In-Depth Cannabis Multiclass Metabolite Profiling Using Sorptive Extraction and Multidimensional Gas Chromatography with Low- and High-Resolution Mass Spectrometry

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Supplementary Table S1. Chemical class, compound name, CAS number (CAS#) and experimental retention index (RI_{exp}) of the 27 chemical standards used for chemical class elution classification in the GC×GC system and method validation.

Chemical class	Name	CAS#	RI_{exp}
Cannabinoids	Cannabidiol (CBD)	13956-29-1	2452
	Cannabidiolic Acid (CBD-A)*	1244-58-2	-
	Δ9-tetrahydrocannabinol (THC)	972-08-3	2552
	Δ9-tetrahydrocannabinolic acid A (THC-A)*	23978-85-0	-
	Cannabinol (CBN)	521-35-7	2623
Monoterpenes	α-pinene	80-56-8	933
	Camphene	79-92-5	950
	β-pinene	18172-67-3	976
	β-myrcene	123-35-3	984
	δ-3-carene	13466-78-9	1009
	α-terpinene	99-86-5	1017
	p-cymene	99-87-6	1028
	Limonene	5989-27-5	1026
	Eucalyptol	470-82-6	1033
	Ocimene	13877-91-3	1049
	γ-terpinene	99-85-4	1060
	Terpinolene	586-62-9	1088
	Linalool	78-70-6	1099
	Isopulegol	89-79-2	1146
	Geraniol	106-24-1	1255
Sesquiterpenes	β-caryophyllene	87-44-5	1423
	α-humulene	6753-98-6	1461
	Nerolidol (cis- isomer)	3790-78-1	1544
	Nerolidol (trans- isomer)	35944-21-9	1564
	Caryophyllene oxide	1139-30-6	1593
	Guaiol	489-86-1	1608
	α-bisabolol	23089-26-1	1695

*Subjected to decarboxylation at the GC inlet into the corresponding neutral form

Supplementary Table S2. Relative standard deviation (RSD%) of the chemical classes of interest in the quality control sample under the final SBSE conditions. For the characteristics ions used for measuring the response, refer to Table 2 in the main text.

Classes	**RSD% (n=8)**
Monoterpenes	18.0
Sesquiterpenes	7.0
IS (d_5-chlorobenzene)	11.6
Hydrocarbons	13.2
Terpenoid alcohols and fatty acids	11.8
Cannabinoids	18.3

Supplementary Table S3. Qualitative information (MS library similarity (MS lib. match) and experimental retention index (RI_{exp})) of the compounds reported in Figure 5. For the retention index search, the tolerance was set to ±20 units. When a reference retention index was not available and high MS similarity (>800) was obtained, the MS library hit is reported.

Label	Tentative ID	MS lib. match*	RI_{exp} #
Mono.1	α-thujene	877	930
Mono.2*	α-pinene*	898	938
Mono.3	Sabinene	790	987
Mono.4*	β-myrcene*	882	989
Mono.5	α-phellandrene	816	1012
Mono.6*	Eucalyptol*	878	1037
Mono.7	α-ocimene	891	1055
Mono.8*	γ-terpinene*	880	1068
Mono.9	Fenchone	840	1098
Mono.10	Trans-limonene oxide	740	1131
Mono.11	Monoterpene - unidentified	798	1152
Mono.12	Monoterpene - unidentified	706	1158
Mono.13	Terpinen-4-ol	865	1187
Mono.14	p-cymen-8-ol	792	1192
Mono.15	Fenchyl acetate	754	1231
Mono.16	Monoterpene - unidentified	-	1321
Sesqui.1	α-copaene	761	1373
Sesqui.2	Ylangene	881	1385
Sesqui.3	γ-elemene	710	1444
Sesqui.4	Alloaromadendrene	829	1467
Sesqui.5	Sesquiterpene - unidentified	-	1471
Sesqui.6	Sesquiterpene - unidentified	-	1473
Sesqui.7	Sesquiterpene - unidentified	-	1540
Sesqui.8	α-calacorene	884	1563
Sesqui.9*	Nerolidol*	901	1569
Sesqui.10	Sesquiterpene - unidentified	-	1576
Sesqui.11*	Caryophyllene oxide*	894	1599
Sesqui.12	Sesquiterpene - unidentified	822	1681
Sesqui.13	Sesquiterpene - unidentified	-	1689
Sesqui.14	Sesquiterpene - unidentified	-	1737
Terp.alc.-fat.ac.1	Fat.ac. ester - unidentified	-	1795
Terp.alc.-fat.ac.2	Benzoic acid, 2-methylbutyl ester	800	1796
Terp.alc.-fat.ac.3	Fat.ac. ester - unidentified	-	1826
Terp.alc.-fat.ac.4	Ester (benzoic acid, hept-3-yl ester)°	803	1832
Terp.alc.-fat.ac.5	Ketone (ketone, methyl 2,2,3-trimethylcyclopentyl)°	801	1895
Terp.alc.-fat.ac.6	Neophytadiene	839	1886
Terp.alc.-fat.ac.7	Unknown	-	1903
Terp.alc.-fat.ac.8	Fat.ac. ester - unidentified	-	1991
Terp.alc.-fat.ac.9	Fat.ac. ester - unidentified	-	1998
Terp.alc.-fat.ac.10	Fat.ac. ester - unidentified	-	2094
Terp.alc.-fat.ac.11	Phytol	893	2095

Terp.alc.-fat.ac.12	9-octadecenoic acid, methyl ester	911	2108
Terp.alc.-fat.ac.13	Fat.ac. ester - unidentified	-	2185
Terp.alc.-fat.ac.14	Isoamyl laurate	838	2251
Terp.alc.-fat.ac.15	Fat.ac. ester (stearic acid, 2-hydroxy-1-methylpropyl ester)°	802	2312
Terp.alc.-fat.ac.16	Fat.ac. ester (9,12,15-octadecatrienoic acid, methyl ester)°	853	2440
Terp.alc.-fat.ac.17	Fat.ac. ester (octadecanoic acid, 3-hydroxy-, methyl ester)°	814	2490
Terp.alc.-fat.ac.18	Fat.ac. ester (decanoic acid, octyl ester)°	800	2576
Hydrocarbon.1	Hydrocarbon (decane, 6-ethyl-2-methyl-)°	834	1146
Hydrocarbon.2	Hydrocarbon (hexane, 3-ethyl-2,5-dimethyl-)°	853	1157
Cannabinoid.1	Cannabidivarol°	842	2245
Cannabinoid.2	Cannabinoid (cannabichromene)°	904	2487
Cannabinoid.3	Cannabinoid (cannabichromene)°	897	2669
Cannabinoid.4	Cannabinoid (11-hydroxy-Δ9-tetrahydrocannabinol)°	708	2666
Cannabinoid.5	Cannabinoid (cannabichromene)°	848	2367
Cannabinoid.6	Cannabinoid analogue (Δ8-tetrahydrocannabinol)°	810	2497
Cannabinoid.7	Cannabinoid analogue (Δ9- tetrahydrocannabinol)°	845	2556
Unclassified.1	Alcohol (2,4,4-Trimethyl-1-pentanol)°	807	1358
Unclassified.2	Ester (2-furanmethanol, tetrahydro-, acetate)°	805	1605
Unclassified.3	Unknown	-	1812
Unclassified.4	Hexadecanal	899	1823
Unclassified.5	Unknown	-	1847
Unclassified.6	Unknown	-	1761
Unclassified.7	Unknown	-	2409
Unclassified.8	Unknown	-	2429
Unclassified.9	Unknown	-	2584
Unclassified.10	Ester (phenethyl tetradecanoate)°	919	2693
Unclassified.11	2-phenylethy linoleate	831	2878
Unclassified.12	Unknown	-	2951
Unclassified.13	Unknown	-	3132

* Confirmed with analytical-grade standards

° Library hit (>800 MS match) for compounds where the reference retention index was not available

Supplementary Table S4. Repeatability and inlet conversion yields (5 replicates) of the acid CBD-A and THC-A to the neutral forms CBD and THC.

	Repeatability* (RSD%)	**Conversion yield**
CBD	5.8	-
CBD-A	15.3	61%#
THC	4.3	-
THC-A	10.2	56%°

* Spiking 1 µL of the standard (100 µg/mL) on the SBSE

Calculated using: $\frac{\text{response } CBD - A \text{ spike}}{response\ CBD\ spike} x\ 100$

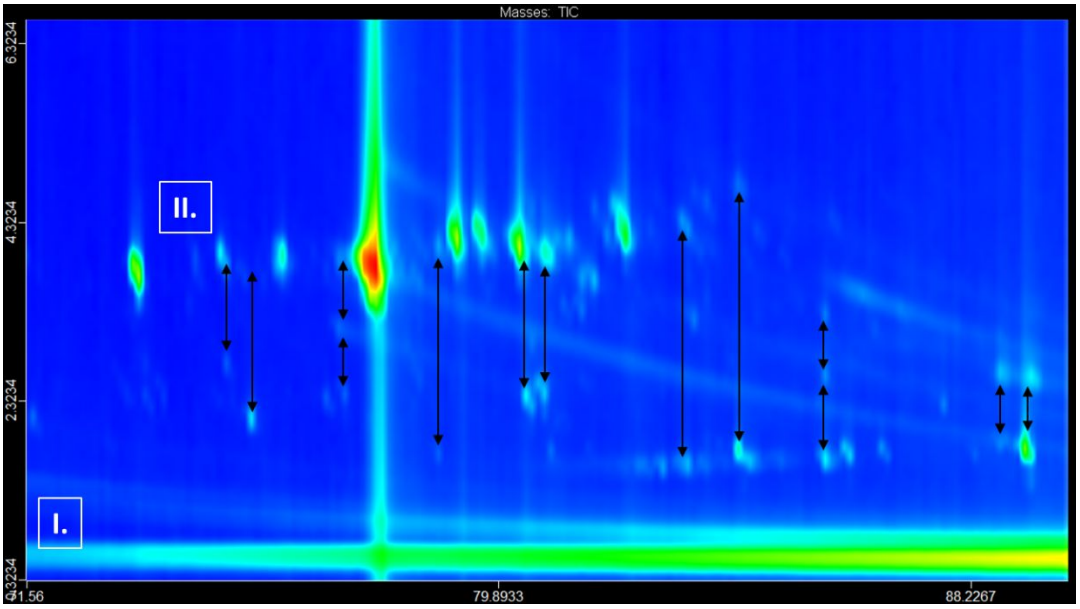
° Calculated using: $\frac{\text{response } THC - A\ spike}{response\ THC\ spike} x\ 100$

Supplementary Table S5. Quantitative information and chemotyping of the analyzed samples. Quantitative values were obtained from 3 replicates and are expressed in *w/w*%.

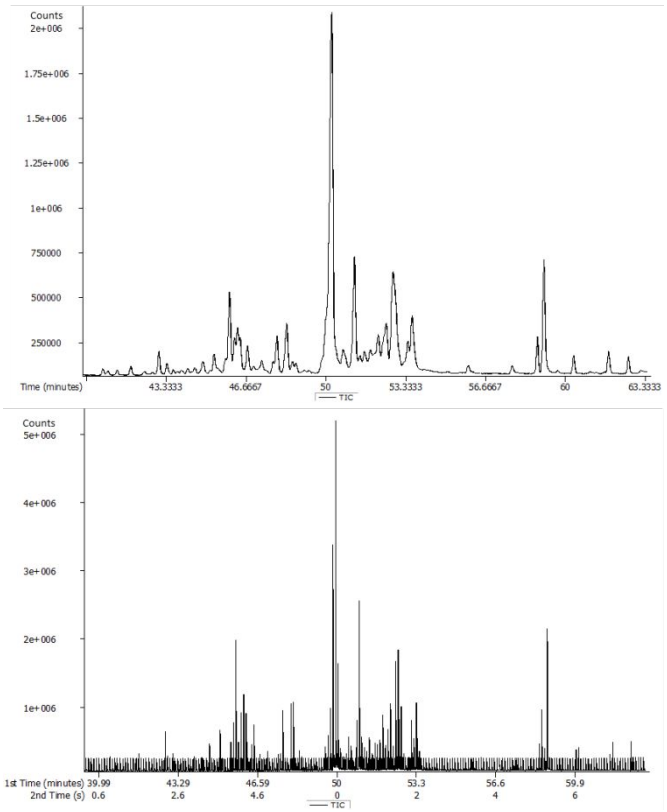
	Total CBD	**Total THC**	**CBN**	**Chemotype***	**Terpene dominance#**
Sample 1	1.80 ± 0.077	0.08 ± 0.008	0.03 ± 0.007	CBD-dominant	β-myrcene/β-caryophyllene
Sample 2	1.92 ± 0.120	0.07 ± 0.010	0.02 ± 0.003	CBD-dominant	β-caryophyllene
Sample 3	1.50 ± 0.053	0.06 ± 0.002	< 0.02	CBD-dominant	β-caryophyllene
Sample 4	1.91 ± 0.042	0.07 ± 0.002	0.02 ± 0.002	CBD-dominant	β-myrcene/Limonene/β-caryophyllene
Sample 5	2.07 ± 0.130	0.08 ± 0.003	0.02 ± 0.001	CBD-dominant	β-myrcene/Limonene/β-caryophyllene
Sample 6	1.75 ± 0.150	0.07 ± 0.006	0.02 ± 0.005	CBD-dominant	β-myrcene/Limonene/β-caryophyllene
Sample 7	5.53 ± 0.190	0.12 ± 0.009	0.07 ± 0.012	CBD-dominant	Limonene/β-caryophyllene
Sample 8	7.94 ± 0.076	0.18 ± 0.007	0.01 ± 0.003	CBD-dominant	Limonene/β-caryophyllene

* based on ref. 22 in the main text

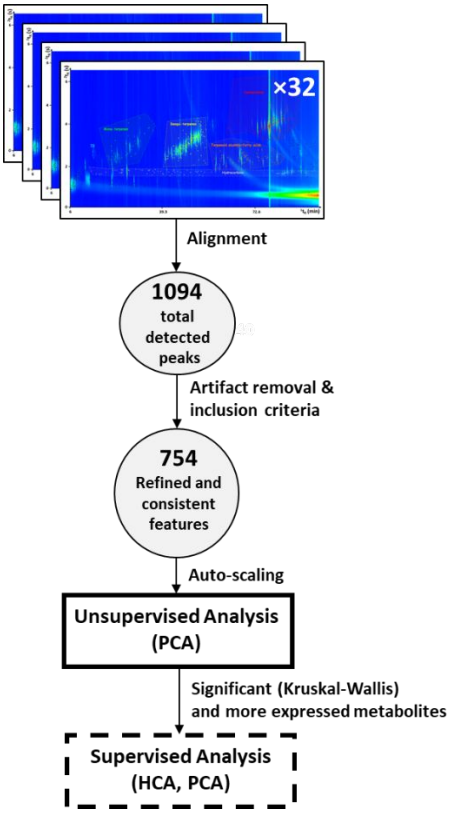
tentative subclassification calculated using the response (TIC) of the selected terpene and the total response of the mono and sesquiterpene elution regions. The obtained values were then compared with the guidelines reported in ref. 22 in the main text



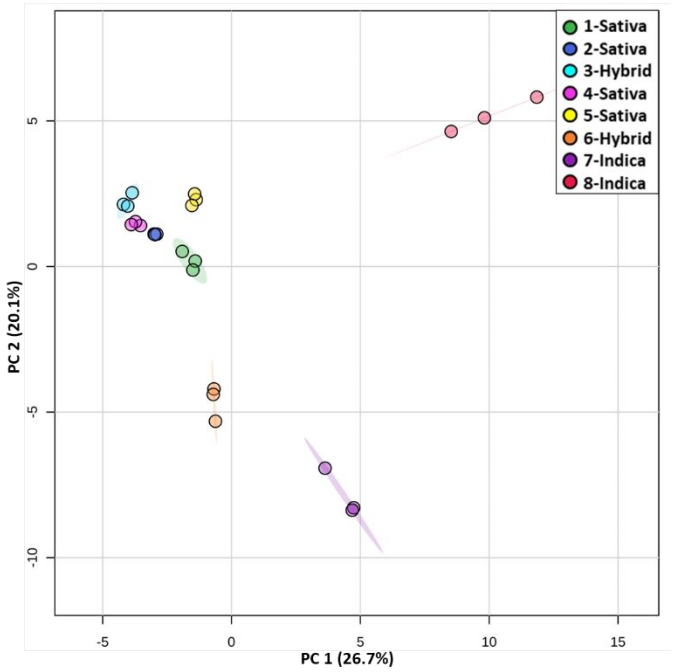
Supplementary Figure S1: The 2D chromatogram expansion of the cannabinoids elution zone (sample 7) highlighting the selectivity of GC×GC. I) Separation between chromatographic artifacts (*i.e.*, column bleed) and analytes of interest. II) Resolution of analyte pairs (arrows) which would co-elute in conventional 1D GC.



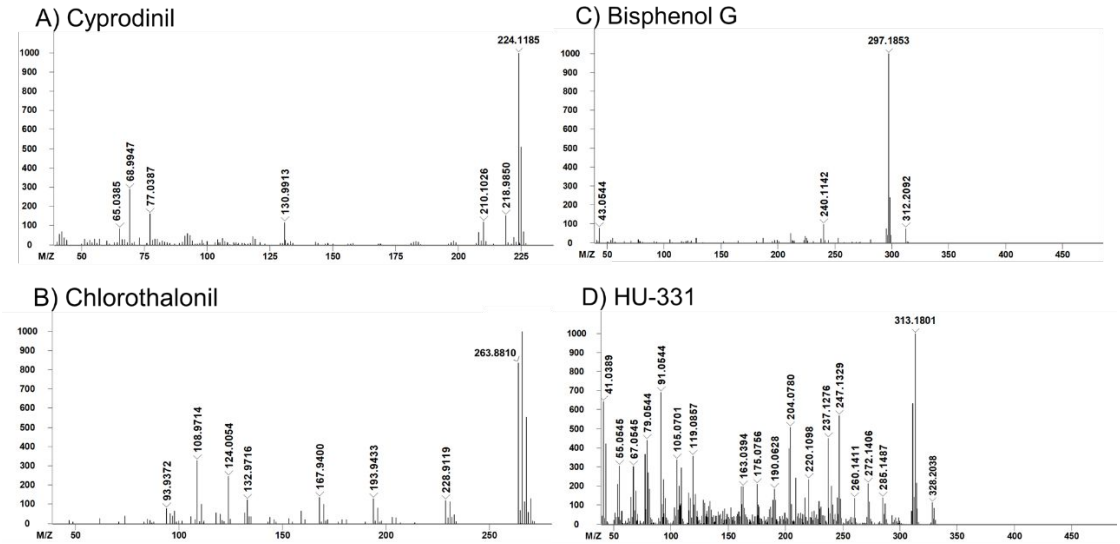
Supplementary Figure S2: Comparison of flow-modulated GC×GC (lower) and unmodulated GC (upper) traces showing the difference in response magnitude (sesquiterpenes elution zone of sample 8).



Supplementary Figure S3: Workflow of data treatment for the cannabis inflorescences analysis.



Supplementary Figure S4: PC scores plot of the selected 70 features from the cannabis inflorescences. Features were selected amongst the most statistically significant ones (Kruskal-Wallis, $p > 0.05$), and more expressed at least in one of the sample type. Ellipses represent the 95% confidence interval for each group.



Supplementary Figure S5A-D: High-resolution mass spectra (≥ 25,000 fwhm) of chlorothalonil (A), cyprodinil (B), bisphenol G (C), and HU-331 (D).