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Chapter 4

Quality Traits of Medical *Cannabis sativa* L. Inflorescences and Derived Products Based on Comprehensive Mass-Spectrometry Analytical Investigation

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Additional information is available at the end of the chapter

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Abstract

Cannabis sativa L. has been cultivated throughout the world for industrial and medical purposes and is the most controversial plant ever exploited, with considerable discrepancies in the praise and disapproval it receives. Medical Cannabis prescriptions are on the increase in several countries where its therapeutic use is authorised due to its positive role in treating several pathologies even if it represents a multifaceted reality in terms of application. There are at least 550 identified compounds in *C. sativa* L., including more than 100 phytocannabinoids and 120 terpenes. The chemical complexity of its bioactive constituents highlights the need for standardised and well-defined analytical approaches able to characterise plant chemotype and herbal drug quality as well as to monitor the quality of pharmaceutical cannabis extracts and preparations. This research highlights the potential of using different analytical procedures involving the combination of headspace-solid-phase microextraction (HS-SPME) coupled to GC–MS and accelerated solvent extraction (ASE) coupled to high resolution mass-spectrometry (HPLC-Q Orbitrap®) for the in-depth profiling of quality traits in authorised medical varieties of *Cannabis sativa* L. flos (Bediol®) and corresponding macerated oil preparations. This approach could add new knowledge to the field of "*omic*" analytical applications which are fundamental nowadays for Cannabis used for therapeutic remedies.

Keywords: Bediol®, terpenes, cannabinoids, GC-MS, HS-SPME, HPLC-Q-Exactive-Orbitrap-MS, *Cannabis sativa* L

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1. Introduction

Cannabis (*Cannabis sativa L.*) is the most controversial plant ever exploited, with considerable discrepancy in the praise and disapproval it receives. It is intriguing that cannabis produces the natural substances that appear to target key protein receptors of important physiological systems quite selectively [1]. Plants containing such secondary metabolites usually belong to unique chemotaxa that induce potent pharmacological effects and have typically been used for recreational and medicinal purposes. *Cannabis sativa* L. has a long history as a medicinal plant and was fundamental in the discovery of the endocannabinoid system.

Over the past decades, considerable research has been carried out to enable a clear distinction to be made between cannabis as a hazardous drug and as a beneficial medicine [2, 3].The authorised medicinal use of cannabis is still associated with doubts on its safe use due to a few ambiguous issues including quantity, dynamics and way of administration [4].

Medications based on cannabis have been used for therapeutic purposes in many cultures for centuries. In Europe, they were used at the end of the nineteenth century to treat pain, spasms, asthma, sleep disorders, depression, and loss of appetite. In the first half of the twentieth century, cannabinoid medications fell into almost complete disuse, partly because scientists were unable to establish the chemical structure of the main cannabis plant ingredients. The emergence of interest in botanical medicinal cannabis is thought by many to be a collateral effect of the opioid abuse epidemic; public perception surrounding the use of medicinal cannabis suggests that this plant-based therapy is viewed as not very different from a botanical drug product or supplement used for health or relief of symptoms if disease persists. Like some herbal preparations or supplements, however, medicinal cannabis may similarly pose health risks associated with its use, including psychoactive, intoxicating, and impairing effects, which have not been completely elucidated through clinical trials.

The method of its application for therapeutic purposes certainly depends on its phytocannabinoid profile: over 70 cannabinoids are defined in *Cannabis sativa L.* They are classified chemically into 10 most important categories where the THC, cannabidiol (CBD), cannabigerol (CBG), cannabichromene (CBC), and cannabinol (CBN)-types are recognised as the most relevant [5].

The main constituent of cannabis is THC, which is responsible for the psychoactive features of cannabis due to its high affinity to cannabinoid receptors. Most of the effects of cannabis preparations are based on the agonistic action of THC on the various cannabinoid receptors. Two primary endocannabinoid receptors have been identified: CB1 and CB2 [6]. CB1 receptors are found predominantly in the brain and nervous system, as well as in peripheral organs and tissues, and are the main molecular target of the endocannabinoid binding molecule, anandamide, as well as its mimetic phytocannabinoid, THC.

Another important component is cannabidiol (CBD) which was proven to possess several pharmacological properties (analgesic, antioxidant and antiepileptic), but not psychotropic activity as THC [7]. The presence and amount of CBD is essential in the therapeutic usage of

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cannabis, because it reduces THC collateral effects. Furthermore, minor constituents such as CBC and CBG exhibit anti-inflammatory, antibacterial and antifungal activity, while CBN has strong sedative properties [5, 7]. As regards cannabidiol (CBD)-based preparations that are becoming extremely popular as CBD has been shown to have beneficial effects on human health, a recent work highlighted a wide variability in the cannabinoid profile that justifies the need for strict and standardised regulations [8].

Although CBD and THC are the key molecules, the plant itself is capable of producing only their acid counterparts: cannabidiolic acid (CBDA) and tetrahydrocannabinolic acid (THCA) [9]. Decarboxylation of these forms leads to the formation of bioactive chemical species, CBD and THC, respectively. CBDA and THCA are the major components of cannabis inflorescence while among other cannabinoid acids, cannabigerolic acid (CBGA) is shown to be essential due to the fact that it is a precursor of all the other cannabinoid acids. It is worth mentioning the other minor acidic cannabinoids such as cannabichromenic acid (CBCA) which also gives corresponding neutral analogues upon decarboxylation.

At present, the international medical and scientific community has widely recognised *Cannabis sativa* L. as a promising source of therapeutic agents for the treatment of certain diseases such as multiple sclerosis, HIV, epilepsy, glaucoma, chemotherapy, chronic pain, nausea/vomiting [10, 11].

Unfortunately, despite the emergence of a huge amount of preclinical literature that describes the actions and effects of some cannabinoids, there have, as yet, been relatively few publications describing the effects produced by cannabinoids in clinical studies performed with human subjects. Importantly, a cannabis-based medication, Sativex®, approved by the European medical association (EMA), was recently licenced in 18 European countries for the treatment of tremor and spasticity symptoms associated with multiple sclerosis [12]. Besides, other cannabinoid drugs, Cesamet® (Nabilone) and Marinol® (synthetic tetrahydrocannabinol (THC)) were successfully applied for the treatment of vomiting and nausea caused by cancer therapy. Some other cannabis-derived substances seem to be on hold. For example, Epidolex®, an experimental drug derived from cannabis-based medicine for the treatment of child epilepsy is on the brink of becoming the first of its kind to obtain FDA government approval [13].

Capsules, cannabis extracts such as mouth spray or oils, dry cannabis for inhalation or as tea are the main medical products approved by the EU, according to the European Monitoring Center for Drugs and Drug Addiction (EMCDDA) 2017 [14].

Within the EU there is no agreement on the legalisation of medical cannabis, but it appears to be moving toward greater use faster than in the past [15, 16]. For the time being, only Austria, the Czech Republic, Finland, Germany, Italy, Portugal, Poland, Spain and Croatia have allowed the use of cannabis in medicine in the EU, while other countries are planning to legalise it. As a confirmation of the blurred legal status of *Cannabis sativa* L. within the EU community, it took a 4-year trial before the Danish Parliament approved the use of medical cannabis for patients suffering from various diseases starting from January 1, 2018. Moreover, in 2017, an increasing number of EU members, such as Greece and Ireland, announced or proposed changes in legislation and the use of medical cannabis. Since November 2017,

cannabis-based medicines in Poland can be sold if they are made in pharmacies with the use of an imported substance.

The current status of cannabis highlights that, since it causes "psychoactive activity," its use in medicine should follow the legal provisions of member states, including "control of the use of narcotics and psychotropic substances" [17]. European countries have an obligation to control cannabis according to the three UN Conventions on Drug Control that require them to restrict drug supplies and use it exclusively for medical and scientific purposes.

At an EU level there are no harmonised laws on the recreational and medical use of cannabis and the member states themselves decide whether to legalise them.

As an example, medical cannabis in Italy represents a multifaceted reality [16, 18]. At present varieties Bedrocan, Bediol, Bedica and Bedrolite produced by company Bedrocan from Netherlands [19] and the new strain FM2 produced by the Military Pharmaceutical Chemical Works of Florence, Italy (authorised in November 2015 with a Ministerial Decree) can be prescribed to treat a wide range of pathological conditions [16]. In relation to this, Italian galenic pharmacies are authorised to prepare precise cannabis doses for vaping, herbal teas, resins, micronised capsules and oils [20]. The latter, prepared by using European Pharmacopoeia olive oil (FU) as extraction solvent has received great attention due to the easiness with which dosage can be modulated or titrated during the treatment period. Also, oil formulations are high-steamed because of the extended bioavailability of the active compounds contained.

As regards *Cannabis sativa* composition, beyond and besides cannabinoids, a substantial amount of the approximately 500 compounds (terpenes, flavonoids, stilbenoids, fatty acids, alkaloids, carbohydrates, and phenols) are described [21]. Terpenes represent the volatile component of the plant and have been proven to have a synergic action with cannabinoids [19]. Cannabis plants produce and accumulate a terpene-rich resin in glandular trichomes, which are abundant on the surface of the female inflorescence [22]. Bouquets of different monoterpenes and sesquiterpenes are important components of cannabis resin as they define some of the unique organoleptic properties and may also influence medicinal qualities of different cannabis strains and varieties [23]. Differences between the pharmaceutical properties of different cannabis strains have been attributed to interactions (or an 'entourage effect') between cannabinoids and terpenes [24]. Terpenes themselves exhibit a wide array of pharmacological properties, including interaction with the mammalian endocannabinoid system: sesquiterpene β-caryophyllene interacts with mammalian cannabinoid receptors [25, 26]. Some terpenes like β-myrcene, limonene and linalool display anxiolytic, antibacterial, anti-inflammatory, and sedative effects, too [27].

The chemical complexity of cannabis makes its pharmaceutical standardisation challenging and must include well-defined methodologies that would characterise the plant chemotype and the herbal drug as well as extraction procedures. As a matter of fact, it was found that the concentrations of target cannabinoids obtained for the same plant chemotype originating from different suppliers varied by more than 25% [28]. This lack of standardisation could be overcome with two distinct approaches.

The first is a botanical issue and points toward strict control of varieties and strains during cultivation in order to assure the highest homogeneity in the final plants, especially if the

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cannabis inflorescence is the final product. The other tactic is focused on extraction and purification procedures, which are fundamental if cannabis-derived formulations such as oils or tinctures are targeted. As recently reviewed by Citti et al. [29] and Calvi et al. [30], the choice of the analytical approach(es) employed represents a pivotal task, with particular emphasis on the need for a comprehensive chemical characterisation of the composition of cannabis and derived products. Nowadays, analytical methods based on gas chromatography-mass spectrometry (GC-MS) and/or high pressure liquid chromatography (HPLC) coupled to the recently introduced high resolution mass spectrometer HRMS-Orbitrap, represent the gold standard techniques for the investigation of the highly complex cannabis composition due to their excellent resolution, precision and sensitivity. Consequently, it is now crucial to complete the chemical and pharmacological characterisation of all phytocannabinoids known to be present in cannabis.

Based on the above-mentioned considerations, in the first part of the here presented research project different analytical procedures involving the combination of headspace-solid-phase microextraction (HS-SPME) coupled to GC-MS and accelerated solvent extraction (ASE) coupled to high resolution mass-spectrometry (HPLC-Q Orbitrap®) were applied for the in-depth profiling and fingerprinting of cannabinoids and terpenes in authorised medical grade varieties of *Cannabis sativa* L. *flos* (Bediol®) and in corresponding macerated oil preparation. Particular emphasis was given to the study of *untargeted* cannabinoids so as to investigate and obtain an exhaustive and realistic profile of medical Bediol® inflorescences and derived macerated oil preparations, since they have so far received less attention compared to target compounds (THC, THC-A, CBD, CBD-A). This approach could add new knowledge to the field of "*omic*" analytical applications as well.

2. Materials and methods

2.1. Chemical and reagents

All HPLC or analytical grade chemicals were from Sigma (Sigma-Aldrich, St. Louis, MO, USA). Formic acid 98–100% was from Fluka (Sigma-Aldrich, St. Louis, MO, USA). Ultrapure water was obtained through a Milli-Q system (Millipore, Merck KGaA, Darmstadt, Germany). For headspace (HS) analysis, the SPME coating fibre (DVB/CAR/PDMS, 50/30 μm) was from Supelco (Bellefonte, PA, USA). Acetonitrile, 2-propanol, formic acid LC-MS grade were purchased from Carlo Erba (Milan, Italy). CBD, THC, CBN, CBG, CBNA, THCA, CBGA were purchased from Sigma Aldrich (Round Rock, Texas). High intensity planetary mill Retsch (model MM 400, Retsch, GmbH, Retsch-Allee, Haan) was used to obtain representative aliquots of cannabis flos samples powder.

2.2. Cannabis plant material and superfine grinding (SFG) sample preparation

Bediol® medical Cannabis chemotype that contains 6.5% THC and 8% CBD as standardised and certified by the company Bedrocan was used for all analyses. It was selected as representative

because it represents the most common medical variety actually prescribed alone or in combination for several pathologies. Superfine cannabis inflorescence powder was prepared using mechanical grinding-activation in an energy intensive vibrational mill. Different samples (1.0 g each) were ground in a high intensity planetary mill. The mill was vibrating at a frequency of 25 Hz for 1 min, using two 50 mL jars with 20 mm stainless steel balls. Prior to use, jars were pre-cooled with liquid nitrogen. The speed differences between balls and jar result in the interaction of frictional and impact forces, releasing high dynamic energies. The interplay of all these forces results in the very effective energy input of planetary ball mills. Mechano-chemical technology has been developed and successfully adopted in different fields (synthesis of superfine powder, surface modification, drug and pharmaceutical applications) and could represent a novel research tool.

2.3. Accelerated Solvent Extraction (ASE) for cannabinoid analysis

All extractions to define the cannabinoid profile of Bediol® medical chemotype were executed using an ASE 350 (Thermo-Fisher Scientific, Waltham, MA, USA). 34-mL stain steel cells were used for the extraction. 100 mg of Cannabis flos powder obtained by using SFG was weighed and then homogenised with an equal weight of diatomaceous earth and transferred into the cell. Then, 100 μL of extraction solution containing the IS (diazepam 1 mg mL^{-1}) was added. Different extraction solvents were tested and were: methanol, methanol:CH_3Cl (9:1), hexane, acetonitrile and ethanol. Diatomaceous earths were added in order to fill the remaining empty part of the cell. Room temperature of 25°C, pressure (1500 psi), number of static cycles (2 cycles, 5 min each), purging time (60 s with nitrogen) and rinse volume (90%) were used for the study. Organic extracts were finally collected in 66 mL vials and treated with sodium sulphate to remove any possible humidity. Afterwards, the extract was collected and dried under vacuum in a centrifugal evaporator. The residue was dissolved in 1 mL of acetonitrile and after proper dilution, 2 μL were submitted to analysis by HPLC-Q-Exactive-Orbitrap-MS. Validation was performed according to the European Union SANTE/2015 guidelines usually adopted to test ASE performance especially for trace residue analysis [31].

The method was completely optimised investigating the typologies of extraction solvents, number of extraction cycles and extraction temperature to define the optimum analytical conditions as well. To realise the matrix-matched calibration curves (MMCs) blank samples (100 mg officinal plant previously analysed for the absences of cannabinoids) were used and spiked with appropriate standard solution of THC, THC-A, CBD, CBD-A and CBN covering the concentration range from 0.1 to 10 μg g^{-1}. Recoveries were calculated by comparing the concentrations of the extracted compounds with those from the MMC calibration curves at two different fortification levels (1.0 and 10 μg g^{-1}).

2.4. HS-SPME and GC-MS analysis for terpenes investigation

One gram of oil or 100 mg of inflorescence previously grinded were weighed and put into 20 mL glass vials along with 100 μL of the IS (4-nonylphenol, 2000 μg/mL in 2-propanol). Each vial was fitted with a cap equipped with a silicon/PTFE septum (Supelco, Bellefonte, PA, USA). A temperature of 37°C was selected as both the extraction and equilibration temperature

according to previous published research, in order to prevent possible matrix alterations ensuring the most efficient adsorption of volatile compounds onto the SPME fibre [15, 16]. To keep the temperature constant during analysis, the vials were maintained in a cooling block (CTC Analytics, Zwingen, Switzerland). At the end of the sample equilibration time (30 min), a conditioned (60 min at 280°C) SPME fibre was exposed to the headspace of the sample for 120 min using a CombiPAL system injector autosampler (CTC Analytics, Zwingen, Switzerland). All analytical parameters had already been validated in our previous research [32].

Analyses were performed with a Trace GC Ultra coupled to a Trace DSQII quadrupole mass spectrometer (MS) (Thermo-Fisher Scientific, Waltham, MA, USA) equipped with an Rtx-Wax column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) (Restek, Bellefonte, PA, USA). The oven temperature program was: from 35°C, held for 8 min, to 60°C at 4°C/min, then from 60 to 160°C at 6°C/min and finally from 160 to 200 at 20°C/min. Helium was the carrier gas, at a flow rate of 1 mL/min. Carry over and peaks originating from the fibres were regularly assessed by running blank samples. After each analysis fibres were immediately thermally desorbed in the GC injector for 5 min at 250°C to prevent contamination. The MS was operated in electron impact (EI) ionisation mode at 70 eV. An alkane mixture (C8-C22, Sigma R 8769, Saint Louis, MO, USA) was run under the same chromatographic conditions as the samples to calculate the Kovats retention indices (RI) of the detected compounds. The mass spectra were obtained by using a mass selective detector, a multiplier voltage of 1456 V, and by collecting the data at a rate of 1 scan/s over the *m*/*z* range of 35–350. Compounds were identified by comparing the retention times of the chromatographic peaks with those of authentic compounds analysed under the same conditions when available, by comparing the Kovats retention indices with the literature data and through the National Institute of Standards and Technology (NIST) MS spectral database. The quantitative evaluation was performed using the internal standard procedure and the results were finally expressed as μg/g or mg/g IS equivalents of each volatile compound. All analyses were done in triplicate.

2.5. Cannabis macerated oil preparations

Three different methods for oil preparation were performed and evaluated. The preparation conditions were selected on the basis of previously published methods [31]. Briefly, common issues for all three methods were the amount of Bediol® inflorescence used (1 g) and the European Pharmacopoeia (FU) olive oil volume (10 mL) that served as extraction matrix. The crucial differences concerning the preheating temperature of the inflorescence to perform the decarboxylation step and extraction process are highlighted in **Table 1.** After extraction and cooling down (methods 1 and 2) the oils were filtrated and subsequently prepared for LC-Q-Exactive-Orbitrap-MS analysis.

2.6. Cannabinoids LC-Q-Exactive-Orbitrap-MS analysis

The cannabinoid profile in plants and the corresponding oil were assessed applying the method recently published with particular emphasis on method development [31]. In order to perform HPLC-Q-Exactive-Orbitrap®-MS analysis, samples extracted with ASE were prepared as indicated in Section 2.4, while oil samples were prepared by dissolving 100 mg of

Preparation's step	Preparation method		
	Romano and Hazekamp [32]	**Pacifici et al. [33]**	**Calvi et al. [30]**
	(1)	(2)	(3)
Decarboxylation step (conversion acid form in neutral form of cannabinoids)	No	Yes/145°C, 30 min static oven	Yes/145°C, 30 min static oven
Amount inflorescence/FU oil volume	1 g:10 mL	1 g:10 mL	1 g:10 mL
Extraction process	Heating in water bath (98°C 120 min)	Heating in water bath (98°C 60 min)	Ultrasound (35 KHz 30 min)
Filtration	Yes/filter paper	Yes/filter paper	Yes/filter paper
Preparation time (min)	150	120	90

Table 1. Preparation procedures details for Bediol® macerated oils.

each oil in 10 mL of isopropanol. After adding 1 μg/mL of IS, 10 μL of each sample were diluted in 890 μL of initial mobile phase from which 2 μL was injected.

Chromatography was accomplished on an HPLC system (Thermo Fisher Scientific, San Jose, CA, USA) that was made up of a Surveyor MS quaternary pump with a degasser, a Surveyor AS autosampler with a column oven and a Rheodyne valve with a 20 μL loop. Analytical separation was carried out using a reverse-phase HPLC column 150 × 2 mm i.d., 4 μm, Synergi Hydro RP, with a 4 × 3 mm i.d. C18 guard column (Phenomenex, Torrance, CA, USA). The mobile phase contained a binary combination of 0.1% aqueous formic acid and acetonitrile. The gradient was initiated with 60% eluent 0.1% aqueous formic acid with a linear decrease up to 95% in 10 min. This condition was maintained for 4 min. The mobile phase was returned to initial conditions at 14 min, followed by a 6-min re-equilibration period. The flow rate was 0.3 mL/min. The column and sample temperatures were 30 and 5°C, respectively. The mass spectrometer Thermo Q-Exactive Plus (Thermo Scientific, San Jose, CA, USA) was equipped with a heated electrospray ionisation (HESI) source. Capillary temperature and vaporiser temperature were set at 330 and 280°C, respectively, while the electrospray voltage was adjusted at 3.50 kV (operating in both positive and negative mode). Sheath and auxiliary gas were 35 and 15 arbitrary units, with S lens RF level of 60. The mass spectrometer was controlled by Xcalibur 3.0 software (Thermo Fisher Scientific, San Jose, CA, USA). The exact mass of the compounds was calculated using Qualbrowser in Xcalibur 3.0 software. The FS-dd-MS^2 (full scan data-dependent acquisition) in both positive and negative mode was used for both screening and quantification purposes. Resolving power of FS adjusted on 140,000 FWHM at m/z 200, with scan range of *m*/*z* 215-500. Automatic gain control (AGC) was set at $3e^6$, with an injection time of 200 ms. A targeted MS/MS (dd-MS^2) analysis operated in both positive and negative mode at 35,000 FWHM (*m*/*z* 200). The AGC target was set to $2e^5$, with the maximum injection time of 100 ms. Fragmentation of precursors was optimised as two-stepped normalised collision energy (NCE) (25 and 40 eV). Detection was based on calculated exact mass of the protonated/deprotonated molecular ions, at least one corresponding fragment and on retention time of target compounds [12]. Extracted ion chromatograms (EICs) were

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obtained with an accuracy of 2 ppm *m/z* from total ion chromatogram (TIC) engaging the *m/z* corresponding to the molecular ions [M+H]+ 315,23145 for CBD and THC, 311,20020 for CBN. 317,24716 for CBG and 311,2024 for CBN. In ESI_ the molecular ions [M_H]_ considered were 357,2164 for CBDA and THCA, while CBGA was detected by 359,22269.

3. Results and discussion

3.1. Quality analysis of Cannabis inflorescences

3.1.1. ASE Cannabis sample preparations from Bediol® medical chemotype

The choice of the appropriate analytical approach for cannabinoid profiling in cannabis inflorescences is extremely important, considering the need for a comprehensive chemical characterisation of cannabis and derived products [34]. For these reasons, analytical techniques based on high resolution mass spectrometer (HRMS-Orbitrap), due to their excellent resolution, precision and sensitivity [35], nowadays represent the gold standard techniques for the investigation of the highly complex cannabis composition. Proper purification and extraction methodology must also be implemented and is considered crucial in order to achieve an in-depth screening of the cannabinoids in *Cannabis sativa L.* inflorescence [32, 33].

The traditional solvent extraction methods often used for the extraction of different bioactive compounds from plants carry certain drawbacks [30]. Often, they are time consuming, laborious, have low selectivity or low extraction yields and usually large amounts of toxic solvents are required. Emphasis has currently shifted toward the use of sub- and supercritical fluids and generally-recognised-as-safe (GRAS) solvents as also detailed elsewhere [34]. Recent advances using accelerated solvent extraction (ASE) systems, as described in several publications [35, 36] include procedures for selective removal of interferences during sample extraction, thus combining extraction and purification into a single step. ASE is considered one of the most promising extraction process because, unlike standard extraction methods, it utilises high temperature and pressure to improve the extraction of the analyte from the solid sample. These conditions enhance the diffusion of the extraction solvent throughout the sample matrix which result in the more complete dissolution and recovery of the investigated compounds. The sample to be extracted is placed in a sealed metal cell that is then allocated automatically in a heated oven chamber and filled with the extraction solvent. The extraction cell is then pressurised, allowing for an increase in the boiling point of the extraction solvent, and for the solubilisation of the analytes at a temperature higher than would be possible at atmospheric pressure. Hereafter, the sample is extracted and collected by the automated filling and voiding of the cell through repeated static cycles. Compared to other solid sample extraction techniques, ASE requires less time, consumes less solvent during extraction and, with the added benefit of automation, has proven effective for several food solid samples.

Evaluation of the performance of ASE for the extraction of natural compounds like curcuminoids, saponins, flavonolignans, terpenes, taxanes, xanthone, flavonoids and artemisinin has already been conducted, as well as the application of ASE for the characterisation of phenolic compounds

from fine Alpine plant roots [37]. The advantage of applying pressure is due to the fact that it is able to force the extracting solvent into the matrix and therefore may improve extraction efficiency dramatically. To the best of our knowledge, the present study reports an ASE-based method applied to the extraction of cannabinoids from cannabis row material (inflorescences) for the first time.

Bediol® chemotype was chosen for the optimisation of the ASE working parameters as it encompasses a combination of balanced amounts of THC and CBD, two cannabinoids responsible for most of the clinical effects that medical cannabis can express. In addition, it has been repeatedly suggested that the effect of isolated THC or of any other single cannabinoid is not equivalent to that of whole cannabis preparations, since some of the bioactivity observed could be related also to the presence of acidic cannabinoids. In this context, the use of an analytical method allowing the qualitative and quantitative exhaustive extraction of neutral cannabinoids and its native, acidic forms (THCA and CBDA) from cannabis plant is fundamental to characterise different cannabis varieties, a particularly relevant point when considering medical varieties. That is why the extraction efficacy of ASE was evaluated also for THCA and CBDA.

However, the optimization of effective extraction from cannabis plant is a strategic and very important issue in cannabinoid determination, as it determines the accuracy of the whole analytical method. Therefore, several extraction solvents for ASE extraction of cannabinoids from Bediol® chemotype were evaluated herein.

The best combination in terms of relative area (area analyte/IS) was obtained using methanol as extraction solvent at room temperature and 2 extraction cycles of 5 min each, with a resulting total extraction time of 15 min **(Figure 1**). These results are in line with a recent study that investigated the use of different extraction methods (dynamic maceration, ultrasound, microwave and supercritical fluid extraction) for the analysis of cannabinoids from fibre-type cannabis varieties [38]. Recoveries calculated by comparing the concentrations of the extracted compounds with those from the MMC calibration curves at two different fortification levels showed an average recovery of 93 and 5.7% as coefficient of variation. Based on obtained MMC calibration curves used for the purpose of validation of ASE procedures the percentage of THC, THCA, CBD and CBDA in Bediol® inflorescence by means of LC-Q-Exactive-Orbitrap-MS analysis was calculated as being: 0.88, 5.7, 0.96 and 7.4%, respectively.

3.1.2. HS-SPME and GC-MS for terpenes fingerprint from Bediol® medical chemotype

In comparison with cannabinoid derivatives, the volatile constituents of *Cannabis sativa* L. have received much less attention. At present, scarce emphasis has been given toward the exhaustive characterisation of the terpenes profile obtained from Cannabis chemotype standardised and certified for medical use [18, 27]. In relation to recent evidence concerning the synergic role of terpenes and cannabinoids (entourage effect) [21], the comprehensive evaluation of terpene compounds especially characterising medical strains is nowadays crucial to correctly managing Cannabis as a complete therapeutic tool. In addition, several medical applications of Cannabis flos involve the vaporisation of inflorescence by using medical vaping equipment to heat the herb thus releasing both cannabinoids and terpenes into the vapour phase. The need

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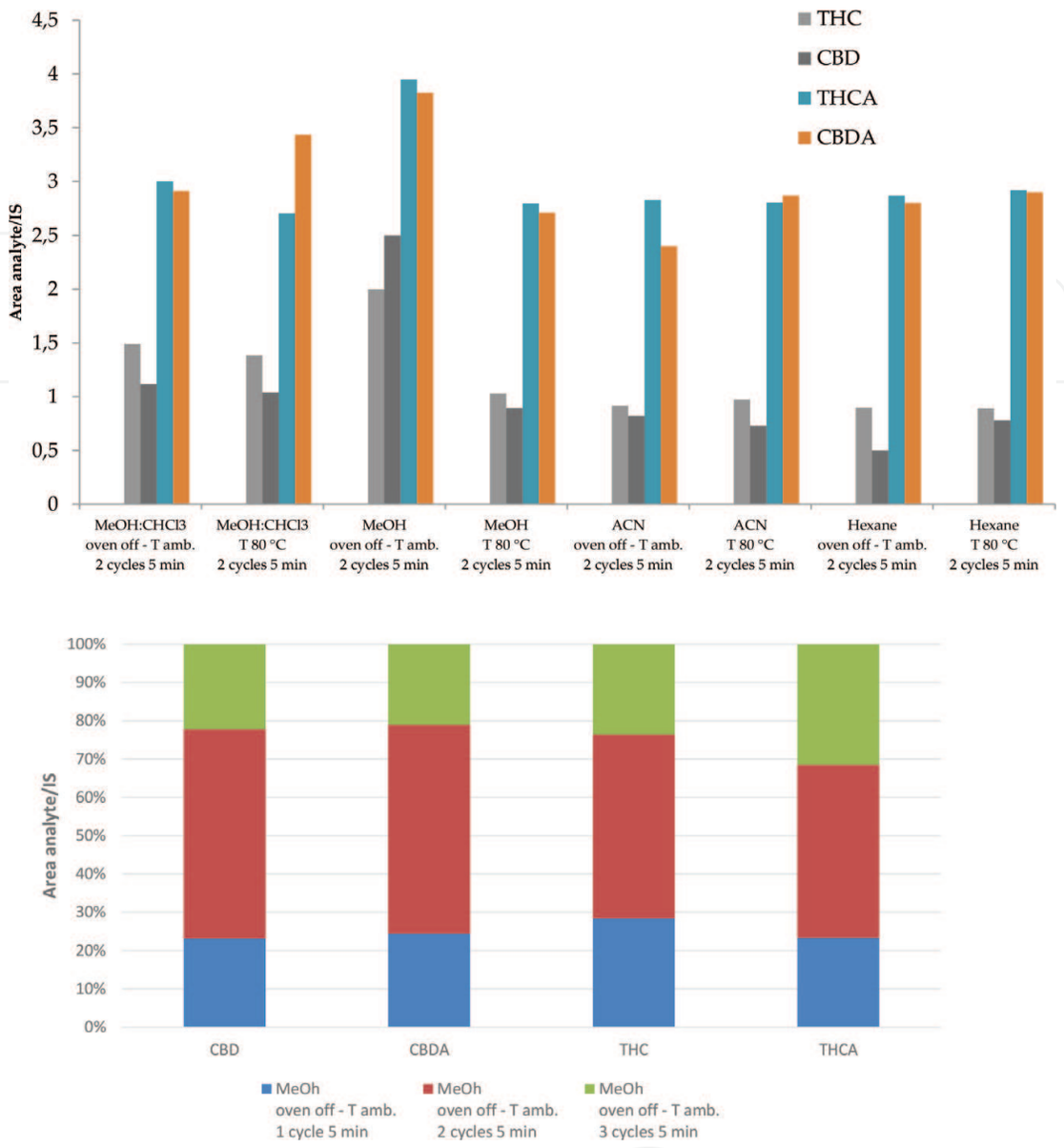

Figure 1. Impact of extraction solvents, temperature and number of extraction cycles on extractability of cannabinoids by using accelerated solvent extraction (ASE) from Bediol® chemotype.

to understand the real terpene profile emitted by medical varieties in order to select the most appropriate varieties for therapeutic use is particularly evident. In the present study, an HS-SPME method was adopted for the preconcentration of the volatile compounds with particular focus on terpenes fraction (mono-di-tri terpenes and sesquiterpenes). HS-SPME is considered a gold analytical technique for the analysis of volatile compounds in general (ref), but scarce data are available about the application of HS-SPME in the analysis of terpenes and in general of the volatile profile from medical cannabis varieties. Nevertheless, a study published recently demonstrates the convenience of HS-SPME in the characterisation of hashish terpene profile [35]. In particular, by the means of HS-SPME, authors were able to isolate and identify a potential volatile marker that might serve as a substance by which the resin and plant material

could be discriminated. Volatiles in some Bedrocan® varieties have been previously investigated for their terpene content by GC-FID [29], a technique that provides only a partial volatile profile and is severely limited, as it does not furnish the identification of unknown volatiles, as is feasible with GC-MS facilities accompanied by adequate, up-dated mass spectrum libraries [31, 40].

Furthermore, the terpenes were extracted using ethanol as an extraction solvent [29] and then quantified by using a calibration curve constructed by using generic internal standard. This approach is usually limitative as the polarity of the solvent could dramatically influence the terpene profile obtained and lead to the underestimation of the complex mixture of secondary metabolites emitted by plants as a result [40]. Methods involving headspace sampling appear to be the most opportune option to investigate cannabis volatile profile to obtain a representative profile of their volatile constituents avoiding interference potentially brought by predominant cannabinoids in the resulting chromatogram [41].

It is worth mentioning that the terpenes family includes a great variety of compounds (mono-di-tri and sesquiterpenes) with pronounced chemical differences which consequentially aggravate the dissimilarities in terms of potential clinical effects. It was possible to identify more than 40 monoterpenes in Bediol® medical chemotype by using the optimised HS-SPME and GC-MS. The most representative are presented in **Figure 2.** As a general consideration, β-myrcene was the predominant terpene in Bediol® chemotype as was reported previously [22, 29, 41]. Moreover, this is an extremely important finding as this monoterpene demonstrates a prominent narcotic-like effect that is seemingly responsible for the 'couch lock' phenomenon frequently associated with modern cannabis phenomenology [24]. Furthermore, five other monoterpenes, namely α-terpinolene, β-ocimene, β-phellandrene α-and β-pinene are the major monoterpenes in Bediol® chemotype, as was revealed for other *Cannabis sativa* L. varieties [42]. Interestingly, our analysis revealed the presence of limonene (930 μg/g), which

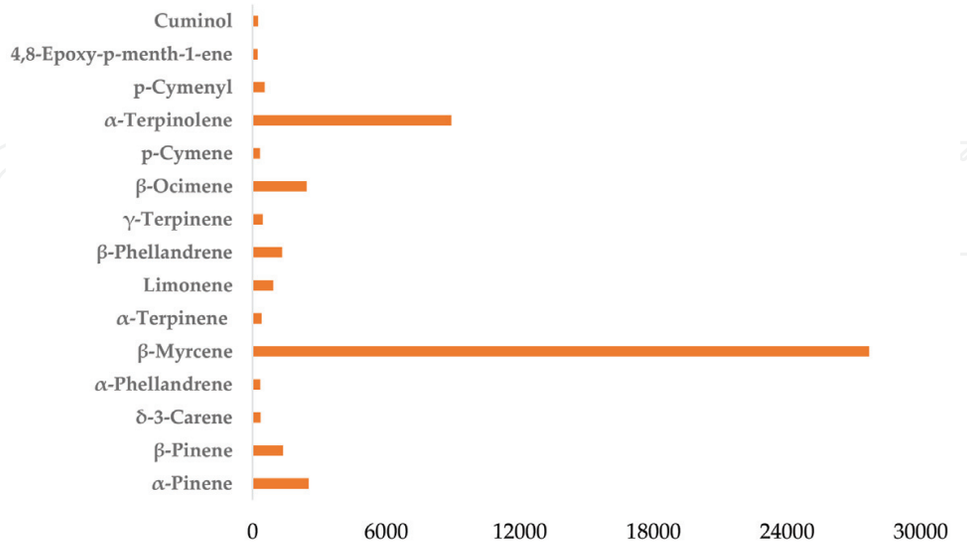

Figure 2. Representative terpenes fraction extracted from Bediol® chemotype by means of HS-SPME and identified using the GC-MS (μg/g).

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is in contrast to previously published data for Bediol® inflorescence [29]. This finding is remarkable because the Bediol® chemotype is obtained by hybridising the Bedrocan variety (high THC content) with CBD-predominant varieties. Although the mechanisms underlying the regulation of terpene synthesis in cannabis plants remain to be elucidated, it is possible that selective, individual breeding could influence terpene proportion profiles [22].

Besides the chemical composition of the terpene fraction of Bediol® inflorescence that is comprehensively documented herein, the sesquiterpene fraction was also investigated in detail (**Figure 3**). This flos was particularly rich in trans-caryophyllene which is typical for most of *Cannabis sativa* L. varieties [19, 41, 42], but the significant amount of selina-3,7(11)-dione might be more specific to the Bediol® chemotype. In addition, by the means of mass spectrometry it was possible to identify a compound with a sesquiterpene structure which does not correspond to any known substance from this class. Considering its abundance, a profound examination of this "new", unknown compound is mandatory, as it could be used as a specific Bediol® marker.

Also, this chemotype was principally rich in esters, volatile compounds responsible for, and associated with, "fruity" flavour notes (**Figure 4**). The most abundant ester found is butanoic acid-hexyl ester, which is recognised by its sweet, apple, and apple peel flavour [43]. Its domination in the ester profile of Bediol® candidates this compound as the principal natural flavouring substance for this *Cannabis sativa* L. chemotype.

3.2. Quality analysis of Bediol® oil formulations: cannabinoids and VOC profile

In line with the approval by the Italian Ministry of Health of a decree that regulates the cultivation, processing, and therapeutic uses of Cannabis [16], there has been increasing request for the medicinal oil extracts obtained from the dried flowers [43]. A standardised protocol for oily preparations is therefore also required, but until now has not been formulated. In this

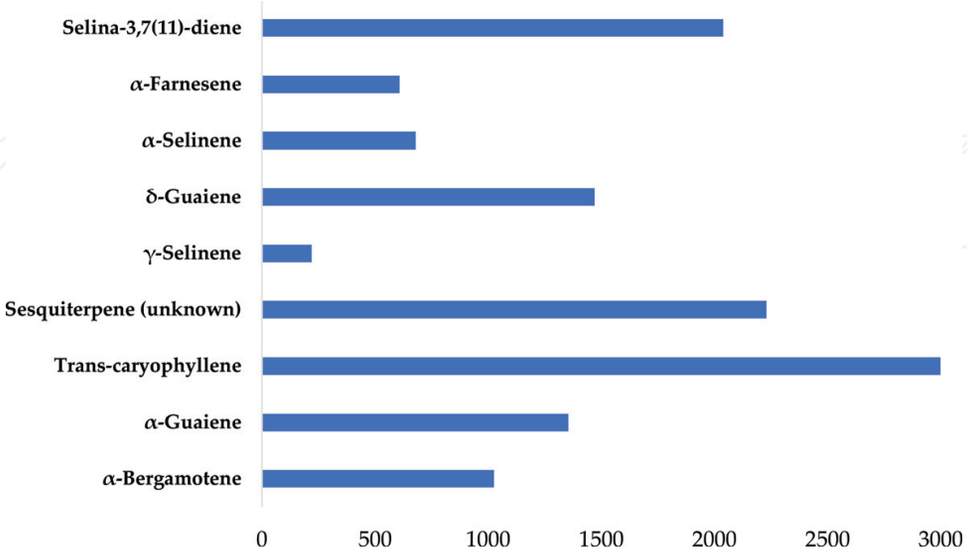

Figure 3. Representative sesquiterpenes fraction extracted from Bediol® chemotype by means of HS-SPME and identified using the GC-MS (μg/g).

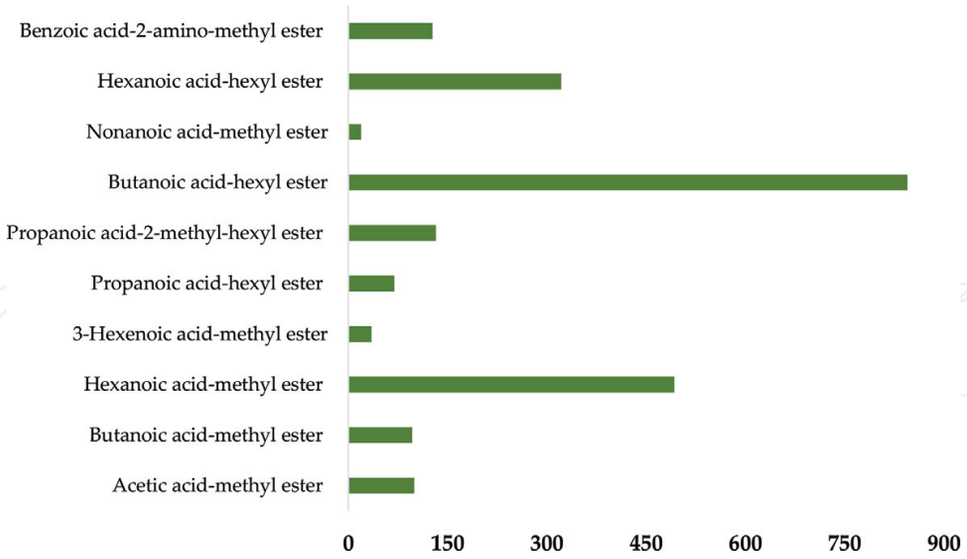

Figure 4. Esters fraction extracted from Bediol® chemotype by means of HS-SPME and identified using the GC-MS (μg/g).

context, cannabis extraction was performed using olive oil and a standardised medicinal cannabis "flos" (according to pharmaceutical standards) [31, 34, 39, 44, 45].

HPLC-MS/MS based analysis has recently been employed for the analysis of cannabinoids in plant materials, extracts and biological matrices [8, 29, 45]. This detection technique has proven to be particularly trustworthy, as there is no risk of native cannabinoids decomposition (decarboxylation of cannabinoid acids during the analysis), which may compromise the accurate assessment of the overall cannabinoids profile. Currently, the most widely used analysers for cannabinoids quantification are the triple quadrupole instruments, which possess excellent sensitivity and selectivity [31, 46]. However, they do not allow structural identification of "*non-target*" compounds.

In this respect, high-resolution accurate mass (HRMS) analyser such as Q-Exactive-Orbitrap-MS, offers the possibility to operate generating an "*in-depth*" qualitative analysis of thousands of compounds in complex biological, environmental or food matrixes providing insights beyond what is currently achievable with classic mass spectrometry instrumentation. Orbitrap mass spectrometer technology is rapidly developing also for cannabinoids profiling in different matrices, because it uniquely provides accurate molecular masses and specific fragmentation patterns for detected species. Moreover, HRMS acquisition mode accumulates all sample data, enabling identification of "unpredicted" compounds with cannabinolic structure and retrospective data analysis without the need to re-run samples.

As an example, a simultaneous identification of 24 synthetic and natural cannabinoids for a wide variety of samples such as herbal cannabis plant material by means of Orbitrap was reported [3]. Moreover, our research group has also recently published results concerning HPLC-Q-Exactive-Orbitrap-MS method for the determination of the seven most important cannabinoids, including four essential cannabinoids (THC, CBD, THCA and CBDA) accompanied with quantification of

CBN, CBG and CBGA [30]. Applying this method, we were able to determine the cannabinoid profile in Bediol® chemotype oils prepared by three different methods, as described in the materials and methods section.

Method 3 (realised by applying a preheating/ultrasounds assisted extraction), showed the highest extraction yields of the neutral cannabinoids CBD and THC. In contrast, method 1 provided the maximal concentrations of THCA, CBDA and CBGA, as a preheating step was not involved. At present, it is important to emphasise that, in the field of the therapeutic uses of cannabinoids related to pharmacological and clinical effects, THC and CBD in their neutral forms are of primary interest, even if there is growing attention toward the acidic forms (**Table 2**) [3].

Furthermore, apart from the targeted compounds revealed, several other untargeted cannabinoids were detected, as well. HRMS analysis has proven to be very useful also in the retrospective evaluation of untargeted isomeric cannabinoids. The structural interpretation of *untargeted* compounds was accomplished from the mass spectra collected in the FS and corresponding dd-MS^2 scan mode, and relied on the information found in the literature [30, 45, 46, 47, 49] and mass spectrum libraries [48]. In this respect, Q-Exactive-Orbitrap-MS analyser is often used in order to obtain structural information of the compounds detected as it provides accurate mass identification for both the precursor and the product ions. Among untargeted molecules, we verified the presence of THCV and CBDV that expressed the same fragmentation behaviour as their C5 equivalents but differed in fragments that contained the C3 side chain [30]. The presence and further quantification of those two compounds seems to be essential as it was revealed that in three models of seizure, cannabis-derived "botanical drug substances" rich in CBDV and CBD exerted significant anticonvulsant effects that were not mediated by the CB1 receptor and were of comparable efficacy with purified CBDV [50]. On the other hand, it is well-known that THCV (also as THC) binds to CB1 and CB2 receptors and acts as a cannabimimetic agonist [50, 51]. Therefore, the pharmacological potency of CBDV and THCV is substantial and, regardless of their relatively small amounts in oil preparations, they may contribute to the physiological efficiency of the overall cannabinoids profile [18], at least as far as Bediol® oil preparation is concerned.

Moreover, in the Bediol® oil extract samples in full scan negative acquisition mode at least four different cannabinoids with the same molecular ions (*m*/*z* 343.1915) but different retention times were noted (**Figure 5**). Their appearance and intensity varies according to the preparation method used. The fragmentation pattern of peaks at retention time (RT) 9.91 and 12.24 min correspond to tetrahydrocannabinolic acid—C4 **(**THCA-C4) and cannabidiolic acid—C4 (CBDA-C4). Those two

Preparation method	THC	CBD	CBN	CBG	THC-A	CBD-A	CBG-A
1 [32]	370 ± 23	2010 ± 56	10 ± 0.5	7 ± 0.8	8300 ± 507	14,120 ± 1002	260 ± 23
2 [33]	4520 ± 102	5503 ± 89	56 ± 7	125 ± 21	1808 ± 201	1208 ± 750	114 ± 15
3 [30]	5214 ± 87	7304 ± 108	47 ± 4	102 ± 12	487 ± 42	29 ± 0.75	18 ± 6

Table 2. Quantitative analysis of main cannabinoids from Bediol®'s macerated oil preparations obtained by three different preparation procedures (μg/g, mean ± SD, n = 3).

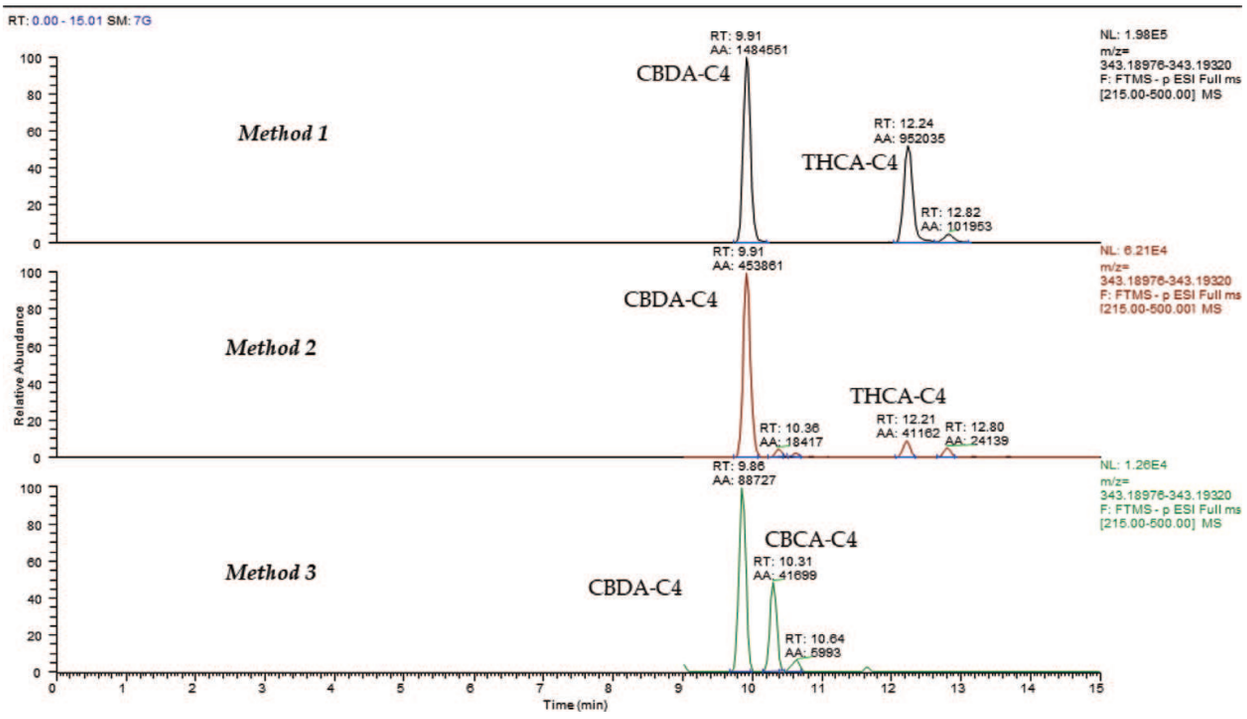

Figure 5. Extracted ion chromatograms from retrospective data analysis which point toward the presence of CBDA-C4; THCA-C4 and CBCA-C4.

acids are respectively homologues of main acids (THCA and CBDA) from which they differ just in the butyl side chain (instead of pentyl). In addition, the presence of the peak 10.31 and its fragmentation profile indicate the presence of cannabichromenic acid C4 (CBCA-C4). In a completely analogous way, the extracted ion chromatograms for *m/z* 329.17580 confirm the occurrence of THCVA and CBDVA, the acidic precursors of the above-mentioned THCV and CBDV, just for the oil samples from methods 1 and 2 (**Figure 6**). Additionally, the oil extract obtained by extraction method 3 revealed the presence of cannabichromevarinic acid (CBCVA). This compound, like its neutral counterpart cannabichromevarin CBCV, is not supported by adequate research work to fully understand its eventual distinctive pharmacological and physiological behaviour. However, the fact that extraction method 3 (preheating/ultrasounds) transfers this compound from the inflorescence to the medicinal oil has to be taken into consideration, especially when the signals of THCVA and CBDVA were practically absent in extract 3. This is most likely due to different kinetics of extraction performed by ultrasound that preserves the benzopiranic structure of CBCVA.

All in all, our retrospective analysis of Bediol® medical oil provides clear evidence of the need to develop a standardised procedure for extraction, especially in terms of time and extraction method, since they unambiguously affect the chemical composition of the final product, thus influencing the pharmacological effect of the medicinal preparation that is eventually dispensed to patients.

As far as VOCs profile is concerned, all three preparation methods extracted substantial amounts of terpenes, resembling the profile obtained for the Bediol® inflorescence. Comparing the three different preparation methods, it can be observed that method 1 extracted the highest

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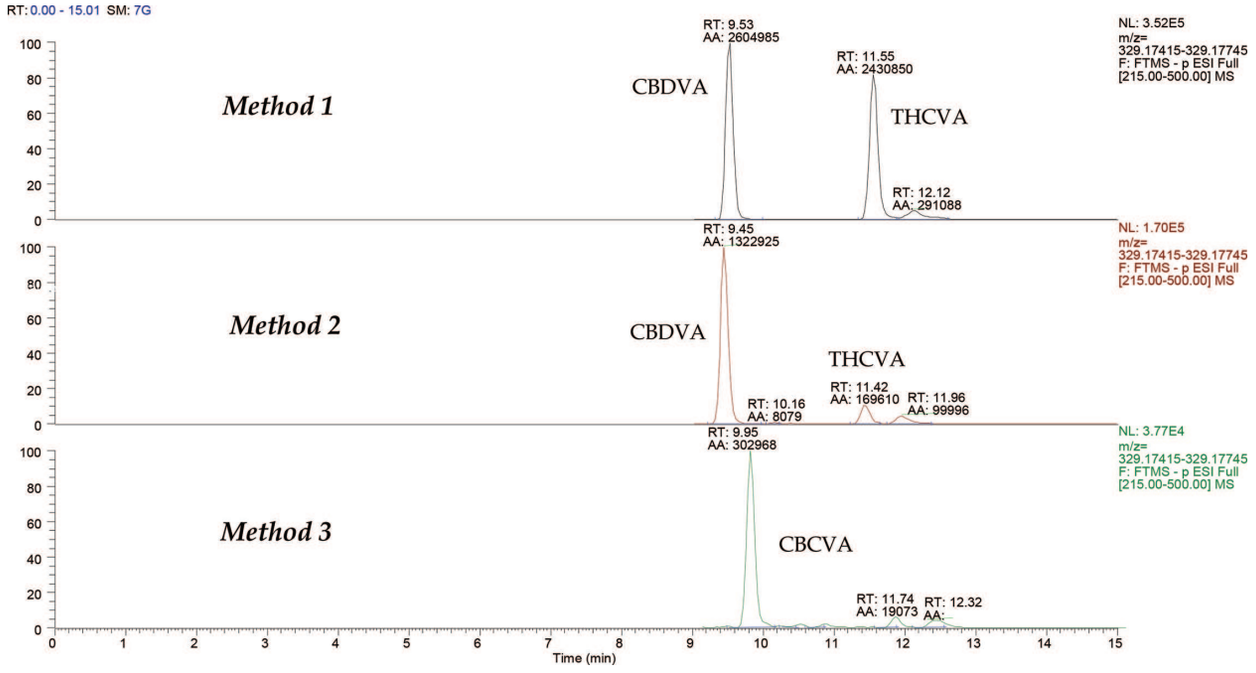

Figure 6. Extracted ion chromatograms from retrospective data analysis which point toward the presence of CBDVA; THCVA and CBCVA.

amount of terpenes, followed by methods 3 and 2 (**Table 3**). This was predictable, as method 1 did not include preheating for decarboxylation, thus the terpene fraction was preserved with evident domination of β-Myrcene. Although preheating the plant material released more of the known active neutral cannabinoids, it simultaneously led to the loss of components such as terpenes by degradation or evaporation.

As regards lipid oxidation products, the opposite trend was shown among the three preparation procedures. In particular, method 3, realised without any heating step, showed minor concentrations of lipid oxidation products. The macerated oil obtained using the method by Romano-Hazekamp (method 1) contained the highest levels of oxidation products, compared with the other two procedures, as expected. This can be related to preparation conditions in which the oil is heated at 98°C for 120 min. The data concerning the formation of lipid oxidation products in cannabis medical oil preparations are extremely limited [30]. The occurrence of aldehydes in the sample obtained by method 1 indicates the initiation of lipid peroxidation of polyunsaturated fatty acids (PUFA) from oils used as a matrix [52, 53]. It is well documented that peroxidation of PUFA leads to the formation of a well-defined series of aldehydes and ketones such as nonenal, hexanal and pentanal, 2-heptenal [54]. The formation rate of lipid oxidation products depends closely on several factors among which the most important are: method preparation temperature, fatty acid composition of oil in which cannabis extract is dissolved and storage conditions [55]. These parameters are crucial to define the ultimate characteristics of the final products to be used for medical treatment. Finally, the presence of 2-furancarboxaldehyde in the oil sample obtained by method 1 confirmed that preheating initiates the series of reactions that leads to the formation of potentially toxic compounds.

Compound class/name	Preparation method		
	1 [32]	2 [33]	3 [30]
Alcohols			
1-Hexanol	31.10 ± 2.8	15 ± 1.3	13.15 ± 2.12
3-Hexen-1-ol	1.10 ± 0.14	0.56 ± 0.12	0.7 ± 0.1
2-Ethyl-1-hexanol	0.22 ± 0.03	n.d.	n.d.
3,3,6-Trimethyl-1,5-heptadien-4-ol	13.1 ± 0.5	7.3 ± 1.93	5.3 ± 0.45
α-Toluenol	0.16 ± 0.03	0.10 ± 0.02	0.08 ± 0.02
Aldehydes			
2-Methyl-butanal	0.42 ± 0.05	n.d.	n.d.
3-Methyl-butanal	0.26 ± 0.03	n.d.	n.d.
Hexanal	1.51 ± 0.13	n.d.	n.d.
Heptanal	1.06 ± 0.29	n.d.	n.d.
2-Hexenal	1.90 ± 0.22	n.d.	n.d.
Octanal	0.54 ± 0.09	0.36 ± 0.01	0.04 ± 0.02
Ketones			
6-Methyl-5 hepten-2 one	1.8 ± 0.15	0.98 ± 0.14	0.28 ± 0.08
3-Methyl-3-cyclohexen-1-one	3.01 ± 0.67	0.58 ± 0.14	0.19 ± 0.05
Esters			
Acetic acid-methyl ester	0.41 ± 0.09	n.d.	n.d.
3-Hexen-1-ol-acetate	0.51 ± 0.02	0.22 ± 0.03	0.18 ± 0.01
Propanoic acid-hexyl ester	1.84 ± 0.01	0.99 ± 0.17	0.90 ± 0.1
Propanoic acid-2-methyl-hexyl ester	2.47 ± 0.01	1.55 ± 0.25	1.70 ± 0.09
Butanoic acid-hexyl ester	21.01 ± 0.21	10.80 ± 2.72	16 ± 0.82
Hexanoic acid-hexyl ester	1.78 ± 0.54	1.23 ± 0.28	1.43 ± 0.22
Benzoic acid-2-amino-methyl ester	0.55 ± 0.04	0.53 ± 0.16	0.53 ± 0.04
Mono/di/triterpenes			
α-Pinene	109 ± 1.4	12.37 ± 2.54	29.0 ± 0.39
α-Thujene	5.41 ± 0.45	2.12 ± 0.34	2.71 ± 0.11
Camphene	2.27 ± 0.15	0.67 ± 0.09	0.30 ± 0.01
β-Pinene	55.04 ± 7.0	14.57 ± 1.54	17.20 ± 0.67
Sabinene	1.82 ± 0.14	0.2 ± 0.07	n.d.
δ-3-Carene	18.4 ± 1.93	6.62 ± 0.90	7.44 ± 0.13
α-Phellandrene	19.00 ± 2.21	10.67 ± 1.93	5.57 ± 0.51
β-Myrcene	1074.2 ± 30	227.77 ± 35.1	458.0 ± 2.74
α-Terpinene	13.90 ± 1.27	10.20 ± 1045	16.56 ± 1.14
Limonene	32.4 ± 4.13	14.39 ± 1.75	18.17 ± 1.38

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Compound class/name	Preparation method		
	1 [32]	2 [33]	3 [30]
Eucalyptol	5.2 ± 0.58	3.14 ± 0.76	4.84 ± 0.46
β-Phellandrene	52.00 ± 7.57	27.25 ± 4.37	35.83 ± 1.57
Cis-ocimene	2.70 ± 0.20	1.47 ± 0.24	0.72 ± 0.11
γ-Terpinene	13.87 ± 1.13	14 ± 2.36	8.50 ± 0.48
β-Ocimene	107.22 ± 6	49.0 ± 6.7	64.88 ± 1.15
p-Cymene	11.86 ± 1.11	6.7 ± 0.63	4.7 ± 0.49
α-Terpinolene	253.3 ± 20.9	157.78 ± 19.46	197.14 ± 1.08
1,3,8-p-Menthatriene	0.63 ± 0.01	0.37 ± 0.03	0.27 ± 0.04
p-Cymenyl	6.3 ± 0.18	6.84 ± 1.46	8.07 ± 0.33
Isomenthone	n.d.	0.16 ± 0.02	0.57 ± 0.08
4,8-Epoxy-p-menth-1-ene	12.11 ± 0.12	4.57 ± 1.01	2.80 ± 0.27
β-Linalool	0.89 ± 0.05	0.83 ± 0.19	0.66 ± 0.05
p-Menth-2-en-1-ol	0.42 ± 0.05	n.d.	n.d.
4-Terpineol	2.60 ± 0.01	2.65 ± 0.78	2.61 ± 0.18
Verbenol	2.41 ± 0.13	1.56 ± 0.63	2.21 ± 0.08
1,8-Menthadien-4-ol	7.00 ± 0.32	5.34 ± 1.55	6.15 ± 0.25
α-Terpineol	4.66 ± 0.15	3.63 ± 1.15	3.45 ± 0.20
Borneol	1.07 ± 0.16	0.89 ± 0.26	0.77 ± 0.02
p-Menth-1-en-3-ol	0.85 ± 0.03	0.39 ± 0.06	0.25 ± 0.03
Trans-3-caren-2-ol	1.00 ± 0.05	0.64 ± 0.11	0.52 ± 0.04
Cuminol	4.60 ± 0.36	3.42 ± 0.66	4.29 ± 0.23
Sesquiterpenes			
α-Santalene	0.94 ± 0.16	0.61 ± 0.08	0.57 ± 0.06
α-Bergamotene	4.66 ± 1.03	3.17 ± 0.63	4.28 ± 0.83
α-Guaiene	8.94 ± 2.17	6.97 ± 1.14	7.05 ± 1.93
Trans-caryophyllene	27.64 ± 4.78	20.60 ± 3.11	21.07 ± 3.13
α-Humulene	10.62 ± 2.35	7.11 ± 1.39	8.00 ± 1.73
δ-Guaiene	7.50 ± 2.11	5.84 ± 0.94	5.90 ± 1.41
β-Selinene	1.15 ± 0.26	0.83 ± 0.11	0.90 ± 0.29
α-Selinene	1.78 ± 0.07	1.07 ± 0.11	1.90 ± 0.45
α-Farnesene	0.63 ± 0.20	0.42 ± 0.06	0.54 ± 0.16
Selina-3,7(11)-diene	7.40 ± 2.30	5.60 ± 0.78	6.65 ± 1.93
Nerolidol	0.37 ± 0.08	0.35 ± 0.11	0.46 ± 0.18
Furans			
2-Furancarboxaldehyde	0.32 ± 0.05	n.d.	n.d.

Compound class/name	Preparation method		
	1 [32]	2 [33]	3 [30]
Dihydro-2(3H)-furanone	0.24 ± 0.06	0.16	n.d.
5-Ethyl-2(5H)-furanone	0.32 ± 0.04	0.27 ± 0.03	n.d.
Miscellaneous			
Dimethyl sulfide	0.63 ± 0.12	n.d.	n.d.
Methyl-pyrazine	n.d.	n.d.	n.d.
2,5-Dimethyl-pyrazine	n.d.	0.22 ± 0.07	0.16 ± 0.09
Dibutylformamide	n.d.	n.d.	n.d.
Acetylpyrrole	0.32 ± 0.07	0.41 ± 0.05	0.38 ± 0.06

Data are expressed in μg/g (mean value ± SD, n = 3).

Table 3. Volatile compounds extracted and identified by HS-SPME-GC/MS in Bediol® oil obtained from different preparation methods.

4. Conclusions

In this study, an analytical protocol involving the combination of HS-SPME coupled to GC–MS and ASE coupled to HPLC-HRMS (Orbitrap®) was applied for the in-depth profiling and fingerprinting of cannabinoids and terpenes in an authorised medical grade variety of *Cannabis sativa* L. (Bediol®). HS-SPME was shown to be an excellent technique to investigate both the cannabis inflorescence and derived macerated oil volatile composition. In particular, HS-SPME extraction provides an accurate profile concerning plausible terpenes fingerprint of different cannabis chemotypes, as presented in this study.

LC-HRMS-Orbitrap, used to investigate cannabinoids extracted from inflorescences and macerated oils, showed high-throughput performances, as it can be used both for quantification of *target* analytes and to investigate *untargeted* fraction to obtain a very complex prolife as an expression of plant phytocomplex at the same time.

These approaches are nowadays essential and pivotal in order to understand the composition of *Cannabis sativa* chemotypes currently used for their role in therapeutic management, as they are able to provide comprehensive information essential to then correlate the phytochemical characteristics of cannabis and the clinical results obtained when managed and administered to patients as well.

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