

Characterization of the Volatile Components of Cannabis Preparations by Solid-Phase Microextraction Coupled to Headspace-Gas Chromatography with Mass Detector (SPME-HSGC/MS)

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Abstract

Solid phase microextraction coupled to headspace sampling and GC/MS technique was applied to the characterization of the volatile components of several Cannabis preparations (hashish). Different parameters of the analytical method (fiber, coating thickness, sampling and exposition temperatures, sample preparation) were evaluated to optimize the characterization of the volatile components. α -Pinene, β -myrcene, limonene, 4-carene, trans-3(10) caren-2-ol, 4,7,7-trimethylbicyclo [4.1.0] heptan-3-ol, caryophyllene, β -humulene, azulene, gurjunene, ledene and caryophyllene oxide were identified among the volatile components of all hashish preparations. Moreover, a suitable internal standard (nonane) was chosen, the reproducibility and linearity of the method were evaluated in order to carry out the quantitative determination of caryophyllene, the most abundant volatile terpene. Its quantity ranged from 800 to 3000 $\mu\text{g/g}$.

Keywords: Hashish; Cannabis; Volatile components; Caryophyllene; Azulene; HS-SPME-GC

Introduction

Cannabis preparations (marijuana and hashish) are still among the most consumed illicit drugs worldwide, due to their low price and the commonly considered low social impact. Hashish is the sticky resin produced by the Cannabis female flowers and it is particularly rich of psychoactive principles [1,2]. The biologically active compounds are a group of terpenoid secondary metabolites, called cannabinoids. Among them, the main constituents are cannabidiol (CBD), cannabinol (CBN) and μ -9-tetra-hydrocannabinol (μ -9-THC) [3,4]. Hashish looks like a hard yellowish-brownish paste that becomes malleable upon heating and different samples can present a high variety in terms of color, density, content of the active principles and flavor [1]. Besides the biologically active cannabinoids, more than 90 phytocannabinoids have been isolated from Cannabis and its essential oil is a complex mixture containing also monoterpenoids and sesquiterpenoids [5-8], giving it the typical organoleptic properties. The volatiles constituents of Cannabis have been extensively studied [9] because they represent a potential for chemically fingerprinting different cultivars [10]. On the other hand, less attention was dedicated to the volatile components of hashish, despite the fact that they could be very useful for characterizing the different preparations and for establishing the origin and eventual links between different seizures [2]. In this frame, we were interested in analyzing the volatile components of different hashish preparations seized by the judicial authority and delivered to our laboratory for the determination of the content of cannabinoids [11]. For this purpose, we chose solid phase microextraction [12] coupled to headspace sampling, which is based on the adsorption of the volatile analytes by the coating of a suitable fiber and their direct injection into a GC/MS system. This method shows several advantages respect to liquid-liquid extraction (LLE), because it is more selective, less time consuming, it does not require the use of solvents and it is particularly suitable for volatile analytes [13]. Different parameters of the analytical method (fiber, coating thickness, sampling and exposition temperatures, sample preparation) were evaluated to optimize it for the determination of the volatile components of hashish, in particular, the parameters were studied taking into account azulene and caryophyllene, two significant

representatives of the monoterpene and sesquiterpene classes present in hashish (Figure 1). Moreover, the quantitative determination of caryophyllene, the most abundant component was carried out choosing nonane as internal standard (IS).

Materials and Methods

Reagents, chemicals, and standards

All reagents were of analytical grade and were stored as indicated by the supplier. Caryophyllene, nonane (IS), β -jonone, trans-inane, methyl oleate, α -Pinene, β -myrcene, limonene, 4,7,7-trimethylbicyclo [4.1.0] heptan-3-ol, β -humulene, azulene, gurjunene, caryophyllene oxide and ledene were purchased by Sigma-Aldrich, (Steinheim, Germany). 4-Carene and trans-3(10) caren-2-ol were from ABI Chem (Germany).

Methanol was obtained by Baker (Deventer, The Netherlands).

A IS standard solution was prepared as follows: 100 μL of nonane were brought to 10 mL with methanol (10 $\mu\text{L/mL}$; 7.2 mg/mL).

Cannabis preparations

Seven different hashish samples were taken into account, seized by the judicial authority and delivered to our laboratory for the determination of the cannabinoid content [11]. The characteristics of the different preparations together with the content of THC, CBD and CBN are reported in Table 1.

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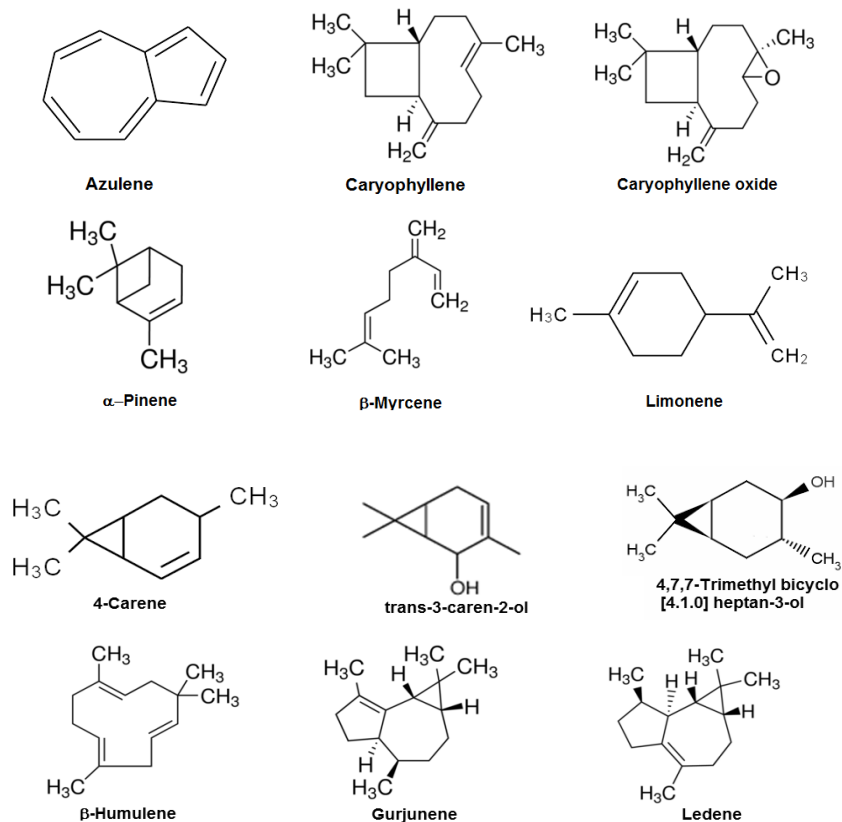


Figure 1: Structures of hashish most abundant terpenes.

Sample	Type	Description	%THC	%CBD	%CBN
1	Hashish	Color: malleable	19.0	5.8	0.5
2	Hashish	Color: dark brown; very hard texture	13.4	6.0	0.6
3	Hashish	Color: black; soft texture, malleable	9.8	5.8	0.6
4	Hashish	Color: light brown; hard texture	11.7	7.9	0.3
5	Hashish	Color: dark brown; compact texture	18.3	7.0	0.4
6	Hashish	Color: brown; soft texture	16.0	6.7	0.9
7	Hashish	Color: greenish-brownish; very hard and compact texture	21.7	9.2	0.6

Table 1: Characteristics of the different preparations and content (% w/w) of THC, CBD and CBN.

Sample 3 was used to evaluate method repeatability and it was considered as a reference because it was the most abundant sample and it was particularly aromatic, soft and malleable and it had a gold punching as a mark of quality.

GC/MS instrument

Analyses were carried on a 3900 Varian GC system (Agilent, Santa Clara, CA), with a split-splitless injection system operated in a split mode and a Varian Saturn 2100T Detector operated in electron impact mode (70 eV). Data acquisition and analysis were performed using standard software supplied by the manufacturer (Varian Workstation 6.0). The GC was equipped with a capillary column DB-5MS (30 m, 0.25 mm i.d., film thickness 0.25 μ m) (Agilent, Santa Clara, CA).

GC/MS conditions

The GC/MS system was operated under following conditions: split ratio, splitless for 2 min then 30:1; solvent delay, 2.0 min; injector temperature, 250°C; interface transfer line, 300°C; ion source, 180°C; oven temperature program, from 200°C to 250°C, at 10°C/min, final isotherm, 15 min. Analysis time 20 min.

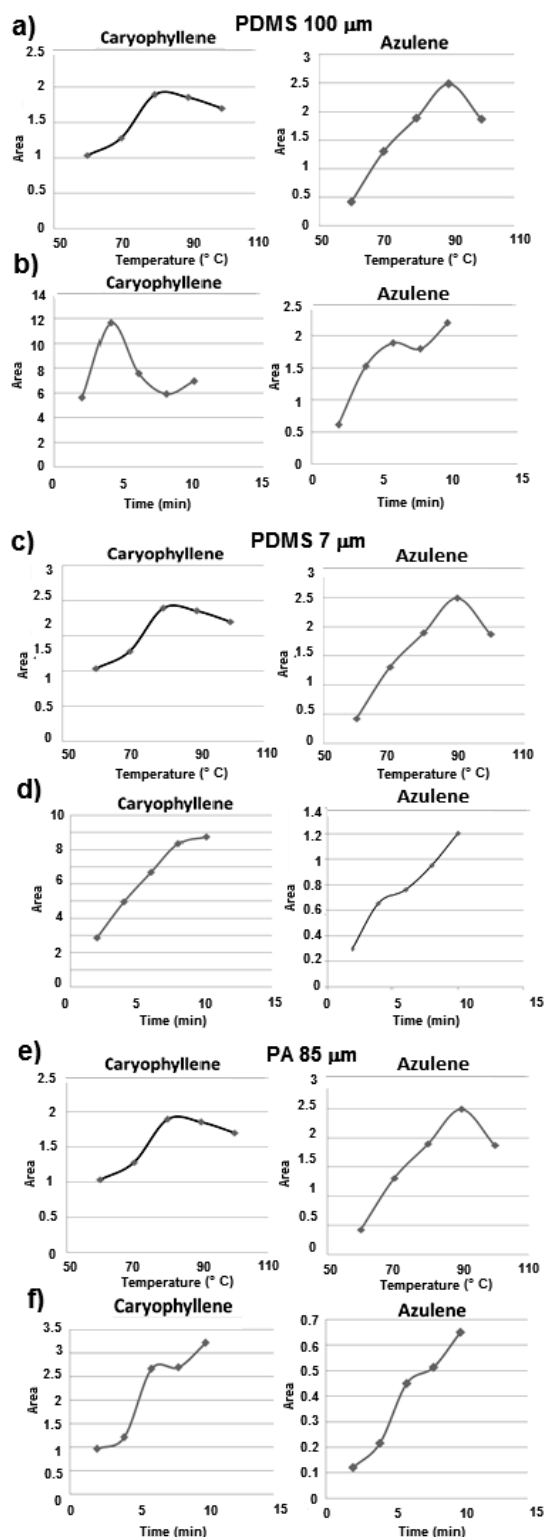
Helium was used as the carrier gas at a flow rate of 1.3 mL/min. The MS detector was operated in SCAN mode, mass range: 70 to 500 m/z.

Linearity

For the study of linearity, a sample of hashish (10 mg of sample 3) was exhausted eliminating the volatile components (heating under vacuum at 100°C for 6 h) and then 10 μ L of a standard solution of caryophyllene (1000 μ g/mL) were added. The chromatographic response was the same as that obtained carrying out the SPME on 10 μ L of the standard solution alone. The calibration curve was then built using 10 μ L of standard solutions of caryophyllene at suitable concentrations (4.3, 8.6, 17.2, 34.4, 68.8, 137.6, 275, 550, 1100, 2200, 4400, 8800 μ g/mL).

Sample preparation for qualitative characterization

A slice was cut from every preparation, frozen and finely chopped. 10.0 mg were weighed, put in a 20 mL headspace vial and immediately sealed using a silicone/PTEFA septum and a magnetic cap. Before the analysis 10 μ L of the IS standard solution (70 mg) were added by a microsyringe.



Kinetics of adsorption of azulene and caryophyllene. (a) PDMS fiber 100 μm; extraction time 5 min; extraction temperature: 60°C, 70°C, 80°C, 90°C, 100°C (b) PDMS fiber 100 μm; extraction time 2, 4, 6, 8 and 10 min; extraction temperature: 80°C (c) PDMS fiber 7 μm; extraction time 5 min; extraction temperature: 60°C, 70°C, 80°C, 90°C, 100°C (d) PDMS fiber 7 μm; extraction time 2, 4, 6, 8 and 10 min; extraction temperature: 80°C (e) PA fiber 85 μm; extraction time 5 min; extraction temperature: 60°C, 70°C, 80°C, 90°C, 100°C (f) PA fiber 85 μm; extraction time 2, 4, 6, 8 and 10 min; extraction temperature: 80°C.

Figure 2: Results obtained are depicted.

SPME extraction

Solid phase microextraction coupled to headspace sampling is based on the adsorption of the volatile analytes by the coating of a suitable fiber and their direct injection into a GC/MS system. The sample vial was equilibrated at 80°C for 5 min. For adsorption, the needle of the SPME device containing the extraction fiber (SPME fiber assembly polyacrylate df 85 μm, Supelco, Sigma Aldrich, Steinheim, Germany) was inserted through the septum of the vial and the fiber was exposed to the headspace in the vial for 5 min. Finally, the SPME fiber with the absorbed compounds was introduced into the injection port of the GC/MS for 5 min to accomplish complete desorption of the analytes.

Results and Discussion

We were interested in analyzing the volatile components of different hashish preparations seized by the judicial authority and delivered to our laboratory for the determination of the content of cannabinoids (Table 1), in order to characterize the different preparations and to establish the origin and eventual links between different seizures.

For this purpose we chose solid phase microextraction coupled to headspace sampling, which shows several advantages because it is more selective, less time consuming and it is particularly suitable for volatile analytes. Different parameters of the analytical method were evaluated to optimize it for this kind of determination. Sample preparation was investigated and we decided to carry out the extraction on the vegetable material as it was without adding a solvent. In this way, the aromatic and volatile component of the hashish preparations is preserved and the chromatograms obtained do not show the peak of the solvent but only peaks related to the matrix. Due to the high sensitivity of this technique, we decided to use 10 mg of hashish preparations.

Three different fibers were evaluated: two of them with a polydimethylsiloxane (PDMS) non polar coating but with a different thickness, respectively 100 μm and 7 μm; the third with a polyacrylate (PA) polar coating and a thickness of 85 μm. These three fibers represent a pool recommended by the suppliers for the analysis of a wide range of analytes; in particular, low molecular weight or volatile compounds usually require a 100 μm polydimethylsiloxane (PDMS)-coated fiber. Larger molecular weight or semivolatile compounds are more effectively extracted with a 7 μm PDMS fiber. To extract very polar analytes from polar samples, an 85 μm polyacrylate-coated fiber is required. The thickness of the coating also shows an influence on the extraction of the analytes: thicker coatings favor the adsorption of low molecular weight volatiles, while lower thicknesses adsorb preferentially semivolatile analytes.

Five different adsorption times (2, 4, 6, 8 and 10 min) and temperatures (60, 70, 80, 90 and 100°C) were taken into account. The adsorption of two representative terpenes (the monoterpene azulene and the sesquiterpene caryophyllene, Figure 1 in the volatile portion of hashish samples) was considered.

Heating favors the evaporation of the volatile analytes from the vegetable material, so the higher the temperature, the higher the vapor pressure of the analyte and consequently the adsorption should be. Temperatures higher than 100°C were not tested because some terpenes are not stable. The different classes of terpenes show different boiling points and consequently adsorption profiles: monoterpenes, such as limonene (b.p.=175,5-176,5°C) are more volatile and an excessive increase of the temperature leads to the desorption from the fibers. On the other hand, sesquiterpenes such as caryophyllene (b.p.=262°C) need a higher temperature to pass to the vapor phase, but above a certain temperature, the desorption process became predominant.

The curves obtained demonstrated that 80°C is the best adsorption temperature for caryophyllene, in the case of azulene, the maximum of adsorption is reached at about 90°C, but it is high also at 80°C, so we decided to set the adsorption temperature at 80°C. As regard as the time of adsorption, 5 min resulted to be the most suitable time. In fact lower times do not allow the establishment of the adsorption equilibrium, higher times likely bring to the desorption of the less adsorbed analytes. So the optimal adsorption conditions were 80°C for 5 min. On the basis of the results obtained reported in Figure 2, the 100 µm PDMS fiber was chosen, because the area of both caryophyllene and azulene resulted higher, indicating a higher adsorption of the analytes.

To evaluate the reproducibility of the method a suitable internal

Analyte	Retention times (min)	KI
C ₁₂ H ₂₆	4.332	1200
C ₁₄ H ₃₀	6.524	1400
C ₁₆ H ₃₄	8.846	1600
C ₁₈ H ₃₈	11.034	1800
C ₂₀ H ₄₂	13.053	2000
Nonane	2.347	950
<i>trans</i> -Pinane	2.839	995
α-Pinene	2.610	1040
β-Myrcene	2.789	1056
Limonene	3.156	1090
4-Carene	3.571	1127
Trans-3(10)caren-2-ol	4.538	1216
4,7,7- trimethylbicyclo[4.1.0]heptan-3-ol	4.708	1231
Caryophyllene	7.229	1461
β-Humulene	7.690	1503
β-Ionone	8.021	1533
Azulene	8.109	1541
Gurjunene	8.690	1594
Caryophyllene oxide	9.274	1647
Ledene	10.100	1722
Methyl oleate	14.294	2104
CBD	17.316	2380
THC	18.174	2458
CBN	18.721	2508

Table 2: Kovats retention indices (KI).

Withdrawal at the same point	IS Area	Caryophyllene Area
A	0.561	6.584
B	0.511	6.637
C	0.479	7.048
D	0.488	8.005
E	0.507	7.381
F	0.535	6.717
Mean	0.51	7.06
Standard deviation	0.03	0.55
%CV	5.89	7.81
Withdrawal at different points	IS Area	Caryophyllene Area
A	0.500	7.677
B	0.481	6.437
C	0.516	7.410
D	0.483	7.645
E	0.484	5.660
F	0.478	7.248
Mean	0.49	7.01
Standard deviation	0.01	0.80
%CV	3.01	11.44

Table 3: Reproducibility of the method evaluated on sample 3.

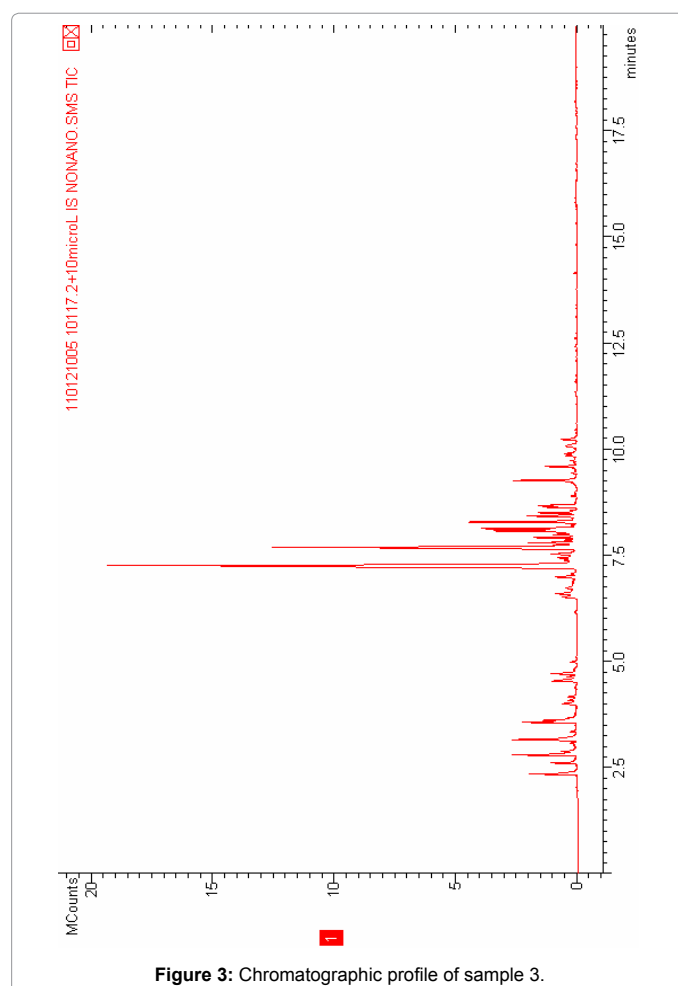


Figure 3: Chromatographic profile of sample 3.

standard was needed. To this end, Kovats retention index (KI) of a series of terpenes evidenced in the analysis of hashish preparations were calculated. As the Kovats index is related to the retention time of the analyte normalized to the retention times of adjacently eluting n-alkanes, decane, dodecane, tetradecane, hexadecane, octadecane and icosane were injected by an autosampler in the same chromatographic conditions used for the analysis of hashish volatile components. THC, CBD and CBN were also analyzed and identified in the hashish volatile components by means of their Kovats retention index. The Kovats retention indices obtained for the most abundant peaks of the volatile components of hashish preparations are reported in Table 2, together with the values obtained for the series of linear alkanes, THC, CBD and CBN. Limonene, β-ionone, *trans*-pinane, nonane and methyl oleate were taken into account as possible internal standards.

Limonene could not be chosen because it is present in the vegetable material, while β-ionone and *trans*-pinane are not present in the matrix but they have retention times too close to those of compounds present in the vegetable material. Methyl oleate is the internal standard routinely used for the analysis of cannabinoids by GC/FID technique, but it has a too high boiling point (218°C) for this kind of application. On the other hand, nonane that was indicated in the literature [10] as internal standard for the analysis of cannabinoids and terpenes in Cannabis, showed the suitable features to be used as internal standard.

To evaluate the reproducibility of the method different analyses were carried out withdrawing six samples from the same portion of the stick of hashish (sample 3) or from different portions of the same

Sample	A	B	C	D	E	F	I	II	III	IV	V	VI	IS
1	0.24	0.57	0.77	0.40	0.32	0.26	7.073	2.865	0.938	0.353	0.541	0.208	0.457
2	1.09	0.80	0.55	0.51	0.20	0.22	7.164	2.385	0.743	0.157	1.229	0.377	0.539
3	0.18	0.09	0.21	0.15	0.10	0.28	7.248	2.206	1.561	2.264	0.533	0.589	0.478
4	0.04	0.03	0.04	0.09	0.22	0.15	6.204	2.652	1.274	0.173	0.587	0.168	0.450
5	0.30	0.67	1.13	0.54	0.37	0.28	8.997	3.395	1.169	0.210	0.827	0.331	0.484
6	0.03	0.02	0.36	0.76	0.17	0.17	3.589	1.731	0.591	0.317	1.271	0.250	0.522
7	0.21	0.31	0.44	0.34	0.36	0.43	1.780	5.430	1.503	0.585	1.218	0.343	0.473

Table 4: Area of the peaks of the terpenes identified in hashish preparations.

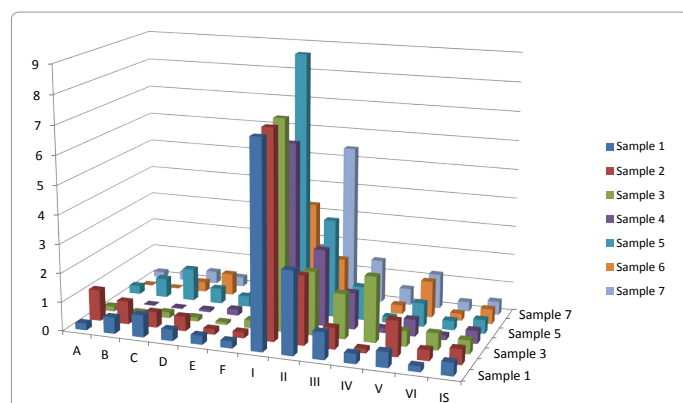


Figure 4: Area of the peaks of the terpenes identified in hashish preparations.

Sample	I (µg/g)
1	1280
2	1688
3	1545
4	1477
5	796
6	2963
7	2945

Table 5: Content of caryophyllene (I, µg/g) in the different Hashish preparations.

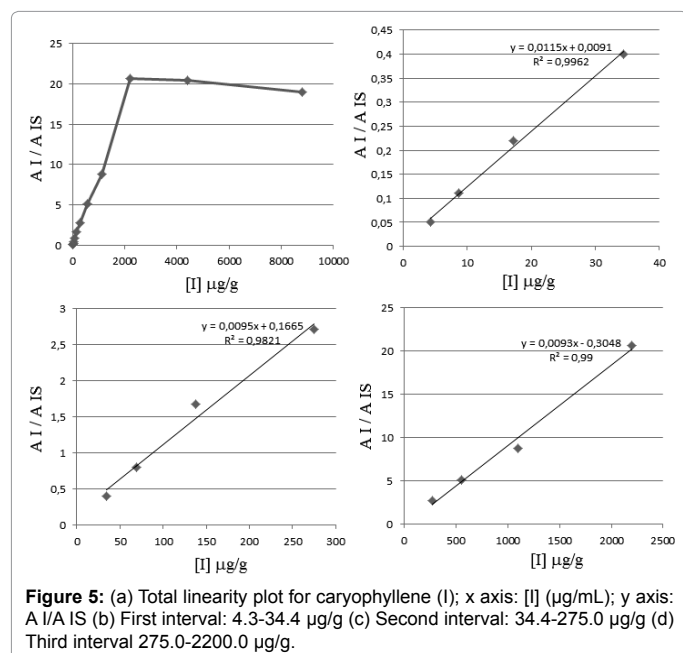


Figure 5: (a) Total linearity plot for caryophyllene (I); x axis: [I] (µg/mL); y axis: A I / A I S (b) First interval: 4.3-34.4 µg/g (c) Second interval: 34.4-275.0 µg/g (d) Third interval 275.0-2200.0 µg/g.

stick. The area of the peaks related to caryophyllene, the most abundant component were compared (Table 3).

Percentage coefficients of variation show that the method has a good reproducibility, this is particularly important for this kind of vegetable material, which has a lower degree of homogeneity, respect to other kind of matrices.

At this point we analyzed all the different preparation of Cannabis (samples 1-7), comparing the chromatographic profile. In Figure 3, the chromatographic profile obtained for sample 3 is reported.

By comparison with the relative standards and the evaluation of Kovats indices, the following terpenes were identified: α -pinene (A), β -Myrcene (B), Limonene (C), 4-Carene (D), *trans*-3(10) caren-2-ol (E), 4,7,7- trimethylbicyclo[4.1.0]heptan-3-ol (F), Caryophyllene (I), β -Humulene (II), Azulene (III), Gurjunene (IV), Caryophyllene oxide (V), Ledene (VI) whose areas are reported in Table 4 and Figure 4.

The most abundant terpenes in all hashish preparations were caryophyllene (I) and β -Humulene (II), two sesquiterpenes, confirming literature data [2]. To carry out quantitative determinations, the linearity of the method was studied for caryophyllene (I), the most abundant component. Twelve standard solution, at different concentrations were analyzed in the range 4.3 µg/mL and 8800.0 µg/mL. The linearity plot obtained plotting the ratio between the area of the peak of caryophyllene (I) and the area of the peak of the internal standard (A I / A I S) against the concentration of caryophyllene ([I]) is reported in Figure 5.

Three different linearity intervals were identified: the first interval from 4.3 to 34.4 µg/g (linearity equation $y=0.0115x+0.091$; $R^2=0,9962$); the second from 34.4 to 275.0 µg/g (linearity equation $y=0.0095x+0.1665$; $R^2=0,9821$) and the third from 275.0 to 2200.0 µg/g (linearity equation $y=0.0093x-0.3048$; $R^2=0,9900$). In the fourth region from 1100.0 to 8800.0 µg/g of the linearity curve a plateau is reached probably due to the saturation of the fiber and the method is not linear any more. The quantitative determination of caryophyllene was carried out using the linearity equation of the third interval. In fact, the concentration of caryophyllene in hashish preparation follows in this range. The results obtained are reported in Table 5. The content of caryophyllene of the last two samples were outside the linearity range, therefore, these samples were determined weighting 5 mg of hashish.

Conclusions

The HS-SPME-GC/MS method studied herein allows the rapid and reproducible determination of the volatile components of Cannabis. This analysis could be particularly useful for the characterization of Cannabis preparations (hashish) and to establish the region of origin and eventual links between different seizures. To this end, the parameters of the SPME and of the analytical method were optimized using azulene and caryophyllene, two representative terpenes of hashish. Ten different hashish preparations were analyzed and the quantitative determination of caryophyllene, the most abundant component was carried out, using nonane as internal standard.

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