CANNABIDIOL (CBD) IN HEMP SEED OIL: "IMPURITY" AS A MARKER OF "PURITY"

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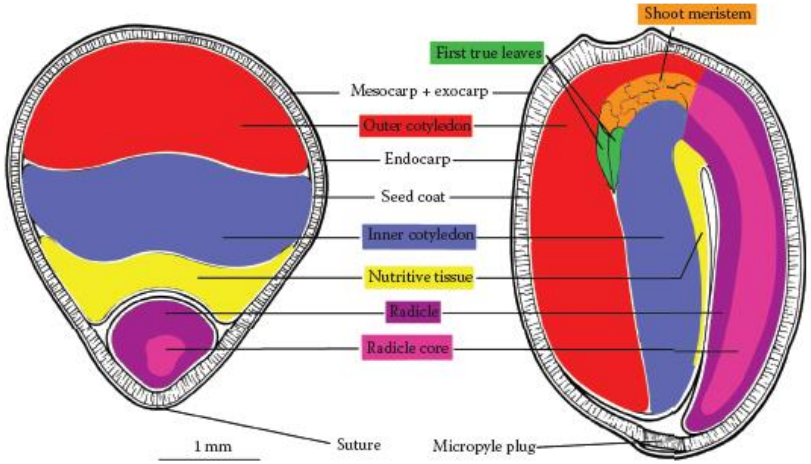
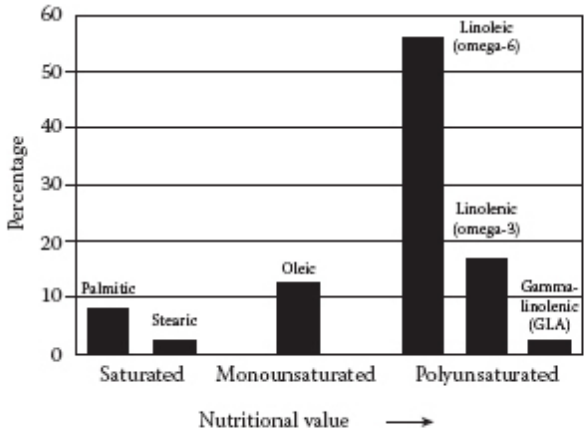
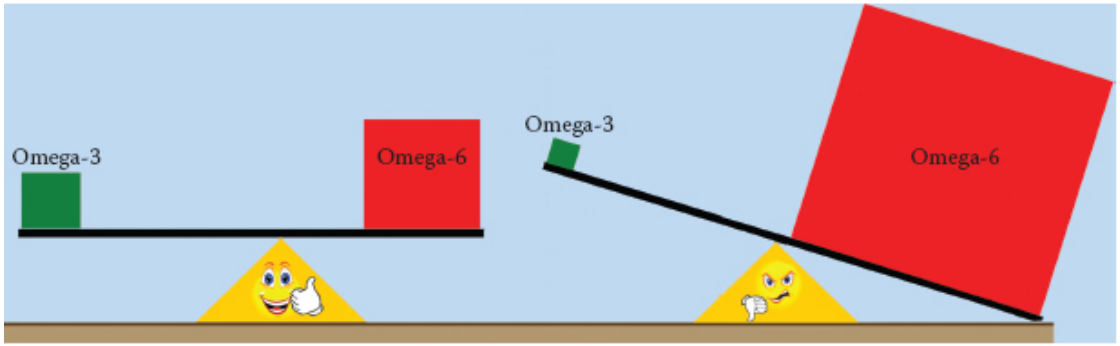
Cannabineae.
Cannabis sativa L.
W. Müller.


Shoot meristem
First true leaves
Mesocarp + exocarp
Outer cotyledon
Endocarp
Seed coat
Inner cotyledon
Nutritive tissue
Radicle
Radicle core
1 mm
Suture
Micropyle plug


(a)
(b)
(c)



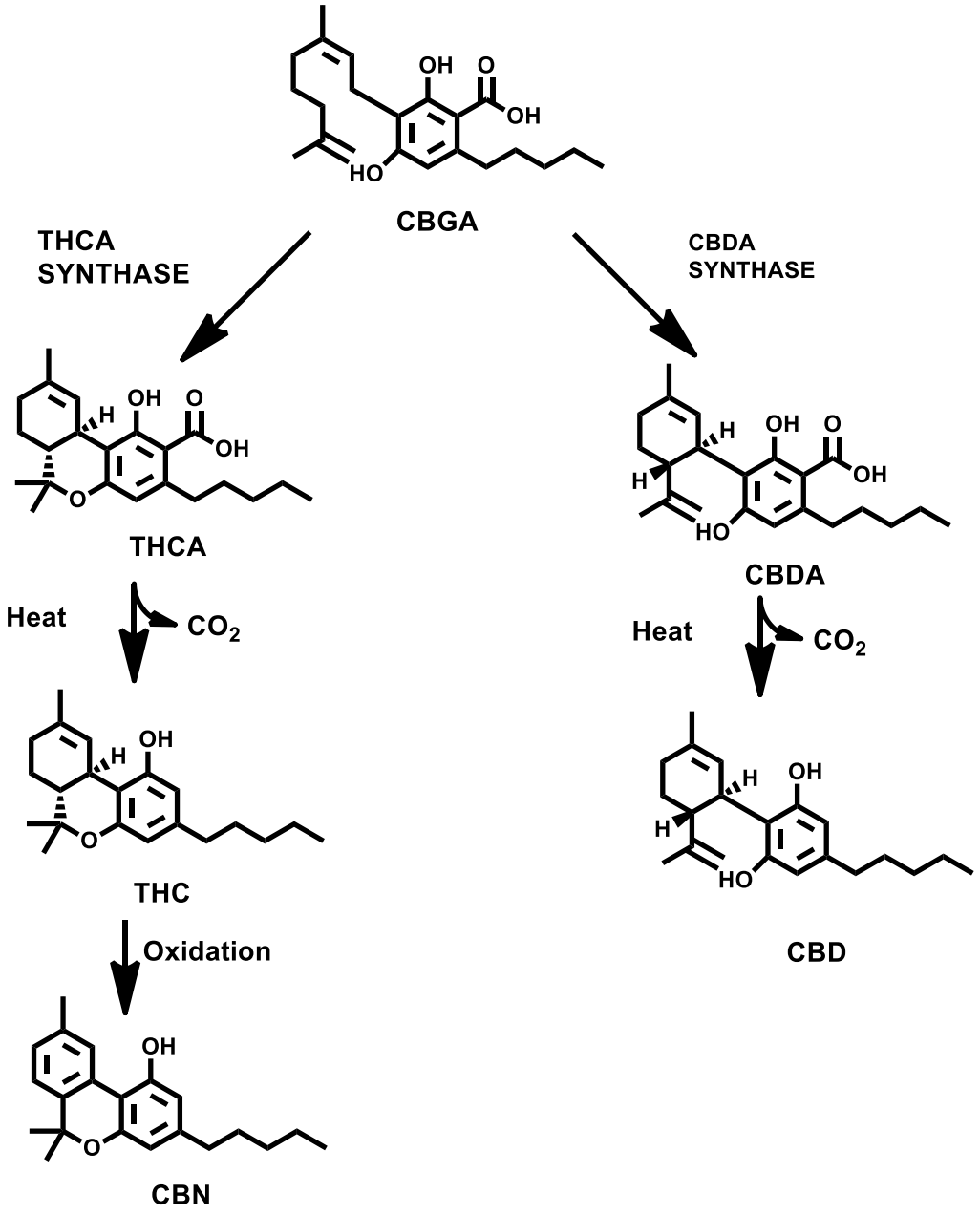


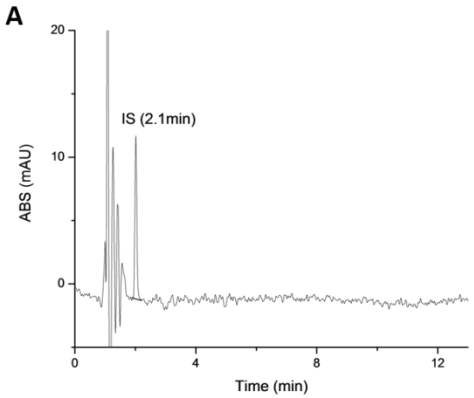
Health significance of relative balance of omega-6 to omega-3 fatty acids. An ideal dietary balance requires two to three times as much omega-6 as omega-3 fatty acids.
The seriously unbalanced diet typical of much of society today has far too much omega-6 compared to omega-3 fatty acids.

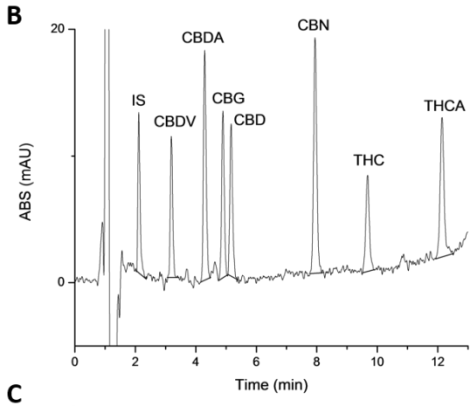
Small, Ernest. Cannabis: A Complete Guide. CRC Press. Kindle Edition.

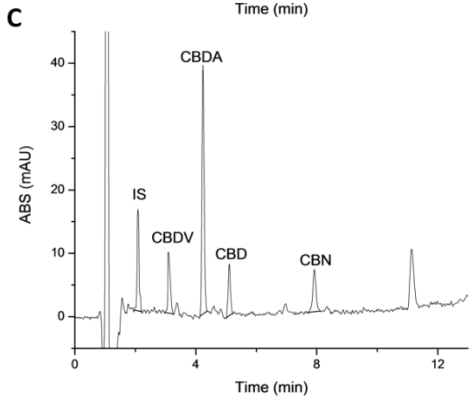
THC (ppm, mg/Kg)

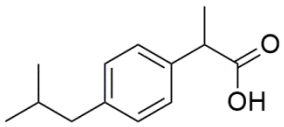
RAW MATERIAL & INTERMEDIATE	NOVA REFERENCE VALUES	THC GUIDELINES	THC LIMITS			INDUSTRIAL PLEDGE	
		GERMANY – BgVV	SWITZERLAND*	BELGIUM	CANADA	AUSTRALIA AND NEW ZEALAND	USA
Whole Hemp Seeds	5.0	-	10	5	10	5	-
Hulled Hemp Seeds	2.5	-	10	5	-	5	1.5
Hemp Oil	10	5	20	10	10	10	5
Hemp Flour/Hemp Protein	3.5	-	-	5	-	5	-


CBGA
THCA SYNTHASE
CBDA SYNTHASE
THCA
CBDA
Heat
CO_2
Heat
CO_2
THC
CBD
Oxidation
CBN

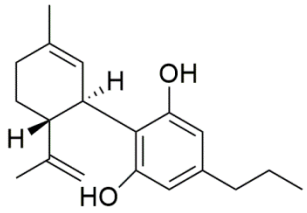




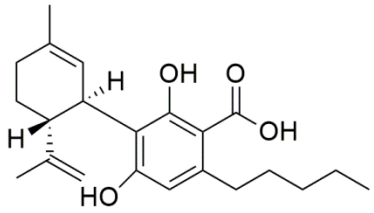




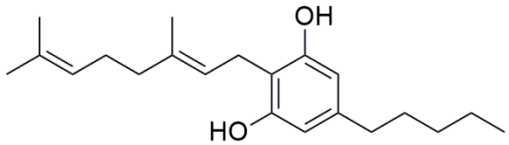
Ibuprofen (IS)



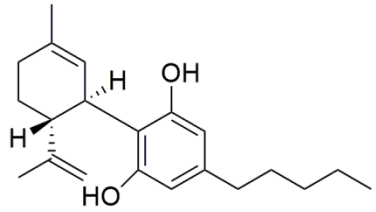
CBDV



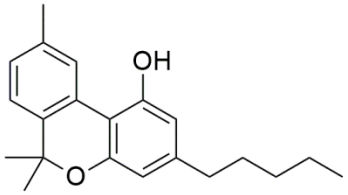
CBDA



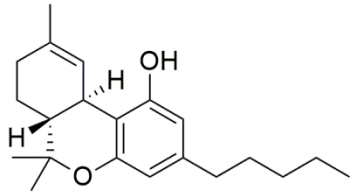
CBG



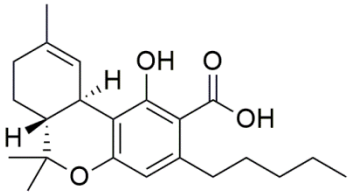
CBD



CBN



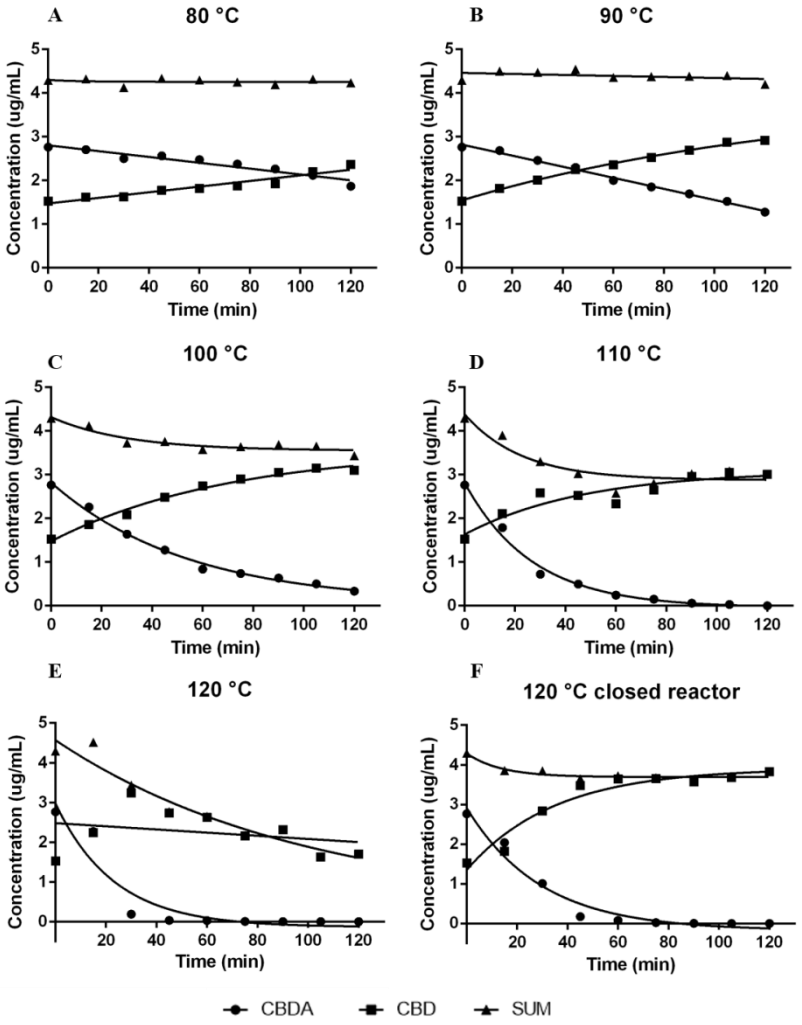
THC



THCA

Amount of the main cannabinoids in 13 different commercial hemp seed oils. The values are expressed in ppm as mean ± standard deviation (n = 3).
[a] When CBD concentration was too low, we considered the LOD value to calculate the CBDA/CBD ratio. mg/Kg (ppm)

Oil	CBDV	CBDA	CBG	CBD	CBN	THC	THCA	CBDA/CBD
H1	8.769±0.080	<LOQ	<LOD	<LOD	1.004±0.199	1.429±0.117	5.525±0.113	2.3±0.5[a]
H2	5.984±0.052	31.93±1.051	<LOD	4.801±0.270	1.460±0.159	<LOQ	<LOQ	6.7±0.5
H3	6.061±0.015	41.59±0.506	<LOD	8.658±0.410	12.41±0.850	<LOD	<LOQ	4.8±0.3
H4	7.741±0.057	2.575±0.442	<LOD	<LOD	2.060±0.340	1.804±0.317	8.883±0.408	12.9±2.2[a]
H5	6.273±0.023	3.641±0.128	<LOD	<LOD	3.227±0.230	<LOQ	4.913±0.674	18.2±0.6[a]
H6	6.352±0.037	2.265±0.203	<LOD	<LOD	1.642±0.134	1.042±0.036	7.074±1.253	11.3±1.0[a]
H7	6.770±0.035	3.178±0.243	<LOD	1.545±0.387	2.046±0.455	<LOQ	9.462±0.514	2.1±0.5
H8	<LOQ	24.33±0.387	<LOD	5.743±0.348	1.217±0.054	<LOQ	1.473±0.093	4.3±0.3
H9	7.448±0.009	3.509±0.151	<LOD	1.637±0.091	<LOQ	<LOQ	<LOQ	2.2±0.2
H10	6.370±0.112	22.26±0.696	<LOQ	6.525±0.027	8.398±0.757	<LOQ	<LOQ	3.4±0.1
H11	75.39±0.596	821.1±13.22	<LOQ	1056±21.84	8.470±0.466	<LOQ	<LOQ	0.7±0.0
H12	64.89±0.156	233.8±10.85	1.283±0.960	60.26±2.716	12.05±1.335	<LOQ	<LOQ	3.9±0.1
H13	66.27±0.345	195.7±2.987	1.381±0.044	56.21±3.985	8.956±0.468	<LOQ	<LOQ	3.5±0.2


A
80 °C
B
90 °C
C
100 °C
D
110 °C
E
120 °C
F
120 °C closed reactor
Concentration (ug/mL)
Time (min)
CBDA
CBD
SUM

Kinetic parameters (k and $t_{1/2}$), linear regressions with R^2, free energy barriers ($\Delta G^{\#}(T)$) and activation entropy ($\Delta S\#(T)$) of the decarboxylation of CBDA in hemp seed oil at each temperature in open reactor (80, 90, 100, 110 and 120 °C) and at 120 °C in closed reactor.

[a] Experiment carried out in closed reactor.

T **(°C)**	Linear regression	R^2	k (s^{-1})	$t_{1/2}$ **(min)**	$\Delta G^{\#}(T)$ ($kJ \cdot mol^{-1}$)	$\Delta S^{\#}(T)$ ($J \cdot K^{-1} \cdot mol^{-1}$)
80	$y = -3.37\times10^{-5}x + 4.61$	0.9695	$3.37\pm0.20 \times 10^{-5}$	**385**	117.2±0.2	–77.1±9.6
90	$y = -1.02\times10^{-4}x + 4.58$	0.9811	$1.01\pm0.04 \times 10^{-4}$	**115**	117.3±0.1	–77.4±9.6
100	$y = -2.55\times10^{-4}x + 4.70$	0.9947	$2.55\pm0.08 \times 10^{-4}$	**38**	117.8±0.1	–77.6±9.6
110	$y = -4.61\times10^{-4}x + 4.52$	0.9943	$4.61\pm0.20 \times 10^{-4}$	**23**	119.1±0.1	–77.8±9.6
120	$y = -7.46\times10^{-4}x + 4.51$	0.9770	$7.46\pm0.41 \times 10^{-4}$	**14**	120.7±0.2	–78.0±9.6
120[a]	$y = -1.21\times10^{-3}x + 4.91$	0.9657	$1.21\pm0.91 \times 10^{-4}$	**14**	119.2±0.4	-

The Arrhenius plot also allowed to extrapolate k and t1/2 of decarboxylation at 25, 20 and 5 °C in order to provide an indication of stability of CBDA in hemp seed oil during common storage conditions.

The decarboxylation rate constant at 25 °C k(25) was 1.66±0.99 × 10-7 s-1 and the **t1/2(25) was about 49 days** (**less than 2 months**). Decreasing the temperature by only 5 °C, the resulting k(20) was one order of magnitude lower than k(25), 9.20±5.97 × 10-8, and **t1/2(20**) was about two times higher than that at 25 °C, i.e**. 87 days (about 3 months)**. If the hemp seed oil is stored in the fridge (5 °C), the decarboxylation rate constant k(5) becomes 1.37±1.07 × 10-8, which is translated in a **t1/2(5) of 587 days (about 20 months)**.

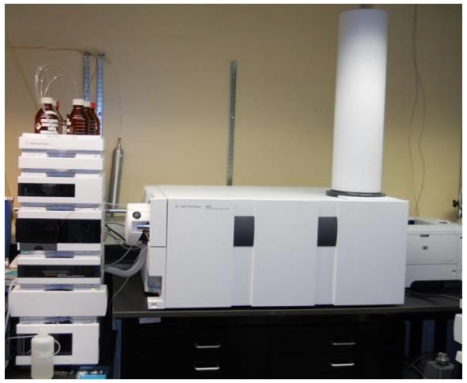
Conclusions

- A highly sensitive and rapid HPLC-UV method was developed and validated for the qualitative and quantitative determination of the main cannabinoids present in commercial hemp seed oils.
- The results suggested that CBDA/CBD ratio can be taken as a marker of purity of the oil (cold pressing and good storage conditions), as CBD is an impurity brought by heat exposure.
- Another aim of this work was to evaluate the decarboxylation kinetics of CBDA, which is the main acid cannabinoid present in hemp seed oil. The results obtained at different temperatures indicated that in open reactor at 100 °C and above, not all the CBDA consumed is converted into CBD, but there is most likely a partial evaporation of the latter. In fact, it was difficult to study CBD formation kinetics due to a more complex chemistry involved. These studies allowed to extrapolate the stability of hemp seed oil at room temperature and at 5 °C, suggesting that a cold storage favors a higher stability of the oil purity.

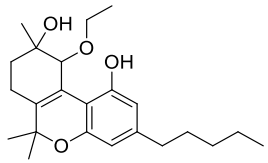
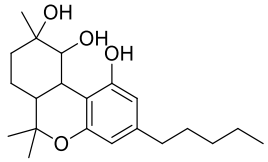
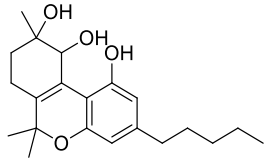
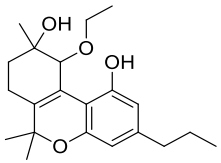
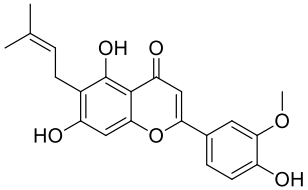
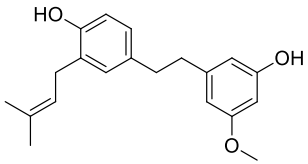
Hemp seeds oil

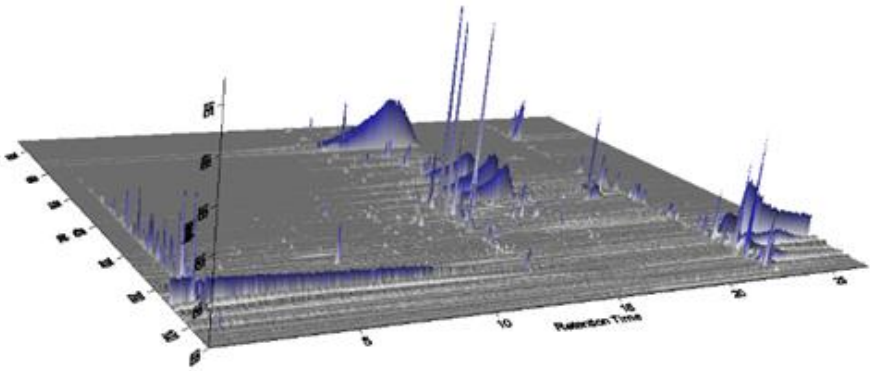
Sample preparation

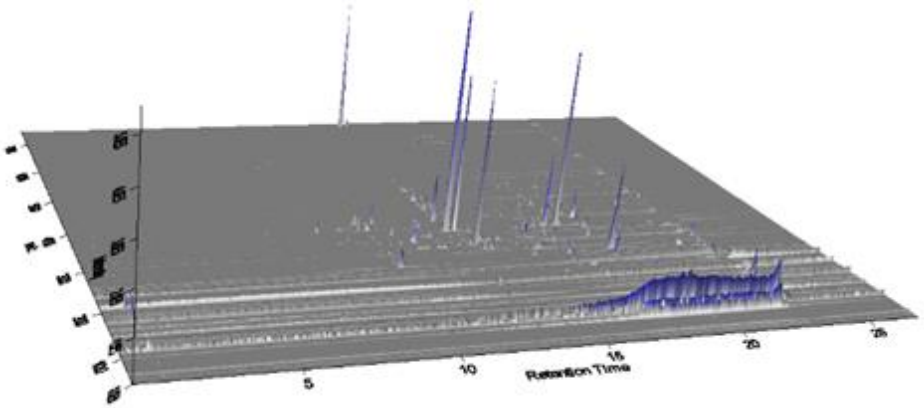
5µL in HPLC-ESI-QTOF
Colonna: Poroshell (2.1 × 10 cm, 3 µm)
Gradiente lineare 5%-95% ACN in 45 min,
Flusso: 0.3 mL/min



ESI+/-, *m/z* 50-1700
AutoMS/MS: 20 eV

R_T (min)	Compound	Mass
8.76	 10-Ethoxy-9-hydroxy-Δ6a-THC	374.2457
9.14	 Cannabiripsol (CBR)	348.2301
9.65		
9.39	CBDA isomer	358.2144
8.48	 Cannabitriol (CBT)	346.2144
9.51		
9.95	 Ethoxy-cannabitriolvarin (CBTVE)	346.2144
10.06	 Cannaflavin B	368.1260
10.35	 Cannabistilbene I	312.1725





THANKS