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Development of GC–MS coupled to GC–FID method for the quantification of cannabis terpenes and terpenoids: Application to the analysis of five commercial varieties of medicinal cannabis

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ABSTRACT

Cannabis terpenes and terpenoids are among the major classes of pharmacologically active secondary metabolites of therapeutic interest. Indeed, these hydrocarbon molecules, responsible for the characteristic aroma of cannabis flowers, are thought to be involved in a synergistic effect known as the "entourage effect", together with cannabinoids. Numerous analytical studies have been carried out to characterize the terpene and terpenoid contents of some cannabis varieties, but they have not proposed any real quantification or have described a limited number of analytical standards or average response factors, which may have led to over- or underestimation of the real content of the cannabis flowers. Real and reliable quantification is necessary to justify the entourage effect. Here, we report a rigorous and precise GC–FID and GC–MS method for the identification and quantification of cannabis terpenes and terpenoids. This method is distinguished by the use of a high number of analytical standards, the determination of retention indices for all compounds studied, an exhaustive comparison of databases and scientific literature, the use of relevant response factors, and internal calibration for reliable results. It was applied to the study of terpenic compounds in five commercial varieties of medicinal cannabis produced by Bedrocan International: a CBD-rich (Bedrolite®), a THC/CBD balanced (Bediol®), and three THC-dominant (Bedrocan®, Bedica® and Bedrobinol®). Two extraction solvents are described (ethanol and hexane) to compare their selectivity towards target molecules, and to describe as exhaustively as possible the terpenic profile of the five pharmaceutical-grade varieties. Twenty-three standards were used for accurate dosages. This work highlights that the choice of solvent and the analysis method reliability are critical for the study of these terpenic compounds, regarding their contribution to the entourage effect.

1. Introduction

Cannabis sativa L. is an annual and mostly dioecious species. It is cultivated, depending on the variety, for its fibers or for the bioactive molecules it contains.

Terpenes and terpenoids are a major secondary metabolite group in cannabis that are of interest to scientists. Their main biosynthesis and accumulation take place in glandular trichomes, which are epidermic growths abundantly covering flowering cannabis bracts [1]. From a structural point of view, terpenes are molecules whose carbon skeleton consists of a succession of isoprenic units [2]: a chain of five carbons atoms containing a double carbon–carbon bond. They are named according to the number of isoprenic units: mono-, sesqui-, and diterpenes contain respectively two, three, and four isoprenic units. Terpenoids are oxidized terpene analogs with chemical functions [2], e.g., ketone, hydroxyl, ether, and epoxide groups. Their structures can vary; some of these compounds are linear carbon chains, some are branched, and others are cyclic or polycyclic. Terpenes and terpenoids are both volatile compound families and are responsible for the characteristic aroma of cannabis, with more than 200 identified molecules [3]. They also play valuable biological roles as pollinator attractants, herbivore repellents, or antibacterial agents [4].

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Terpenes and terpenoids from *C. sativa* are being increasingly studied for their potential contribution to the therapeutic effects of cannabinoids, as major secondary metabolites of cannabis known for their interaction with the human endocannabinoid system [5]. This synergy called the "entourage effect", which was described in 1999 by Mechoulam et al. [6], could explain why in some cases "plants are better drugs than the natural products isolated from them" meaning that isolated cannabinoids could not lead to same biological response as when native plant is administrated. Russo et al. [7] published a review paper listing numerous synergistic effects illustrating this phenomena.

Phytoterpenes and terpenoids are apolar molecules that can be recovered by means of extraction directly from plant biomass. The classical methods to obtain terpene-rich fractions in large scale include liquid extraction by organic solvents, steam distillation or supercritical fluid extraction [8], but technologies such as solid-phase microextraction (SPME) [9] and static headspace (SHS) [10] have also been highlighted by some authors for analytical purposes.

Volatile terpenes and terpenoids are usually analyzed by means of gas chromatography (GC), and the use of apolar chromatographic columns allows their separation according to vaporization temperature. The most described columns for the analysis of these hydrocarbon compounds are apolar capillary chromatographic columns, whose stationary phases consist of 95 % dimethyl polysiloxane groups and 5 % phenyl groups. Several detectors allow the identification and quantification of volatile molecules. The flame ionization detector (FID) has been widely described for quantification due to its sensitivity and low cost of use [9,11–13]. The mass spectrometer detector (MSD) is a more complex detector, since it allows molecule structural determination.

In the field of cannabis terpenic compounds, several studies have already described the volatile compound content of different varieties. Nevertheless, there is a lack of complete quantification of these molecules. Indeed, many publications give only relative profiles [9,12,14–16], which permit a quick overview of the global composition but give no indication of their quantities in the inflorescences. Others propose quantification methods limited by the number of standards [17] or by the use of approximative response factors for a large number of compounds [18], which are easier to implement and less costly, but could lead to over- or underestimation of dosages. However, to understand and to justify the role of these compounds to the "entourage effect", it is essential to describe the actual volatile compound content of cannabis flowers and to know their real composition. It is also common practice to use headspace technology for the analysis of volatile compounds, as demonstrated by the numerous studies describing it for the analysis of cannabis terpenes [9,19–21]. Nevertheless, contrary to direct injection, the sensitivity of this methodology is highly dependent on the matrix analyzed, the partition coefficients of the analytes between the phases, and the ratio of sample to vapor [22], which could also lead to over- or underestimates.

At present, in France, the acquisition of cannabis flowers is restricted due to regulation. Bedrocan® International is one of the few producers that can provide EU GMP-certified pharmaceutical-grade medicinal cannabis inflorescences to accredited laboratories. Bedrocan® varieties are highly studied because of their varied profiles in cannabinoids (i.e., THC-dominant type, CBD-dominant type, or THC/CBD-intermediate type). These flowers are also rich in terpenic compounds, but the study of these has been limited, and little quantitative information is available.

The aim of this study was to propose GC–FID and GC–MS methods for the identification and the quantification of terpenes and terpenoids, and to execute them in five different commercial cultivars of medicinal cannabis produced by Bedrocan International. Two commonly used organic solvents of different polarity were chosen to compare their selectivity in the recovery of volatile compounds: absolute ethanol and *n*-hexane. Particular attention was paid to (1) the use of a large number of analytical standards for accurate quantification, (2) the determination of retention indices for all analyzed compounds for a more accurate comparison with the scientific literature, (3) comparison with databases, literature, and reference works for compound identification, (4) the determination and use of more relevant response factors for the quantification of analytes without standards, and (5) internal calibration for more reliable results.

2. Materials and methods

2.1. Reagents and materials

Five cultivars of medicinal *C. sativa* dried female inflorescence, including THC-dominant type Bedrocan® variety (batch: 20C30EY20E13), CBD-dominant type Bedrolite® variety (batch: 20I14FR20L02), THC/CBD-intermediate type Bediol® variety (batch: 19L16FB20K04), THC-dominant type Bedrobinol® variety (batch: 19H19FA20A29), and THC-dominant type Bedica® variety (batch: 20K09FP21A06), were purchased from Bedrocan International (Veendam, Netherlands) according to OMC (Office of Medicinal Cannabis, Netherlands) and ANSM (Agence Nationale de Sécurité du Médicament et des produits de santé, France) requirements and authorization. Samples were stored in airtight containers in the dark at 25 °C.

Mixes of analytical standards of terpenes and terpenoids were acquired from Restek, including α-pinene (purity 99 %), camphene (purity 98 %), (−)-β-pinene (purity 99 %), β-myrcene (purity 95 %), δ-3-carene (purity 96 %), α-terpinene (purity 94 %), *p*-cymene (purity 99 %), D-limonene (purity 99 %), β-ocimene (purity 91 %, sum of enantiomers *cis*-β-ocimene (31 %) and *trans*-β-ocimene (69 %)), γ-terpinene (purity 99 %), terpinolene (purity 99 %), linalool (purity 97 %), (−)-isopulegol (purity 99 %), geraniol (purity 98 %), β-caryophyllene (purity 98 %), α-humulene (purity 97 %), nerolidol (purity 99 %), (−)-guaiol (purity 99 %), (−)-α-bisabolol (purity 94 %), eucalyptol (purity 99 %), and (−)-caryophyllene oxide (purity 98 %).

A commercial standard of tridecane (purity > 99 %) and an analytical mixture of *n*-alkanes (from C7 to C30, purity > 99 %) were purchased from Sigma-Aldrich. *n*-hexane (LC grade, purity > 97 %), cyclohexane (LC grade, purity > 99,5%), and absolute ethanol (LC grade, purity > 99 %) were obtained from VWR.

2.2. Terpene and terpenoid extraction

2.2.1. Preparation of extraction solvent

Solutions of known concentrations of tridecane in solvents were prepared as extraction solvents. Briefly, 50 mg of tridecane was precisely weighed and brought to volume with solvent in a 200 mL volumetric flask. The solutions were kept at −20 °C in capped amber-glass bottles until further usage.

2.2.2. Extraction procedure and sample preparation

For each variety studied, 1 g of inflorescence was precisely weighed, frozen with liquid nitrogen, and then hand-crushed using a mortar and pestle. Milled frozen samples were extracted at 25 °C in capped amber-glass vials with 10 mL of extraction solvent solution (whether absolute ethanol or *n*-hexane) and stirred at 250 rpm on an orbital shaking table (Thermo Scientific) for 1.5 h. The supernatants were pooled, filtered through a 0.45 μm pore size syringe filter (13 mm diameter, PTFE, Phenomenex), and kept at −20 °C in capped amber-glass vials until further analysis.

2.3. Gas chromatography–mass spectrometry (GC–MS) for identification of volatile compounds

2.3.1. Instrumental conditions

Terpenes and terpenoids were identified using a gas chromatograph (6890 Series GC System, Agilent) coupled with a mass selective detector (5973 Network MSD, Agilent).

Samples (1 μL) were injected at appropriate dilution in liquid

injection mode, in split mode (1/10) at 270 °C. Chromatographic separation was performed through a DB-5MS column (30 m × 0.25 mm × 0.25 μm, Agilent) with helium as the carrier gas at a 1.3 mL/min gas flow. The oven temperature program was defined as follows: isotherm at 50 °C for 5 min, temperature gradient from 50 °C to 180 °C at 6 °C/min, temperature gradient from 180 °C to 260 °C at 20 °C/min, and final isotherm at 260 °C for 10 min.

The MSD was operated in electron impact (EI) ionization mode (70 eV) for compounds with mass in the range of 35–350 Da. The temperature of the ion source and quadrupole were set, respectively, at 230 °C and 150 °C.

2.3.2. Qualitative analysis

Data were processed using MSD ChemStation software (version E.02.02.1431, Agilent). Mass spectra were compared with the NIST database (version 2.2). Identifications were confirmed by comparison with spectra of available standards. For compounds whose standards were not available, identification was possible by comparison with retention index (RI) values between the experimental RI and the literature [23].

Experimental RI values were calculated by injection under the same instrumental conditions of a mixture of *n*-alkanes and by applying the following formula [23]:

$$RI = 100 \times A_n + 100 \times \frac{RT(i) - RT(A_n)}{RT(A_{n+1}) - RT(A_n)} \quad (1)$$

where RI is the retention index of the studied compound, A_n is the previous *n*-alkane, A_{n+1} is the following *n*-alkane, *n* is the number of carbon atoms of an *n*-alkane, RT is the retention time, and *i* is the studied compound.

Identification hypotheses were retained if values of Match and Reverse match given by the NIST database were above 800.

2.4. Gas chromatography–flame ionization detector (GC–FID) for quantification of volatile compounds

2.4.1. Preparation of standards and construction of calibration curves

Analytical standard solutions in cyclohexane were prepared for the construction of the calibration curves at the nominal concentrations of 25 μg/mL, 50 μg/mL, 100 μg/mL, 125 μg/mL, 150 μg/mL, 200 μg/mL, and 250 μg/mL (concentrations were corrected using values of purity given by the supplier). A mass of 110.88 μg of tridecane was introduced to each calibration solution as the internal standard (ISTD).

The seven-point standard curves were obtained by plotting the analyte area/ISTD area against the amount of analyte/ISTD amount.

2.4.2. Instrumental conditions

Terpenes and terpenoids were quantified using a gas chromatograph (3900 Series GC System, Varian) equipped with an autosampler (CP-8400, Varian) and a flame ionization detector (FID). Samples (5 μL) were injected in liquid injection mode, in split mode (1/10) at 270 °C. Chromatographic separation was performed through a VF-5MS column (30 m × 0.25 mm × 0.25 μm, Agilent) with helium as the carrier gas at a 1.3 mL/min gas flow. The oven temperature program was defined as follows: isotherm at 50 °C for 5 min, temperature gradient from 50 °C to 180 °C at 6 °C/min, temperature gradient from 180 °C to 300 °C at 30 °C/min and final isotherm at 300 °C for 9.33 min. The FID detection was operated at 300 °C with dihydrogen and air flows at, respectively, 30 mL/min and 300 mL/min.

2.4.3. Quantitative analysis

Data were processed using Star software (version 6.41, Varian). Calibration curves were used for compounds with available standards. For compounds identified via GC–MS whose standards were not available, quantification was conducted by expressing results as the β-myrcene equivalent and β-caryophyllene equivalent for monoterpenes and sesquiterpenes, respectively.

Correspondences between GC–MS and GC–FID chromatograms for the unknown compounds were possible by comparison of the RI. Retention index values in GC–FID were obtained by applying the same protocol already described for the GC–MS identification method.

2.4.4. Method validation

Limits of detection (LOD) and quantification (LOQ) were determined for all compounds whose standards were available by injecting all calibration solutions five times. LOD and LOQ values were obtained in accordance with the ANSES validation guide [24], applying the following formulas for each compound:

$$LOQ = 9.9x(S_{a0}/a_1) \quad (2)$$

$$LOD = 3.3x(S_{a0}/a_1) \quad (3)$$

where S_{a0} is the standard deviation of the y-intercepts and a_1 is the slopes, both determined by linear regression calculation with Excel software (version 16.66, Microsoft).

2.4.5. Statistics and data analysis

All extractions were conducted in triplicate (n = 3), and data are expressed as their mean value (m) and standard deviation (sd).

3. Results and discussion

3.1. Extraction procedure

Before extraction, the inflorescences were meticulously crushed by hand with liquid nitrogen in a mortar. Therefore, no decarboxylation step was performed. These precautions were taken to avoid any heating that could lead to evaporation of volatile compounds or their degradation [9].

The extraction process applied was maceration and the fixed operating parameters were the extraction solvents, solid-to-liquid ratio, temperature, stirring speed, and extraction time. To highlight the influence of solvent polarity in the recovery of compounds, absolute ethanol (LogP = −0.1 [25]) and *n*-hexane (LogP = 3.9 [26]) were selected as polar and non-polar solvents. Ethanol is often used for the simultaneous recovery of cannabis terpenes, terpenoids, and cannabinoids as it is the most suitable green solvent for the extraction of cannabinoids [27–29]. *n*-hexane, however, has been shown to be a better solvent than ethanol for the recovery of cannabis volatile compounds [15]. Jin et al. [18], who carried out their extraction with methanol for the recovery of different families of compounds (including terpenes and terpenoids), described low amounts of volatile compounds. Methanol is a highly polar solvent (logP = −0.5 [32]) that is not suitable for the recovery of hydrocarbon molecules.

We introduced the ISTD directly into the extraction solvents. This methodology facilitates the quantification procedure as the ratio ISTD/biomass is known from the beginning, avoiding sample spiking before injection. This also compensates for errors related to solvent evaporation and injection. Tridecane was selected as the internal standard in this study for several reasons: it was not present in any of the samples studied and it was eluted between the monoterpenes group and the sesquiterpenes. Tridecane-containing solvents have already been described [18,33]. Other alkanes, such as nonane and dodecane, were mentioned in spiking samples [13,34].

3.2. Identification of volatile compounds in the five cannabis varieties by GC–MS

In this study, five commercial varieties of medicinal *Cannabis sativa* L. were studied by extracting their content with two commonly used

Table 1
Identified terpenes and terpenoids in the five commercial *Cannabis sativa* L. varieties. Data are expressed as μg/100 mg of dry inflorescence (m, n = 3) ± sd.

No	Compound	Synonyms	CAS number	FID RI (exp.)	MS RI (exp.)	RI (lit.)	S.	μg/100 mg of dry plant matter									
								Bedica®		Bediol®		Bedrobinol®		Bedrocan®		Bedrolite®	
								m	sd	m	sd	m	sd	m	sd	m	sd
						Monoterpenes and monoterpenoids											
1	α-thujene [b,e]	Origanene [g]	2867–05-2	928	n.d	924[c]	H							1.20	0.07	0.53	0.03
							E							0.99	0.12	0.41	0.04
2	α-pinene [a]	2-pinene [g]	80–56-8	935	933	932[c]	H	23.31	0.47	13.23	0.40	276.41	3.79	59.68	2.57	27.36	0.90
							E	13.46	0.79	11.63	0.11	178.39	3.03	45.47	2.17	18.97	0.63
3	Camphene [a]	/	79–92-5	953	950	946[c]	H	0.38	0.01	0.29	0.05	5.26	0.09	2.23	0.13	0.69	0.06
							E	0.19	0.03	0.16	0.10	3.56	0.26	2.10	0.09	0.37	0.02
4	Sabinene [b,e]	4(10)-thujene [g]	3387–41-5	978	n.d	969[c]	H										
							E			0.05		0.06		0.34	0.13	0.36	0.03
5	β-pinene [a]	Nopinene; terebenthene; pseudopinene [g]	127–91-3	981	977	974[c]	H	2.74	0.10	2.54	0.03	24.91	0.41	23.65	0.79	8.62	0.27
							E	1.79	0.05	2.30	0.08	16.43	1.07	17.98	0.73	5.55	0.20
6	β-myrcene [a]	β-geraniolene [g]	123–35-3	992	990	988[c]	H	27.37	0.85	125.80	3.44	149.23	2.76	390.29	12.96	143.19	4.84
							E	17.54	0.49	108.38	2.51	59.32	3.87	295.70	12.30	89.31	3.89
7	α-phellandrene [b,e]	Menthadiene; *p*-mentha-1,5-diene; 1,3-cyclohexadiene [g]	99–83-2	1009	1005	1002[c]	H	0.23		2.78	0.04	0.71	0.09	32.41	1.27	5.01	0.20
							E			1.66	0.01	0.38	0.02	24.43	1.05	2.89	0.04
8	δ-3-carene [a]	/	13466–78-9	1012	1007	1008[c]	H			1.52	0.06			33.54	1.20	4.47	0.16
							E			1.36	0.11			25.72	1.06	2.76	0.13
9	α-terpinene [a]	*p*-mentha-1,3-diene; terpilene [g]	99–86-5	1020	1017	1014[c]	H	0.19		3.24	0.46	0.30	0.02	30.27	1.29	5.96	0.20
							E	0.10	0.07	2.72	0.20	0.20	0.09	22.21	0.86	3.93	0.13
10	*p*-cymene [a]	*p*-isopropyltoluene; *p*-cymol; camphogene; dolcymene [g]	99–87-6	1030	1025	1020[c]	H			2.35	0.16	0.30	0.02	24.99	0.78	6.50	0.22
							E			1.52	0.03	0.17	0.02	19.02	0.76	3.85	0.21
11	D-limonene [a]	Cajeputene; cinene; kautschin; nesol; *p*-mentha-1,8-diene; dipentene [g]	138–86-3	1034	1029	1024[c]	H	1.03	0.06	2.26	0.07	7.05	0.17	55.51	1.88	7.51	0.24
							E	0.79	0.03	1.98	0.05	4.26	0.21	42.93	1.69	5.13	0.24
12	β-phellandrene [b,e]	*p*-menth-1(7),2-diene [g]	555–10-2	1035	1031	1025[c]	H	0.61	0.04	3.30	0.08	1.83	0.06	39.84	1.32	8.33	0.27
							E	0.43	0.02	2.68	0.00	0.88	0.33	30.75	1.61	5.50	0.28
13	Eucalyptol [a]	1,8-cineole; cajeputol; 1,8-epoxy-*p*-menthane; zedoary oil; eucapur; terpan [g]	470–82-6	1037	1033	1026[c]	H	0.28	0.03	3.03	0.13	0.54	0.02	4.38	0.24	4.67	0.16
							E	0.17				0.82	0.04	1.83	0.07	0.22	0.08
14	*cis*-β-ocimene [a]	(*Z*)-β-ocimene [g]	3338–55-4	1040	1037	1032[c]	H	0.35	0.14	0.40	0.23	1.97	0.10	3.30	0.10	0.61	0.10
							E									0.35	
15	*trans*-β-ocimene [a]	(*E*)-β-ocimene [g]	3779–61-1	1051	1047	1044[c]	H	7.53	0.12	3.72	0.01	4.56	0.25	109.58	3.54	8.45	0.36
							E	4.55	0.12	2.79	0.06	1.88	0.11	83.97	3.39	6.08	0.23
16	γ-terpinene [a]	Crithmene; moslene; *p*-mentha-1,4-diene [g]	99–85-4	1063	1059	1054[c]	H	0.15	0.02	1.90	0.02	0.24	0.04	21.39	0.49	4.23	0.19
							E	0.18	0.02	1.47	0.02	0.11	0.02	16.55	0.83	2.89	0.16
17	Cresol <*p*-> or <*m*-> [d,e]	4-methylphenol; 4-cresol; p-tolyl alcohol or 3-methylphenol; 3-cresol; m-cresylic acid [g]	106–44-5 or 108–39-4	1077	n.d	1071[c] or 1072[c]	H	1.26	0.04	1.17	0.00	0.43	0.05	5.02	0.13	2.00	0.08
							E	1.20	0.03	0.94	0.04	0.31	0.11	4.42	0.32	1.60	0.10
18	Camphor	Kampfer; bornan-2-one; formosa [g]	76–22-2	1086	n.d	1141	H							0.47	0.06	0.18	0.08
							E										
19	Terpinolene [a]	*p*-mentha-1,4(8)-diene; isoterpinene [g]	586–62-9	1090	1085	1086[c]	H	0.32	0.07	25.75	0.53	0.27	0.01	764.99	27.18	86.40	2.46
							E	0.12	0.04	20.05	0.19	0.15	0.04	583.27	22.63	57.62	2.02
20	*p*-cymenene [b,e]	α-*p*-dimethylstyrene; dehydro-*p*-cymene; 4-isopropenyltoluene [g]	1195–32-0	1096	1090	1089[c]	H			3.84	0.15	0.23	0.01	42.02	1.48	9.10	0.28
							E			2.31		0.06	0.01	31.37	1.38	6.19	0.26
21	Linalool [a]	Phantol; linalyl alcohol [g]	78–70-6	1106	1099	1095[c]	H	39.50	0.92	3.80	0.21	0.94	0.07	23.39	1.03	10.14	0.65
							E	35.33	0.32	2.30		0.40	0.20	19.81	1.08	8.17	0.21
22	Compound 22 [e]	/	/	1150	n.d	/	H	1.53	0.10	7.06	0.38	2.63	0.00	21.56	0.84	5.92	0.40
							E	1.18	0.04	5.38	0.10	1.12	0.35	18.57	1.00	4.42	0.32
23	(−)-isopulegol [a]	/	89–79-2	1157	1150	1145[c]	H							0.66			
							E			0.74		0.66	0.18			0.87	
24	Compound 24 [e]	/		1182	n.d	/	H	2.96	0.08	3.64	0.07	6.64	0.13	9.46	0.57	3.40	0.18
							E	1.85	0.09	2.27	0.13	3.95	0.57	6.17	0.42	2.20	0.11
25	Compound 25 [e]	/	/	1186	n.d	/	H			0.59	0.02			2.61	0.03	0.64	0.09

(*continued on next page*)

Table 1 (*continued*)

No	Compound	Synonyms	CAS number	FID RI (exp.)	MS RI (exp.)	RI (lit.)	S.	µg/100 mg of dry plant matter									
								Bedica®		Bediol®		Bedrobinol®		Bedrocan®		Bedrolite®	
								m	sd	m	sd	m	sd	m	sd	m	sd
26	Terpinen-4-ol [b,e]	4-terpineol; *p*-menth-1-en-4-ol; 4-carvomenthenol [g]	562–74-3	1187	1181	1174[c]	E			0.88	0.10			3.32	0.27	1.04	0.06
							H			12.45	0.22			26.22	0.94	11.04	0.68
27	*p*-cymen-8-ol [b,e]	8-hydroxy-*p*-cymene; dimethyl-*p*-tolyl carbinol [g]	1197–01-9	1195	1187	1183 [i]	E			10.02	0.41	0.66		23.30	1.06	9.23	0.43
							H	0.73	0.03	1.24	0.12	0.37		5.55	0.61		
28	Hexyl butanoate [b,e]	Hexyl butyrate; butanoic acid, hexyl ester [g]	2639–63-6	1197	1191	1191[c]	E	0.72	0.17	1.33	0.07	0.38	0.04	5.93	0.57		
							H			13.64	0.61			65.79	2.21	17.84	1.21
29	α-terpineol [b,e]	*p*-menth-1-en-8-ol [g]	98–55-5	1204	1195	1186	E			10.35	0.38			57.57	2.26	15.51	0.76
							H	1.77	0.07	5.90	0.09	1.59	0.01	26.78	0.98	5.94	0.36
30	Nerol [b,e]	*cis*-geraniol; (*Z*)-geraniol; neryl alcohol [g]	106–25-2	1233	1224	1227	E	1.45	0.05	4.49	0.12	1.16	0.04	23.43	0.95	4.66	0.36
							H			2.85	0.10			5.93	0.14	1.74	0.25
31	Geraniol [a]	Lemonol; geranyl alcohol; (*E*)-nerol; Guaniol [g]	106–24-1	1260	1250	1249[c]	E			2.01	0.09	0.07		5.44	0.24	1.49	0.03
							H	2.15	0.14	0.62	0.16	0.27		2.69	0.21	0.51	0.06
32	Compound 32 [e]	/	/	1291	n.d	/	E			0.42							
							H	0.14		0.70	0.08	0.36	0.13	5.82	0.30	1.33	0.10
33	Compound 33 [e]	/	/	1327	n.d	/	E	0.17	0.02	0.79	0.17	0.22	0.02	4.97	0.30	1.30	0.08
							H	0.48		7.13	0.01	0.09	0.06	24.15	0.73	6.85	0.40
							E	0.17		6.19	0.55			22.27	1.15	5.08	0.25
		Sesquiterpenes and sesquiterpenoids															
34	β-caryophyllene [a]	*trans*-caryophyllene [g]	87–44-5	1429	1423	1417[c]	H	75.59	1.50	64.21	0.20	55.92	1.26	162.99	4.77	89.00	3.32
							E	66.95	0.71	48.89	1.42	49.49	2.73	139.56	5.60	70.26	2.47
35	*trans*-α-bergamotene [b,f]	/	13474–59-4	1438	1435	1432[c]	H	6.96	0.09	14.00	0.15	4.30	0.36	30.55	0.86	11.27	0.42
							E	5.92	0.08	10.76	0.38	4.14	0.23	25.04	1.22	9.04	0.27
36	α-guaiene [b,f]	Guaia-1(5),11-diene [g]	01/12/3691	1442	1438	1437[c]	H	0.40	0.03	26.34	0.27	0.28	0.18	43.54	1.22	16.75	0.61
							E	0.24	0.04	20.22	0.51	0.18	0.04	38.74	1.73	13.25	0.40
37	*trans*-β-farnesene [b,f]	*trans*-β-farnesene [g]	18794–84-8	1456	1453	1454[c]	H	4.92	0.09	19.42	0.43	1.97	0.25	45.29	1.16	20.35	0.80
							E	4.11	0.04	16.03	0.55	1.62	0.11	39.32	1.65	16.86	0.63
38	α-humulene [a]	α-caryophyllene; 3,7,10-humulatriene [g]	6753–98-6	1466	1459	1452[c]	H	26.08	0.37	25.12	0.20	24.65	0.85	63.07	1.94	34.89	1.33
							E	22.95	0.24	19.34	0.68	21.59	1.16	54.28	2.27	28.34	1.09
39	Compound 39[f]	/	/	1487	n.d	/	H	0.42	0.09	3.53	0.05	0.72	0.00	2.91	0.06	2.39	0.20
							E	0.32	0.04							0.35	0.01
40	Compound 40[f]	/	/	1499	n.d	/	H	4.17	0.10	13.57	0.06	15.00	0.78	35.85	1.08	13.36	0.57
							E	4.78	0.10	10.60	0.47	13.72	0.88	31.70	1.32	10.87	0.34
41	Compound 41[f]	/	/	1507	1499	/	H	5.95		16.13	0.13	11.66	0.28	40.74	1.23	14.11	0.62
							E	4.77	0.07	12.15	0.49	10.28	0.84	35.17	1.39	11.21	0.39
42	δ-guaiene [b,f]	Bulnesene; guaia-1(10),11-diene [g]	3691–11-0	1511	1504	1509[c]	H	5.91		52.46	0.03			87.11	3.16	31.63	0.58
							E			34.43	2.61	10.59		73.69	2.63	23.85	0.61
43	Compound 43[f]	/	/	1514	n.d	/	H	10.15	0.02	2.37	0.04	3.33	0.14	10.45	0.12	2.37	0.08
							E	8.42	0.09	2.09	0.11	2.54	0.33	9.80	0.48	3.45	0.09
44	Compound 44[f]	/	/	1525	n.d	/	H	3.57	0.01	2.10	0.00	7.34	0.15	19.06	0.67	1.15	0.07
							E	1.90	0.11	1.47	0.13	5.38	0.34	16.27	0.70	0.92	0.02
45	Compound 45[f]	/	/	1531	n.d	/	H	4.63	0.03	5.51	0.05	6.65	0.13	22.17	0.60	3.23	0.18
							E	4.13	0.06	4.36	0.25	5.91	0.33	19.39	0.81	2.66	0.13
46	*cis*-nerolidol [a,f]	Peruviol; (*Z*)-nerolidol [g]	3790–78-1	1537	1530	1531[c]	H	1.12	0.00	1.09	0.02	1.25	0.06	6.11	0.20		
							E	1.02	0.04	0.89	0.08	1.06	0.07	5.33	0.30		
47	δ-selinene [b,f]	/	473–14-3	1542	1535	1492[c]	H	5.05	0.06	3.26	0.02	7.54	0.19	26.05	0.89	1.53	0.21
							E	4.31	0.13	2.61	0.18	5.69	0.38	22.41	0.97	1.11	0.25
48	Compound 48[f]	/	/	1547	n.d	/	H	14.08	0.29								
							E	11.35	0.42			5.94					
49	Compound 49[b,f]	/	58893–88-2	1549	1541	1544[h]	H	26.33	0.47	20.55	0.04	31.01	0.75	113.52	3.44	1.10	0.08
							E	22.49	0.68	15.23	0.88	26.57	1.36	99.46	3.94	0.51	0.07

(*continued on next page*)

Table 1 (*continued*)

No	Compound	Synonyms	CAS number	FID RI (exp.)	MS RI (exp.)	RI (lit.)	S.	µg/100 mg of dry plant matter							
								Bedica®		Bediol®		Bedrobinol®		Bedrocan®		Bedrolite®	
								m	sd	m	sd	m	sd	m	sd	m	sd
50	Selina-3,7(11)-diene [b,f]	Eudesma-3,7(11)-diene [g]	6813–21-4	1553	1546	1545[c]	H	31.05	0.47	24.50	0.12	34.35	0.66	143.17	4.52	1.32	0.05
							E	24.36	0.87	17.53	1.10	27.65	1.36	124.14	4.75	1.08	0.05
51	*trans*-nerolidol [a]	(*E*)-nerolidol [g]	40716–66-3	1568	1561	1561[c]	H	11.79	0.30	19.16	0.09	7.35	0.07	13.99	0.50	6.57	0.24
							E	11.40	0.17	16.13	1.05	5.94	0.84	12.55	0.56	5.86	0.14
52	(−)-caryophyllene oxide [a]	β-caryophyllene epoxide [g]	1139–30-6	1597	1587	1582[c]	H	10.88	0.27	8.51	0.06	12.36	0.18	24.48	0.75	10.92	0.53
							E	9.02	0.09	7.92	0.48	11.84	0.38	22.95	0.95	10.06	0.32
53	(−)-guaiol [a]	Guai-1(5)-en-11-ol; champacol; champaca camphor; guaiac alcohol [g]	489–86-1	1610	1599	1600[c]	H	56.07	1.33	0.21	0.07	0.56	0.43	0.98	0.02	0.29	0.12
							E	45.91	0.67	0.47	0.07	1.01	0.09	1.27	0.11	0.27	0.11
54	Compound 54[f]	/	508–55-4	1639	1628	/	H	53.03	1.24	1.63	0.05	5.93	0.09	8.05	0.25	0.76	0.02
							E	42.92	0.43	1.74	0.08	5.96	0.52	8.21	0.36	0.86	0.07
55	Compound 55[f]	/	/	1671	n.d	/	H	63.16	1.52	16.46	0.02	8.62	0.07	21.51	0.83	7.13	0.44
							E	53.77	0.40	14.56	0.97	7.72	0.64	20.74	0.96	7.04	0.78
56	(−)-α-bisabolol [a]	Levomenol; kamillosan [g]	23089–26-1	1698	1689	1685[c]	H	87.79	2.18	1.25	0.10	4.98	0.07	6.07	0.10	0.57	0.04
							E	74.87	0.98			5.41	0.21	5.87	0.37		

RI (exp.): experimental Retention Index, RI (lit.): Retention Index from the literature, [a]: standard available, [b]: identified by GC–MS, [c]: [23], [d]: supposed with RI value, n.d: not detected, [e]: expressed as equivalent of β-myrcene, [f]: expressed as equivalent of β-caryophyllene, [g]: [45], [h]: [46], m: mean value, sd: standard deviation, [i]: [30], H: *n*-hexane, E: absolute ethanol.

solvents for the recovery of terpenic compounds (i.e., *n*-hexane and absolute ethanol). In total, 56 compounds were found, among which 23 were strictly identified by comparing their RT, mass spectra, and RI with authentic analytical standards and 19 were putatively identified by comparing their mass spectra and RI with reference works in the literature. All compounds are listed in Table 1, sorted according to their elution order. Their synonyms, CAS number, calculated RI, and literature RI are given for better comprehension. The results are presented as the mean value and its standard deviation, in µg/100 mg of dry inflorescence, for each solvent, allowing the comparison of solvent selectivity. Fig. 1 displays a GC–FID chromatogram from the Bedrocan® variety extracted by *n*-hexane. A full range GC–FID chromatogram is given in supplementary material (SM2). Fig. 2 illustrates all the identified compounds by their chemical structure.

In our work, a DB-5MS column was chosen for identification by GC–MS. Indeed, in the field of analysis of volatile compounds by gas chromatography, apolar columns are commonly described, with stationary phases consisting of 95 % dimethylpolysiloxane groups and 5 % phenyl groups, such as the two columns mentioned in this study (i.e., DB5-MS and VF5-MS). Similar or identical columns have also been reported in the literature for the analysis of cannabis extracts [33,35–38,14,17,39]. Adams et al. [23] and Babushok et al. [40] compilated a large amount of data concerning volatile compounds frequently recovered in plants; their work were exploited in this study for the putative identification. Intermediate and high polarity columns (i.e., ZB-35HT [41] and Rtx-Wax [42]) have also been described in the literature, but these types of columns are less relevant for the analysis of terpene hydrocarbons [43,44].

The GC–MS identification of volatile compounds in cannabis extracts was first performed by injecting, under the same conditions, all the extracts, as well as the standard solutions and the mixture of linear alkanes. First, RI values were determined for all detected peaks by applying Eq (1). Then, attributions using standards were possible by comparing the RT and confirmed by RI and database matching. For some compounds with no standard, RI could not be determined in GC–MS for several reasons such as no detection due to dilution, ionization issues or co-elution phenomena. The obtained values of RI could be compared with the values compiled in the Adams library [23], since all compounds described were obtained with identical chromatographic columns (DB-5 MS). Finally, the remaining peaks were putatively identified by mass spectra comparison, the NIST database, and RI matching with the literature [23,40]. Mass spectra of the studied components were selected at the peak apexes on their corresponding chromatograms. The identifications proposed by the database were critically examined with a focus on the highest Match and Reverse Match values, and by comparing the RI values proposed by the NIST database with those in the literature [23,40] and those calculated. RI comparisons were preferred to RT, since the latter are highly dependent on the analytical system (e.g., column age and condition) as opposed to RI, which remains constant and is comparable even if determined on different analytical devices as long as they are calculated using the same type of column. Thus, all the RI values mentioned in this study could be reemployed and compared to RI values obtained under similar chromatographic conditions.

3.3. Comparison of the terpenic content of the five medicinal varieties depending on the extraction solvent

The quantitative analysis of volatile compounds in the cannabis extracts was executed by GC–FID. All extracts, calibration solutions, and alkane mixtures were injected for RI calculation. All previously identified peaks were assigned in GC–FID using the RI values obtained in GC–MS and those obtained in GC–FID. For quantification, seven-point calibration curves were plotted for each of the 23 standard compounds. An internal standard (tridecane) was introduced in the same quantity to each calibration solution, and the calibration curves were constructed by plotting the analyte area/ISTD area against the amount

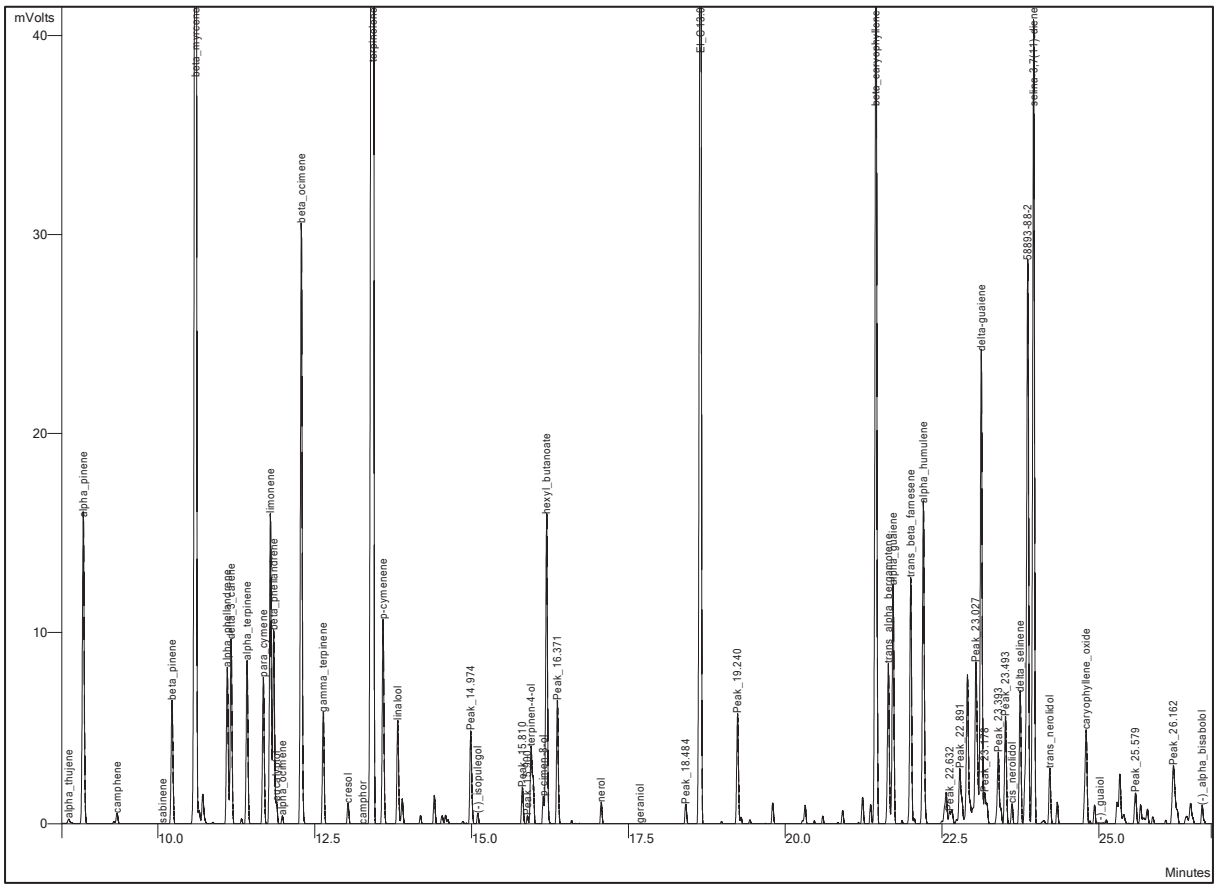

Fig. 1. GC–FID chromatogram of *n*-hexane extract from the Bedrocan® variety. Total peaks = 97, integrated peaks = 56.

of analyte/ISTD amount. A GC–FID chromatogram of analytical standards is given in supplementary material (SM1). Several previously published studies only presented cannabis terpene profiles by expressing the results as a percentage of the total area of the studied compounds [9,12,14–16]. This type of protocol gives a global perspective of the extract, as no calibration is carried out. Nevertheless, several elements must be considered. Firstly, the value of the total area depends essentially on the number of integrated peaks. Secondly, this approach neglects the differences of the response factors (RF) of the compounds by considering that all respond in the same way. Indeed, even though the FID response is proportionate to the number of carbon atoms in molecules, the output is influenced by the presence of heteroatoms and the interaction between ions in the hydrogen flame, and this may explain why, in many cases, the FID response is not equal to the carbon count [47,48]. Therefore, the values reported in Table 2 illustrate the variations of RF within the same group of molecules. For instance, the RF of *p*-cymene (RF = 1.0982) is similar to the RF of d-limonene (RF = 1.0307) but different from the RF of *cis*-β-ocimene (RF = 0.6338), which means that the first two compounds respond in the same way but differently from the third compound, even though these three compounds have the same molecular formula. It was confirmed that the use of analytical standards is the most precise method of quantification.

For studied compounds whose standard were not available, values of quantification were expressed as equivalent to a standard from the same molecular family. For this purpose, the average value of the RF of the monoterpene and monoterpenoid standards was calculated, as well as that of the sesquiterpene and sesquiterpenoid standards. Then, for each group of compounds, the standard whose RF was the closest to the average value was chosen as the reference. Consequently, monoterpenes and monoterpenoids were expressed as β-myrcene equivalents, and sesquiterpenes and sesquiterpenoids were expressed as β-caryophyllene equivalents. Other strategies have been reported in the literature. Jin et al. [18] calculated the average RF of all standards, whether mono- and sesqui-, in order to express results as the "average terpene" equivalent. This strategy is more straightforward, since the same RF can be employed for the quantification of the analytes. However, as described above, this technique is usually limited, because they are based on the assumption that all compounds respond in the same way. Another approach is to utilize a single analytical standard to quantify all identified peaks. For instance, Hazekamp et al. [17] published a comparative dosage of terpenes and terpenoids in three commercial varieties using γ-terpinene as a unique standard for the quantification of 20 compounds. The authors justified their choice by the prohibitive costs of acquiring analytical standards, which is not scientifically acceptable and certainly led to an under- or overestimation of dosages.

Particular attention was given to the peak separations by varying the temperature programming. However, due to the complexity of the matrices and the very close chemical structures of some compounds, non-optimal resolutions were observed, such as limonene (RT = 11.795 min) and β-phellandrene (RT = 11.847 min). The area integrations and by extension the quantification results are strictly linked to the peak resolution.

Sequentially, the Bedica® variety extracted using *n*-hexane exhibits a higher content of sesquiterpenes than monoterpenes and monoterpenoids: (−)-α-bisabolol (87.79 μg/100 mg), β-caryophyllene (75.59 μg/100 mg), compound 55 (63.16 μg/100 mg), (−)-guaiol (56.07 μg/100 mg), and compound 54 (53.03 μg/100 mg) were the most abundant compounds among sesquiterpenes and sesquiterpenoids, whereas linalool (39.50 μg/100 mg), β-myrcene (27.37 μg/100 mg), and α-pinene (23.31 μg/100 mg) were the most prevalent among monoterpenes and monoterpenoids. The same extract obtained by means of ethanolic maceration showed the same terpenic profile with these same

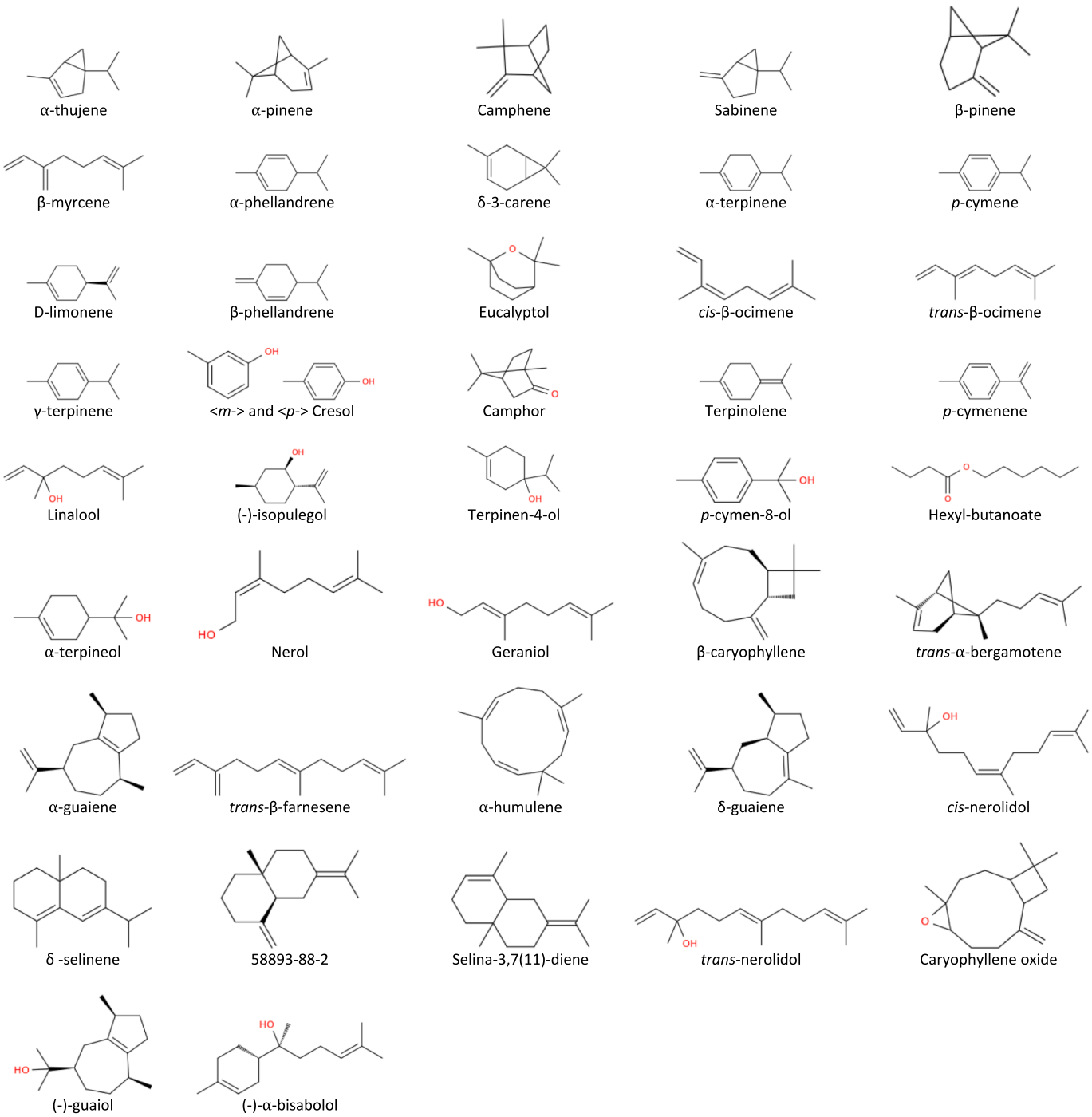

Fig. 2. Structures of identified terpenes and terpenoids in C. sativa (all structures were obtained from the OPSIN database [31]).

eight major compounds, but with a less important recovery of all compounds, including other minor terpenes: (−)-α-bisabolol (74.87 μg/100 mg), β-caryophyllene (66.95 μg/100 mg), compound 55 (53.77 μg/100 mg), (−)-guaiol (45.91 μg/100 mg), compound 54 (42.92 μg/100 mg), linalool (35.33 μg/100 mg), β-myrcene (17.54 μg/100 mg), and α-pinene (13.46 μg/100 mg). The most abundant volatile compound in the Bediol® variety was β-myrcene, with a better recovery with *n*-hexane compared to ethanol (125.80 μg/100 mg versus 108.38 μg/100 mg). The sesquiterpene profile obtained with *n*-hexane was more varied, among which β-caryophyllene (64.21 μg/100 mg), δ-guaiene (52.46 μg/100 mg), α-guaiene (26.34 μg/100 mg), α-humulene (25.12 μg/100 mg), and selina-3,7(11)-diene (24.50 μg/100 mg) were the most abundant compounds. The ethanolic extracts displayed the same profile with less effectiveness in the recovery of β-caryophyllene (48.89 μg/100 mg), δ-guaiene (34.43 μg/100 mg), α-guaiene (20.22 μg/100 mg), α-humulene (19.34 μg/100 mg), and selina-3,7(11)-diene (17.53 μg/100 mg). The extracts generated from the Bedrobinol® variety in *n*-hexane showed higher amounts of α-pinene and β-myrcene (276.41 μg/100 mg and 149.23 μg/100 mg, respectively), and in lower quantities β-caryophyllene (55.92 μg/100 mg). These three compounds were less effectively extracted by ethanol, with the following values: 178.39 μg/100 mg, 59.32 μg/100 mg, and 49.49 μg/100 mg for α-pinene, β-myrcene, and β-caryophyllene, respectively. The terpenic profile of the Bedrocan® variety was the most diverse among all strains. The *n*-hexane macerates exhibited the following values: terpinolene (764.99 μg/100 mg) and β-myrcene (390.29 μg/100 mg); thus, most of the mono- and

Table 2
Response factors and determination coefficients of analytical standards.

Standard	Slope	R^2	Standard	Slope	R^2
α-pinene	1.0066	0.999913	Terpinolene	0.98913	0.999954
Camphene	1.0319	0.999926	Linalool	0.86037	0.999931
β-pinene	1.0085	0.999930	(−)-isopulegol	0.83843	0.999860
β-myrcene	0.91247	0.999953	Geraniol	0.71156	0.997336
δ-3-carene	1.0146	0.999948	β-caryophyllene	1.0625	0.999943
α-terpinene	1.0223	0.999803	α-humulene	1.0350	0.999939
p-cymene	1.0982	0.999974	*cis*-nerolidol	0.88374	0.999900
D-limonene	1.0307	0.999985	*trans*-nerolidol	0.89762	0.999869
Eucalyptol	0.86374	0.999906	(−)-caryophyllene oxide	0.96693	0.999960
cis-β-ocimene	0.63386	0.999919	(−)-guaiol	0.89893	0.999925
trans-β-ocimene	0.987	0.999930	(−)-α-bisabolol	0.90055	0.999932
γ-terpinene	0.99504	0.999959			

sesqui- occurred in lower quantities, such as β-caryophyllene (162.99 μg/100 mg), selina-3,7(11)-diene (143.17 μg/100 mg), compound 49 (113.52 μg/100 mg), *trans*-β-ocimene (109.58 μg/100 mg), and δ-guaiene (87.11 μg/100 mg). As reported for the previous varieties, the ethanolic extraction showed reduced recovery of these same compounds, as with terpinolene (583.27 μg/100 mg) and β-myrcene (295.70 μg/100 mg). Finally, the Bedrolite® variety also showed an equilibrate profile, with most of studied compounds having higher contents of β-myrcene (143.19 μg/100 mg with *n*-hexane and 89.31 μg/100 mg with absolute ethanol), terpinolene (86.40 μg/100 mg versus 57.62 μg/100 mg), and β-caryophyllene (89.00 μg/100 mg and 70.26 μg/100 mg).

In other words, three monoterpenes were consistently detected in the five varieties: α-pinene, β-myrcene, and β-caryophyllene. Their abundance in each variety varied, with a maximum of α-pinene in Bedrobinol® and of β-myrcene and β-caryophyllene in Bedrocan®. Terpinolene was another monoterpene found in relatively high quantities in Bediol® and Bedrolite®, with a maximum in Bedrocan®. Sesquiterpenes were well represented in Bedica®, such as selina-3,7(11)-diene, (−)-guaiol, and (−)-α-bisabolol. The comparison between the extractions conducted with *n*-hexane and ethanol underlined that the alkane is a better solvent for the recovery of volatile compounds, regardless of the variety.

Some of these varieties commercialized by Bedrocan International have already been described, but an exhaustive comparison of all five varieties has yet to be proposed in the literature. Hazekamp et al. [17] proposed a quantitative analysis of 20 terpenes and terpenoids from Bedrocan®, Bedrobinol®, and Bedica® in ethanolic extracts. Their analysis of the Bedrocan® extract showed that terpinolene is the major compound (580 μg/100 mg), followed by β-myrcene (290 μg/100 mg) and *cis*-ocimene (200 μg/100 mg), which was consistent with the values presented in our work. The results reported for the Bedrobinol® variety are different; β-myrcene was shown to be the major compound of their samples (590 μg/100 mg), with a value ten times greater than that in our samples, whereas the dosage of α-pinene (190 μg/100 mg) was in accordance with our measurements. Finally, their ethanolic extraction of the Bedica® variety exhibited a higher content of β-myrcene (370 μg/100 mg) and β-caryophyllene (130 μg/100 mg); however, no data regarding linalool were given, while we reported the latter as a major monoterpenoid in this variety. These differences in values could be explained by dissimilarities in the extraction protocol (i.e., the solid/liquid ratio and the extraction time), or especially differences in the biomass used (i.e., cultivation conditions, age, storage conditions, sampling, etc.), which can greatly impact the composition of the final plant [28,35,49–52]. Indeed, Aizpurua-Olaizola et al. [35] and Booth et al. [51] pointed out that the terpene content is unequally distributed within the plant itself: the inflorescences exhibit a higher concentration of terpenes and terpenoids than other parts, such as the leaves. Additionally, Namdar et al. [28] explained that the amount of terpenic compounds is subordinate to the position of the flowers along the flowering stem. Thus, the inflorescences harvested in the uppermost position generate extracts with higher contents of terpenes than flowers sampled in middle and lower positions. Finally, Booth et al. [52] also demonstrated that the amount of terpenes was higher in mature flowers than in juvenile flowers.

Table 3
Limits of detection (LOD) and quantification (LOQ). Data are expressed as μg/mL.

Standard	LOD (μg/mL)	LOQ (μg/mL)	Standard	LOD (μg/mL)	LOQ (μg/mL)
α-pinene	1.45	4.36	Terpinolene	2.15	6.46
Camphene	1.49	4.47	Linalool	2.39	7.18
β-pinene	1.69	5.07	(−)-isopulegol	2.49	7.46
β-myrcene	1.73	5.18	Geraniol	2.76	8.28
δ-3-carene	1.81	5.42	β-caryophyllene	2.55	7.65
α-terpinene	1.80	5.41	α-humulene	2.50	7.50
p-cymene	1.91	5.73	*cis*-nerolidol	1.11	3.34
D-limonene	1.92	5.77	*trans*-nerolidol	1.50	4.50
Eucalyptol	2.21	6.63	(−)-caryophyllene oxide	2.88	8.65
cis-β-ocimene	0.60	1.79	(−)-guaiol	2.24	6.72
trans-β-ocimene	1.31	3.92	(−)-α-bisabolol	2.39	7.16
γ-terpinene	2.08	6.24			

3.4. LOD, LOQ, and linearity study

The values of the limit of detection (LOD) and the limit of quantification (LOQ) were calculated using Eqs. (2) and (3) in accordance with the ANSES validation guide [24], and are listed in Table 3. The compounds are sorted according to their elution order and results are presented in μg/mL. The LOD represents the lowest concentration of an analyte that can be detected and the LOQ corresponds to the lowest concentration of an analyte that can be quantified. The LOD values ranged from 0.60 μg/mL for *cis*-β-ocimene to 2.88 μg/mL for (−)-caryophyllene oxide. LOQ values ranged from 1.79 μg/mL for δ-3-carene to 8.65 for β-caryophyllene.

Values of the coefficients of determination and the slopes are listed in Table 2.

4. Conclusions

In this research, two extraction solvents were tested for the recovery of terpenes and terpenoids from five commercial varieties of medicinal *C. sativa*. Volatile compounds were identified by GC–MS and quantified by GC–FID. Special attention was given to quantification using 23 analytical standards, internal calibration, RI calculation, use of the relevant RF, and multiple sources for identification, allowing the elucidation of a very complete distribution of terpenes and terpenoids.

The utilization of many analytical standards and the quantification of

unknown compounds with standards with similar structures allowed a more accurate determination of the content of an extract. The methodology was validated with the analysis of five pharmaceutical grade varieties produced by Bedrocan International. It was found to be suitable to discriminate cultivars based on their terpenoid content.

Our results highlighted that the choice of the solvent and the analysis method reliability are critical for the study of these terpenic compounds, regarding their contribution to the entourage effect. Indeed, as mentioned by Namdar et al. [53], the ratio of cannabinoids to volatile compounds in pharmaceutical preparations is different from those natively produced in plants and could lead to unsatisfactory results, justifying the need for reliable quantification.

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CRediT authorship contribution statement

Victor Pereira Francisco: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Muriel Cerny:** Writing – review & editing, Methodology, Conceptualization. **Romain Valentin:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Franck Milone-Delacourt:** Writing – review & editing, Funding acquisition. **Alexandra Paillard:** Writing – review & editing. **Marion Alignan:** Writing – review & editing, Supervision, Project administration, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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