak in the sample and extrapolation of the calibration curve.

3.4. Discussion

Many of the volatile compounds identified in this study can be linked back to basic plant chemistry. For example, β-caryophyllene, α-pinene, β-pinene, β-myrcene, and limonene are all dominant

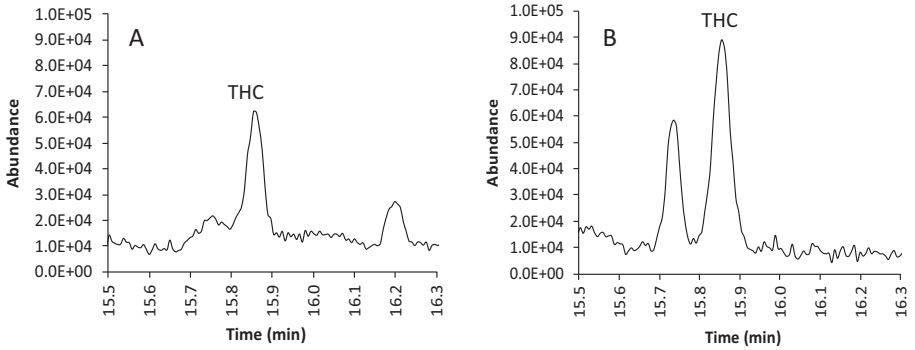

Fig. 3. (A) Total ion chromatogram of the VOC profile of marijuana headspace sample #8, emphasizing THC peak at 15.85 min and (B) the direct spike response of 1 ppm THC in a MeOH solution on CMV; peak at 15.85 min.

biogenic isoprenoids emitted by plants [25]. The monoterpene linalool is also typically found in plants; it is associated with flowering fragrances and is used to attract pollinators [25]. Although these six compounds were common in most of the plants analyzed, variability in the emissions of these VOCs was evident as a result of the complex relationship between each plant and its environment [25]. VOC emissions by plants can be influenced by internal factors such as genetics, and external factors such as temperature, light, and water availability [26]. Plant competition also plays a role in what compounds plants emit, as variability in terpene production correlates to the amount of nutrients in a plant's soil [27]. Further, monoterpenes are often emitted as a defense mechanism against attacks from herbivores or pathogens, and such compounds are stored in plant secretory organs [25]. All of these environmental factors must be taken into account when analyzing the headspace of plants, as any change in a plant's condition could drastically change the types and amounts of volatiles they emit.

Wards hierarchical clustering of the total VOC profiles of samples was not successful at differentiating marijuana samples from other samples analyzed during this study. We propose that the variation in the observed VOC profile of each marijuana sample is a result of artifacts of the different packaging materials and potential adulterants in the marijuana samples. To mimic in field examples of concealed drugs, we did not control for the packaging material. The presented VOC profiles are therefore a combination of both the VOCs emitted directly by marijuana and those produced by the different packaging materials. The close association and similarity in the total VOC profile for all hops varieties and also for the *Salvia* spp. demonstrates that a consistent volatile organic compound profile is evident for related plant material. However, due to the complex nature of VOC profiles in real life situations, such as in field detection of concealed drug, total VOC profiles are likely to be difficult to decipher. As such, targeted compound identification is more suitable.

Out of all samples analyzed in this study, hops samples collectively showed the most similar volatile emissions to marijuana. α-caryophyllene, was found in all hops samples, and is reported as being highly abundant in the essential oil of hops [28]. β-caryophyllene is also known to be highly abundant in the essential oil of hops, but can be found in the essential oils of many other plants as well, specifically cloves [28]. Both α-caryophyllene and β-caryophyllene were found in hops and marijuana samples, eliminating them as markers for marijuana detection. Another peak of interest in hops is β-myrcene, which is abundant in the headspace of fresh hops and disappears as the sample increases in age [29]. This compound was present in both hops and marijuana headspace, also eliminating it as a possible marker.

A total of 44 compounds were identified in the headspace of marijuana with a targeted profile of 16 compounds used for comparison to other plants. Thirty-five of those compounds identified in marijuana headspace (Table 3) have been previously reported in studies on cannabis volatile constituents [30,31]. It is of interest to note that the identification of cannabinoids THC and CBN in the marijuana samples occurred under room temperature sampling conditions as described in this study. Cannabinoids occur mainly in their carboxylic acid derivative form in the plant and are not usually released until the sample is heated since they decarboxylate slowly at room temperature [33]. Since the direct detection of cannabinoids is unlikely at room temperature based on their low vapor pressure, it is hypothesized that THC and CBN entered the CMV adsorbed on ambient particles (possibly pollen particles). Rothschild et al previously reported that benzyl alcohol was found in marijuana pollen. Of the six occurrences of THC identified in the grab samples where the headspace above the plant was directly sampled, three of those also contained benzyl alcohol (ie. Samples 10, 13 and 14).

Higher concentrations of the monoterpenes, α-pinene and β-pinene, β-myrcene, and sesquiterpenes, β-caryophyllene and α-caryophyllene, have been found in marijuana headspace in numerous studies [32,33]. Because of their abundance it the headspace, these compounds are commonly the targets for canine detection [34]. However, these high abundance compounds in marijuana headspace are also commonly emitted by non-marijuana plants, as previously stated, which brings into question their reliability as markers for marijuana detection. Of the 16 compounds found in the headspace of marijuana samples in this study only α-santalene, valencene, β-bisabolene, THC and Cannabinol were found to be specific to marijuana samples.

The ability to detect the VOC components from marijuana headspace has been demonstrated by coupling CMV sampling to GC–MS analysis. The technique facilitates the identification of a collection of compounds that may be indicative of the presence of marijuana in a room or concealed space. The results not only show the wide range of volatile emissions from marijuana and related products and plants, but also that the age and nature of each marijuana sample significantly impacts the types and amount of volatiles. Fresher and larger samples of marijuana, such as seen with Marijuana sample 1, emitted a wider array of volatiles and in higher concentrations. Older samples and trace amounts of marijuana, such as marijuana sample 2–6, may provide difficulties with detection in larger spaces than that demonstrated here, due to their low emissions of characteristic volatile compounds. However, the detection of many marijuana specific compounds outside a small wrapped marijuana sample (marijuana sample 2), from double-sealed zip lock bags, and from traces left on a grinder, show promise for the detection of the illicit material when secured in bags or other packaging. Although some marijuana samples only emitted a smaller range of volatiles, most did emit the key volatiles that were found

Table 4
Comparison of the key volatile organic compounds detected in the headspace of marijuana and other related plants and products with CMV–GC–MS. The presence of a given compound i

Sample	α-Pinene	β-Pinene	β-Myrcene	Limonene	β-Linalool	β-Caryophyllene	α-Caryophyllene	α-Selinene	α-Bergamotene	3,7(11)-Selinadiene	α-Terpineol	α-Santalene	β-Bi
Marijuana sample 1	X	X	X	X	X	X	X	X	X	X	X	X	X
Marijuana sample 2	X	X	X	X	X	X	X	X	X	X	X		
Marijuana sample 3	X	X	X	X	X	X	X	X	X	X	X	X	X
Marijuana sample 4	X	X	X	X	X	X	X	X	X	X	X	X	X
Marijuana sample 5	X	X	X	X	X	X	X	X	X	X	X	X	X
Marijuana sample 6	X	X	X	X	X	X	X	X	X	X	X	X	X
Marijuana sample 7	X	X	X	X	X	X	X	X	X	X	X	X	X
Marijuana sample 8	X	X	X	X	X	X	X	X	X	X	X	X	X
Marijuana sample 9	X	X	X	X	X	X	X	X	X	X	X	X	X
Marijuana sample 10	X	X	X	X	X	X	X	X	X	X	X	X	X
Marijuana sample 11	X	X	X	X	X	X	X	X	X	X	X	X	X
Marijuana sample 12	X	X	X	X	X	X	X	X	X	X	X	X	X
Marijuana sample 13	X	X	X	X	X	X	X	X	X	X	X	X	X
Marijuana sample 14	X	X	X	X	X	X	X	X	X	X	X	X	X
Marijuana sample 15	X	X	X	X	X	X	X	X	X	X	X	X	X
Czech Saaz hops	X	X	X	X	X	X	X	X	X				
Columbus hops	X	X	X	X		X	X	X		X			
Chinook hops	X	X	X	X		X	X	X		X			
Williamette hops	X	X	X	X	X	X	X	X					
German Hallertau hops	X	X	X	X	X	X	X						
Kent Golding hops	X	X	X	X	X	X	X						
US Northern Brewer hops	X	X	X	X	X	X	X						
Citra hops		X	X	X	X	X	X						
Sirachi Ace hops	X	X	X	X		X	X						
Cascade hops		X	X	X		X	X						
Whole sterilized hemp seed	X					X					X		
100% Combed hemp fiber				X									
100% degummed hemp fiber				X									
100% Raw hemp long fiber				X									
Handmade hemp paper													
Hemp rope													
100% Raw hemp bark fiber													
100% Short hemp fiber													
Cigar				X									
Ficus perforata	X		X	X	X	X	X						
Lantana involuciata	X	X			X	X	X		X				
Pilea nummularifolia	X	X		X		X	X						
Salvia coccinea		X		X	X	X							
Rhaphiolepis umbellata		X		X	X	X							
Trema micrantha	X			X		X	X						
Salvia miniata	X					X	X						
Veronia berrata				X	X	X							
Myriocarpa longpipes						X	X						
Ficus aurea						X							
Ficus montana						X							
St Augustine Grass	X	X											

to characterize marijuana samples. The threshold of the detection of these volatiles should be further studied in order to better understand the limitations to detecting trace amounts of marijuana.

Slight variations in the volatile organic compound profile of marijuana samples investigated in this study were observed. Variations could occur due to differences in geographic origin [32,33], due to variety, or potentially due to the processing of the sample into hashish, in which photo-oxidation affects many of the volatiles emitted [30]. Further analysis into variation of the headspace composition between marijuana varieties, and processing procedures will be important to establish how the VOC profile changes with processing, cultivation type and aging.

4. Conclusion

The capillary microexctractor of volatiles (CMV) was found to be a viable complement to canine detection of illicit drugs, specifically marijuana. Its ability to collect trace amounts of volatile compounds within a short (~1 min.) extraction time facilitates the development of a technique for filed testing for the presence of marijuana plants. The CMV was shown as a suitable investigative tool and it was also found that such a device could detect a collection of volatile constituents that are characteristic to marijuana plants. These identified compounds provide marijuana a distinct chemical signature that can be compared to other samples. In order to determine that the volatile compounds identified are "unique" to marijuana headspace, a more diverse set of samples must be collected and analyzed. This will help establish a collective chemical profile of marijuana that may be used for comparisons. A database can then be created and utilized for detecting marijuana in multiple environments through use of the CMV sampling.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.forc.2016.08.004.

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