Journal of Chromatography B 1221 (2023) 123682

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/jchromb

Enantioseparation of chiral phytocannabinoids in medicinal cannabis

Fabiana Russo [a,b], Francesco Tolomeo [c], Maria Angela Vandelli [d], Giuseppe Biagini [b], Aldo Laganà [e], Anna Laura Capriotti [e], Andrea Cerrato [e], Luigi Carbone [c], Elisabetta Perrone [c], Alberto Cavazzini [f], Vincenzo Maiorano [c], Giuseppe Gigli [c], Giuseppe Cannazza [c,d,*], Cinzia Citti [c,d,*]

[a] Clinical and Experimental Medicine PhD Program, University of Modena and Reggio Emilia, 41125 – Modena, Italy
[b] Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, 41125 – Modena, Italy
[c] Institute of Nanotechnology – CNR NANOTEC, Campus Ecotekne, Via Monteroni, 73100 – Lecce, Italy
[d] Department of Life Sciences, University of Modena and Reggio Emilia, Via Campi 103, 41125 – Modena, Italy
[e] Department of Chemistry, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 – Rome, Italy
[f] Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Via L. Borsari 46, 44121 – Ferrara, Italy

ARTICLE INFO

Keywords:
Chiral cannabinoids
Chiral chromatography
High-resolution mass spectrometry
Enantioseparation
Decarboxylation

ABSTRACT

The evaluation of the chiral composition of phytocannabinoids in the cannabis plant is particularly important as the pharmacological effects of the (+) and (-) enantiomers of these compounds are completely different. Chromatographic attempts to assess the presence of the minor (+) enantiomers of the main phytocannabinoids, cannabidiolic acid (CBDA) and *trans*-Δ^9-tetrahydrocannabinolic acid (*trans*-Δ^9-THCA), were carried out on heated plant extracts for the determination of the corresponding decarboxylated species, cannabidiol (CBD) and *trans*-Δ^9-tetrahydrocannabinol (*trans*-Δ^9-THC), respectively. This process produces an altered phytocannabinoid composition with several new and unknown decomposition products. The present work reports for the first time the stereoselective synthesis of the pure (+) enantiomers of the main phytocannabinoids, *trans*-CBDA, *trans*-Δ^9-THCA, *trans*-CBD and *trans*-Δ^9-THC, and the development and optimization of an achiral-chiral liquid chromatography method coupled to UV and high-resolution mass spectrometry detection in reversed phase conditions (RP-HPLC-UV-HRMS) for the isolation of the single compounds and evaluation of their actual enantiomeric composition in plant. The isolation of the peaks with the achiral stationary phase ensured the absence of interferences that could potentially co-elute with the analytes of interest in the chiral analysis. The method applied to the Italian medicinal cannabis variety FM2 revealed no trace of the (+) enantiomers for all phytocannabinoids under investigation before and after decarboxylation, thus suggesting that the extraction procedure does not lead to an inversion of configuration.

1. Introduction

Since the discovery of phytocannabinoids and their biological properties, *Cannabis sativa* L. has assumed a key role in a number of scientific areas, especially in the cosmetic [1] and nutraceutical [2] industry with the use of the non-intoxicating species, as well as in the pharmaceutical industry and clinical practice including the adoption of drug-type varieties [3,4]. The latter typically show higher concentration of Δ^9-tetrahydrocannabinol (Δ^9-THC), a phytocannabinoid with psychotropic and euphoriant effects [5], while non-intoxicating compounds are generally present in higher levels in fiber-type cannabis varieties. These non-intoxicating compounds include the majority of the members of the phytocannabinoids class since only Δ^9-THC, its regioisomers and their stereochemical variants have been recognized as responsible for the cannabimimetic and narcotic activity, thus under international control (Schedule I and II of the 1971 Convention on Psychotropic Substances [6]). Notwithstanding the side effects of the use of cannabis, its therapeutic benefits are considerably higher than the potential risks so that the Expert Committee on Drug Dependence (ECDD) of the WHO recommended to remove cannabis from Schedule IV of 1961 Single Convention, which is the most restrictive category for a psychotropic substance, thus recognizing its medical value [7].

Hence, *Cannabis sativa* L. has become impressively attractive for the vast range of phytocannabinoids produced in the trichomes of the

* Corresponding authors at: Institute of Nanotechnology – CNR NANOTEC, Campus Ecotekne, Via Monteroni, 73100 – Lecce, Italy.
E-mail addresses: giuseppe.cannazza@unimore.it (G. Cannazza), cinzia.citti@unimore.it (C. Citti).

https://doi.org/10.1016/j.jchromb.2023.123682
Received 16 January 2023; Received in revised form 13 March 2023; Accepted 15 March 2023
Available online 21 March 2023

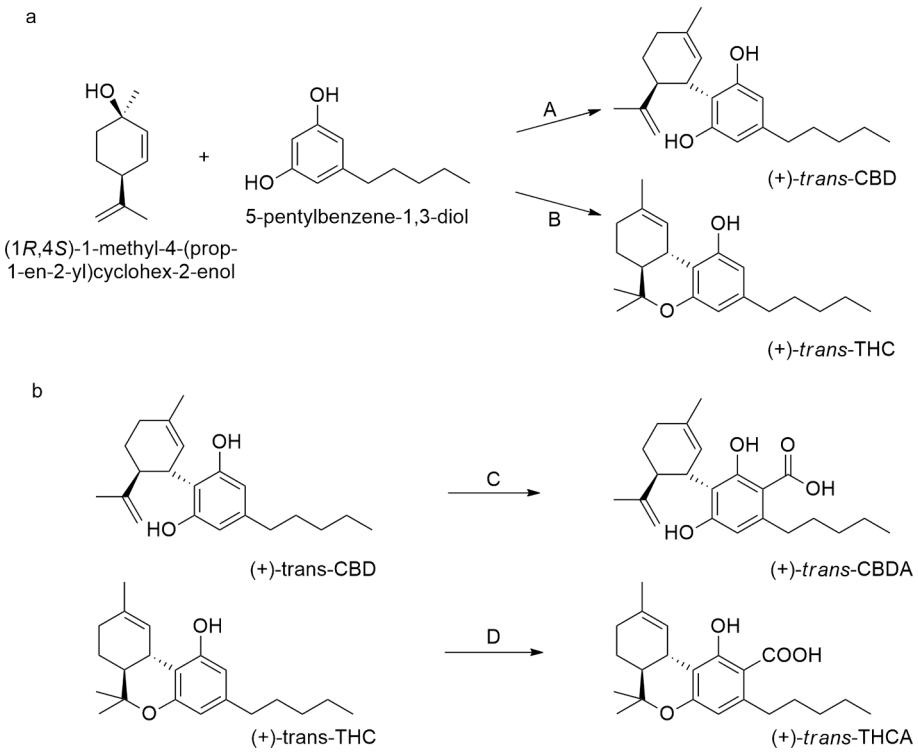

Fig. 1. Synthetic procedure for the (+)-*trans* enantiomers of CBD, Δ^9-THC, CBDA and Δ^9-THCA. a) Synthesis of the decarboxylated forms (+)-*trans*-CBD and (+)-*trans*-Δ^9-THC; b) Synthesis of the carboxylated forms (+)-*trans*-CBDA and (+)-*trans*-Δ^9-THCA. Reagents and conditions: A) pTSA (2%w/w.), dry DCM, room temperature, N_2 atmosphere, 4.5 h stirring. B) *p*TSA (10%mol), dry DCM, room temperature, N_2 atmosphere, 4.5 h stirring. C) MMC (Methyl Magnesium Carbonate) 2 M in DMF, DMF, 120 °C stirring for 3 h in a closed vessel. D) MMC, 2 M in DMF, DMF, 120°C overnight in a closed vessel.

female inflorescences reaching a number as high as 150 and over [8–14] thanks to the great advances in the analytical and software technologies [15,16]. Cannabigerolic acid (CBGA) is the father of the three main phytocannabinoids, namely Δ^9-tetrahydrocannabinolic acid (Δ^9-THCA), cannabidiolic acid (CBDA) and cannabichromenic acid (CBCA), so far known to be produced by an enzyme-driven conversion [17]. These carboxylated species are then converted to the well-known cannabigerol (CBG), Δ^9-THC, cannabidiol (CBD), and cannabichromene (CBC), respectively, via a decarboxylation reaction occurring during heating, drying or storage [18,19]. All other phytocannabinoids originate from either oxidation, cyclization or isomerization reactions of the main phytocannabinoids [20].

Although specific pharmacological properties have been recognized to all phytocannabinoids, only Δ^9-THC and CBD have been approved for some therapeutic indications [21,22]. These two phytocannabinoids possess two chiral centers, which are responsible for the potential existence of four stereoisomers: (-)-*trans*-, (+)-*trans*-, (-)-*cis*-, and (+)-*cis*-isomer. The *cis* isomers of CBD have never been reported to occur naturally in cannabis, while the *cis* isomers of Δ^9-THC have been recently found as a scalemic mixture in amounts as high as the (-)-*trans* counterpart in fiber-type cannabis plants [23], meaning that their levels do not exceed 0.3% of the dry weight of the raw material. As regards to CBD, the (-)-*trans* isomer is the one found in the plant, while no literature record mentions the presence of the (+)-*trans* isomer. While nature seems to prefer the (-)-*trans* isomer also for Δ^9-THC, trace amounts of the (+)-*trans* isomer (0.135% enantiomeric fraction) have been found in the medicinal cannabis variety Bedrocan (Bedrocan B.V., The Netherlands) by Mazzoccanti et al. [24]. However, no other studies have been carried out to confirm the presence of this minor stereoisomer in other varieties. From a pharmacological point of view, the stereoisomers of a chiral compound are not equivalent and often show completely different activities [25,26]. With particular attention to *trans*-CBD and *trans*-Δ^9-THC, the (+)- and (-)- isomers proved to have contrasting biological activities. For example, (-)-*trans*-CBD has no affinity for either CB1 or CB2 receptors, whereas in comparison unnatural (+)-*trans*-CBD showed a strong binding affinity for CB2 and enhanced affinity for CB1 receptors [27], though not as high as (-)-*trans*-Δ^9-THC [28]. In light of the above, it is extremely important to evaluate the stereoisomeric composition of the phytocannabinoids especially in medicinal cannabis extracts.

To this end, a few papers report the development of chiral chromatographic methods for the separation of phytocannabinoids diastereoisomers, in particular by means of liquid chromatography (HPLC) [23,29–31] and supercritical fluid chromatography (SFC) [24] with chiral stationary phases (CSPs). Some authors exploited the advantages of the inverted chirality column approach (ICCA), which proved to be very useful to calculate the enantiomeric excess by both HPLC and SFC [23,24]. In particular, the ICCA consists of running an enantiomeric pair on two CSPs with the same bound selector but opposite configuration; in this way, the elution order of the given enantiomeric pair is inverted on the two columns according to the reciprocal principle of selectand-selector-systems [24,32]. This approach is particularly useful when one enantiomer of the pair is not available as a standard. In order to assess the stereoisomeric composition of phytocannabinoids in cannabis extracts, the original plant material is usually heated to convert the phytocannabinoid precursors (e.g. Δ^9-THCA) into the corresponding decarboxylated derivatives (e.g. Δ^9-THC). This procedure is generally undertaken in order to avoid the presence of the carboxylated phytocannabinoids for two main reasons: they are not easy to handle because of their thermal instability and no commercial standard is available for all their diasteroisomers apart from the (-)-*trans*.

To the best of the authors' knowledge, no analytical method has been published on the chiral separation of the phytocannabinoid carboxylated precursors, which instead are the real picture of what the plant produces enzymatically. Taking into account that a stereoconversion may occur during the extraction process, including the decarboxylation step, as well as in biological fluids after administration [33], it becomes important to establish whether the enzymatic production of phytocannabinoids in the plant is stereoselective or the minor stereoisomeric forms can be also formed. In order to shed some light on this aspect, which has never been investigated, it is important to work with pure enantiomerically enriched compounds. Since no analytical standard is commercially available for the (+)-*trans* diasteroisomers of Δ^9-THCA and CBDA, as well as for the decarboxylated counterparts Δ^9-THC and CBD, a stereoselective synthesis was performed in order to develop and

Table 1
Optimization of the chiral chromatographic conditions for the enantiomeric separation of *trans*-CBDA, *trans*-Δ^9-THCA, *trans*-CBD and *trans*-Δ^9-THC with different CSPs, organic modifiers, column temperatures and flow rates. For each analysis the retention time (R_T) of the enantiomers (t_1 and t_2), the retention factors (k_1 and k_2), the separation factor (α) and the resolution factor (R_S) were calculated and reported. Best conditions found are reported in bold.

Compound	Column	Organic modifier		T (°C)	Flow rate (mL/min)	R_T (min)		k_1	k_2	α	R_S
		Type	%			t_1	t_2				
trans-CBDA	CHIRALCEL OJ-H	ACN	50	30	0.75	35.9	39.9	8.16	9.18	1.11	0.9
		ACN	50	45	0.75	31.4	34.4	6.92	7.78	1.11	1.0
		ACN	70	30	0.75	7.3	7.7	0.82	0.92	1.05	0.7
	CHIRALCEL AD	ACN	60	30	1.5	9.8	9.8	3.67	3.67	1.00	0.0
	CHIRALCEL AD-RH	ACN	60	30	1.5	3.6	3.6	2.00	2.00	1.00	0.0
		EtOH	80	30	1	3.5	3.8	2.16	0.89	1.08	0.8
		i-PrOH	60	30	1	3.6	4.2	2.16	1.33	1.17	0.6
		***i*-PrOH**	**50**	**30**	**1**	**14.5**	**16.4**	**7.43**	**8.53**	**1.15**	**1.3**
		i-PrOH	50	25	1	16.1	17.5	8.20	9.00	1.10	0.9
		i-PrOH	45	30	1	–	–	–	–	–	–
trans-Δ^9-THCA	CHIRALCEL OJ-H	ACN	70	30	0.75	11.3	11.9	1.83	1.98	1.05	0.6
		ACN	60	30	0.75	20.8	22.6	4.17	4.62	1.09	0.9
	CHIRALCEL AD-RH	ACN	60	30	1.5	10.9	10.9	8.08	8.08	1.00	0.0
		EtOH	80	30	0.5	24.5	38.1	1.16	8.52	1.55	4.5
		EtOH	80	30	1	12.3	19.3	1.16	8.65	1.57	2.9
		i-PrOH	50	30	1	–	–	–	–	–	–
		***i*-PrOH**	**75**	**30**	**1**	**3.3**	**4.0**	**0.9**	**1.3**	**1.4**	**2.3**
trans-CBD	CHIRALCEL OD-R	ACN	70	30	1	7.5	7.5	1.53	1.61	1.03	0.2
		ACN	70	10	1	7.6	7.9	2.06	2.19	1.04	0.2
	CHIRALCEL OB-H	ACN	70	10	1	4.9	4.9	1.30	1.30	1.00	0.0
		ACN	60	30	0.75	9.7	9.7	1.32	1.32	1.00	0.0
	CHIRALCEL OJ-H	ACN	70	30	0.75	6.4	6.7	1.14	1.19	1.02	0.3
		ACN	70	10	1	8.7	8.9	1.33	1.44	1.05	0.5
	CHIRALPAK AD	ACN	70	30	1	6.9	8.6	1.16	1.69	1.25	1.0
		ACN	70	30	1.5	4.5	5.6	1.06	1.57	1.24	1.0
		ACN	50	30	1.5	31.0	31.9	13.49	13.91	1.03	0.4
		EtOH	100	30	0.5	6.8	6.8	0.18	0.18	1.00	0.0
		EtOH	90	30	0.5	8.8	8.8	0.32	0.32	1.00	0.0
		EtOH	80	30	0.5	13.4	14.8	1.02	1.23	1.10	0.9
	CHIRALPAK AD-RH	ACN	70	30	1.5	2.6	3.3	1.16	1.73	1.27	2.3
		ACN	**60**	**30**	**1.5**	**5.6**	**8.0**	**1.16**	**1.73**	**1.27**	**3.3**
trans-Δ^9-THC	CHIRALCEL OD	ACN	70	10	1	11.5	11.5	1.16	1.73	1.27	0.0
	CHIRALCEL OB	ACN	70	10	1	6.1	6.5	1.86	2.05	1.07	0.9
		ACN	60	30	0.75	12.9	13.7	2.09	2.28	1.06	0.9
	CHIRALCEL OJ-H	ACN	70	30	0.75	11.6	12.5	1.86	2.08	1.08	0.8
	CHIRALCEL AD	ACN	70	30	1	12.2	14.7	2.81	3.59	1.20	0.9
		EtOH	100	30	0.5	7.6	9.8	0.32	0.71	1.29	2.1
		EtOH	90	30	0.5	11.5	16.4	0.73	1.47	1.43	2.9
		EtOH	80	30	0.5	22.2	34.1	2.36	4.16	1.54	3.0
		EtOH	80	30	1	11.2	17.3	2.38	4.23	1.54	2.9
	CHIRALCEL AD-RH	ACN	70	30	1.5	4.9	6.1	1.16	4.08	1.24	1.2
		ACN	**60**	**30**	**1.5**	**9.8**	**12.8**	**1.158**	**9.64**	**1.31**	**3.0**

optimize a chiral HPLC method coupled to a diode array detector (DAD) for the separation of all stereoisomers of these carboxylated and decarboxylated analytes. The method was then applied to the Italian medicinal cannabis variety FM2 to evaluate the chiral composition of these main phytocannabinoids. In order to do so, the plant material was extracted without a preventive decarboxylation step and the two phytocannabinoids *trans*-Δ^9-THCA and *trans*-CBDA were isolated, each in an individual fraction, by achiral reversed phase (RP) HPLC-DAD. After decarboxylation of the starting plant material, the corresponding decarboxylated species *trans*-CBD and *trans*-Δ^9-THC were isolated and the four phytocannabinoids were analysed by chiral HPLC-DAD. The addition of the high-resolution mass spectrometry (HRMS) detector after the DAD ensured that all analytes were unambiguously identified thus avoiding misinterpretation of the peaks.

2. Materials and methods

2.1. Chemicals and reagents

LC-MS grade acetonitrile and formic acid and analytical grade ethanol 96% (v/v) were purchased from Honeywell (Charlotte, North Caroline, USA). Ultrapure water was obtained with a water purification system (Direct-Q 3UV, Merck Millipore, Milan, Italy). Stock solutions of pure certified analytical standards of (-)-*trans*-Δ^9-THCA, (-)-*trans*-CBDA, (-)- *trans*-Δ^9-THC, and (-)-*trans*-CBD, were bought from Cerilliant (Sigma-Aldrich Merck, Milan, Italy), while (+)-*trans*-Δ^9-THCA, (+)-*trans*-Δ^9-THC, (+)-*trans*-CBDA and (+)-*trans*-CBD were synthesized in house.

All reagents and solvents for the syntheses were purchased and used without further purification. Reactions were monitored by thin-layer chromatography on silica gel (60F-254, E. Merck) and checked by $KMnO_4$ aqueous solution or UV light. When necessary, reaction products were purified, by normal phase chromatography on silica gel (40–63 μm) with the solvent system indicated. The chemical structures were ensured through Nuclear Magnetic Resonance (NMR). ^{1}H NMR spectra were recorded on a Bruker 400 spectrometer working at 400.134 MHz. Chemical shifts (δ) are in parts per million (ppm) and they were referenced to the solvent residual peaks ($CDCl_3$ $\delta = 7.26$ ppm). One-dimentional spectra were acquired with a spectral width of 8278 Hz. Optical rotation (α) was measured with a Polarimeter 241 (cell length 100 mm, volume 1 mL) from PerkinElmer (Milan, Italy).

3. Plant material

The FM2 medicinal cannabis variety (batch n. 6A32/1) was supplied by the Military Chemical Pharmaceutical Institute (Florence, Italy) with

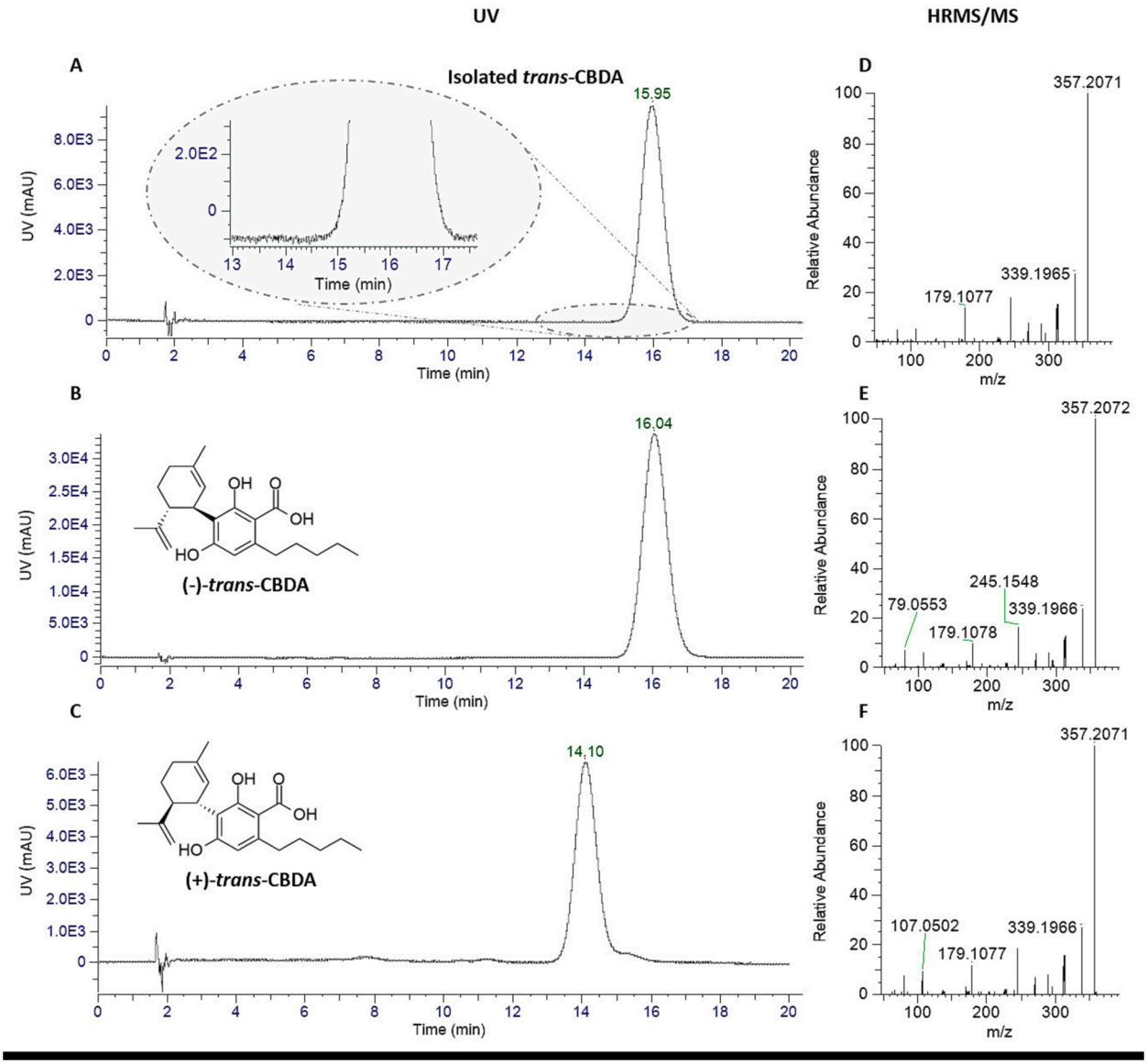

Fig. 2. Comparison of the UV traces and tandem HRMS spectra of the isolated *trans*-CBDA and the pure synthetic (-) and (+) enantiomers of *trans*-CBDA. UV traces at 306 nm of the isolated peak of *trans*-CBDA (A), pure (-)-*trans*-CBDA (B) and pure (+)-*trans*-CBDA (C); HRMS/MS spectra in HESI- mode of the isolated peak of *trans*-CBDA (D), pure (-)-*trans*-CBDA (E) and pure (+)-*trans*-CBDA (F).

the authorization of the Italian Ministry of Health (prot. n. SP/062). The raw plant material (100 mg) was finely grinded and a crude extract was obtained following the protocol reported in the German Pharmacopoeia for *Cannabis flos* [34]. Briefly, the plant material was extracted with ethanol 96% (EtOH) in three cycles (5 mL, 2 mL, 2 mL) and the extracts were combined in a volumetric flask and brought to 10 mL final volume with fresh ethanol. A small aliquot (1 mL) was centrifuged at 2000 × *g* and the pellet removed. After filtration with a 0.45 μm PTFE filter, 10 μL of the sample were injected without further dilution into the achiral RP-HPLC system for the isolation of *trans*-CBDA and *trans*-Δ^9-THCA. Five injections provided 500 μL of the former and 350 μL of the latter. The solvent was reduced under a gentle stream of nitrogen gas and the residue reconstituted with acetonitrile (ACN) to get the final concentration of 100 μg/mL of each phytocannabinoid.

About 500 mg of the FM2 plant material were placed in a beaker and heated for 2 h at 130 °C. Extraction with ethanol was performed on 100 mg of decarboxylated material as previously described for the crude extract. After filtration with a 0.45 μm PTFE filter, 10 μL of the sample were injected without any further dilution into the achiral RP-HPLC system for the isolation of *trans*-CBD and *trans*-Δ^9-THC. Five injections provided 350 μL of the former and 250 μL of the latter. The solvent was reduced under a gentle stream of nitrogen gas and the residue reconstituted with ACN to get the final concentration of 100 μg/mL of each phytocannabinoid.

4. Synthesis and characterization of the (+)-*trans* isomers

(1′*S*,2′*S*)-5′-methyl-4-pentyl-2′-(prop-1-en-2-yl)-1′,2′,3′,4′-tetrahydro-[1,1′-biphenyl]-2,6-diol, (+)-*trans*-CBD

To a solution of (1*R*,4*S*)-1-methyl-4-(prop-1-en-2-yl)cyclohex-2-enol (0.9 eq., 1.4 mmol) and 5-pentylbenzene-1,3-diol (1 eq., 1.7 mmol) in dry dichloromethane (DCM, 15 mL) at room temperature, in the dark and under nitrogen atmosphere, *p*-toluenesulfonic acid (*p*TSA) (2% w/w of 5-pentylbenzene-1,3-diol) was added. The reaction was stirred in the same conditions for 5 h. After that, the reaction was quenched with 10 mL of a saturated solution of $NaHCO_3$. The organic phase was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under

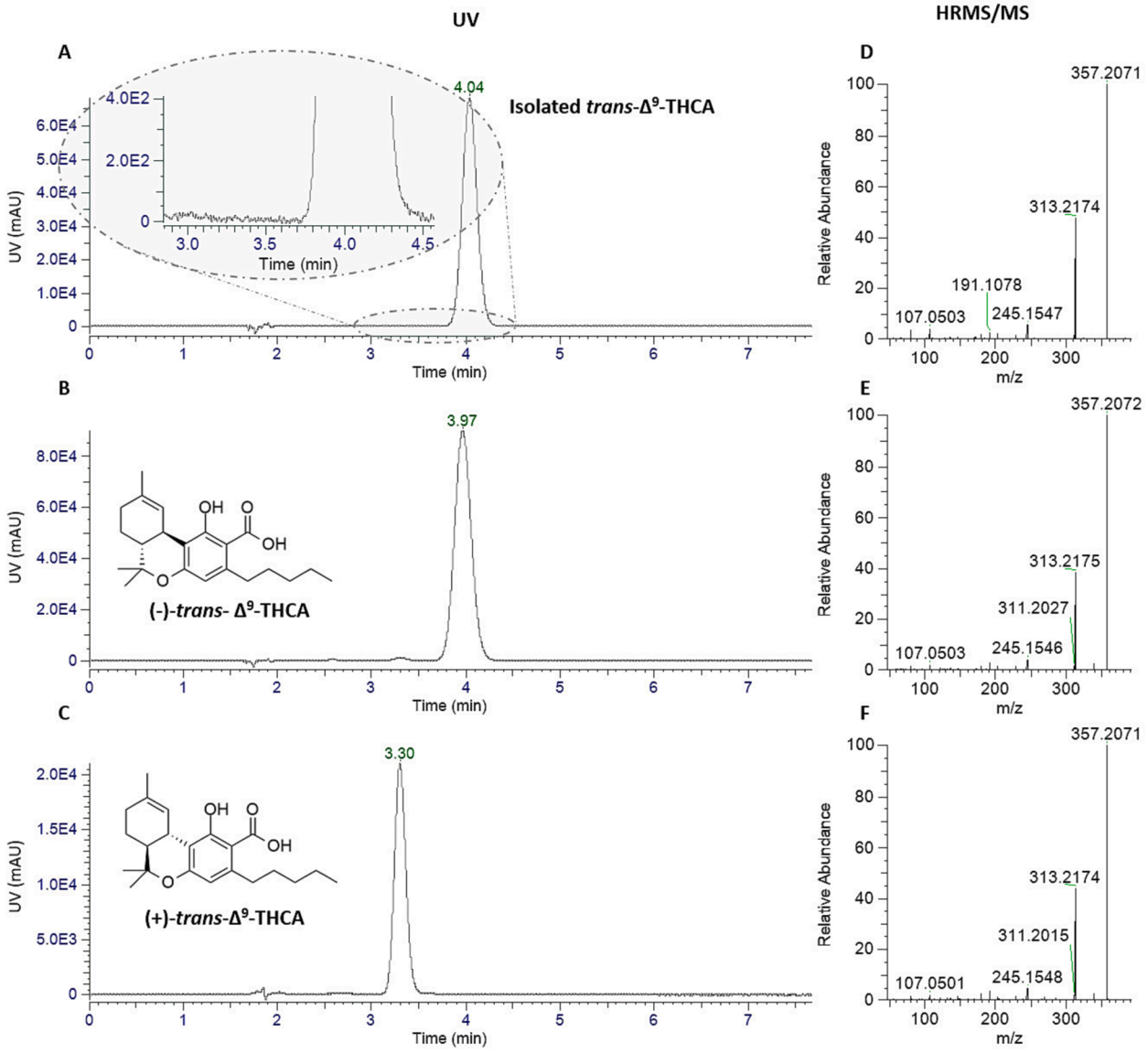

Fig. 3. Comparison of the UV traces and tandem HRMS spectra of the isolated *trans*-Δ^9-THCA and the pure synthetic (-) and (+) enantiomers of *trans*-Δ^9-THCA. UV traces at 306 nm of the isolated peak of *trans*-Δ^9-THCA (A), pure (-)-*trans*-Δ^9-THCA (B) and pure (+)-*trans*-THCA (C); HRMS/MS spectra in HESI- mode of the isolated peak of *trans*-Δ^9-THCA (D), pure (-)-*trans*-Δ^9-THCA (E) and pure (+)-*trans*-Δ^9-THCA (F).

vacuum. The obtained crude (500 mg) was chromatographed (crude: silica 1:80, eluent: cyclohexane (CE):DCM, 6:4) to give 150 mg of colourless liquid (40% yield, 92% *ee* by HPLC-UV). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.25 (brs, 1H), 6.18 (brs,1H),5.97 (s, 1H), 5.57 (s, 1H), 4.66 (s, 1H), 4.56 (brs, 1H), 3.89–3.77 (m, 1H), 2.47–2.35 (m, 3H), 2.29–2.15 (m, 1H), 2.15–2.03 (m, 1H), 1.88–1.73 (m, 5H), 1.65 (s, 3H), 1.60–1.51 (m, 2H), 1.37–1.22 (m, 4H), 0.88 (t, J = 7.0 Hz, 3H) (Figure S1). $[\alpha]_D^{25}$=+126 (*c*. 0.05, 96% EtOH).

5. (6a*S*,10a*S*)-6,6,9-trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6*H*-benzo[*c*]chromen-1-ol, (+)-*trans*-Δ^9-THC

To a solution of resorcinol (1 eq.) and (1*R*,4*S*)-1-methyl-4-(prop-1-en-2-yl)cyclohex-2-enol (0.9 eq.) in dry DCM (10 mL), *p*TSA (10% mol) was added. The reaction was stirred at room temperature and in the dark for 4 h. After that, it was quenched with a saturated solution of $NaHCO_3$. The organic phase was washed with brine, dried over Na_2SO_4 and concentrated under vacuum. The obtained crude (350 mg) was purified over silica gel (ratio crude:silica 1:100, eluent CE:DCM 6:4) to give 70 mg of colourless liquid. (20% yield, 96% *ee* by HPLC-UV). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.31–6.28 (m, 1H), 6.27 (d, J = 1.6 Hz, 1H), 6.14 (d, J = 1.6 Hz, 1H), 4.73 (s, 1H), 3.25–3.14 (m, 1H), 2.50–2.37 (m, 2H), 2.23–2.12 (m, 2H), 1.94–1.85 (m, 1H), 1.71–1.64 (m, 4H), 1.59–1.52 (m, 2H), 1.43–1.39 (m, 4H), 1.32–1.28 (m, 4H), 1.09 (s, 3H), 0.88 (t, J = 7.0 Hz, 3H) (Figure S2). $[\alpha]_D^{25}$=+160 (*c*. 0.1, $CHCl_3$).

6. (1′*S*,2′*S*)-2,6-dihydroxy-5′-methyl-4-pentyl-2′-(prop-1-en-2-yl)-1′,2′,3′,4′-tetrahydro-[1,1′-biphenyl]-3-carboxylic acid, (+)-*trans*-CBDA

The conversion of (+)-*trans*-CBD into (+)-*trans*-CBDA has already been in a previous work by our group (39% yield, 99% *ee* by HPLC-UV) [13]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.93 (brs,1H), 6.61 (s, 1H), 6.25 (s, 1H), 5.55 (s, 1H), 4.54 (s, 1H), 4.39 (s, 1H), 4.08 (brd, 1H), 2.97–2.87 (m, 1H), 2.87–2.76 (m, 1H), 2.45–2.32 (m, 1H), 2.31–2.20 (m, 1H), 2.16–2.07 (m, 1H), 1.85–1.81 (m, 2H), 1.80 (s, 3H), 1.71 (s, 3H), 1.61–1.53 (m, 2H), 1.36–1.30 (m, 4H), 0.88 (t, J = 6.9 Hz, 3H) (Figure S3). $[\alpha]_D^{20}$=+60 (*c*. 0.05, methanol (MeOH)).

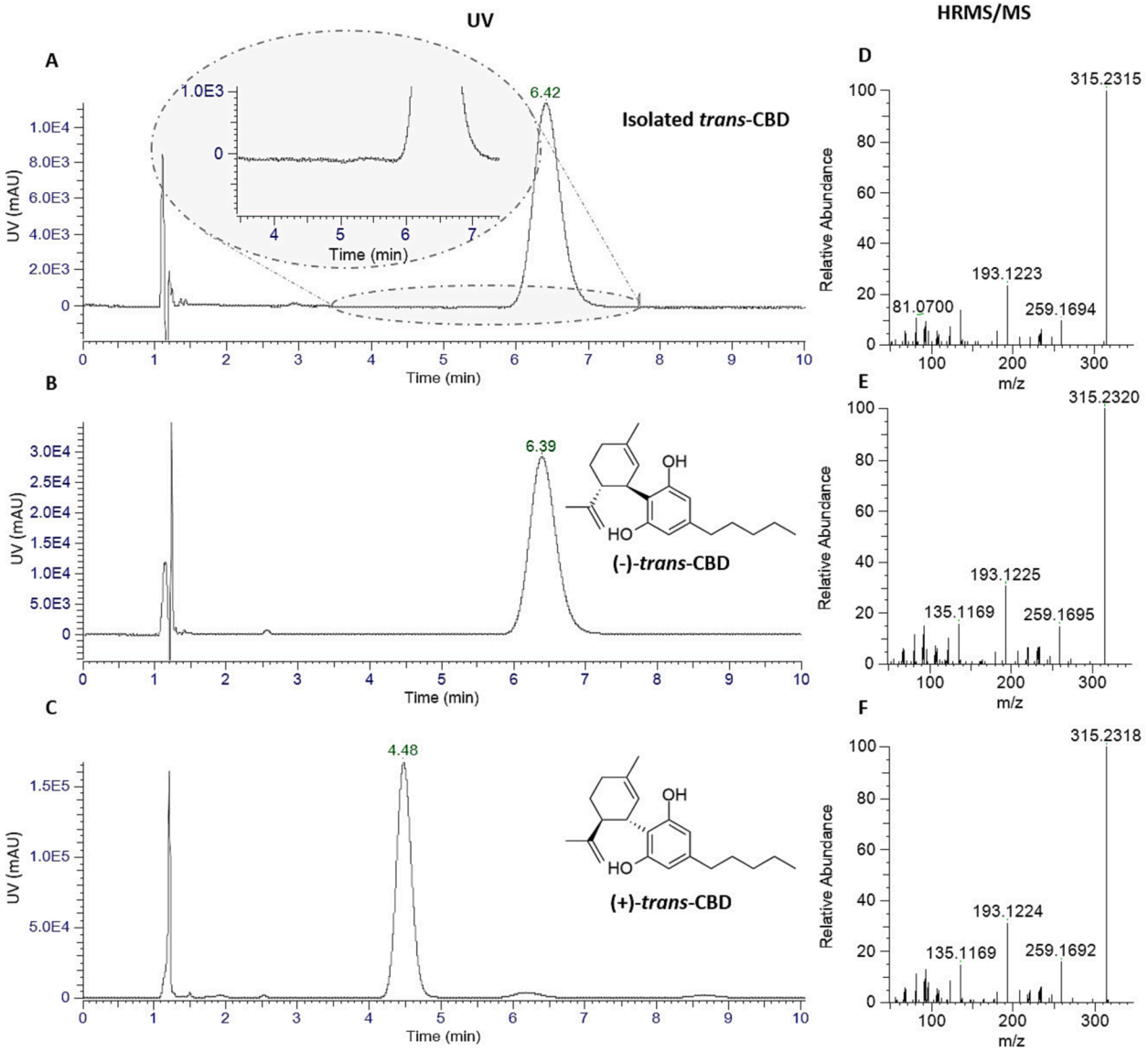

Fig. 4. Comparison of the UV traces and tandem HRMS spectra of the isolated *trans*-CBD and the pure synthetic (-) and (+) enantiomers of *trans*-CBD. UV traces at 270 nm of the isolated peak of *trans*-CBD (A), pure (-)-*trans*-CBD (B) and pure (+)-*trans*-CBD (C); HRMS/MS spectra in HESI + mode of the isolated peak of *trans*-CBD (D), pure (-)-*trans*-CBD (E) and pure (+)-*trans*-CBD (F).

7. (6a*S*,10a*S*)-1-hydroxy-6,6,9-trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6*H*-benzo[*c*]chromene-2-carboxylic acid, (+)-*trans*-Δ^9-THCA

As for (+)-*trans*-CBDA, (+)-*trans*-Δ^9-THC was converted into (+)-*trans*-THCA (10% yield, >99% *ee* by HPLC-UV) [13]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.23 (s, 1H), 6.39 (s, 1H), 6.25 (s, 1H), 3.22 (d, J = 7.0 Hz, 1H), 2.97–2.89 (m, 1H), 2.81–2.73 (m, 1H), 2.19–2.15 (m, 2H), 1.94–1.89 (m, 1H), 1.70–1.66 (m, 4H), 1.57 (s, 2H), 1.44 (s, 3H), 1.37–1.31 (m, 5H), 1.11 (s, 3H), 0.90 (t, J = 7.0 Hz, 3H) (Figure S4). $[\alpha]_D^{20}$=+206 (*c*. 5.0, chloroform ($CHCl_3$)).

8. Isolation of phytocannabinoids from FM2 extracts and HPLC-DAD-HRMS/MS analysis

HPLC analyses were performed on a Vanquish Core System equipped with a binary pump, a vacuum degasser, a thermostated autosampler, a thermostated column compartment and a DAD and interfaced to an Exploris 120 Orbitrap HRMS (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Achiral analysis was carried out with a Poroshell 120 EC-C18 (100 × 3.0 mm I.D., 2.7 μm) with guard (5 × 3 mm I.D., 2.7 μm) (both from Agilent Technologies, Milan, Italy) eluting water and ACN (both with 0.1% formic acid) as mobile phase. The following chromatographic conditions were applied: linear gradient from 60% to 95% ACN (with 0.1% formic acid) in 15 min, followed by isocratic elution at 95% ACN, washing step at 98% ACN for 3 min and re-equilibration with the initial conditions (total run time 26 min), maintaining the flow rate at 0.5 mL/min throughout the entire run. A post-column T-connection was added in order to split the eluent flow (Figure S5). The fractions corresponding to the phytocannabinoids of interest were collected from an output of the T-connection at a flow rate of 0.4 mL/min, while the eluent from the other output flowed at 0.1 mL/min towards the UV and MS detectors. The isolated fractions were then checked for purity under the same chromatographic conditions. The Orbitrap mass analyzer was equipped with a heated electrospray ionization source operating in both positive (HESI+) and negative (HESI-) mode. The former was employed for the detection of the decarboxylated species, while the latter was applied to the carboxylated compounds based on the best signal response obtained, which was compound-specific [16]. Simultaneous full scan (FS) and data-dependent

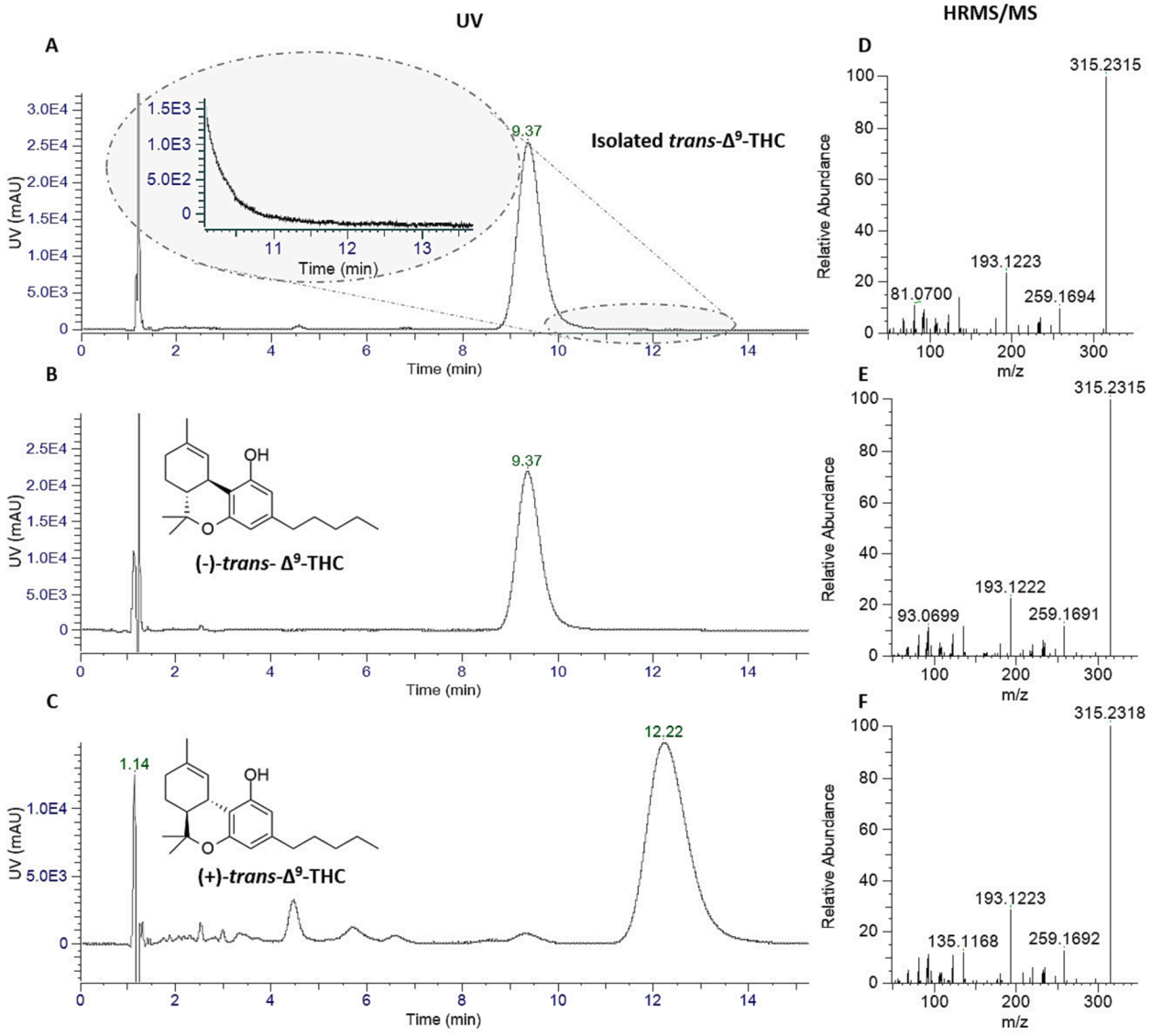

Fig. 5. Comparison of the UV traces and tandem HRMS spectra of the isolated *trans*-Δ^9-THC and the pure synthetic (-) and (+) enantiomers of *trans*-Δ^9-THC. UV traces at 270 nm of the isolated peak of *trans*-Δ^9-THC (A), pure (-)-*trans*-Δ^9-THC (B) and pure (+)-*trans*-Δ^9-THC (C); HRMS/MS spectra in HESI + mode of the isolated peak of *trans*-Δ^9-THC (D), pure (-)-*trans*-Δ^9-THC (E) and pure (+)-*trans*-Δ^9-THC (F).

acquisition (DDA) experiments were run with the parameters optimized by direct infusion of the pure analytes from a syringe pump. For the HESI source: capillary temperature, 390 °C; vaporizer temperature, 150 °C; electrospray voltage, 4.2 kV (HESI +) and 3.8 kV (HESI-); sheath gas, 70 arbitrary units (au); auxiliary gas, 5 au; sweep gas, 70au; S lens RF level, 70%. For the analyzer: resolution, 60,000 FWHM (full width at half maximum) at *m/z* 200 FS mode and 30,000 FWHM for DDA mode; scan range, *m/z* 75–750; maximum injection time, 54 ms for FS mode and 22 ms for DDA mode; isolation window, *m/z* 0.7 for FS mode and *m/z* 1.2 for DDA mode; stepped NCE (normalized collision energy), 20–40-100.

The sample volume injected for the analyses was 5 μL. The analytes precursor ions $[M-H]^-$ and $[M+H]^+$ were extracted with a 5-ppm mass tolerance from the total ion current. The analyses were acquired with Xcalibur 3.0 (Thermo Fisher Scientific) and processed using FreeStyle 1.7 (Thermo Fisher Scientific).

9. Development and optimization of the chiral HPLC-DAD method

The following CSPs were tested to achieve the optimal chromatographic resolution of the (+) and (-) enantiomers of the four phytocannabinoids under investigation: CHIRALCEL OD-R [cellulose tris (3,5-dimethylphenylcarbamate)] (250 × 4.6 mm I.D., 10 μm), CHIRALCEL OB-H [cellulose tribenzoate] (250 × 4.6 mm I.D., 5 μm), CHIRALCEL OJ-H [cellulose tris (4-methylbenzoate)] (250 × 4.6 mm I. D., 5 μm), CHIRALPAK AD [amylose tris (3,5-dimethylphenylcarbamate)] (250 × 4.6 mm I.D., 10 μm), and CHIRALPAK AD-RH [amylose tris (3,5-dimethylphenylcarbamate)] (150 × 4.6 mm I.D., 5 μm) (all from Daicel supplied by Chiral Technologies Europe S.A.S, France). Also, the effect of the nature and percentage of the organic modifier, as well as the temperature of the column oven, were screened in order to find the optimal separation conditions. Either ACN, EtOH or 2-propanol (*i*-PrOH) were alternatively tested as organic modifier. The chromatographic output was monitored by DAD at two wavelengths, 274 nm for the decarboxylated species and at 306 nm for the carboxylated ones. During the development and optimization step the chromatographic parameters were calculated for each compound in each analytical run: the retention factors (k_1 and k_2) were calculated as $k_1 = (t_1 - t_0)/t_0$, where t_1 and t_2 are the retention times of the first and second eluted enantiomers, the separation factor (α) was calculated as k_2/k_1 and the resolution factor (R_s)

was calculated using the formula $R_s = (t_2 - t_1)/(w_1 + w_2)$, where w_1 and w_2 are the peak widths at the base for the first and second eluted enantiomer.

10. Results and discussion

10.1. Synthesis and characterization of (+)-trans-CBDA, (+)-trans-Δ⁹-THCA, (+)-trans-CBD and (+)-trans-Δ⁹-THC

The evaluation of the chiral composition of phytocannabinoids in plant material requires the use of proper standards for all the stereoisomeric forms of the analytes under investigation. The lack of commercially available standards for the (+) enantiomers has so far limited the extent of such investigation. The ICCA, for example with the Pirkle-type CSPs with both HPLC and SFC, has been explored to evaluate the presence of the (+)-*trans* enantiomer of Δ^9-THC in the Bedrocan medicinal cannabis variety [24], as well as for the evaluation of the stereoisomeric composition of *cis*-Δ^9-THC in different plant samples [23]. This approach proved to be useful also when only one enantiomer is available [23,24]. Unfortunately, the studies on the chiral composition of phytocannabinoids in plants are very few and all deal with the decarboxylated forms obtained after heating the samples at high temperatures. As the extraction process could affect the configurational stability, it becomes important to understand the original stereoisomeric composition in the plant material. To this end, the (+) enantiomers of *trans*-CBDA, *trans*-Δ^9-THCA, *trans*-CBD and *trans*-Δ^9-THC were obtained by a stereoselective synthetic procedure. In particular, a Friedel-Craft allylation of 5-pentylbenzene-1,3-diol with (1*R*,4*S*)-1-methyl-4-(prop-1-en-2-yl)cycloex-2-enol, using *p*TSA as catalyst (Fig. 1a) allowed to access the decarboxylated forms, which were then used to obtain the carboxylated ones (Fig. 1b) following a procedure reported in previous works [13,35].

The chemical structure of synthetic standards was confirmed by ^{1}H NMR spectroscopy and polarimetric analysis. The perfect match between the monodimensional ^{1}H NMR spectrum of synthetic (+)-*trans*-Δ^9-THC and the spectroscopic data reported by Schafroth et al. [36], confirmed its chemical structure. Similarly, the comparison of ^{1}H NMR spectra of (+)-*trans*-CBD, (+)-*trans*-CBDA and (+)-*trans*-Δ^9-THCA with those of the corresponding (-)-enantiomers reported in the literature [37] ensured their chemical structure (Supporting information). Lastly, the (+) configuration was confirmed by measuring the optical rotation power for each compound and comparing it to that of the (-) enantiomers.

11. Development and optimization of the chiral HPLC-DAD method

With the pure stereoisomers in hand, we tested various CSPs varying different chromatographic parameters to find the optimal separation conditions. First, both cellulose and amylose based CSPs were employed in the method development step and different column lengths and particle sizes were screened. In general, amylose based CSPs performed better than the cellulose-based ones. Moreover, a smaller particle size (5 μm) guaranteed a better resolution compared to the larger one (10 μm). Therefore, CHIRALPAK AD-RH resulted the optimal solution for all analytes. Several mobile phases were screened for the separation and we could not find one mobile phase optimal for all phytocannabinoids. All chiral chromatographic methods reported in the literature involve only normal phase conditions (NP-HPLC), while RP-HPLC is generally employed for achiral separation of cannabinoids [38]. It is noteworthy that NP conditions can be also used to separate phytocannabinoids with the advantage of using higher flow rates compared to RP conditions thanks to low-viscosity solvents [39]. On the other hand, RP conditions have also been used with CSPs to improve separation of phytocannabinoids and study their retention behaviour on different carbamate polysaccharide-based CSPs [40,41]. Only one record presents a successful chiral resolution of *trans*-CBD enantiomers using RP conditions [42]. The advantage of working in RP conditions is the compatibility of the solvents with the ESI-MS detector, which is particularly useful in the analysis of complex matrices, such as cannabis extracts. In this work, ACN, EtOH and *i*-PrOH (all with 0.1% of formic acid) were used as organic modifier; their percentage with respect to water was decided according to the lipophilicity of the compound: for example, CBD generally elutes earlier than THC, which requires higher percentages of the organic component, and this applies also to their carboxylated counterpart. As a general trend, the decrease in temperature did not improve the enantiomeric resolution. Eventually, the enantiomers of *trans*-CBD and *trans*-Δ^9-THC were separated with 60% ACN setting the column compartment temperature at 30 °C and the flow rate at 1.5 mL/min. The presence of the additional carboxylic group in CBDA and THCA makes these compounds difficult to separate with ACN, thus shifting the attention to either EtOH or *i*-PrOH. A high percentage of EtOH (80%) gave good results for the separation of the enantiomers of *trans*-Δ^9-THCA, while it was unable to separate those of *trans*-CBDA. The attempt to use 50% *i*-PrOH instead of EtOH proved to be beneficial for *trans*-CBDA, but was insufficient for *trans*-Δ^9-THCA, whose enantiomers eluted in the washing step. The optimal conditions were found in 50% *i*-PrOH for *trans*-CBDA and 75% *i*-PrOH for *trans*-Δ^9-THCA, both at 30 °C and 1 mL/min. All the experimental conditions are reported in Table 1.

12. Evaluation of the enantiomeric composition of *trans*-CBDA and *trans*-Δ⁹-THCA in *cannabis sativa* L.

The assessment of the enantiomeric composition of the main phytocannabinoids *trans*-CBDA and *trans*-Δ^9-THCA in plants has never been investigated. Attempts were made only on their decarboxylated derivatives and only after a heating step at high temperatures. In order to avoid the variable of the extraction methodology, which could affect the stereostability of the analytes, the analyses in this work were carried out on the unheated ethanol extract of the FM2 variety. Additionally, the chiral evaluation was not performed on the total crude extract since potential interfering compounds could elute at the same retention time of the enantiomers of the analytes. This is particularly important in phytocannabinoids analysis as many compounds are isobaric and present the same MS fragmentation spectrum [43]. Therefore, the compounds of interest *trans*-CBDA and *trans*-Δ^9-THCA were isolated by analytical achiral chromatography by adding a post-column T-connection, which allowed to collect the pure compounds and bring the eluent at a lower flow rate to the UV and MS detectors. Starting from the unheated and undiluted FM2 extract characterized by 55 mg/g of *trans*-CBDA and 33 mg/g of *trans*-Δ^9-THCA, five 10 μL injections were sufficient to obtain 500 μL of *trans*-CBDA and 350 μL of *trans*-Δ^9-THCA. Each phytocannabinoid solution was brought to the final concentration of 100 μg/mL and checked on the C_{18} column with both UV and HRMS detectors in order to discard the potential presence of interferences. The purified compounds were then analysed with the CSP using the previously optimized conditions. The corresponding pure enantiomers were always injected for comparison and the match of UV and MS/MS spectra were useful for the unambiguous identification. At the limit of detection of the method (0.005 μg/mL), both isolated phytocannabinoids resulted enantiomerically pure with the (-)-*trans* being the detected enantiomer (>99% *ee*) (Figs. 2 and 3).

In order to understand whether the decarboxylation process could lead to a stereoconversion of the pure enantiomers of *trans*-CBDA and *trans*-Δ^9-THCA, the plant material was heated at 130 °C for 2 h. The decarboxylated undiluted extract, characterized by 37 mg/g of *trans*-CBD and 24 mg/g of *trans*-Δ^9-THC, was injected into the achiral chromatographic system to isolate the single peaks of *trans*-CBD and *trans*-Δ^9-THC as previously done for their carboxylated precursors. The chiral chromatographic analysis was then performed to assess their enantiomeric composition. Both samples containing either *trans*-CBD or *trans*-Δ^9-THC at the concentration of 100 μg/mL did not show the presence of

the (+) enantiomer at least at the limit of detection of the method (0.05 μg/mL) (Figs. 4 and 5), suggesting that the decarboxylation in the conditions applied did not affect the stereostability of the investigated compounds.

13. Conclusions

The extent of the evaluation of the chiral composition of phytocannabinoids in the plant material has always been limited due to the unavailability of the analytical standards of the (+) enantiomers of carboxylated phytocannabinoids. This work addresses for the first time the synthesis of the pure (+) enantiomers of the main carboxylated and decarboxylated phytocannabinoids, *trans*-CBDA, *trans*-Δ^9-THCA, *trans*-CBD and *trans*-Δ^9-THC. These were used to develop and optimize an HPLC-UV-HRMS/MS method in RP conditions, which allowed convenient coupling with the MS detection. Moreover, the achiral-chiral chromatographic method for the isolation and chiral analysis of the single peak proved to be useful to avoid the presence of interfering compounds co-eluting with the enantiomers peaks that could lead to misinterpretation and erroneous identification. At the limit of the sensitivity of the developed method we found that the main phytocannabinoids, *trans*-CBDA, *trans*-Δ^9-THCA, *trans*-CBD and *trans*-Δ^9-THC, are all present as single (-) enantiomers and no trace of the (+) enantiomers was detected in the Italian medicinal cannabis variety FM2. Lastly, the great advantage of the developed achiral-chiral chromatographic method is the possibility to disclose the presence of enantiomeric impurities of single peaks without heating the starting material, which would otherwise lead to an altered phytocannabinoid composition.

Funding

This work was partially funded by UNIHEMP research project "Use of iNdustrIal Hemp biomass for Energy and new biocheMicals Production" (ARS01_00668) funded by Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR) and Fondo Europeo di Sviluppo Regionale (FESR) (within the PON R&I 2017–2020 – Axis 2 – Action II – OS 1.b). Grant decree UNIHEMP prot. n. 2016 of 27/07/2018; CUP B76C18000520005.

The work was also partially supported by the MIUR project "ECOTEC - ECO-sustainable and intelligent fibers and fabrics for TEChnic clothing", PON «R&I»

2014–2020, project N° ARS01_00951, CUP B66C18000300005.

CRediT authorship contribution statement

Fabiana Russo: Investigation, Formal analysis. **Francesco Tolomeo:** Investigation, Formal analysis. **Maria Angela Vandelli:** Data curation. **Giuseppe Biagini:** Data curation. **Aldo Laganà:** Investigation, Formal analysis. **Anna Laura Capriotti:** Investigation, Formal analysis. **Andrea Cerrato:** Investigation, Formal analysis. **Luigi Carbone:** Data curation. **Elisabetta Perrone:** Data curation. **Alberto Cavazzini:** Investigation, Formal analysis. **Vincenzo Maiorano:** Data curation, Resources. **Giuseppe Gigli:** Resources. **Giuseppe Cannazza:** Conceptualization, Project administration, Methodology, Writing – original draft, Funding acquisition, Writing – review & editing. **Cinzia Citti:** Conceptualization, Project administration, Methodology, Writing – original draft.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jchromb.2023.123682.

References

[1] P.R. Cohen, Therapeutic and Cosmetic Uses of Cannabis: Cannabinoids for Acne Treatment and Skin -Rejuvenation, Skinmed (2021) 45–47.
[2] P. Cerino, C. Buonerba, G. Cannazza, J. D'Auria, E. Ottoni, A. Fulgione, A.D. Stasio, B. Pierri, A. Gallo, A Review of Hemp as Food and Nutritional Supplement, Cannabis and cannabinoid research 6 (2021) 19–27.
[3] P. Kumar, D.K. Mahato, M. Kamle, R. Borah, B. Sharma, S. Pandhi, V. Tripathi, H. S. Yadav, S. Devi, U. Patil, J. Xiao, A.K. Mishra, Pharmacological properties, therapeutic potential, and legal status of Cannabis sativa L.: An overview, Phytother. Res. 35 (2021) 6010–6029.
[4] C. Pagano, G. Navarra, L. Coppola, G. Avilia, M. Bifulco, C. Laezza, Cannabinoids: Therapeutic Use in Clinical Practice, International Journal of Molecular Sciences 23 (2022) 3344.
[5] R.G. dos Santos, J.E.C. Hallak, J.A.S. Crippa, Neuropharmacological Effects of the Main Phytocannabinoids: A Narrative Review, in: E. Murillo-Rodriguez, S.R. Pandi-Perumal, J.M. Monti (Eds.), Cannabinoids and Neuropsychiatric Disorders, Springer International Publishing, Cham, 2021, pp. 29–45.
[6] United Nations Convention on Psychotropic Substances, Austria, 1971.
[7] WHO Expert Committee on Drug Dependence, fortieth report, WHO Technical Report Series, N. 1013, Geneva: World Health Organization, 2018, pp. 25-27.
[8] C. Citti, P. Linciano, F. Forni, M.A. Vandelli, G. Gigli, A. Laganà, G. Cannazza, Analysis of impurities of cannabidiol from hemp. Isolation, characterization and synthesis of cannabidibutol, the novel cannabidiol butyl analog, J. Pharm. Biomed. Anal. 175 (2019), 112752.
[9] C. Citti, P. Linciano, F. Forni, M.A. Vandelli, G. Gigli, A. Laganà, G. Cannazza, Chemical and spectroscopic characterization data of 'cannabidibutol', a novel cannabidiol butyl analog, Data in Brief 26 (2019), 104463.
[10] F. Tolomeo, F. Russo, M.A. Vandelli, G. Biagini, A.L. Capriotti, A. Laganà, L. Carbone, G. Gigli, G. Cannazza, C. Citti, HPLC-UV-HRMS analysis of cannabigerovarin and cannabigerobutol, the two impurities of cannabigerol extracted from hemp, J. Pharm. Biomed. Anal. 203 (2021), 114215.
[11] P. Linciano, C. Citti, F. Russo, F. Tolomeo, A. Laganà, A.L. Capriotti, L. Luongo, M. Iannotta, C. Belardo, S. Maione, F. Forni, M.A. Vandelli, G. Gigli, G. Cannazza, Identification of a new cannabidiol n-hexyl homolog in a medicinal cannabis variety with an antinociceptive activity in mice: cannabidihexol, Sci. Rep. 10 (2020) 22019.
[12] P. Linciano, C. Citti, L. Luongo, C. Belardo, S. Maione, M.A. Vandelli, F. Forni, G. Gigli, A. Laganà, C.M. Montone, G. Cannazza, Isolation of a high-affinity cannabinoid for the human CB1 receptor from a medicinal Cannabis sativa variety: Δ9-Tetrahydrocannabutol, the butyl homologue of Δ9-Tetrahydrocannabinol, J. Nat. Prod. 83 (2020) 88–98.
[13] P. Linciano, F. Russo, C. Citti, F. Tolomeo, R. Paris, F. Fulvio, N. Pecchioni, M. A. Vandelli, A. Laganà, A.L. Capriotti, G. Biagini, L. Carbone, G. Gigli, G. Cannazza, The novel heptyl phorolic acid cannabinoids content in different Cannabis sativa L. accessions, Talanta 235 (2021), 122704.
[14] C. Citti, P. Linciano, F. Russo, L. Luongo, M. Iannotta, S. Maione, A. Laganà, A. L. Capriotti, F. Forni, M.A. Vandelli, G. Gigli, G. Cannazza, A novel phytocannabinoid isolated from Cannabis sativa L. with an in vivo cannabimimetic activity higher than Δ9-tetrahydrocannabinol: Δ9-Tetrahydrocannabiphorol, Sci. Rep. 9 (2019) 20335.
[15] C.M. Montone, A. Cerrato, B. Botta, G. Cannazza, A.L. Capriotti, C. Cavaliere, C. Citti, F. Ghirga, S. Piovesana, A. Laganà, Improved identification of phytocannabinoids using a dedicated structure-based workflow, Talanta 219 (2020), 121310.
[16] A. Cerrato, C. Citti, G. Cannazza, A.L. Capriotti, C. Cavaliere, G. Grassi, F. Marini, C.M. Montone, R. Paris, S. Piovesana, A. Laganà, Phytocannabinomics: Untargeted metabolomics as a tool for cannabis chemovar differentiation, Talanta 230 (2021), 122313.
[17] M.N. Tahir, F. Shahbazi, S. Rondeau-Gagné, J.F. Trant, The biosynthesis of the cannabinoids, Journal of Cannabis Research 3 (2021) 7.
[18] O.-E. Schultz, G. Haffner, Zur Frage der Biosynthese der Cannabinole, Arch. Pharm. 293 (1960) 1–8.
[19] T. Yamauchi, Y. Shoyama, H. Aramaki, T. Azuma, I. Nishioka, Tetrahydrocannabinolic acid, a genuine substance of tetrahydrocannabinol, Chem. Pharm. Bull. (Tokyo) 15 (1967) 1075–1076.
[20] L.O. Hanuš, S.M. Meyer, E. Muñoz, O. Taglialatela-Scafati, G. Appendino, Phytocannabinoids: a unified critical inventory, Nat. Prod. Rep. 33 (2016) 1357–1392.
[21] M. Maccarrone, R. Maldonado, M. Casas, T. Henze, D. Centonze, Cannabinoids therapeutic use: what is our current understanding following the introduction of THC, THC:CBD oromucosal spray and others? Expert Rev. Clin. Pharmacol. 10 (2017) 443–455.

[22] A. Talwar, E. Estes, R. Aparasu, D.S. Reddy, Clinical efficacy and safety of cannabidiol for pediatric refractory epilepsy indications: A systematic review and meta-analysis, Exp. Neurol., DOI https://doi.org/10.1016/j.expneurol.2022.114238(2022) 114238.
[23] M.A. Schafroth, G. Mazzoccanti, I. Reynoso-Moreno, R. Erni, F. Pollastro, D. Caprioglio, B. Botta, G. Allegrone, G. Grassi, A. Chicca, F. Gasparrini, J. Gertsch, E.M. Carreira, G. Appendino, Δ9-cis-Tetrahydrocannabinol: Natural Occurrence, Chirality, and Pharmacology, J. Nat. Prod. 84 (2021) 2502–2510.
[24] G. Mazzoccanti, O.H. Ismail, I. D'Acquarica, C. Villani, C. Manzo, M. Wilcox, A. Cavazzini, F. Gasparrini, Cannabis through the looking glass: chemo- and enantio-selective separation of phytocannabinoids by enantioselective ultra high performance supercritical fluid chromatography, Chemical Communications 53 (2017) 12262–12265.
[25] U.M. Battisti, C. Citti, M. Larini, G. Ciccarella, N. Stasiak, L. Troisi, D. Braghiroli, C. Parenti, M. Zoli, G. Cannazza, "Heart-cut" bidimensional achiral-chiral liquid chromatography applied to the evaluation of stereoselective metabolism, in vivo biological activity and brain response to chiral drug candidates targeting the central nervous system, J. Chromatogr. A 1443 (2016) 152–161.
[26] U.M. Battisti, C. Citti, G. Rastelli, L. Pinzi, G. Puja, F. Ravazzini, G. Ciccarella, D. Braghiroli, G. Cannazza, An unexpected reversal in the pharmacological stereoselectivity of benzothiadiazine AMPA positive allosteric modulators, MedChemComm 7 (2016) 2410–2417.
[27] I. González-Mariscal, B. Carmona-Hidalgo, M. Winkler, J.D. Unciti-Broceta, A. Escamilla, M. Gómez-Cañas, J. Fernández-Ruiz, B.L. Fiebich, S.-Y. Romero-Zerbo, F.J. Bermúdez-Silva, J.A. Collado, E. Muñoz, (+)-trans-Cannabidiol-2-hydroxy pentyl is a dual CB1R antagonist/CB2R agonist that prevents diabetic nephropathy in mice, Pharmacological Research 169 (2021), 105492.
[28] A.C. Howlett, F. Barth, T.I. Bonner, G. Cabral, P. Casellas, W.A. Devane, C. C. Felder, M. Herkenham, K. Mackie, B.R. Martin, R. Mechoulam, R.G. Pertwee, International Union of Pharmacology. XXVII. Classification of Cannabinoid Receptors, Pharmacol. Rev. 54 (2002) 161–202.
[29] S. Levin, S. Abu-Lafi, J. Zahalka, R. Mechoulam, Resolution of chiral cannabinoids on amylose tris (3, 5-dimethylphenylcarbamate) chiral stationary phase: Effects of structural features and mobile phase additives, J. Chromatogr. A 654 (1993) 53–64.
[30] S. Levin, M. Sterin, S. Abu-lafi, Structural features affecting chiral resolution of cannabimimetic enantiomers by amylose 3, 5-dimethylphenylcarbamate chiral stationary phase, Chirality 7 (1995) 140–146.
[31] S. Abu-Lafi, M. Sterin, S. Levin, Role of hydroxyl groups in chiral recognition of cannabinoids by carbamated amylose, J. Chromatogr. A 679 (1994) 47–58.
[32] V. Schurig, The Reciprocal Principle of Selectand-Selector-Systems in Supramolecular Chromatography †, Molecules 21 (2016) 1535.
[33] C. Citti, D. Braghiroli, M.A. Vandelli, G. Cannazza, Pharmaceutical and biomedical analysis of cannabinoids: A critical review, J. Pharm. Biomed. Anal. 147 (2018) 565–579.
[34] Cannabis Flos; New Text of the German Pharmacopoeia, Bonn, Germany, 2018.
[35] R. Mechoulam, Z. Ben-Zvi, Carboxylation of resorcinols with methylmagnesium carbonate. Synthesis of cannabinoid acids, J. Chem. Soc., Chem. Commun., DOI 10.1039/C29690000343(1969) 343-344.
[36] M.A. Schafroth, G. Zuccarello, S. Krautwald, D. Sarlah, E.M. Carreira, Stereodivergent Total Synthesis of Δ9-Tetrahydrocannabinols, Angewandte Chemie International Edition 53 (2014) 13898–13901.
[37] Y.H. Choi, A. Hazekamp, A.M.G. Peltenburg-Looman, M. Frédérich, C. Erkelens, A. W.M. Lefeber, R. Verpoorte, NMR assignments of the major cannabinoids and cannabiflavonoids isolated from flowers of Cannabis sativa, Phytochem. Anal. 15 (2004) 345–354.
[38] W.J. Umstead, Polysaccharide Chiral Stationary Phases for the Achiral and Chiral Separation of Cannabinoids, DOI.
[39] C. De Luca, A. Buratti, Y. Krauke, S. Stephan, K. Monks, V. Brighenti, F. Pellati, A. Cavazzini, M. Catani, S. Felletti, Investigating the effect of polarity of stationary and mobile phases on retention of cannabinoids in normal phase liquid chromatography, Anal. Bioanal. Chem. 414 (2022) 5385–5395.
[40] C. De Luca, A. Buratti, W. Umstead, P. Franco, A. Cavazzini, S. Felletti, M. Catani, Investigation of retention behavior of natural cannabinoids on differently substituted polysaccharide-based chiral stationary phases under reversed-phase liquid chromatographic conditions, J. Chromatogr. A 1672 (2022), 463076.
[41] T. Onishi, W.J. Umstead, The Separation of Cannabinoids on Sub-2 μm Immobilized Polysaccharide Chiral Stationary Phases, Pharmaceuticals (Basel) 14 (2021).
[42] J.U. Weston, The Chiral Separation of the (+) and (-) Enantiomers of Cannabidiol, Cannabis, Science and Technology 5 (2022) 30–34.
[43] A.L. Capriotti, G. Cannazza, M. Catani, C. Cavaliere, A. Cavazzini, A. Cerrato, C. Citti, S. Felletti, C.M. Montone, S. Piovesana, A. Laganà, Recent applications of mass spectrometry for the characterization of cannabis and hemp phytocannabinoids: From targeted to untargeted analysis, J. Chromatogr. A 1655 (2021), 462492.