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A sustainable approach for the reliable and simultaneous determination of terpenoids and cannabinoids in hemp inflorescences by vacuum assisted headspace solid-phase microextraction

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ABSTRACT

Cannabis sativa L. is an intriguing plant that has been exploited since ancient times for recreational, medical, textile and food purposes. The plant's most promising bioactive constituents discovered so far belong to the terpenoid and cannabinoid classes. These specialised metabolites are highly concentrated in the plant aerial parts and their chemical characterisation is crucial to guarantee the safe and efficient use of the plant material irrespective of which use it is. This study investigates for the first time the use of vacuum assisted HS-SPME as a sample preparation process in an analytical protocol based on HS-SPME combined to fast GC-MS analysis that aims at comprehensively characterising both the terpenoid and cannabinoid profiles of *Cannabis* inflorescences in a single step. The results proved that vacuum in the HS should be preferred over atmospheric pressure conditions as it ensures the fast recovery of cannabinoid markers at relatively lower sampling temperatures (i.e., 90°C) that do not discriminate the most volatile fraction nor cause the formation of artefacts when the sampling time is minimised.

1. Introduction

Cannabis sativa L. can be considered as one of the most studied plants in reason of its relevance in the illicit drug market and in the textile and food industry [1] as well as of its potential medical usage. Whether the plant is intended for recreational purposes, fiber production (hemp) or medical use, it depends on the content of two major cannabinoids in the aerial parts of the plant: the psychoactive (-)-*trans*-Δ^9-tetrahydrocannabinol (Δ^9-THC) and cannabidiol (CBD), the latter displaying several biological activities but not the psychotropic one [2,3]. Illicit drug chemotype, also known as Type 1, contains an excess of Δ^9-THC and a limited amount of CBD. Contrary, in the *Cannabis* chemotype used in manufacturing (industrial hemp or type III) the ratio is reversed and Δ^9-THC content cannot exceed 0.2%. Finally, type II chemotype, which is used for medical purposes, is defined as having high mean contents of both CBD and Δ^9-THC (i.e., Bedrocan®: 22% THC, <1% CBD; Bediol® 6.5% THC, 8% CBD) [3–5].

Other than Δ^9-THC and CBD, the plant may synthesise several specialised metabolites, including additional phytocannabinoids and terpenes, amongst others [6], which are both produced by stalked glandular trichomes that are highly concentrated on female inflorescences [7]. Phytocannabinoids are C21 compounds known as terpenophenolic compounds. They are produced by the plant in their acidic form which under heating or during storage is decarboxylated into the active neutral form [6]. At least 104 cannabinoids have been isolated so far [8]: the predominant ones are Δ^9-tetrahydrocannabinolic acid (Δ^9-THCA), cannabidiolic acid (CBDA), cannabichromenic acid (CBCA) and cannabigerolic acid (CBGA), which is the precursor of the former compounds. Other minor cannabinoids include cannabinolic acid (CBNA) and Δ^8-THCA, which are artefacts of Δ^9-THCA, and cannabielsoin acid (CBEA) and cannabinodiolic acid (CBNDA) which derive from CBDA [9]. Terpenes are *Cannabis* most abundant specialised metabolites including at least 120 identified terpenoids [8]. Literature data suggest that varying pharmaceutical properties between different *Cannabis* varieties can be attributed to synergistic interactions, known as the 'entourage effect', between cannabinoids and terpenes [9]. A comprehensive qualitative characterisation of both the cannabinoid and terpene profiles of the plant raw material is therefore of utmost importance not only to define its rational use (i.e., whether the plant under investigation was cultivated for fiber production, medical or drug purposes) but also to guarantee the efficacy and safety of its potential pharmaceutical application.

Abbreviations: HS-SPME, headspace solid-phase microextraction; Reg-HS-SPME, HS-SPME under regular conditions; Vac-HS-SPME, HS-SPME under reduced pressure; CBD, cannabidiol; Δ^9-THC, Δ^9-tetrahydrocannabinol; CBC, cannabichromene.

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The most employed method of extraction of cannabinoids from plant raw material is solid-liquid extraction (SLE) using ethanol or acetone as extracting solvents due to their affinity and consequent high extracting efficiency for cannabinoids [10,11]. High performance liquid chromatography (HPLC) and gas chromatography (GC) coupled to mass spectrometry are the analytical techniques of choice for the following qualitative and quantitative analysis [11]. HPLC is usually employed when the acid and the neutral form of the investigated cannabinoids must be measured separately, while GC analyses enable the characterisation of the "total-cannabinoid content" (e.g. the combined amount of THC and THCA) as GC systems, by definition, work with high temperatures that lead unavoidably to the decarboxylation of the cannabinoid acids [11]. The "total-cannabinoid content" is usually measured as it best represents the pharmacological activity of the material, unless differently stated by legislation [12].

Thanks to their volatile nature, the isolation of terpenes, and in particular of mono and sesquiterpenes, from plant raw material is straightforward and their profiling can be performed by headspace solid-phase microextraction (HS-SPME) online combined to GC-MS analysis [5].

The recovery of cannabinoids from solid matrices by HS-SPME is also feasible but requires long sampling times in combination to high sampling temperatures due to their low volatility and low tendency to escape to the headspace. In 2004 Lachenmeier et al. optimised a successful HS-SPME method followed by on-coating derivatisation of the cannabinoids with *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) for the extraction of cannabinoids from hemp food products using 90°C as sampling temperature and 30 min of extraction time [13] while in 2005 Ilias et al. showed that cannabinoids extraction should be performed at 150°C to maximise their recovery in short sampling times (i.e., 5 min) [14]. However, in a very recent work, Czégény et al. investigated the effect of temperature on the composition of pyrolysis products of CBD in e-cigarettes. They tested different operating temperatures (250–400°C) and they proved that, depending on the temperature and atmosphere (i.e., inert of oxidative condition), 25–52% of CBD can be converted into other cannabinoids amongst which Δ^9-THC, Δ^8-THC, cannabinol and cannabichromene (CBC) are the predominant pyrolysates [15]. Even though when performing HS-SPME it is usually unlikely to reach such extreme temperatures, the results of Czégény et al. suggest that (1) reduced sampling temperature should be preferred to obtain a truthful cannabinoid fingerprint profile in the plant raw material, (2) CBD potential degradation should be investigated during the optimisation of the sampling temperature.

As thoroughly described by Psillakis et al. [16–19], vacuum is a powerful experimental parameter to consider to increase the extraction kinetic of semi-volatile compounds during the HS-SPME process. This is because in the case of semivolatiles and under non-equilibrium conditions, a reduced pressure inside the sample container decreases the resistance to mass transfer in the gas zone at the solid-headspace interface. As a consequence, higher extraction efficiencies for semi-volatile compounds can be achieved in shorter sampling time and potentially at milder extraction temperatures [20,21].

This study investigates the advantages and disadvantages of using vacuum assisted HS-SPME (Vac-HS-SPME) over regular HS-SPME (Reg-HS-SPME) as sample preparation process to be exploited in analytical protocols aiming at comprehensively characterising both the terpene and cannabinoid profiles of *Cannabis* inflorescences in a single step employing a total analysis system.

2. Materials and methods

2.1. Chemicals and samples

Cannabidiol (CBD) and cannabichromene (CBC) standard solutions 1.0 mg mL^{-1} in methanol were purchased from Merck KGaA, Darmstadt, Germany.

Dried *Cannabis* inflorescences from type III chemotype were purchased from an authorised local hemp shop.

The dried plant material was pulverised by an electric blender and stored at -18°C.

2.2. HS-SPME procedures under reduced (Vac-HS-SPME) and atmospheric pressure conditions (Reg-HS-SPME)

Divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) 50/30 μm (2 cm length) and over coated PDMS/DVB 75 μm (coating thickness includes 65 μm coating + 10 μm OC (overcoating)) fibers were employed for the experiments. The fibers were purchased from Merck KGaA, Darmstadt, Germany and conditioned following the manufacturer's instructions.

Reg-HS-SPME experiments were performed using conventional headspace 20 mL crimp vials provided by Restek, Bellefonte, USA and 20 mm magnetic ring crimp cap, fitted with 20.6 mm septa (Buty red/PTFE grey, 55 shore A, 1.3 mm).

Vac-HS-SPME experiments were performed in the same commercial headspace 20 mL crimp vials hermetically sealed with a stainless-steel closure (provided by Prof. Elefteria Psillakis) having a hole that could tightly accommodate a Thermogreen® LB-1 septum with half-hole (Supelco, Bellefonte, USA) through which the air evacuation step and the SPME sampling were performed.

For the inflorescences, 10 mg of the pulverised sample were placed inside the vial which was again stored at −18°C for one hour and then air-evacuated [20]. The air-evacuation step was performed with a 22 gauge hypodermic needle sealed to a 5 mL syringe tightly secured to the tubing of a N 820.3 FT.18 (7 mbar ultimate vacuum) pumping unit manufactured by KNF Lab (Milan, Italy). The needle was inserted through the septum and the vial was air-evacuated for one minute. For Reg-HS-SPME the procedure was the same, while omitting the air evacuation step.

For CBD standard sampling, 10 µL of the 1.0 mg mL^{-1} solution were introduced through the closure septum after the air evacuation step. For Reg-HS-SPME experiments, the liquid sample was introduced in the vial by the open vial-technique [22].

After the sampling the fiber was withdrawn and the SPME device moved to the GC-MS system for analysis. GC desorption lasted 10 min to minimise carry-over.

10 mg of pulverised plant material were sampled at three different extraction temperatures (i.e., 80, 90 and 150°C) under both Vac- and Reg-HS-SPME experiments. Sampling-time profiles were obtained for all the above mentioned conditions by sampling for 5, 15, and 30 min. 10 µL of CBD standard solution 1.0 mg mL^{-1} were sampled at 90 and 150°C for 5 min, under both pressure conditions. All extractions were run in triplicate.

2.3. Instrumental set-up

2.3.1. GC-MS systems and columns

Analyses were carried out on two different instruments: (1) a MPS-2 multipurpose sampler (Gerstel, Mülheim a/d Ruhr, Germany) installed on a Shimadzu GC-FID-MS system consisting of a Shimadzu GC 2010 system, equipped with FID, in parallel with a Shimadzu QP2010-PLUS GC–MS mass spectrometer (Shimadzu, Milan, Italy); (2) a MPS-2 multipurpose sampler (Gerstel, Mülheim a/d Ruhr, Germany) installed on an Agilent 6890 N GC system coupled to a 5975 MSD mass spectrometer (Agilent Technologies, Santa Clara, CA).

GC analyses were carried out using two MEGA-5 95% methylpolysiloxane 5%-phenyl (MEGA, Legnano, MI, Italy) columns: a conventional 30 m × 0.25 mm d_c, 0.25 µm d_f column installed on the Agilent 6890 N GC - 5975 MSD and a narrow bore 15 m × 0.18 mm d_c, 0.18 µm d_f column installed on the Shimadzu GCMS-QP2010.

Data was processed with the ChemStation Version E.02.02.1431 data processing system (Agilent Technologies, Santa Clara, CA).

2.3.2. GC-MS conditions

Analyses were carried out under the following conditions. Temperatures: injector: 250 °C, transfer line: 270 °C, ion source: 200 °C; carrier gas: He; flow control mode: constant linear velocity; flow rate: 1.00 mL min^{-1} (conventional column), 0.72 mL min^{-1} (narrow bore column); injection mode: split; split ratio: 1:20. The MS was operated in electron ionisation mode (EI) at 70 eV, scan rate: 666 u/s, mass range: 35–350 m/z. Temperature programs: (i) 50°C (one minute)// 3°C/min//250°C (5 min) for conventional MEGA-%; (ii) 50°C (30 s) //7.2°C/min// 250°C (two minutes) for the narrow bore column. The chromatographic conditions for the narrow bore columns were obtained by translating the method parameters through the Agilent method translator software [23]. Identification was performed via comparisons of linear retention indices and mass spectra either with those of authentic standards, or with data stored in commercial [24] and in-house libraries.

3. Results and discussion

Table S1 in the supplementary materials provides the list of the target compounds together with their physicochemical properties (i.e., $LogK_{ow,}$ boiling point and vapour pressure). The mono and sesquiterpene markers to be investigated were chosen according to the results of Jin et al. who comprehensively profiled reference specialised metabolites, in *Cannabis* inflorescences, leaves, stem barks and roots for the three *Cannabis* chemotypes (i.e., Type I, II and III) [8].

3.1. Preliminary optimisation of the fiber coating and chromatographic conditions

The first set of experiments aimed at selecting (1) the optimum fiber coating that could extract all the investigated analytes with acceptable sensitivity and (2) the best chromatographic conditions providing an acceptable resolution of all the investigated markers in a reasonable time for high throughput analyses. In our study two fiber coatings were tested: the PDMS/DVB and the DVB/CAR/PDMS fibers. Fig. 1A and B shows the profiles obtained with the two investigated coatings when sampling 10 mg of matrix with Reg-HS-SPME at 90°C for 30 min of extraction. The chromatographic analyses were performed employing a conventional 30 m × 0.25 mm d_c, 0.25 μm d_f MEGA-5 column. Irrespective of the fiber coating, the most abundant compounds amongst the recovered mono and sesquiterpenes were *β*-myrcene (3), *trans*-*β*-caryophyllene (11) and selina-3,7(11)-diene (20) while only one cannabinoid (i.e., CBD (29)) was recovered. In agreement with the results of Ilias et al. [14], the PDMS/DVB fiber proved to be more efficient for the recovery of CBD compared to the triphasic fiber which, however, extracted a quantitative richer mono and sesquiterpene profile. The PDMS/DVB fiber was chosen for the following experiments because of its higher recovery of CBD while still providing an acceptable sensitivity for the investigated mono and sesquiterpene metabolites.

The employed conventional MEGA-5 column proved to separate the main selected markers with an acceptable resolution. The possibility to improve the analysis speed and to decrease the use of gas with a greener analytical protocol was then investigated. The chromatographic analysis

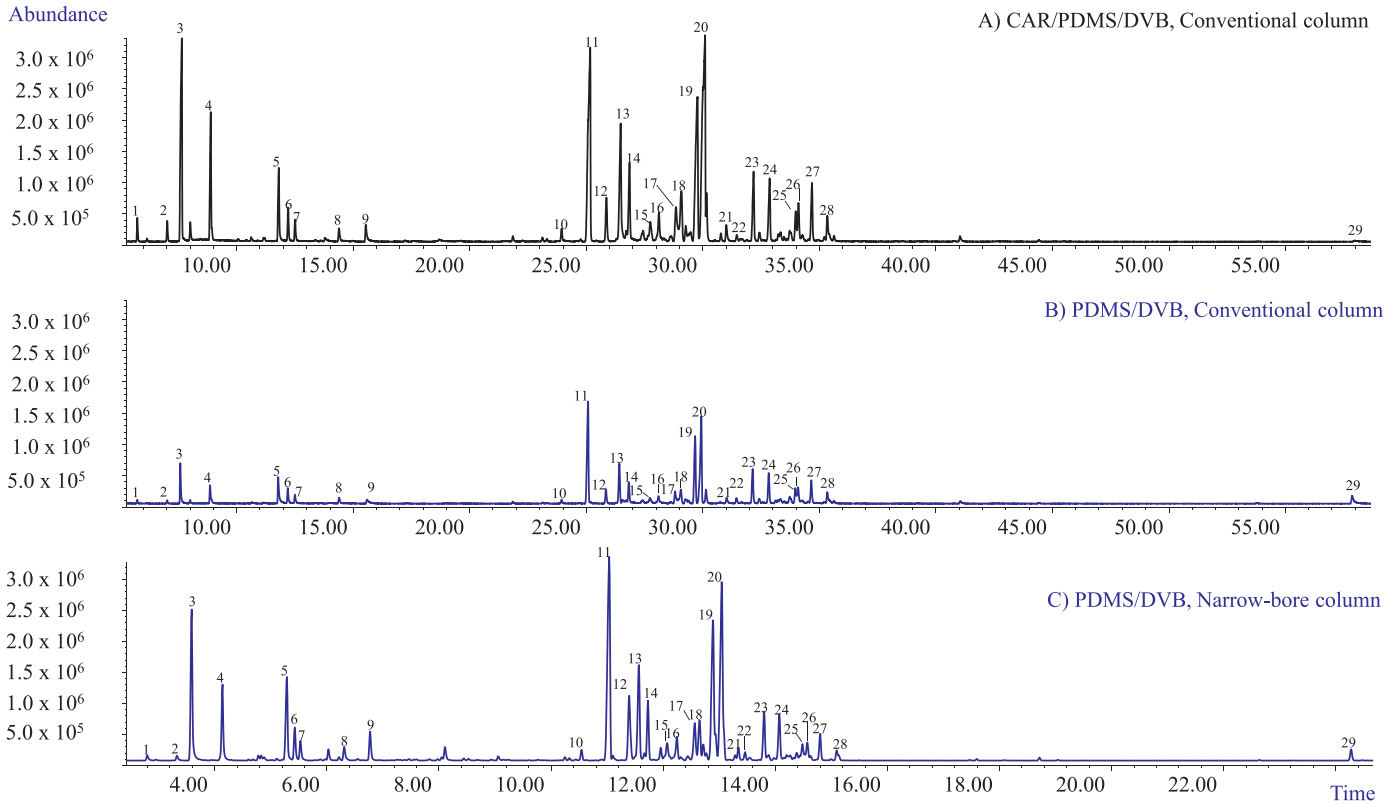

Fig. 1. Reg-HS-SPME GC-MS profiles of the investigate hemp inflorescences obtained with different polymer coatings and chromatographic conditions. (A) Polymer coating: CAR/PDMS/DVB; Column: conventional MEGA-5; GC-MS instrument: Agilent 6890 N GC coupled to a 5975 MSD. (B) Polymer coating: PDMS/DVB; Column: conventional MEGA-5; GC-MS instrument: Agilent 6890 N GC coupled to a 5975 MSD. (C) Polymer coating: PDMS/DVB; Column: narrow-bore MEGA-5; GC-MS instrument: Shimadzu QP2010-PLUS GC–MS system. GC-MS Analysis conditions: see experimental section. Reg-HS-SPME parameters: sampling temperature 90°C, extraction time 30 min. Legend: (1) *α*-Pinene, (2) *β*-Pinene, (3) *β*-Myrcene, (4) Limonene, (5) Linalool, (6) Fenchol, (7) *cis*-Pinene hydrate, (8) Borneol, (9) *α*-Terpineol, (10) *α*-Patchoulene, (11) *trans*-*β*-Caryophyllene, (12) *trans*-*α*-Bergamotene, (13) *α*-Humulene, (14) *trans*-*β*-Farnesene, (15) *β*-Selinene, (16) *α*-Selinene, (17) *α*-Farnesene, (18-19) Sesquiterpenes (MW 204), (20) Selina-3,7(11)-diene, (21) trans-Nerolidol, (22) Caryophyllene oxide, (23) Guaiol, (24) 10-epi-*γ*-Eudesmol, (25) *β*-Eudesmol, (26) *α*-Eudesmol, (27) Bulnesol, (28) *α*-Bisabolol, (29) Cannabidiol

was speeded up by translating the method to a 15 m × 0.18 mm d_c, 0.18 μm d_f column using the method translation approach [23,25,26]. Fig. 1C reports the translated GC-MS profile of the investigated hemp inflorescences with the narrow-bore MEGA-5 column. The analysis time was reduced from 72.67 to 30.28 min, while maintaining the separation and the elution pattern of the investigated markers.

3.2. Vacuum HS-SPME and regular HS-SPME

Due to their relative high molecular weights and boiling points (e.g. CBD 428.52°C, 760 mmHg, [27]) cannabinoids have a low tendency to escape to headspace and require high sampling temperatures to be recovered by HS-SPME [14]. However, in view of a reliable and comprehensive HS-SPME method suitable for the simultaneous qualitative characterisation of both the terpenoid and the cannabinoid fractions of *Cannabis* inflorescences, high sampling temperatures are not advisable for two reasons. First, they may decrease the distribution coefficient between the fiber and the headspace (i.e., K_{fs}) of the most volatile components reducing their recovery [28]. In addition, high sampling temperature, especially when combined with relatively long extraction times, may induce decomposition of some compounds and/or creation of other components or artefacts [29]. According to the theory, a reduced pressure inside the HS sample container increases the compounds' molecular diffusion coefficient in air and favours their vapour flux at the solid surface. As a result, reducing the total headspace pressure is an alternative and complementary strategy, compared to the adoption of high sampling temperatures, to speed up the extraction kinetic of semi-volatile compounds [30].

In this study we compared the performances of Vac-HS-SPME to those of Reg-HS-SPME under different sampling temperatures (i.e., 150°C, 90°C and 80°C) and extraction times (i.e., 5, 15, 30 min) and we explored whether Vac-HS-SPME could be a more suitable sample preparation technique for the simultaneous characterisation of both the terpenoid and cannabinoid profiles of *Cannabis* inflorescences. For the following discussion CBD will be considered as representative of the plant cannabinoids, while *β*-myrcene and *trans*-*β*-caryophyllene of the mono and sesquiterpene markers, respectively.

Tables 1, 2 and 3 report (1) the average peak area and % RSD ($n = 3$) of all the investigated compounds when testing 10 mg of matrix under the different experimental conditions and (2) the relative analyte abundance (RAA) defined as the ratio between the average peak area obtained under vacuum to that measured at atmospheric pressure conditions at 150°C, 90°C and 80°C, respectively. Fig. 2 shows the extraction temperature profiles of *β*-myrcene, *trans*-*β*-caryophyllene and CBD acquired when sampling 10 mg of the matrix for 5 min at the different investigated temperatures. Finally, Fig. 3 provides the extraction times profiles for CBD for each sampling temperature (i.e., 150, 90 and 80°C). In all the cases, the results of both reduced and atmospheric pressure conditions are reported.

In good agreement with the outcomes of Ilias et al. [14], sampling under extremely high temperatures (i.e., 150°C) maximised the recovery of CBD even within a short sampling time (i.e., 5 min) and under both pressure conditions as stands out in Fig. 2. In addition to CBD, two other less abundant cannabinoids were recovered. One of them is cannabichromene (CBC), whose identity was confirmed by comparing its retention time and mass spectrum to those of a certified standard, while the other one is supposed to be Δ^9-THC, as its mass spectrum matches NIST and WILEY MS data as well as its linear retention index [24]. Figs. S1, S2 and S4 in the supplementary materials report the mass spectra of the detected cannabinoids.

The positive effect of vacuum on the recovery of CBD was more important after 5 min of sampling (i.e., CBD RAA equal to 3) compared to 15 and 30 min (i.e., CBD RAA equal to 1.6) suggesting that equilibrium was being approached after 15 min of extraction. In contrast, irrespective of the pressure conditions, sampling at 150°C significantly discriminated against the recovery of the more volatile markers (i.e., mono and sesquiterpenes). This trend was probably due to the combination of two phenomena: (1) a decrease of the distribution coefficient between the fiber and the headspace (i.e., K_{fs}) [28], (2) an intensification of competitive adsorption and displacement of low molecular weight analytes given the extremely high amount of extracted CBD [31]. Therefore, 150°C as sampling temperature proved to be inappropriate for the simultaneous characterisation of the terpene and cannabinoid profiles.

The recovery of CBD was significantly reduced when sampling at relatively lower temperature (i.e., 80 and 90°C) compared to 150°C. Despite this, very promising results were obtained at 90°C especially when sampling under reduced pressure conditions. What is striking in Fig. 3 is the steep rise in the extraction kinetic of CBD obtained when sampling at reduced pressure conditions. At 90°C, irrespective of the sampling time, the amount of CBD extracted with vacuum was at least sixty times greater than that recovered under regular conditions and in just 5 min, vacuum ensured the extraction of a sufficient amount of CBD to meet the instrument sensitivity and to provide a good picture of CBD abundance in the inflorescences. As regards the more volatile markers (i.e., *β*-myrcene, *trans*-*β*-caryophyllene), at 90°C their recovery was on average 10 times higher than that obtained at 150°C irrespective of the pressure conditions. As evidenced by the RAA values reported in Table 2 and in Fig. 5, with 5 min of sampling, lowering the total pressure in the headspace did not improve the extraction of the monoterpene markers (RAA close to one) while, on average, it doubled the recovery of sesquiterpenes, as was expected by their lower volatilities. With longer sampling times (i.e., 15 and 30 min) the RAA significantly dropped for both mono and sesquiterpenes indicating decreased mass loadings under vacuum conditions.

The results obtained when sampling at 80°C were very similar to those observed at 90°C. Under regular conditions, the amount of CBD extracted after 5 min was below the instrument limit of detection (signal to noise ratio below three) while when sampling with vacuum the amount of CBD recovered was far above the instrument limit of quantification (signal to noise ratio close to 1000). When sampling at 80°C the amount of CBD extracted after 5 min of sampling was four times lower than that recovered at 90°C and the repeatability of the extraction of the minor cannabinoids drops down to RSD values higher than 40% while there were no significant differences in the recovery of the most volatile markers, irrespective of the extraction times. These results suggest that under vacuum conditions, 90°C is a more suitable extraction temperature compared to 80°C for the simultaneous optimal recovery of both the volatile (i.e., mono and sesquiterpenes) and semi-volatile markers (i.e., cannabinoids) of *Cannabis* inflorescences.

3.4. Thermal stability of CBD under the investigated sampling conditions

In light of the results obtained by Czégény et al. [15], the thermal stability of CBD at 150°C and 90°C was studied. First, to ensure that no CDB degradation occurs within the analytical instrument, one μL of a CBD standard solution 1 mg mL^{-1} was directly injected and analysed by GC-MS. Fig. 4A reports the obtained chromatogram which proved a standard purity in-line with that declared by the manufacturer and excluded the possibility of CBD degradation within the gas chromatograph.

10 μL of the same 1.0 mg mL^{-1} CBD standard solution were then sampled by HS-SPME, under both pressure conditions for 5 min at 150 and 90°C. Three replicates for each sampling temperature were performed. The chromatograms reported in Fig. 4B and C demonstrate that after 5 min of extraction at 150°C, under both pressure conditions, CBD undergoes degradation forming three cannabinoids. The first one is CBC whose identity was again confirmed by comparing its retention time and mass spectrum to those of a certified standard. The other two cannabinoids are supposed to be Δ^9-THC, Δ^8-THC as their mass spectra matched NIST and WILEY MS data as well as their linear retention index [24]. The relative amount of CBD undergoing to degradation was measured

Table 1
Mean peak area, %RSD (n=3) and Relative Analyte Abundance (RAA) of investigated markers sampled by Reg and Vac-HS-SPME at 150°C. RAA defines the ration between Vac-HS-SPME and Reg-HS-SPME area. Legend: red triangle RAA < 0.8, yellow line 0.8 < RAA < 1.2, green triangle RAA > 1.2

Compound	5 minutes		15 minutes			30 minutes									
	Reg-HS-SPME Average Area	% RSD	Vac-HS-SPME Average Area	% RSD	RAA	Reg-HS-SPME Average Area	% RSD	Vac-HS-SPME Average Area	% RSD	RAA	Reg-HS-SPME Average Area	% RSD	Vac-HS-SPME Average Area	% RSD	RAA
α-Pinene	4.7E+04	18.9	8.1E+04	9.4	▲1.7	4.2E+04	44.4	4.8E+04	4.0	▬ 1.2	4.1E+04	44.7	4.7E+04	7.3	▼ 1.1
β-Pinene	4.1E+04	18.6	7.6E+04	16.2	▲ 1.8	4.9E+04	35.5	4.1E+04	5.7	▬ 0.8	6.1E+04	21.4	3.5E+04	6.6	▬ 0.6
β-Myrcene	1.0E+06	15.7	1.3E+06	20.4	▲ 1.3	9.0E+05	49.5	6.7E+05	7.5	▬ 0.7	7.8E+05	43.3	5.4E+05	4.8	▼ 0.7
Limonene	5.5E+05	10.7	8.0E+05	12.7	▲ 1.5	4.8E+05	39.0	4.7E+05	19.1	▼ 1.0	4.6E+05	32.8	4.3E+05	9.4	▼ 0.9
Linalool	6.2E+05	8.6	9.4E+05	6.4	▲ 1.5	7.0E+05	8.4	5.8E+05	10.5	▬ 0.8	6.4E+05	6.7	4.7E+05	11.2	▬ 0.7
Fenchol	2.8E+05	6.7	4.1E+05	6.3	▲ 1.5	3.2E+05	8.7	2.8E+05	9.2	▬ 0.9	3.2E+05	10.0	2.2E+05	5.3	▼ 0.7
cis-Pinene hydrate	2.3E+05	6.6	3.6E+05	7.7	▲ 1.6	2.6E+05	9.1	2.5E+05	6.8	▬ 1.0	2.6E+05	2.4	2.1E+05	5.0	▼ 0.8
Borneol	1.7E+05	4.8	2.4E+05	5.6	▲ 1.4	2.0E+05	5.9	1.7E+05	11.0	▬ 0.8	2.0E+05	6.4	1.3E+05	2.1	▬ 0.6
α-Terpineol	3.3E+05	7.3	3.8E+05	6.6	▲ 1.1	3.8E+05	8.7	2.8E+05	11.4	▬ 0.7	4.0E+05	15.5	2.1E+05	2.3	▼ 0.5
α-Patchoulene	1.1E+05	5.5	1.3E+05	10.8	▬ 1.2	1.4E+05	14.7	1.1E+05	12.4	▬ 0.8	1.8E+05	5.0	6.6E+04	16.7	▼ 0.4
trans-*β*-Caryophyllene	3.5E+06	5.9	4.2E+06	17.9	▬ 1.2	4.3E+06	3.9	2.7E+06	13.0	▬ 0.6	5.1E+06	8.1	1.5E+06	8.0	▼0.3
trans-*α*-Bergamotene	2.0E+06	7.3	2.0E+06	11.0	▬1.0	2.3E+06	7.9	1.2E+06	10.8	▼0.5	2.6E+06	15.6	7.2E+05	4.8	▼0.3
α-Humulene	1.5E+06	5.3	1.6E+06	16.2	▬1.1	1.8E+06	2.8	1.1E+06	11.4	▼0.6	2.1E+06	9.8	6.3E+05	15.4	▼0.3
trans-*β*-Farnesene	5.8E+05	5.4	6.3E+05	17.1	▬1.1	6.8E+05	12.4	3.5E+05	11.0	▼0.5	7.6E+05	9.1	1.8E+05	20.2	▼0.2
β-Selinene	4.1E+05	6.0	3.0E+05	16.9	▬0.7	4.4E+05	21.2	2.2E+05	13.6	▼0.5	5.4E+05	34.8	1.3E+05	10.5	▼0.2
α-Selinene	3.7E+05	5.6	3.8E+05	12.0	▬1.0	4.2E+05	4.4	3.3E+05	6.9	▬0.8	4.5E+05	7.5	3.1E+05	2.7	▼0.7
α-Farnesene	7.7E+05	6.8	7.5E+05	19.0	▬1.0	8.5E+05	3.7	4.1E+05	15.8	▬0.5	1.0E+06	13.0	2.0E+05	6.2	▼0.2
Sesquiterpene (MW 204)	4.1E+06	6.4	4.2E+06	16.9	▬1.0	4.9E+06	2.2	2.7E+06	11.6	▼0.5	5.5E+06	13.2	1.4E+06	17.0	▼0.3
Selina-3,7(11)-diene	5.0E+06	12.7	4.9E+06	29.9	▬1.0	5.9E+06	9.6	2.8E+06	12.3	▼0.5	6.9E+06	20.8	1.6E+06	7.4	▼0.2
Caryophyllene oxide	4.6E+05	13.8	5.0E+05	9.2	▬1.1	6.0E+05	9.1	3.3E+05	10.1	▼0.6	6.8E+05	14.3	2.1E+05	5.5	▼0.3
Guaiol	3.7E+06	11.1	5.0E+06	13.3	▲1.3	4.7E+06	14.5	2.9E+06	11.0	▼0.6	5.3E+06	21.7	1.8E+06	5.7	▼0.3
10-epi-*γ*-Eudesmol	3.6E+06	10.6	4.4E+06	12.1	▼1.2	4.5E+06	13.3	2.8E+06	11.1	▼0.6	5.2E+06	21.7	1.7E+06	3.6	▼0.3
β-Eudesmol	1.9E+06	10.3	3.2E+06	10.9	▲1.7	2.7E+06	37.0	1.3E+06	9.8	▼0.5	3.6E+06	17.9	7.4E+05	4.0	▼0.2
α-Eudesmol	1.5E+06	11.8	3.5E+06	6.7	▲2.3	2.6E+06	57.1	1.1E+06	11.9	▼0.4	4.0E+06	23.5	7.1E+05	5.7	▼0.2
Bulnesol	3.5E+06	13.3	5.0E+06	10.3	▲1.4	4.6E+06	19.2	3.0E+06	9.7	▼0.6	5.1E+06	23.2	1.8E+06	6.4	▼0.3
α-Bisabolol	2.6E+06	14.1	3.9E+06	12.1	▲1.5	3.1E+06	21.6	1.9E+06	8.5	▼0.6	3.5E+06	23.0	1.1E+06	4.1	▼0.3
CBD	2.5E+07	28.9	7.4E+07	5.5	▲**3.0**	6.7E+07	7.7	1.1E+08	2.3	▲**1.6**	7.8E+07	22.6	1.2E+08	0.9	▲**1.6**
CBC	1.2E+06	19.8	4.3E+06	3.2	▲3.5	4.0E+06	8.5	5.8E+06	2.2	▲1.5	4.7E+06	24.0	5.9E+06	17.4	▲1.3
Supposed Δ^9-THC	9.5E+05	39.2	4.0E+06	9.6	▲4.2	2.9E+06	16.8	7.4E+06	2.4	▲2.5	3.5E+06	52.2	9.0E+06	2.7	▲2.6

n.d.: Not detectable
n.c.: Not calculable because the compound was not detected in Reg-HS-SPME analyses

Table 2
Mean peak area, %RSD (n=3) and Relative Analyte Abundance (RAA) of investigated markers sampled by Reg and Vac-HS-SPME at 90°C. RAA defines the ration between Vac-HS-SPME and Reg-HS-SPME area. Legend: red triangle RAA < 0.8, yellow line 0.8 < RAA < 1.2, green triangle RAA > 1.2

Compound	5 minutes					15 minutes					30 minutes				
	Reg-HS-SPME Average Area	% RSD	Vac-HS-SPME Average Area	% RSD	RAA	Reg-HS-SPME Average Area	% RSD	Vac-HS-SPME Average Area	% RSD	RAA	Reg-HS-SPME Average Area	% RSD	Vac-HS-SPME Average Area	% RSD	RAA
α-Pinene	4.9E+05	58.9	4.7E+05	12.6	1.0	4.2E+05	9.0	3.2E+05	1.0	0.8	5.1E+05	2.3	2.4E+05	0.4	▼0.5
β-Pinene	4.6E+05	43.1	4.4E+05	11.1	0.9	4.4E+05	6.2	2.2E+05	4.8	▼0.5	4.8E+05	12.8	1.7E+05	1.1	▼0.4
β-Myrcene	9.4E+06	43.1	6.6E+06	17.0	▼0.7	9.9E+06	4.2	4.7E+06	3.6	▼0.5	1.1E+07	1.0	3.5E+06	2.3	▼0.3
Limonene	5.2E+06	36.8	3.8E+06	14.0	▼0.7	5.5E+06	3.5	2.8E+06	6.1	▼0.5	6.0E+06	0.2	2.1E+06	2.1	▼0.3
Linalool	6.8E+06	7.3	7.0E+06	8.0	1.0	6.3E+06	2.7	4.4E+06	5.2	▼0.7	6.7E+06	4.8	2.9E+06	4.8	▼0.4
Fenchol	2.7E+06	8.8	3.1E+06	9.9	1.2	2.7E+06	3.7	2.0E+06	4.5	▼0.7	3.0E+06	0.5	1.3E+06	4.8	▼0.4
cis-Pinene hydrate	1.7E+06	6.0	2.4E+06	6.8	▲1.4	1.8E+06	3.9	1.5E+06	6.0	0.9	2.0E+06	2.2	1.0E+06	5.0	▼0.5
Borneol	1.2E+06	5.3	1.7E+06	7.0	▲1.4	1.3E+06	4.0	1.2E+06	6.6	0.9	1.4E+06	3.7	7.6E+05	1.5	▼0.5
α-Terpineol	2.3E+06	4.9	3.1E+06	11.6	▲1.3	2.5E+06	3.7	2.0E+06	4.7	0.8	2.7E+06	4.7	1.4E+06	2.4	▼0.5
β-Patchoulene	8.4E+05	8.3	9.2E+05	10.6	1.1	1.0E+06	3.0	5.0E+05	5.5	▼0.5	1.1E+06	5.0	3.3E+05	27.5	▼0.3
trans-β-Caryophyllene	1.7E+07	6.7	2.0E+07	13.1	1.1	2.0E+07	3.4	1.2E+07	4.4	▼0.6	2.2E+07	3.5	7.3E+06	2.0	▼0.3
trans-α-Bergamotene	5.6E+06	7.2	1.1E+07	4.6	▲1.9	9.0E+06	5.1	1.0E+07	0.8	1.1	1.1E+07	7.4	7.3E+06	1.0	▼0.6
α-Humulene	7.5E+06	6.8	9.5E+06	9.2	▲1.3	9.5E+06	3.4	6.3E+06	2.0	▼0.7	1.0E+07	4.8	4.0E+06	2.1	▼0.4
trans-β-Farnesene	3.9E+06	6.5	4.7E+06	15.8	1.2	5.3E+06	2.1	2.9E+06	5.1	▼0.6	5.6E+06	5.5	1.6E+06	2.5	▼0.3
β-Selinene	2.0E+06	6.6	2.1E+06	13.9	1.1	2.8E+06	3.5	1.2E+06	4.5	▼0.5	3.1E+06	7.4	6.9E+05	2.0	▼0.2
α-Selinene	1.8E+06	7.0	2.7E+06	7.8	▲1.5	2.4E+06	3.7	2.0E+06	1.5	0.8	2.8E+06	6.7	1.9E+06	4.7	▼0.7
α-Farnesene	3.2E+06	9.7	5.3E+06	15.0	▲1.7	5.0E+06	2.5	3.6E+06	6.2	▼0.7	5.7E+06	8.0	1.8E+06	0.5	▼0.3
Sesquiterpene (MW 204)	1.2E+07	11.7	2.0E+07	8.6	▲1.7	1.7E+07	9.6	1.3E+07	6.1	0.8	2.0E+07	3.5	7.5E+06	0.3	▼0.4
Selina-3,7(11)-diene	1.5E+07	6.3	2.4E+07	8.0	▲1.6	2.1E+07	3.4	1.5E+07	1.7	▼0.7	2.4E+07	4.2	8.7E+06	1.7	▼0.4
Caryophyllene oxide	6.3E+05	18.7	1.5E+06	5.4	▲2.3	1.2E+06	8.5	1.5E+06	2.3	▲1.3	1.7E+06	22.3	1.4E+06	3.4	0.8
Guaiol	3.3E+06	5.9	1.0E+07	10.3	▲3.1	6.6E+06	7.7	1.3E+07	1.2	▲1.9	1.0E+07	6.0	1.2E+07	1.3	1.2
10-epi-γ-Eudesmol	2.9E+06	4.1	8.5E+06	7.5	▲2.9	5.5E+06	6.8	9.8E+06	1.6	▲1.8	8.0E+06	8.0	9.3E+06	1.7	1.2
β-Eudesmol	1.5E+06	18.8	4.1E+06	7.9	▲2.8	2.9E+06	6.1	6.4E+06	13.1	▲2.2	4.6E+06	7.4	5.2E+06	0.6	1.1
α-Eudesmol	1.2E+06	20.4	3.2E+06	5.9	▲2.6	2.2E+06	7.8	5.9E+06	32.4	▲2.6	3.3E+06	7.8	3.9E+06	1.9	1.2
Bulnesol	1.8E+06	9.2	7.2E+06	9.4	▲4.0	4.4E+06	8.3	9.9E+06	2.0	▲2.2	7.2E+06	7.0	1.0E+07	3.3	▲1.4
α-Bisabolol	1.1E+06	10.6	5.0E+06	9.5	▲4.7	3.1E+06	8.3	8.3E+06	2.0	▲2.7	5.4E+06	11.0	9.1E+06	1.2	▲1.7
CBD	4.5E+04	13.9	4.1E+06	9.3	▲**91.1**	7.5E+04	13.1	9.8E+06	8.7	▲**131.0**	2.0E+05	15.0	1.6E+07	5.5	▲**80.5**
CBC	n.d.	n.c.	1.7E+05	15.0	n.c.	n.d.	n.c.	3.8E+05	20.9	n.c.	n.d.	n.c.	9.6E+05	14.2	n.c.
Supposed Δ^9-THC	n.d.	n.c.	2.3E+05	21.9	n.c.	n.d.	n.c.	4.7E+05	14.9	n.c.	n.d.	n.c.	8.3E+05	28.8	n.c.

n.d.: Not detectable
n.c.: Not calculable because the compound was not detected in Reg-HS-SPME analyses

Table 3
Mean peak area, %RSD (n=3) and Relative Analyte Abundance (RAA) of investigated markers sampled by Reg and Vac-HS-SPME at 80°C. RAA defines the ration between Vac-HS-SPME and Reg-HS-SPME area. Legend: red triangle RAA < 0.8, yellow line 0.8 < RAA < 1.2, green triangle RAA > 1.2

Compound	5 minutes					15 minutes					30 minutes				
	Reg-HS-SPME Average Area	% RSD	Vac-HS-SPME Average Area	% RSD	RAA	Reg-HS-SPME Average Area	% RSD	Vac-HS-SPME Average Area	% RSD	RAA	Reg-HS-SPME Average Area	% RSD	Vac-HS-SPME Average Area	% RSD	RAA
α-Pinene	6.7E+05	13.1	3.9E+05	8.2	▼0.6	5.9E+05	13.8	2.8E+05	10.9	▼0.5	6.3E+05	8.0	2.3E+05	15.0	▼0.4
β-Pinene	6.6E+05	6.0	3.4E+05	8.6	▼0.5	5.8E+05	12.9	3.2E+05	9.4	▼0.6	5.8E+05	8.2	1.8E+05	12.6	▼0.3
β-Myrcene	1.4E+07	3.9	5.5E+06	5.6	▼0.4	1.2E+07	14.5	4.0E+06	9.6	▼0.3	1.3E+07	6.8	3.4E+06	13.0	▼0.3
Limonene	7.2E+06	3.9	3.5E+06	5.1	▼0.5	6.5E+06	13.5	2.3E+06	8.3	▼0.4	7.0E+06	7.0	2.1E+06	9.9	▼0.3
Linalool	6.1E+06	5.6	7.5E+06	5.9	▲1.2	6.6E+06	4.2	5.4E+06	6.0	▬0.8	7.2E+06	3.0	3.8E+06	10.2	▼0.5
Fenchol	2.4E+06	5.1	3.1E+06	5.1	▲1.3	2.7E+06	5.4	2.3E+06	5.9	▬0.8	3.1E+06	5.1	1.5E+06	11.5	▼0.5
cis-Pinene hydrate	1.4E+06	6.2	2.4E+06	5.6	▲1.7	1.7E+06	3.9	1.8E+06	4.5	▬1.0	1.9E+06	5.0	1.2E+06	8.0	▼0.6
Borneol	8.4E+05	7.4	1.7E+06	5.5	▲2.0	1.2E+06	3.0	1.2E+06	3.5	▬1.1	1.3E+06	6.5	9.1E+05	5.3	▼0.7
α-Terpineol	1.4E+06	8.1	3.1E+06	4.9	▲2.2	2.2E+06	0.9	2.3E+06	2.1	▬1.1	2.5E+06	6.4	1.7E+06	4.0	▼0.7
α-Patchoulene	6.1E+05	14.9	9.3E+05	7.3	▲1.5	1.0E+06	3.0	5.1E+05	4.8	▼0.5	1.1E+06	5.6	3.5E+05	10.1	▼0.3
trans-β-Caryophyllene	1.3E+07	12.0	1.9E+07	6.2	▲1.5	2.1E+07	1.0	1.2E+07	4.2	▼0.6	2.2E+07	4.2	8.8E+06	8.0	▼0.4
trans-α-Bergamotene	2.9E+06	14.2	8.7E+06	3.0	▲3.0	6.8E+06	2.9	9.8E+06	2.7	▲1.5	9.0E+06	5.5	9.1E+06	3.9	▬1.0
α-Humulene	4.5E+06	6.3	8.9E+06	4.9	▲2.0	8.5E+06	2.5	6.1E+06	3.9	▼0.7	1.0E+07	5.7	4.7E+06	6.1	▼0.5
trans-β-Farnesene	2.0E+06	8.4	4.3E+06	8.4	▲2.1	4.7E+06	0.4	2.8E+06	10.0	▼0.6	5.7E+06	6.2	2.2E+06	14.6	▼0.4
β-Selinene	8.8E+05	9.8	1.9E+06	7.9	▲2.1	2.0E+06	15.8	1.2E+06	8.3	▼0.6	2.9E+06	5.4	8.8E+05	11.2	▼0.3
α-Selinene	9.4E+05	9.7	2.5E+06	4.9	▲2.7	2.0E+06	1.8	1.9E+06	5.5	▬0.9	2.5E+06	5.9	1.6E+06	9.0	▼0.6
α-Farnesene	1.5E+06	11.7	4.7E+06	2.2	▲3.2	3.6E+06	10.9	3.3E+06	11.5	▬0.9	5.2E+06	7.0	2.7E+06	14.2	▼0.5
Sesquiterpene (MW 204)	6.7E+06	7.1	1.7E+07	4.5	▲2.6	1.5E+07	3.5	1.3E+07	6.3	▬0.9	1.9E+07	5.3	1.0E+07	9.0	▼0.6
Selina-3,7(11)-diene	7.9E+06	10.4	2.2E+07	2.6	▲2.8	1.7E+07	3.9	1.8E+07	5.5	▬1.0	2.3E+07	5.5	1.3E+07	25.3	▼0.6
Caryophyllene oxide	1.8E+05	10.6	1.1E+06	3.3	▼6.1	6.4E+05	8.8	1.4E+06	2.0	▼2.1	1.0E+06	9.1	1.4E+06	2.1	▼1.3
Guaiol	1.2E+06	9.5	7.0E+06	4.9	▼5.9	3.9E+06	3.1	1.1E+07	8.5	▼2.7	6.2E+06	7.8	1.1E+07	9.1	▼1.8
10-epi-γ-Eudesmol	1.1E+06	9.8	6.1E+06	4.3	▼5.5	3.4E+06	2.9	8.3E+06	3.8	▼2.4	5.3E+06	7.3	8.7E+06	3.9	▼1.6
β-Eudesmol	4.6E+05	11.1	2.7E+06	5.3	▼5.9	1.4E+06	26.8	4.4E+06	9.1	▼3.2	2.8E+06	10.8	5.3E+06	20.4	▼1.9
α-Eudesmol	3.9E+05	10.7	2.2E+06	4.7	▼5.7	1.3E+06	10.9	3.3E+06	6.4	▼2.6	2.2E+06	13.8	4.9E+06	47.6	▼2.3
Bulnesol	6.3E+05	8.9	4.8E+06	4.6	▼7.6	2.5E+06	4.9	7.8E+06	7.2	▼3.2	4.2E+06	8.7	8.9E+06	7.1	▼2.1
α-Bisabolol	3.1E+05	8.1	3.0E+06	5.2	▼9.5	1.5E+06	8.0	6.1E+06	10.6	▼4.1	2.7E+06	12.1	7.8E+06	10.7	▼2.8
CBD	n.d.	n.c.	1.1E+06	6.7	n.c.	7.0E+04	46.4	6.2E+06	6.6	▼**88.8**	2.4E+05	2.1	1.3E+07	12.5	▼**53.8**
CBC	n.d.	n.c.	n.d.	n.c.	n.c.	n.d.	n.c.	2.2E+05	64.3	n.c.	n.d.	n.c.	4.2E+05	49.4	n.c.
Supposed Δ^9-THC	n.d.	n.c.	3.7E+04	51.9	n.c.	n.d.	n.c.	3.2E+05	64.2	n.c.	n.d.	n.c.	5.1E+05	43.4	n.c.

n.d.: Not detectable
n.c.: Not calculable because the compound was not detected in Reg-HS-SPME analyses

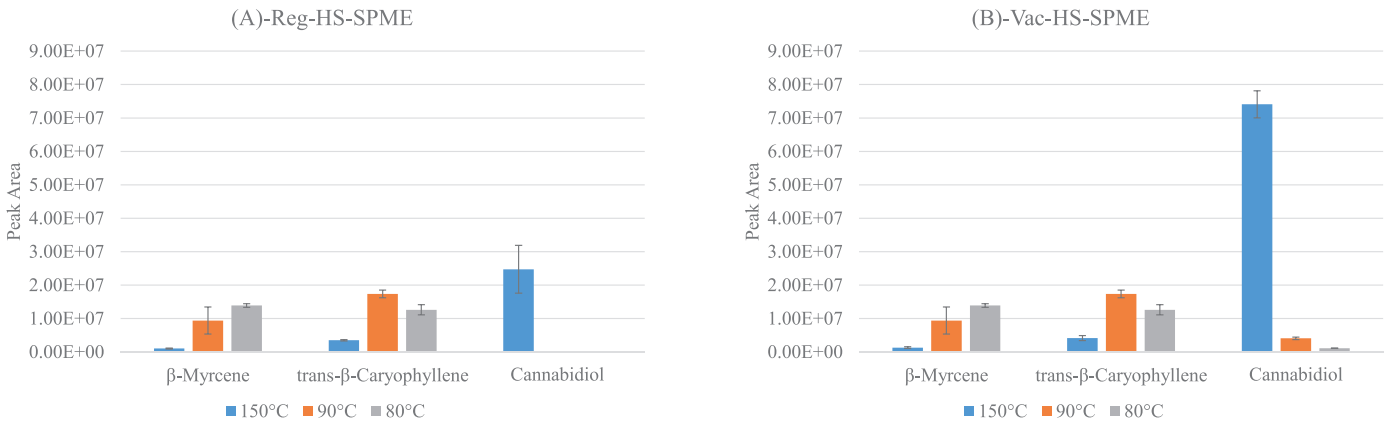

Fig. 2. Extraction temperature profiles of CBD, *β*-myrcene and *trans-β*-caryophyllene obtained under (A) regular (Reg-HS-SPME) and (B) reduced (Vac-HS-SPME) pressure conditions. Experimental parameters: PDMS/DVB fiber; 10 mg of sample; 5 min of extraction time.

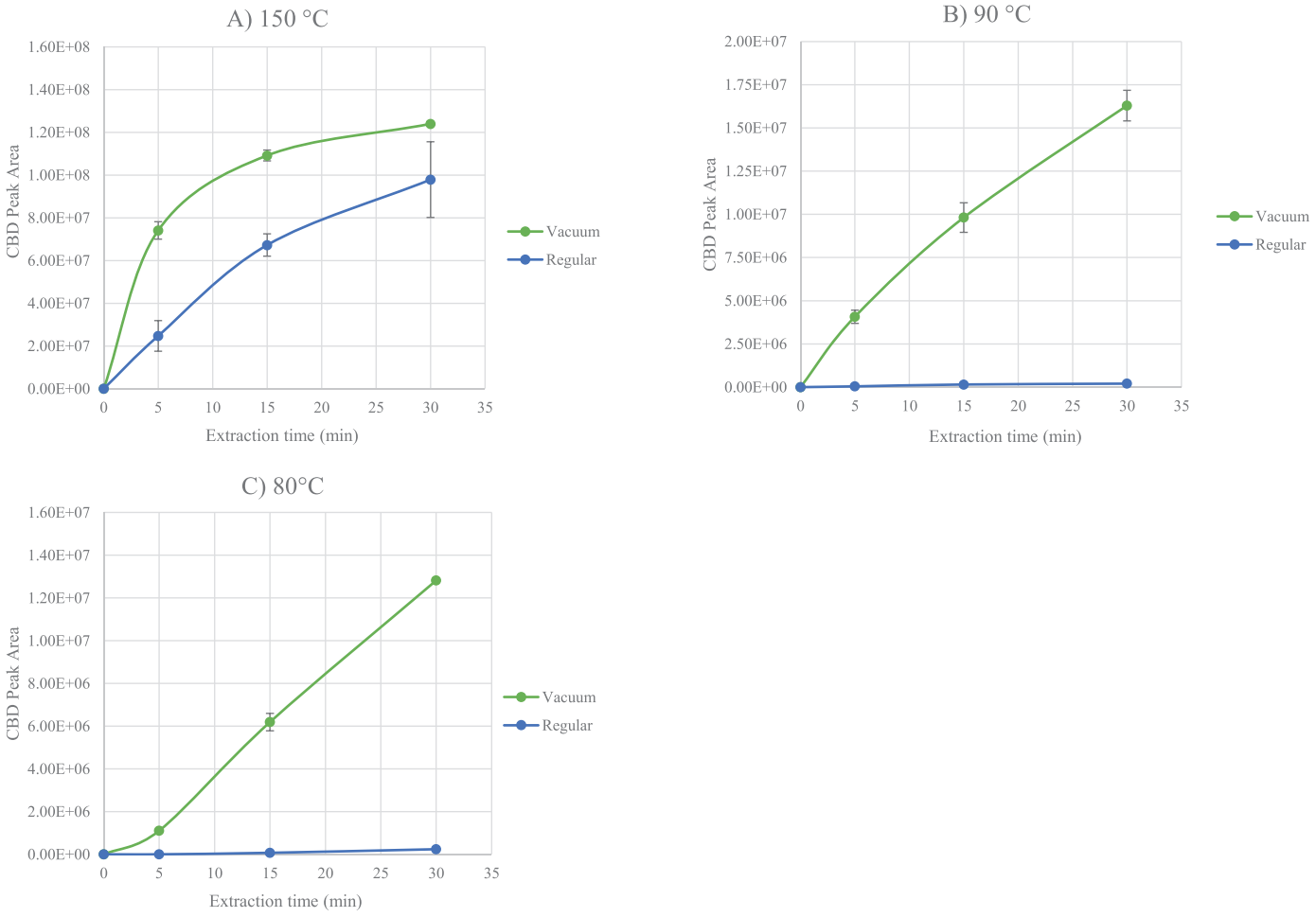

Fig. 3. Extraction time profiles for CBD obtained under reduced (Vac-HS-SPME, green profile) and regular (Reg-HS-SPME, blue profile) conditions. Experimental parameters: PDMS/DVB fiber; 10 mg of sample; extraction times 5, 15, 30 min; Sampling temperature: (A) 150°C; (B) 90°C; (C) 80°C.

according to the following formula

$$\%\ of\ degraded\ CBD = \left(\frac{Total\ area - Area\ CBD}{Total\ area}\right) \times 100$$

where the total area is the sum of the areas of all the detected cannabinoids. The results proved that the percentage of the degraded CBD was not constant amongst the replicates and accounted for 19.5 ± 17.1% and 38.7 ± 17.4% under vacuum and regular conditions respectively. Cannabinoid 2 (i.e., supposed Δ9-THC) was the most abundant degradation products with a relative abundance of 14.6 ± 12.6% and 20.9 ± 15%, under vacuum and regular conditions, respectively. The same degradation trend is not observed by direct injection into the gas chromatograph, even though the CBD is exposed to even higher temperature (i.e., injection port temperature 250°C), probably because the exposure time is too short to cause the degradation.

Fig. 4D and E report the chromatograms obtained when sampling10 μL of the same 1.0 mg mL^{-1} CBD standard solution at 90°C.

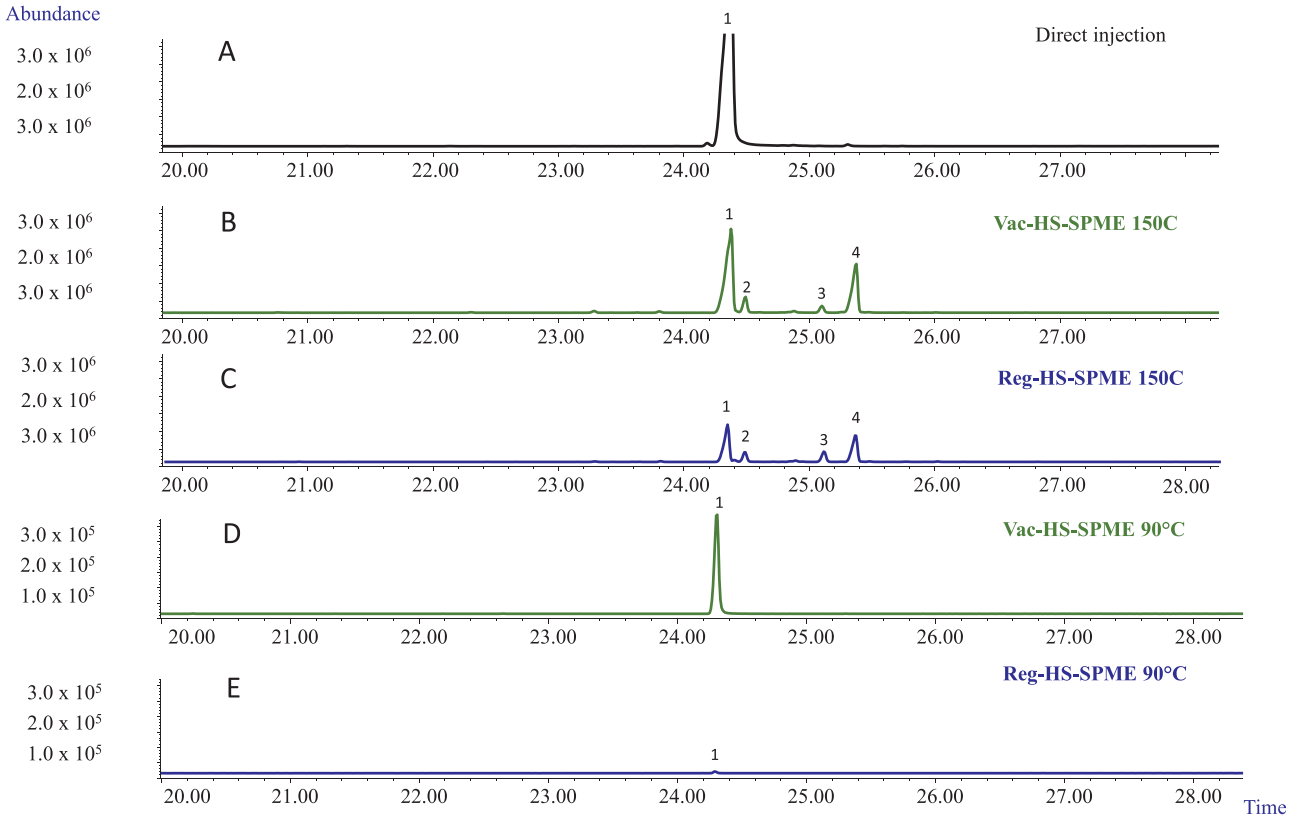

Fig. 4. GC-MS profiles of CBD standard solution obtained with MEGA-5 narrow-bore column under the following conditions: (A) injection of 1 µL of CBD standard solution 1 mg mL^{-1}; (B) 10 µL of CBD standard solution 1 mg mL^{-1} recovered by Vac-HS-SPME after 5 min at 150°C; (C) 10 µL of CBD standard solution 1 mg mL^{-1} recovered by Reg-HS-SPME after 5 min at 150°C; (D) 10 µL of CBD standard solution 1 mg mL^{-1} recovered after 5 min by Vac-HS-SPME at 90°C; (E) 10 µL of CBD standard solution 1 mg mL^{-1} recovered by Reg-HS-SPME after 5 min at 90°C. GC-MS Analysis conditions: see experimental section. Legend: (1) Cannabidiol, (2) Cannabichromene, (3) Cannabinoid 1 (supposed Δ^8-tetrahydrocannabinol), (4) Cannabinoid 2 (supposed Δ^9-tetrahydrocannabinol).

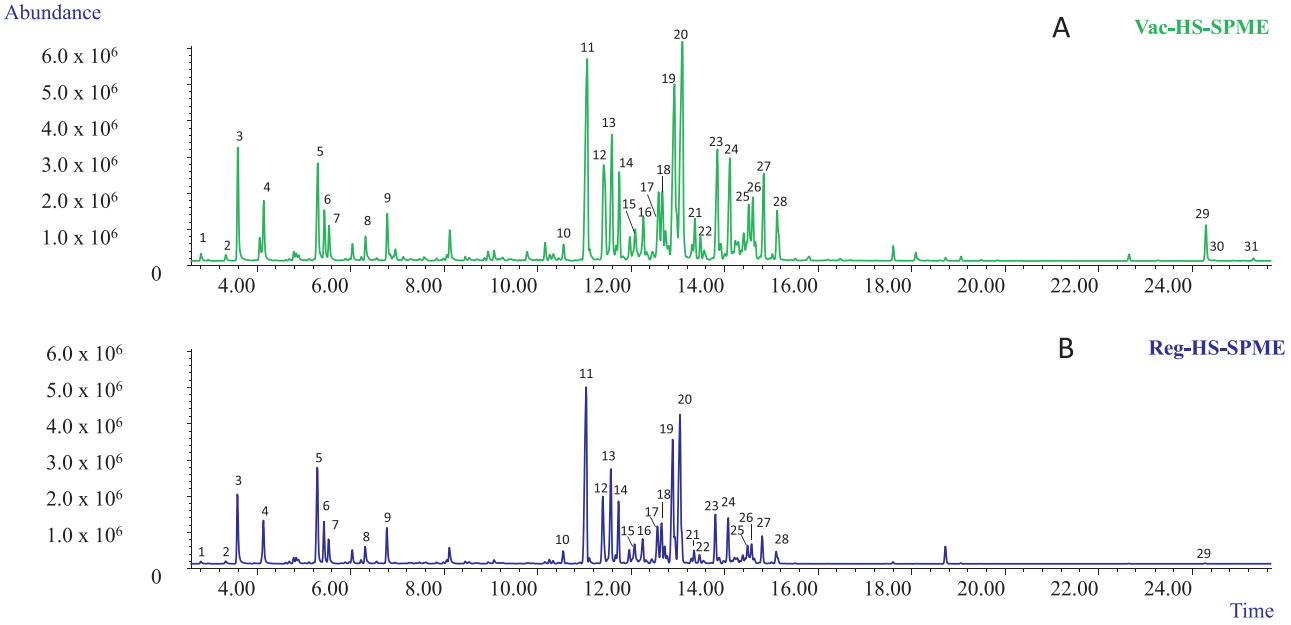

Fig. 5. HS-SPME GC-MS profiles obtained when sampling 10 mg of matrix at 90°C for 5 min under reduced pressure (A) and atmospheric pressure (B) conditions. Legend: (1) α-Pinene, (2) β-Pinene, (3) β-Myrcene, (4) Limonene, (5) Linalool, (6) Fenchol, (7) cis-Pinene hydrate, (8) Borneol, (9) α-Terpineol, (10) β-Patchoulene, (11) trans-β-Caryophyllene, (12) trans-α-Bergamotene, (13) α-Humulene, (14) trans-β-Farnesene (15) β-Selinene, 16) α-Selinene17) α-Farnesene, (18-19) Sesquiterpenes (MW 204), 20) Selina-3,7(11)-diene, (21) trans-Nerolidol, (22) Caryophyllene oxide (23) Guaiol, (24) 10-epi-γ-Eudesmol, (25) β-Eudesmol, (26) α-Eudesmol, (27) Bulnesol, (28) α-Bisabolol, (29) Cannabidiol, (30) Cannabichromene, (31) Cannabinoid 2 (Supposed Δ9-THC)

The results show that, at the investigated temperature, no degradation products were generally detected irrespective of the pressure conditions. Cannabinoid 2 (i.e., supposed Δ^9-THC) was detected only in one replicate under vacuum condition, but its relative abundance was in any case below 0.6%. These results provide further evidence that relative low sampling temperatures (i.e., 90°C) should be preferred for a more reliable characterisation of *Cannabis* inflorescences volatile and semi-volatile fractions.

4. Concluding remarks

The primary aim of this study was to investigate whether Vac-HS-SPME could be a suitable sample preparation technique to be combined to fast GC-MS analysis for the simultaneous characterisation of both the volatile (i.e., mono and sesquiterpenes) and semi-volatile (i.e., cannabinoids) fractions of *Cannabis sativa* inflorescences. The results proved that compared to Reg-HS-SPME, vacuum conditions in the HS ensure the fast recovery of cannabinoid markers at considerably lower sampling temperature (i.e., 90°C) that do not discriminate the most volatile fraction nor cause the formation of artefacts when the sampling time is minimised. The possibility of accelerating the following GC-MS analysis, by the use of a short narrow bore column, was also assessed and a satisfactory resolution of all the investigated markers was obtained in less than 30 min. Overall, since it is fast, totally automatable and solvent-free, the combination of Vac-HS-SPME and fast GC-MS should be considered as a green alternative analytical approach for the characterisation of *Cannabis sativa* inflorescences.

Despite these very promising results, further experiments are still required to concretize the use of Vac-HS-SPME and fast GC-MS analysis for the reliable qualitative and quantitative characterisation of *Cannabis* samples. These experiments should aim at (1) validating a protocol, based on vac-HS-SPME and fast GC-MS analysis, to quantify specific cannabinoid markers (i.e., CBD and Δ^9-THC) and (2) at proving that the quantification results, obtained at relatively low temperatures, describe the absolute total amount of the investigated cannabinoids (i.e., the sum of the acidic and neutral form in the inflorescence) rather than the only neutral form.

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Declaration of Competing Statement

The authors declare no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.sampre.2022.100014.

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