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Victor Pereira-Francisco, Muriel Cerny, Romain Valentin, Franck Milone-Delacourt, Marion Alignan. An alternative easy method for the analysis of volatile compounds from lipid matrices: analysis of terpenes and terpenoids in medical-grade Cannabis sativa L. inflorescences in macerated oils. Journal of Chromatography Open, 2026, 9, pp.100316. <10.1016/j.jcoa.2026.100316>. <hal-05716030>

HAL Id: hal-05716030

<https://hal.inrae.fr/hal-05716030v1>

Submitted on 12 Aug 2026

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An alternative easy method for the analysis of volatile compounds from lipid matrices: analysis of terpenes and terpenoids in medical-grade *Cannabis sativa* L. inflorescences in macerated oils

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ARTICLE INFO

Keywords:

Cannabis sativa L.

Terpenes

Terpenoids

Lipid matrix

Saponification

GC-FID

ABSTRACT

The terpenes and terpenoids present in cannabis are the subject of increasing research, as their interaction with phytocannabinoids appears to enhance their biological activity. In addition, both for health reasons and to meet consumer demand for more natural products, cannabis flowers are increasingly being extracted in vegetable oils, and cannabis extracts are being formulated in vegetable oils. The number of people using cannabis for medical purposes is increasing. In addition to optimizing the combination of active pharmaceutical ingredients (APIs) for the specific indication being treated, it is necessary to know precisely the dose of each important API administered. To achieve this goal, we have developed a new sample preparation method for determining the terpene and terpenoid content of lipid-based plant extracts by GC-FID. The method described has the advantages of being easy to implement, inexpensive, reproducible, and suitable for the quantification of terpenes and terpenoids in oily matrices, an analysis that was generally performed by HS until now. We confirm that the composition of lipid matrices can influence the extraction yields of terpene compounds from medical cannabis. Sesame oil was the most effective lipid matrix (925 µg total terpene compounds/100 mg dry matter) and rapeseed oil was the least effective (762 µg/100 mg dry matter).

1. Introduction

Cannabis terpenes and terpenoids are increasingly studied because of their involvement in the so-called “inter-entourage effect” [1], suggesting that interactions with phytocannabinoids enhance their biological activity. Raz et al. [2] went further by highlighting that synergistic effects between specific terpenes and cannabinoids were more apparent at the natural compound ratio present in extracts derived from cannabis inflorescences.

Furthermore, both for health reasons and due to a consumer desire for more natural products, cannabis flowers are increasingly being extracted in vegetable oils and cannabis extracts are formulated in vegetable oils. The number of medical cannabis users is growing. In addition to optimizing the combination of active pharmaceutical ingredients (APIs) for the specific indication treated, there is a need for precise knowledge of the dose of each important API administered.

Cannabis terpenes and terpenoids from oil matrices are currently analyzed by headspace (HS) sampling. However, multiple experimental parameters must be taken into account in this analytical method, which is therefore relatively complex to implement and presents various disadvantages [3]. Conversely, the analysis of terpenes and terpenoids by gas chromatography-flame ionization detection (GC-FID) on cannabis oil extracts has significant technical limitations. Indeed, the low terpene compound:oil ratio characteristic of these extracts would require the injection of large volumes incompatible with the capacity of the column. We therefore propose a precise alternative method for the analysis of terpenes and terpenoids in oily cannabis matrices. The application of this method to extracts of a commercial variety of medicinal cannabis in various edible oils made it possible to assess the impact of oil composition on the extraction yield of terpene compounds.

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<https://doi.org/10.1016/j.jcoa.2026.100316>

Received 25 November 2025; Received in revised form 12 February 2026; Accepted 17 February 2026

Available online 18 February 2026

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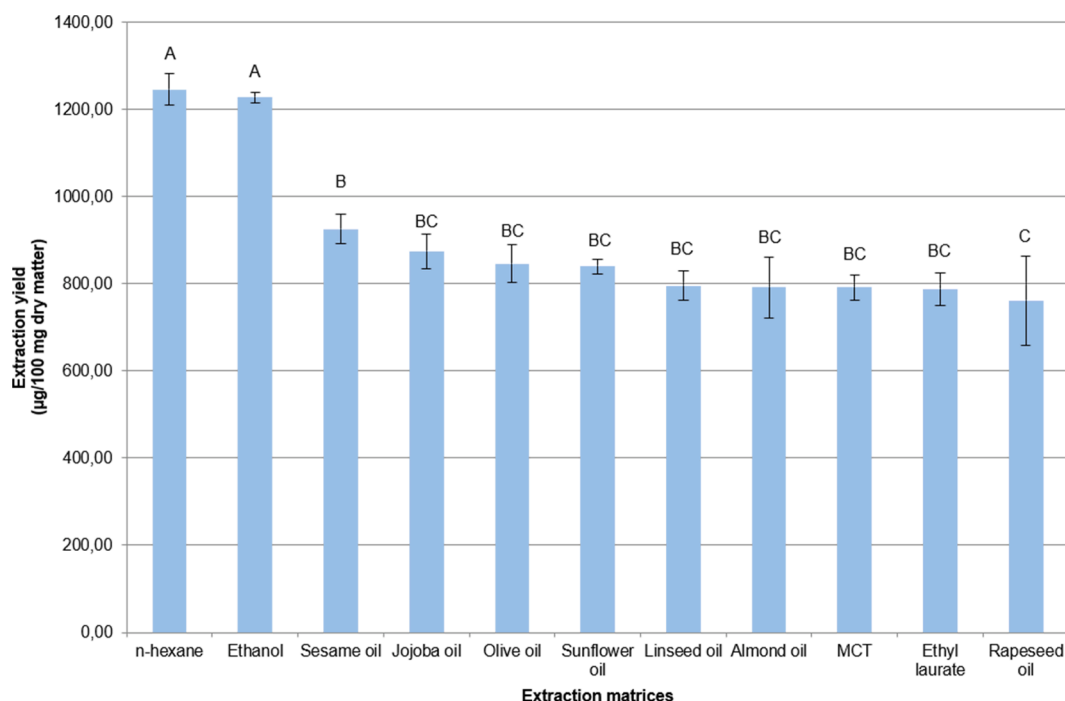


Fig. 1. Total terpene and terpenoid content in THC/CBD-intermediate type Bediol® variety of medicinal cannabis extracted with biological lipid-based matrices, hexane or ethanol ; ($n = 3$, $p < 0,05$). MCT: Medium Chain Triglycerides.

2. Materials and methods

2.1. Reagents and materials

The THC/CBD-intermediate type Bediol® variety of medicinal cannabis (batch: 19L16FB20K04, dried and ground) was purchased from Bedrocan International (Veendam, Netherlands) in accordance with OMC (Office of Medicinal Cannabis, Netherlands) and ANSM (French National Agency for the Safety of Medicines and Health Products) requirements and authorizations. Samples were stored in airtight containers in the dark at 25 °C.

The 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 %), ν -limonene (purity 99 %), cis- β -ocimene (purity 31 %), trans- β -ocimene (purity 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 %), (-)-guaiaol (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

We weighed out 1 g of cannabis inflorescence precisely in an amber glass vial containing 800 µg of the internal standard (ISTD) tridecane. The sample was then subjected to extraction at 20 °C with 10 mL vegetable oil, ethyl laurate or medium-chain triglycerides (MCT), with stirring at 250 rpm on an orbital shaking table (Thermo Fisher Scientific) for 1.5 h.

2.3. Sample preparation

Each extract was filtered through a glass fiber filter (Agela Technologies) and saponified with 6 mL of a 1 M ethanolic solution of potassium hydroxide in a glass tube. The tube was tightly closed with a PTFE screw cap, vortexed and left in the dark at 20 °C for 48 h before the addition of 2 mL pure water and 2 mL cyclohexane. The final preparation was gently mixed and left to stand to allow the phases to separate. The upper organic layer was transferred to an analytical vial for chromatography analysis.

2.4. Gas chromatography–Flame ionisation detector (GC-FID) for quantification of volatile compounds

Terpenes and terpenoids were quantified with a gas chromatograph (3900 Series GC System, Varian) equipped with an autosampler (CP-8400, Varian) and a flame ionization detector (FID). We injected 1 µL of sample in the liquid injection, split mode (1/10) at 270 °C. Chromatographic separation was performed on a VF-5MS column (30 m x 0.25 mm x 0.25 µm, Agilent) with helium as the carrier gas at a flow rate of 1.3 mL/min. The oven temperature program was defined as follows: 50 °C isotherm for 5 min, temperature gradient from 50 °C to 180 °C at a rate of 6 °C/min, temperature gradient from 180 °C to 300 °C at a rate of 30 °C/min and a final 300 °C isotherm for 9.33 min. The FID was operated at 300 °C with H₂ at 30 mL/min and air at 300 mL/min. Data were processed with Star software (version 6.41, Varian). Instrumental calibration was performed and calibration curves were used for compounds for which standards were available. Mass spectral identification of compounds and robustness of GC–MS coupled to GC-FID method were presented in a previous study [4]. Compound identification, quantification strategy, and validation parameters refer to this previously published method. It allowed to exempt of no relevant co-elutions occur in the chromatographic region of interest.

3. Results and discussion

Eight vegetable oils and one liquid wax (jojoba oil) were chosen

Table 1

Mono- and sesqui- terpenes and terpenoids of the Bediol® medicinal cannabis variety extracted with non-polar biobased matrices, hexane or ethanol (n = 3, p < 0,05, the data are expressed as the mean and standard deviation).

	n-hexane	Ethanol	Sesame oil	Jojoba oil	Olive oil	Sunflower oil
Extraction yields (µg/100 mg dry matter)						
α-pinene	71,88 ± 3,04	68,39 ± 1,24	45,46 ± 1,10	41,37 ± 2,97	43,77 ± 1,69	42,02 ± 1,18
Camphene	1,88 ± 0,22	1,68 ± 0,06	0,93 ± 0,05	0,96 ± 0,06	0,93 ± 0,01	0,85 ± 0,02
β-pinene	12,72 ± 0,58	11,56 ± 0,13	7,82 ± 0,31	11,51 ± 0,56	7,49 ± 0,22	7,14 ± 0,20
β-myrcene	781,65 ± 8,69	750,74 ± 7,00	504,77 ± 16,78	503,16 ± 30,79	475,55 ± 24,13	465,37 ± 10,41
α-phellandrene	4,47 ± 0,08	5,33 ± 0,11	3,29 ± 0,11	3,08 ± 0,17	2,86 ± 0,19	2,87 ± 0,10
δ-3-carene	7,33 ± 0,45	5,94 ± 0,10	4,86 ± 0,20	5,19 ± 0,30	4,62 ± 0,26	4,51 ± 0,11
α-terpinene	2,25 ± 0,05	1,98 ± 0,05	2,27 ± 0,06	1,89 ± 0,27	1,89 ± 0,20	2,00 ± 0,32
p-cymene	8,51 ± 0,14	7,77 ± 0,02	3,91 ± 0,11	5,79 ± 0,22	3,76 ± 0,12	3,73 ± 0,15
Limonene	10,85 ± 0,24	11,40 ± 0,09	6,55 ± 0,24	7,09 ± 0,17	6,23 ± 0,25	5,96 ± 0,15
β-phellandrene	12,65 ± 0,34	11,05 ± 0,06	7,60 ± 0,31	8,57 ± 1,20	7,20 ± 0,37	6,85 ± 0,33
Eucalyptol	2,76 ± 0,04	3,04 ± 0,03	0,65 ± 0,24	0,82 ± 0,29	0,58 ± 0,01	0,84 ± 0,14
α-ocimene	1,09 ± 0,11	1,07 ± 0,08	0,56 ± 0,14	0,35 ± 0,09	0,41 ± 0,07	0,35 ± 0,04
β-ocimene	13,53 ± 0,42	12,92 ± 0,15	7,31 ± 0,27	7,33 ± 0,18	7,01 ± 0,34	6,46 ± 0,24
γ-terpinene	6,31 ± 0,24	5,96 ± 0,05	3,86 ± 0,17	3,62 ± 0,16	3,60 ± 0,18	3,40 ± 0,12
Terpinolene	62,49 ± 1,49	67,14 ± 1,52	77,06 ± 2,62	60,15 ± 3,09	63,48 ± 5,07	66,43 ± 0,72
Total monoterpenic compounds	1000,36 ± 32,97	965,97 ± 10,20	676,92 ± 22,20	660,88 ± 37,64	629,37 ± 32,71	618,78 ± 12,30
β-caryophyllene	84,58 ± 2,13	86,65 ± 0,14	64,66 ± 3,03	61,49 ± 2,74	62,28 ± 3,10	61,52 ± 1,81
trans-α-bergamotene	17,90 ± 0,43	18,06 ± 0,08	13,94 ± 0,68	12,99 ± 0,85	13,58 ± 0,71	13,34 ± 0,45
α-guaiene	32,30 ± 0,57	33,47 ± 0,25	27,91 ± 1,41	24,96 ± 1,63	26,70 ± 1,38	26,60 ± 0,75
trans-β-farnesene	34,05 ± 1,37	35,48 ± 0,28	30,22 ± 2,68	38,72 ± 12,2	29,58 ± 2,31	27,50 ± 0,90
α-humulene	31,14 ± 0,77	32,14 ± 0,17	23,81 ± 2,68	22,82 ± 0,67	22,77 ± 1,27	22,62 ± 0,60
δ-guaiene	9,38 ± 1,16	14,34 ± 0,99	34,00 ± 4,31	19,36 ± 3,19	26,90 ± 2,60	32,15 ± 0,62
[58,893–88–2]	15,80 ± 0,42	17,22 ± 0,24	17,47 ± 4,31	14,17 ± 1,18	15,47 ± 0,90	16,31 ± 0,36
Selina-3,7(11)-diène	12,00 ± 0,69	15,69 ± 0,48	19,42 ± 1,70	14,10 ± 1,67	17,02 ± 1,18	19,04 ± 0,77
Caryophyllene oxide	8,73 ± 0,42	8,69 ± 0,32	2,72 ± 0,43	5,34 ± 1,78	2,43 ± 0,59	2,67 ± 0,14
Total sesquiterpenic compounds	245,88 ± 4,84	261,73 ± 2,18	248,48 ± 11,56	213,95 ± 1,82	216,72 ± 12,04	221,75 ± 4,58
Total terpenic compounds	1246,24 ± 35,88	1227,70 ± 2,31	925,40 ± 33,68	874,84 ± 39,22	846,10 ± 42,86	840,53 ± 16,83

	Linseed oil	MCT	Almond oil	Ethyl laurate	Rapeseed oil
Extraction yields (µg/100 mg dry matter)					
α-pinene	36,15 ± 1,95	38,82 ± 1,53	36,89 ± 3,89	42,10 ± 2,07	36,88 ± 5,31
Camphene	0,74 ± 0,04	0,81 ± 0,07	0,73 ± 0,09	0,84 ± 0,04	0,72 ± 0,08
β-pinene	6,44 ± 0,25	6,99 ± 0,37	6,51 ± 0,64	7,24 ± 0,27	6,44 ± 0,98
β-myrcene	416,14 ± 22,89	449,99 ± 22,87	426,24 ± 42,39	442,99 ± 24,16	414,58 ± 66,87
α-phellandrene	2,67 ± 0,17	2,01 ± 0,15	2,60 ± 0,028	2,40 ± 0,14	2,57 ± 0,47
δ-3-carene	4,02 ± 0,24	4,46 ± 0,20	4,21 ± 0,43	4,35 ± 0,23	4,04 ± 0,55
α-terpinene	2,05 ± 0,24	1,05 ± 0,08	1,80 ± 0,22	1,10 ± 0,11	1,69 ± 0,12
p-cymene	3,40 ± 0,19	4,01 ± 0,18	3,59 ± 0,39	3,67 ± 0,12	3,27 ± 0,56
Limonene	5,49 ± 0,28	6,10 ± 0,28	5,64 ± 0,62	5,76 ± 0,28	5,44 ± 0,8
β-phellandrene	6,62 ± 0,40	7,41 ± 0,41	6,91 ± 0,72	6,82 ± 0,33	6,43 ± 1,02
Eucalyptol	0,85 ± 0,02	0,88 ± 0,07	0,57 ± 0,20	0,84 ± 0,25	0,61 ± 0,08
α-ocimene	0,25 ± 0,03	0,31 ± 0,01	0,23 ± 0,10	0,37 ± 0,09	0,39 ± 0,01
β-ocimene	6,21 ± 0,30	6,98 ± 0,67	6,47 ± 0,89	6,24 ± 0,35	6,33 ± 0,92
γ-terpinene	3,26 ± 0,17	3,57 ± 0,07	3,29 ± 0,11	3,23 ± 0,05	3,17 ± 0,54
Terpinolene	67,68 ± 2,29	47,60 ± 1,23	63,29 ± 5,53	54,02 ± 3,18	59,54 ± 9,68
Total monoterpenic compounds	561,98 ± 29,13	580,97 ± 26,95	568,98 ± 55,77	581,98 ± 31,19	551,97 ± 88,03
β-caryophyllene	63,13 ± 2,25	67,78 ± 1,51	63,09 ± 4,74	61,70 ± 1,85	59,66 ± 3,7
trans-α-bergamotene	13,78 ± 0,43	14,51 ± 0,37	13,77 ± 1,06	13,39 ± 0,34	12,94 ± 0,49
α-guaiene	27,26 ± 0,90	27,05 ± 0,48	27,20 ± 1,99	25,76 ± 0,65	25,91 ± 1,10
trans-β-farnesene	28,03 ± 0,65	28,41 ± 1,78	26,68 ± 1,98	25,52 ± 0,78	26,14 ± 1,80
α-humulene	23,08 ± 0,73	24,90 ± 0,65	22,95 ± 1,62	22,77 ± 0,50	21,50 ± 1,46
δ-guaiene	37,38 ± 1,29	14,55 ± 1,30	31,55 ± 1,22	21,27 ± 2,27	28,32 ± 2,73
[58,893–88–2]	17,25 ± 0,52	15,14 ± 0,21	16,73 ± 1,14	14,96 ± 0,33	15,75 ± 1,12
Selina-3,7(11)-diène	20,64 ± 0,45	14,13 ± 0,90	18,26 ± 1,09	15,34 ± 0,50	16,97 ± 1,45
Caryophyllene oxide	2,84 ± 0,04	4,27 ± 0,45	2,73 ± 0,03	5,33 ± 0,50	2,61 ± 0,55
Total sesquiterpenic compounds	233,39 ± 4,69	210,73 ± 2,75	222,96 ± 14,33	206,05 ± 6,99	209,81 ± 14,28
Total terpenic compounds	795,37 ± 33,81	791,70 ± 29,40	791,93 ± 69,83	788,03 ± 38,18	761,78 ± 102,17

based on their composition, the length of their carbon chains and the presence or absence of unsaturations. The choice fell on a variety of oils ranging from MCT, rich in fully saturated medium-chain triglycerides (C8 and C10), to linseed oil containing long unsaturated chains (C18:1, C18:3 and C20:0).

All extractions with saponification steps were conducted in triplicate (n = 3), and the data are expressed as the mean (m) and standard deviation (sd). We analyzed the results by analysis of variance followed by Tukey's multiple comparison test.

Vapor-phase headspace sampling coupled with gas chromatography is the most widely used method for analyzing volatile compounds in oily

matrices. However, this approach could be limited by the high extraction temperatures required, low sensitivity, complex instrumentation, and low extraction kinetics or different fiber-terpenes selectivities, depending on the method used [3]. We have developed an alternative method for the identification and quantification of terpene and terpenoid compounds in cannabis extracts in oil, based on classical instrumental GC-FID. We obtained extracts of cannabis inflorescences in vegetable oils, which were then subjected to saponification to remove triglycerides and to concentrate terpenes and terpenoids facilitating their analysis. We reproduced the conditions and method applied by Alignan et al. [5] to sterol analysis, modifying only the saponification

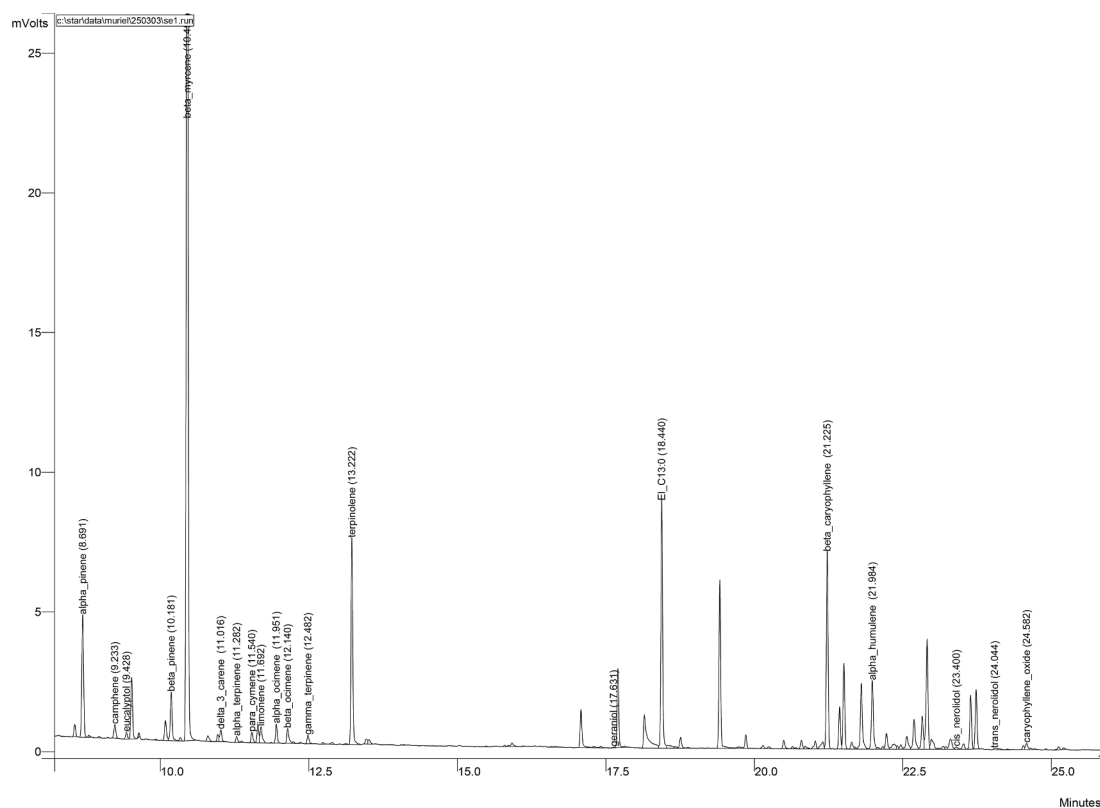


Fig. 2. GC-FID chromatogram of sesame oil extract from the Bediol® variety.

temperature. Saponification is generally carried out with heating to accelerate the chemical reaction. By contrast, here, with the aim of preserving the volatile compounds present, we subjected our extracts to cold saponification (20 °C). Due to their chemical structure, terpene compounds are not saponifiable and remain in the cyclohexane unsaponifiable fraction obtained after the liquid/liquid extraction. The purpose of the saponification we propose is to rid the analysed sample of molecules with high boiling points, such as triglycerides. These are the main constituents of oils and can otherwise pollute the chromatogram, leading to integration inaccuracies. In our study, no such lipid contamination was observed in the GC-FID profiles, confirming the efficacy of the process. We applied this method to the analysis of a medical-grade variety of cannabis subjected to extraction in seven different vegetable oils, medium-chain triglycerides and ethyl laurate. Ethanol and hexane extracts were analyzed in the same conditions as controls. The results are shown in Fig. 1.

Sesame oil was the most effective biological lipid-based matrices (925 µg of total terpene compounds (terpenes and terpenoids) /100 mg dry matter) and rapeseed oil the least effective (762 µg/100 mg dry matter). The hexane and ethanol controls had extraction efficiencies similar to or greater than those of all the other matrices (mean of 1237 µg/100 mg dry matter) for total terpene compounds. This result was expected, as hexane and ethanol are established solvents for terpene extraction in the literature [6,7]. The performance variations observed between the different lipid matrices are also in line with the work of Ramella et al. [8], who attributed such differences to the intrinsic physicochemical properties of the oils rather than to procedural biases. We performed separate analyses of monoterpenes and sesquiterpenes. The total monoterpene content of the Bediol® medicinal cannabis variety ranged from 1000 µg/100 mg dry matter (hexane extract) to 552 µg/100 mg dry matter (rapeseed oil extract). Total sesquiterpene content ranged from 261 µg/100 mg dry matter (ethanol extract) to 206 µg/100 mg dry matter (ethyl laurate extract) (Table 1). The differences in extraction efficiency between the controls and the lipid matrices were

therefore smaller for sesquiterpenes compounds than for monoterpenes ones. This difference may be due to the molecular structures and chain lengths of sesquiterpenes, which render these molecules more apolar than monoterpenes, thereby promoting their extraction in lipid matrices. Fig. 2 displays a GC-FID chromatogram from the Bediol® variety extracted by sesame oil.

4. Conclusion

In summary, the aim of this work was to develop a new method for the determination of terpenes and terpenoids in lipid-based plant extracts by GC-FID. The method described here has the advantages of being easy to implement, cheap, reproducible, and suitable for the quantification of terpenes and terpenoids in oily matrices, an analysis generally performed by HS until now. We confirm here that the composition of lipid matrices can influence the extraction yields of terpene compounds from medical cannabis.

Funding

This research was supported by DelleD/La Fleur, the French National Association for Research in Technology (ANRT) through the CIFRE 2021/0916 and Toulouse INP.

CRediT authorship contribution statement

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

Acknowledgements

We would like to thank the ANSM (French National Agency for the Safety of Medicines and health products) and the Toulouse Blagnac customs office (France) for the authorizations granted for the acquisition and use of narcotics for scientific purposes.

Data availability

Data will be made available on request.

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