Electronic Supplementary Material (ESI) for ChemComm.

Cannabis through the looking glass: chemo- and enantio-selective separation of phytocannabinoids by enantioselective Ultra High Performance Supercritical Fluid Chromatography

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ELECTRONIC SUPPLEMENTARY INFORMATION (ESI)

EXPERIMENTAL SECTION

All the analyses were performed on a Waters Acquity UPC2 (Ultra Performance Convergence Chromatography) instrument. The system was supplied with a binary solvent delivery pump compatible with flow rates up to 4 ml/min and maximum system pressure of 1600 psi. The delivery system was composed of a 250 µL mixing chamber. The system also included an autosampler with a 10 µL loop, a column oven that reached temperatures up to 90 °C, a UV detector equipped with an 8 µL flow–cell and an automated back pressure regulator (ABPR). The injector/column inlet and column/detector connection tubes were 600 mm long and had an I.D. of 0.175 mm. The extra-column and dwell volumes of this instrument were estimated to be 60 µL and 440 µL, respectively. [1S] Data acquisition and control of the UHPSFC system was performed with the Empower 3 software by Waters.

Cannabinoids reference standards, namely (–)-cannabidivarin (CBDV), (–)-cannabidiol (CBD), (–)-Δ^8-tetrahydrocannabinol (Δ^8-THC), (–)-Δ^9-tetrahydrocannabinol (Δ^9-THC), (±)-Δ^9-tetrahydro-cannabinol (Δ^9-THC), (±)-cannabichromene (CBC), cannabigerol (CBG), and cannabinol (CBN) were purchased from Cerilliant (Round Rock, Texas, USA) as methanol solutions (0.1–1.0 mg/ml) with a ≥ 99 % purity . HPLC quality methanol was purchased from Sigma Aldrich (St. Louis, MO, USA), whereas grade 5.5 carbon dioxide was from Gruppo SAPIO (Milan, Italy). All the solvents were filtered before use on a 0.2 µm filter.

Analytical liquid chromatography was performed on a JASCO chromatograph equipped with a Rheodyne model 7725i 20 µL loop injector, PU-1580-CO2 and PU-980 HPLC pumps, a Jasco Mod 975 single wavelength absorbance detector and a Jasco Mod 995-CD circular dichroism detector. Chromatographic data were collected and processed using Borwin software (Jasco Europe, Italy).

[1S] A. Grand-Guillaume Perrenoud, C. Hamman, M. Goel, J.-L. Veuthey, D. Guillarme, Comparison of ultra-high performance supercritical fluid chromatography and ultra-high performance liquid chromatography for the analysis of pharmaceutical compounds, J. Chromatogr. A, 2012, 1266, 158–167.

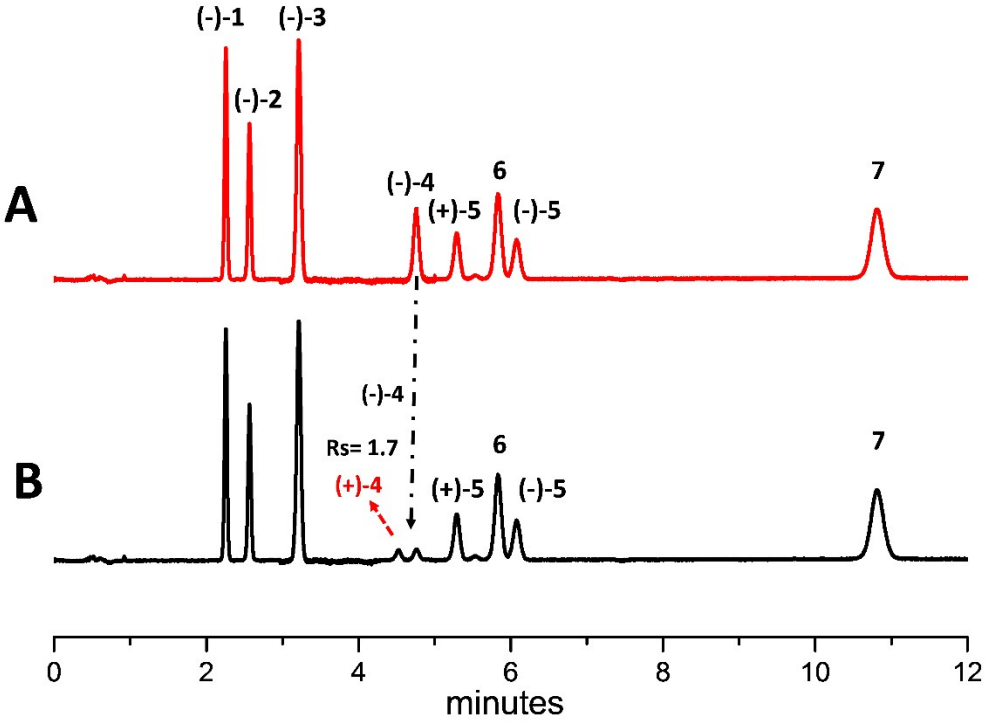

Figure S1. Chemo- and enantio-selective separation of seven phytocannabinoid standards by *e*-UHPSFC on the UHPC-(*S*,*S*)-Whelk-O1 column 1.8 µm (100 × 4.6 mm I.D.).

Mobile phase: CO_2/MeOH = 98:2; flow-rate: 3.5 ml/min; T = 30 °C; ABPR = 1500 psi; detection: UV at 214 nm.

Trace A = standards mixture made by (–)-**1**, (–)-**2**, (–)-**3**, (–)-**4**, (±)-**5**, **6**, **7**.

Trace B = standards mixture made by (–)-**1**, (–)-**2**, (–)-**3**, (±)-**4**, (±)-**5**, **6**, **7**.

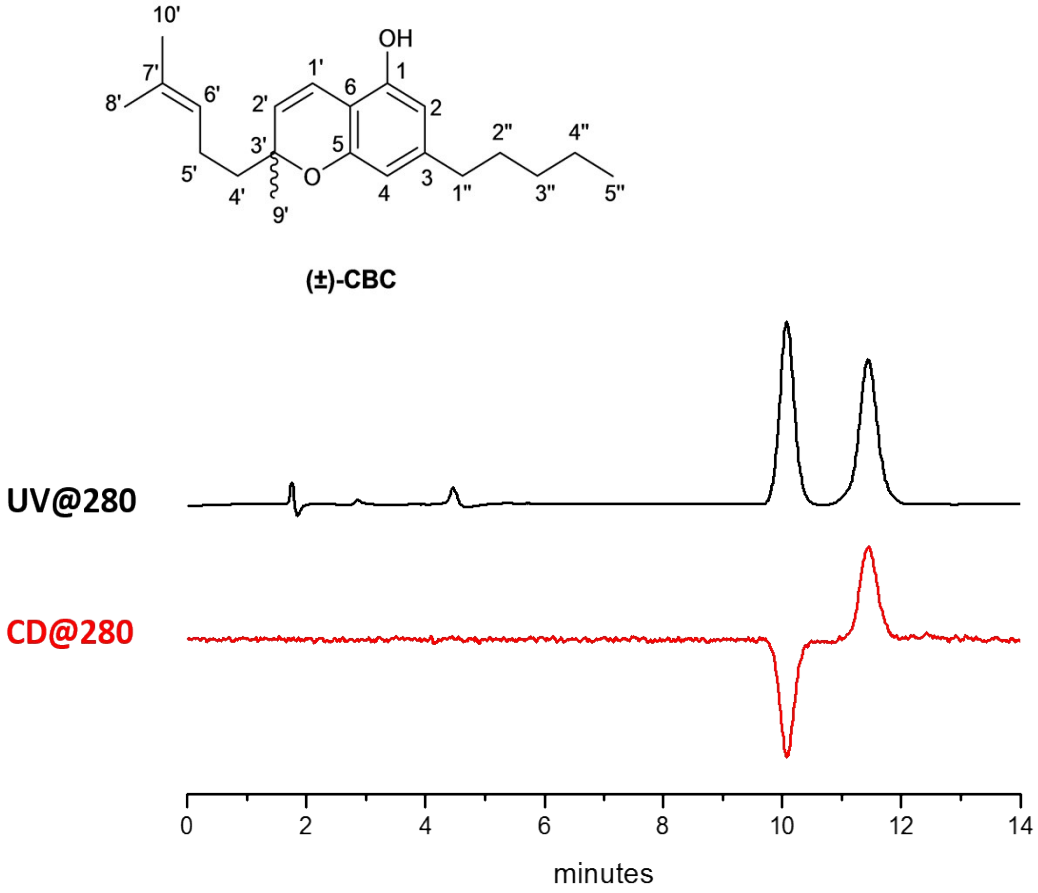

Figure S2. Simultaneous UV and CD detection for racemic cannabichromene (CBC) by enantioselective HPLC on the (*R*,*R*)-Whelk-O1 column 2.5 µm (150 × 4.6 mm I.D.).
Mobile phase: *n*-hexane/ethanol = 99.5:0.5 (v/v) + 0.05% methanol; flow-rate: 1.2 ml/min; T = 30 °C; detection: UV (top) and CD (bottom) at 280 nm.

The use of a different column with respect to that used under UHPSFC conditions is due to the larger volume shown by the UV/CD detector.

Table S1. Chromatographic data for the chemo- and enantio-selective separation of six phytocannabinoid standards by *e*UHPSFC on the UHPC-Whelk-O1 columns 1.8 µm (100 × 4.6 mm I.D.).

CSP config.	Peak name	R_t (min)	*k'*	α	R_s
(*R*,*R*)	(–)-**1**	2.13	5.47	–	–
(*S*,*S*)	(–)-**1**	2.20	5.69	–	–
(*R*,*R*)[a]	(–)-**1**	2.13	5.47	–	–
	(+)-**1**	2.20	5.69	1.04	1.10
(*R*,*R*)	(–)-**2**	2.40	6.29	–	–
(*S*,*S*)	(–)-**2**	2.51	6.63	–	–
(*R*,*R*)[a]	(–)-**2**	2.40	6.29	–	–
	(+)-**2**	2.51	6.63	1.05	1.30
(*R*,*R*)	(–)-**4**	4.43	12.47	–	–
	(+)-**4**	4. 70	13.28	1.06	1.72
(*S*,*S*)	(+)-**4**	4.44	12.50	–	–
	(–)-**4**	4.67	13.19	1.06	1.69
(*R*,*R*)	[CD(–)280]-**5**[b]	5.20	14.81	–	–
	[CD(+)280]-**5**[b]	5.96	17.12	1.16	5.11
(*S*,*S*)	[CD(+)280]-**5**[b]	5.20	14.81	–	–
	[CD(–)280]-**5**[b]	5.97	17.15	1.16	4.44
(*R*,*R*)	**6**	5.84	16.75	–	–
(*S*,*S*)	**6**	5.87	16.84	–	–
(*R*,*R*)	**7**	10.64	31.34	–	–
(*S*,*S*)	**7**	10.64	31.34	–	–

[a] The elution order has been simulated on the CSP with the indicated configuration.

[b] Plus and minus signs refer to the signs of the circular dichroism (CD) band at the indicated wavelength.

Table S2. Chromatographic data for the analysis of a crude plant ethanol extract (Bedrocan®) by *e*UHPSFC on the UHPC-Whelk-O1 columns 1.8 µm (100 × 4.6 mm I.D.).

CSP config.	Peak name		R_t (min)	*k'*	α	R_s
(*R*,*R*)	(–)-**2**		2.38	6.23	–	–
(*S*,*S*)	(–)-**2**		2.50	6.60	–	–
(*R*,*R*)[a]	Simulated (±)-**2**	(–)-**2**	2.38	6.23	–	–
		(+)-**2**	2.50	6.60	1.05	1.30
(*R*,*R*)	(–)-**4**		4.38	12.32	–	–
	(+)-**4**		n.d.	n.d.	–	–
(*S*,*S*)	(+)-**4**		4.37	12.28	–	–
	(–)-**4**		4.63	13.07	1.06	2.60
(*R*,*R*)	[CD(–)280]-**5**[b]		5.17	14.71	–	–
	[CD(+)280]-**5**[b]		5.95	17.09	1.16	5.11
(*S*,*S*)	[CD(+)280]-**5**[b]		5.18	14.74	–	–
	[CD(–)280]-**5**[b]		5.97	17.15	1.16	4.44
(*R*,*R*)	**6**		5.71	16.35	–	–
(*S*,*S*)	**6**		5.71	16.35	–	–
(*R*,*R*)	**7**		10.65	31.38	–	–
(*S*,*S*)	**7**		10.66	31.40	–	–

[a] The elution order has been simulated on the CSP with the indicated configuration.

[b] Plus and minus signs refer to the signs of the circular dichroism (CD) band at the indicated wavelength.