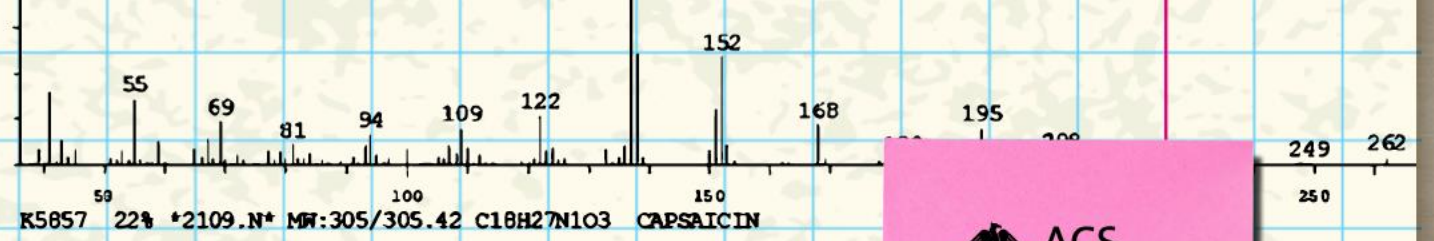

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GC-MS as a straightforward approach to evaluate cannabinoids stability in smokable *Cannabis* preparations.

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Finanziato dall'Unione europea NextGenerationEU
Ministero dell'Università e della Ricerca
Italiadomani PIANO NAZIONALE DI RIPRESA E RESILIENZA

UNIVERSITÀ DI PAVIA

OVERVIEW

- ✓ First part: *Cannabis & GC-MS*
- ✓ Second part: e-cig (CBD)
- ✓ Third part: photostability of *C. sativa* (flowers)

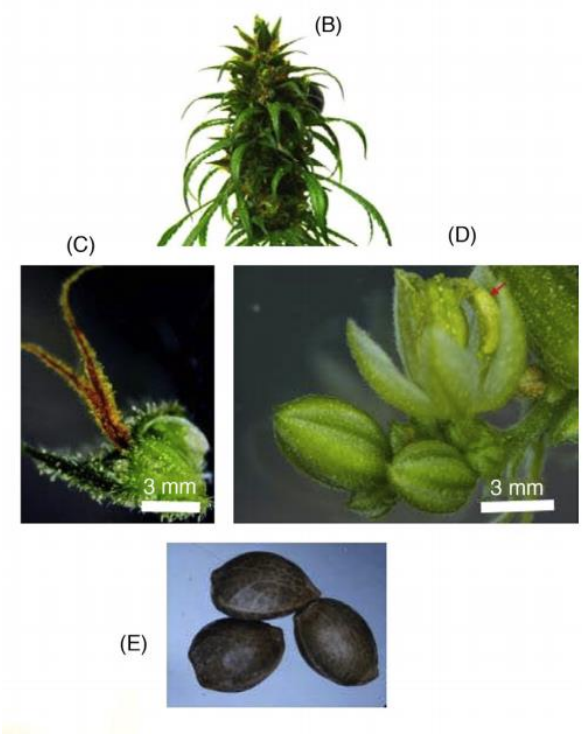
CANNABIS - A flowering plant of the Cannabaceae family.

- *C. sativa*
- *C. indica*
- *C. ruderalis*

→ *Cannabis sativa L.*

Dioecious plant with reproductive organs
(female, male or both)

Possible cultivation both indoors and outdoors



CANNABINOIDS

- Common precursor: cannabigerol (in acidic form, CBGA)
- Possible interconversion in vivo & in vitro

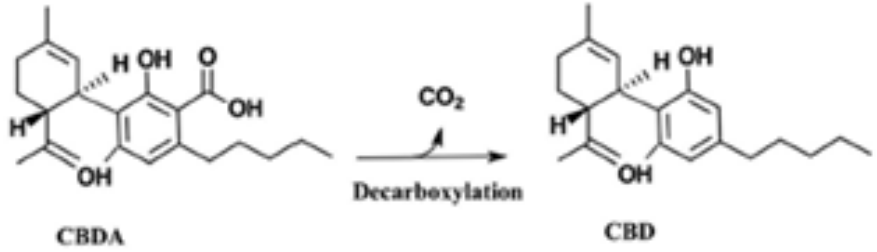
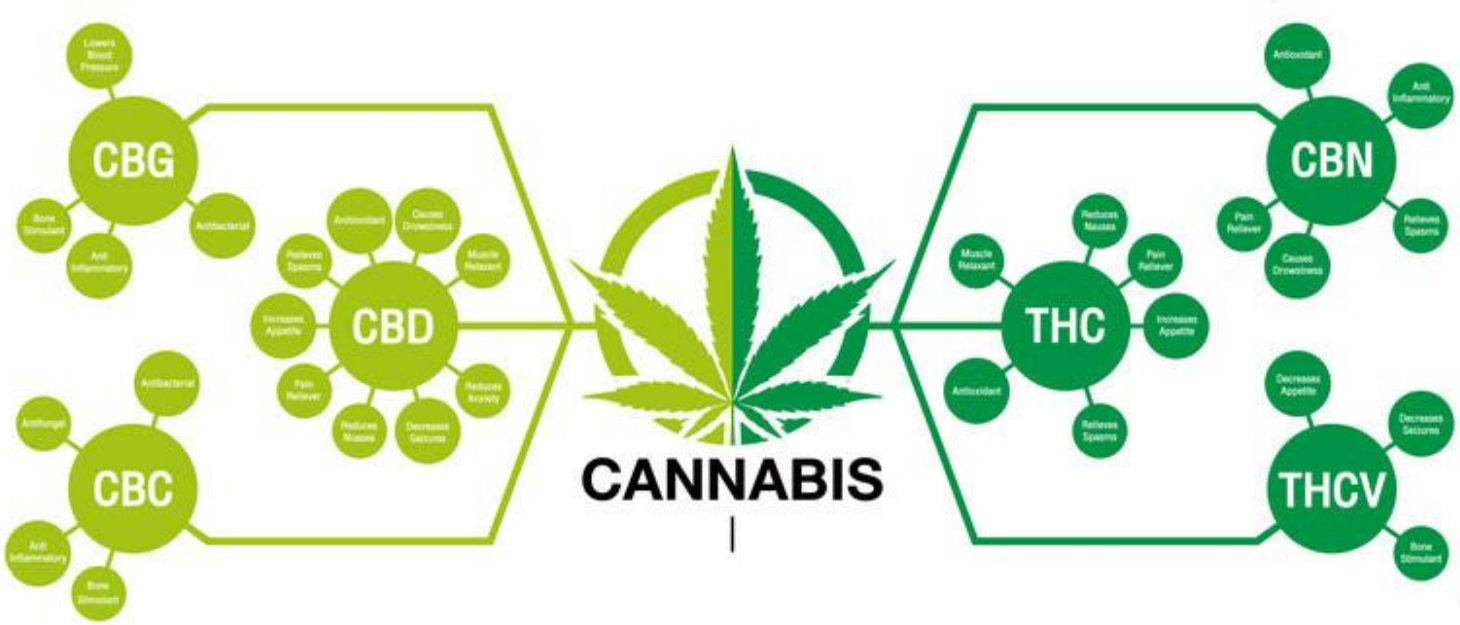


Table 1 An overview of compounds identified in Cannabis

Compound class	*Compounds identified*
Terpenoids	>120
Cannabinoids	>70
Hydrocarbons	50
Sugars and related compounds	34
Nitrogenous compounds	27
Noncannabinoid phenols	25
Flavonoids	23
Fatty acids	22
Simple acids	21
Amino acids	18
Simple ketones	13
Simple esters and lactones	13
Simple aldehydes	12
Proteins, glycoproteins, and enzymes	11
Steroids	11
Elements	9
Simple alcohols	7
Pigments	2
Vitamin	1 (vitamin K)

BIOLOGICAL EFFECTS OF CANNABINOIDS

- CBD (cannabidiol)
  - Analgesic/anti-inflammatory
  - Anxiolytic
  - Antiepileptic
- Δ^9-THC (Δ^9-tetrahydrocannabinol)
  - **Psychotropic**
  - Antiemetic
  - Appetite stimulant
  - Immunomodulatory
  - Biphasic effects: possible anxiety and paranoia

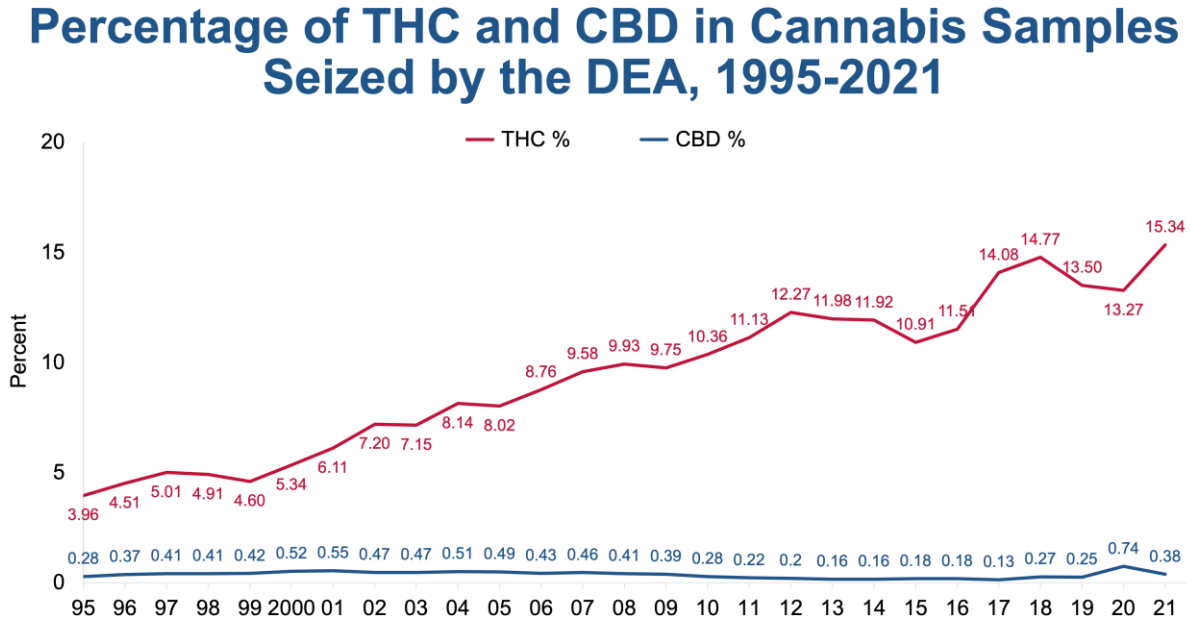


Different Chemotypes

POTENCY TRENDS

- Δ^9-THC: Consistently increased over time from ~4% (1995) to ~15% (2021)
- CBD: decreased from ~.28% (2001) to <.16% (2014). Today: more balanced chemotypes (0.38%).
- Change in the ratio of Δ^9-THC to CBD from 14 times in 1995 to ~80 times in 2014 (~ 40 times in 2021)

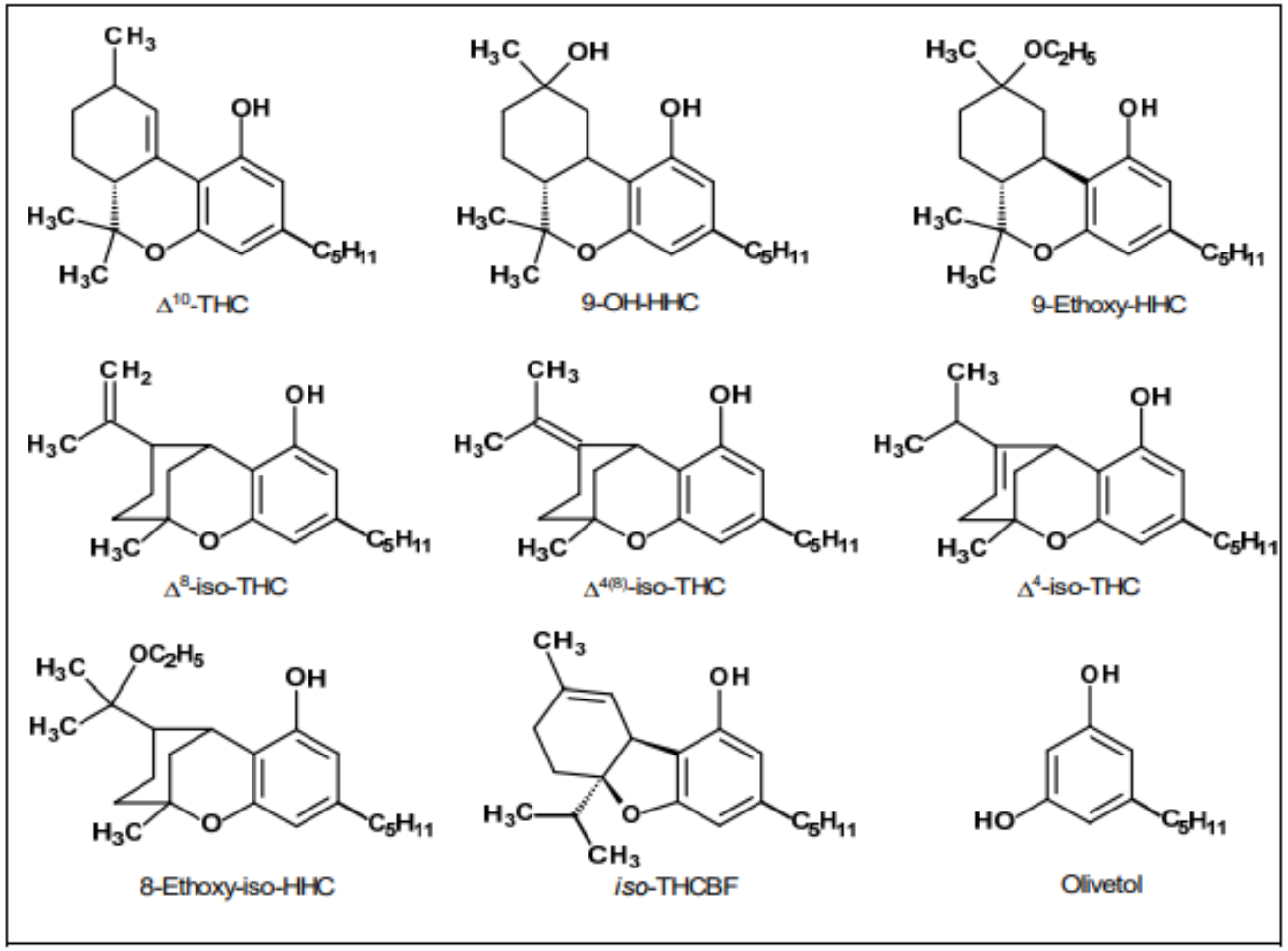
Pure CBD and THC preparation available on the market (recreational or pharmaceutical use)



https://nida.nih.gov/research/research-data-measures-resources/cannabis-potency-data

SOURCE: U Miss, Potency Monitoring Project

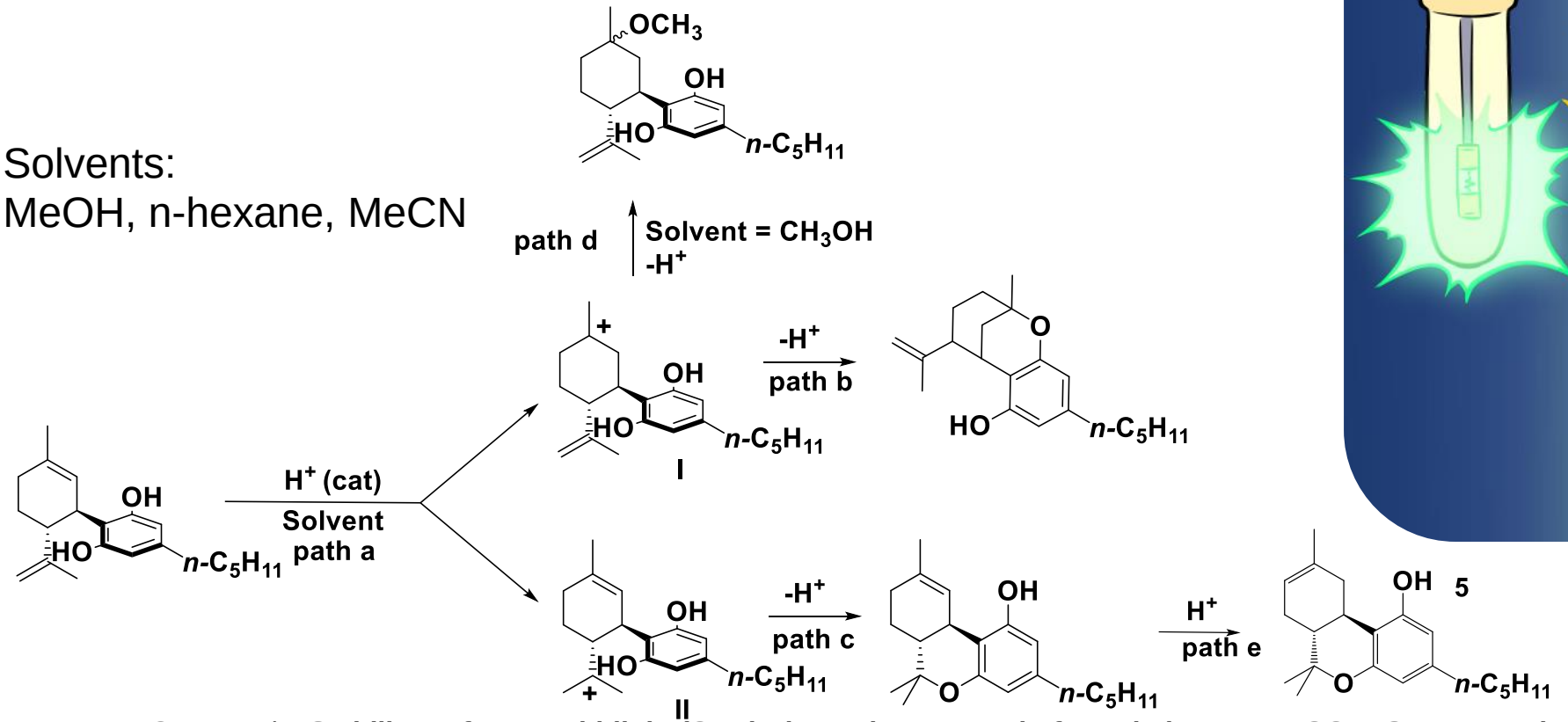
SYNTHETIC THCs AVAILABLE ON THE MARKET

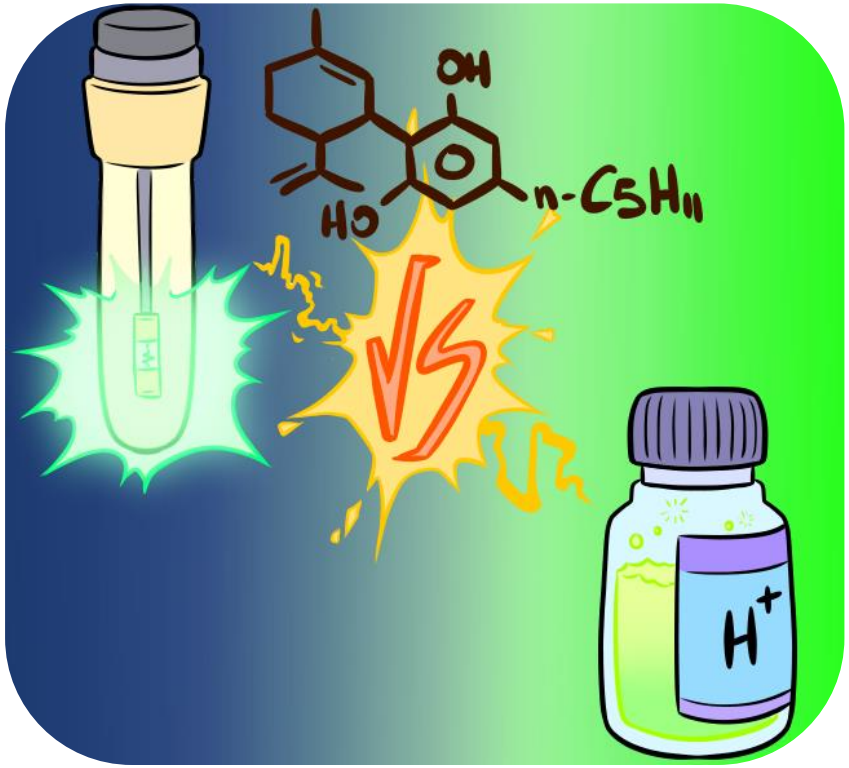


Representative by-products of CBD-based acid catalysed syntheses of Δ^9-THC (Kiselak *et al*, 2020)

REACTIVITY OF CBD ON ACID CATALYSIS

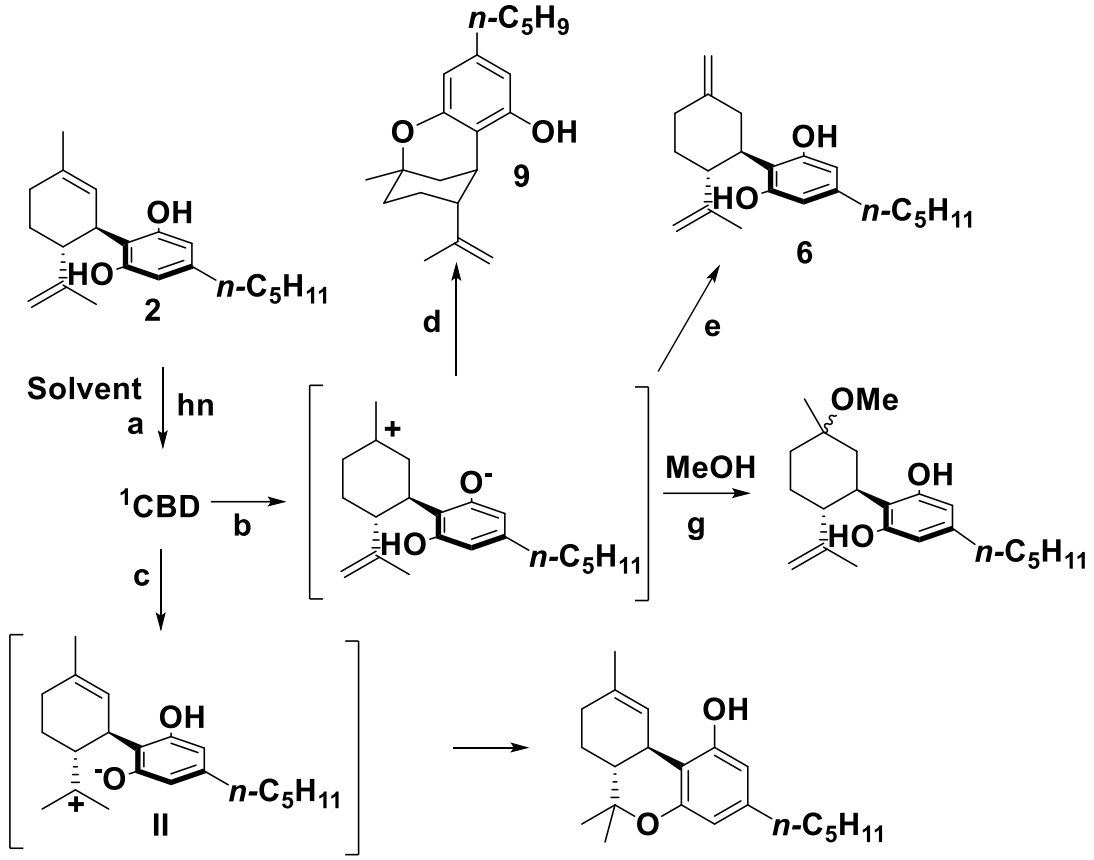
Solvents:
MeOH, n-hexane, MeCN

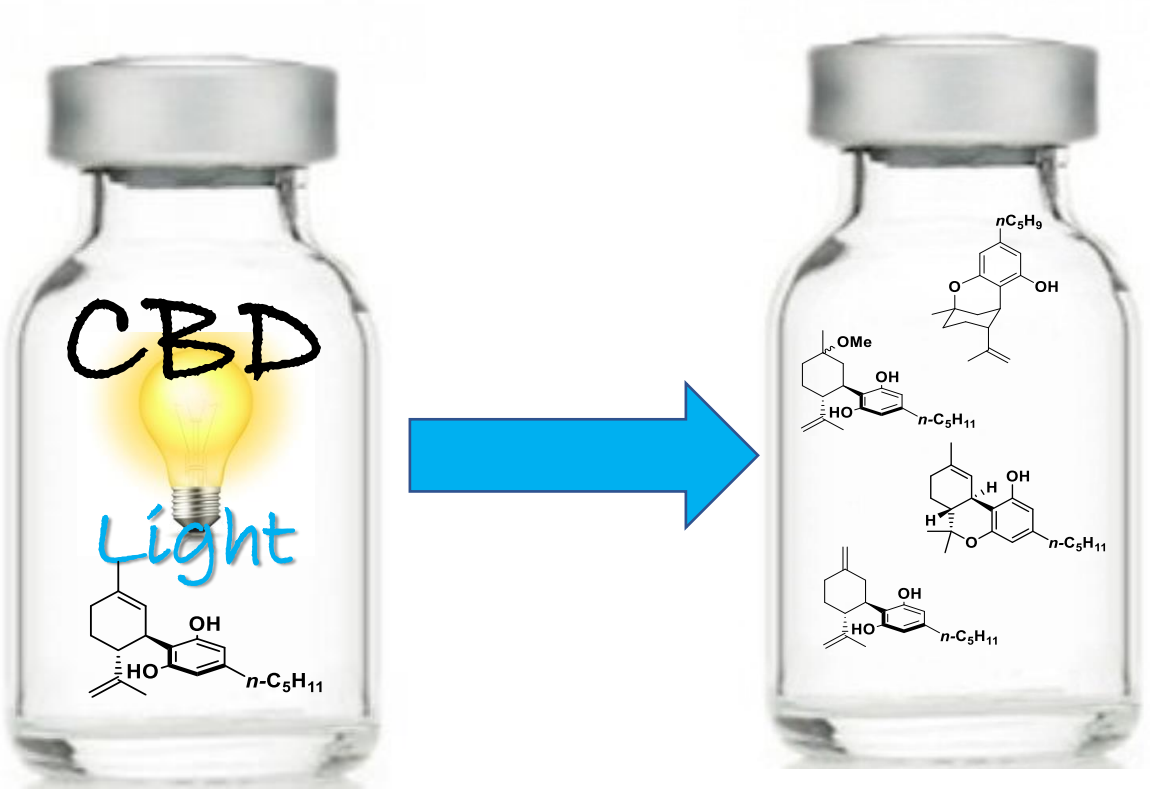




Franco, C. *et al.* **Stability of cannabidiol (CBD) in solvents and formulations: A GC–MS approach**, *Results Chem.* **2022**, *4*, 100465. https://doi.org/10.1016/j.rechem.2022.100465.

PHOTOCHEMICAL REACTIVITY OF CBD

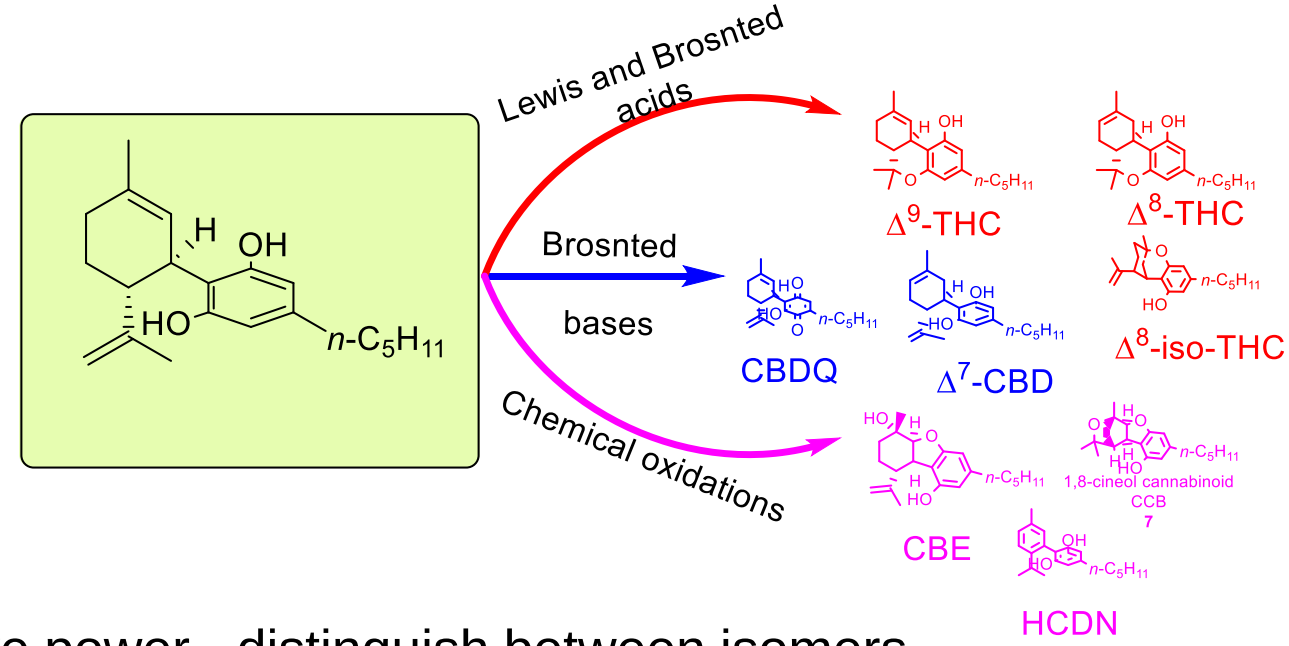




Seccamani, P. *et al.* **Photochemistry of Cannabidiol (CBD) Revised. A Combined Preparative and Spectrometric Investigation**, *J. Nat. Prod.* **2021**, *84*, 2858-2865. https://doi.org/10.1021/acs.jnatprod.1c00567.

ANALYTICAL APPROACH- STANDARD SYNTHESES

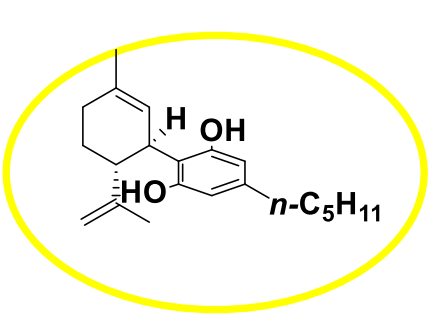
- Common analytical techniques:
  - GC-MS
  - HPLC-MS

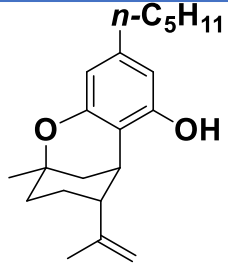


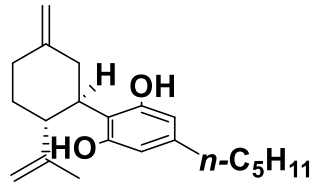
- GC-MS: high resolutive power - distinguish between isomers
- HPLC-MS: well-suited for biological matrices (plasma, urine)

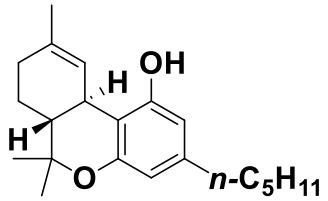
Capucciati, A *et al.* **. CBD-Containing Liquids for e-Cigarettes: Formation of Psychotropic and Secondary Cannabinoids and Amount of CBD Surviving the Smoking Procedure**. *Forensic Sci.* **2023**, *3*, 258-272. https://doi.org/10.3390/forensicsci3020019

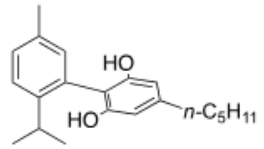
Cannabis e-cig Photostability *C. sativa*



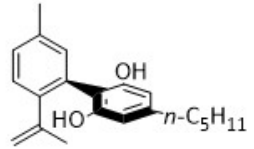




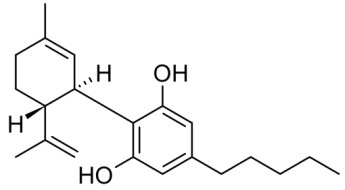


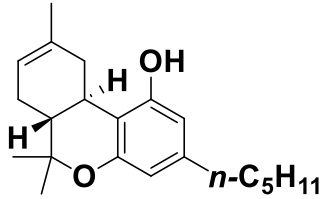


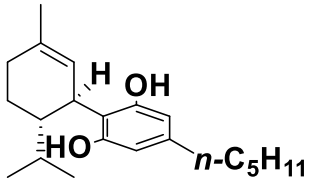
HCDN

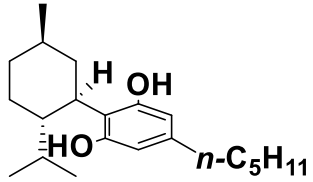


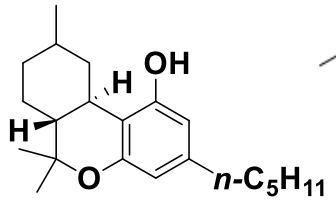
CBND

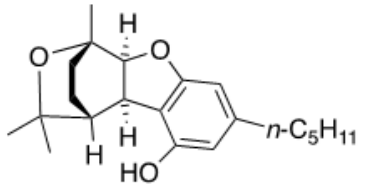




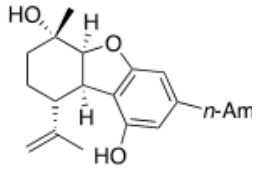




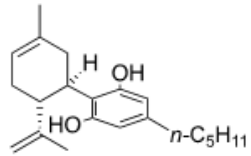




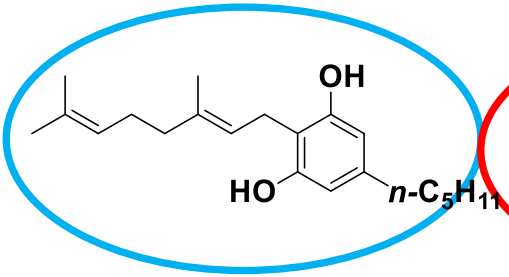
CBS

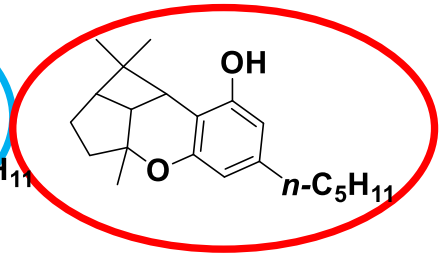


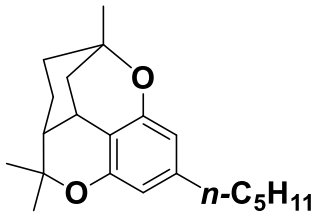
CBE

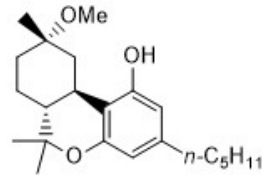


iso-CBD

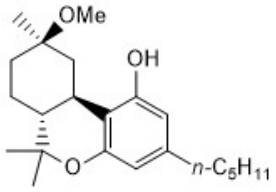




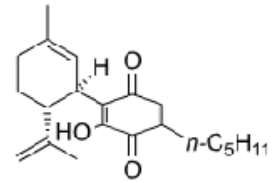




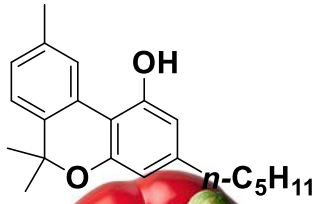
α-MeO-THC

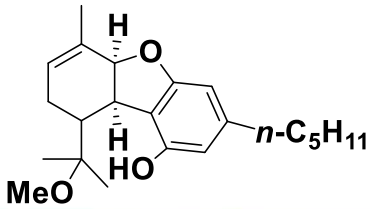


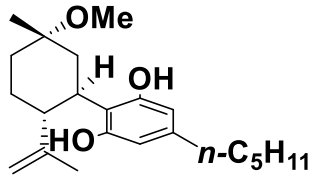
β-MeO-THC

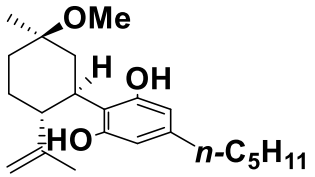


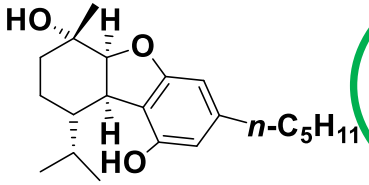
CBQN

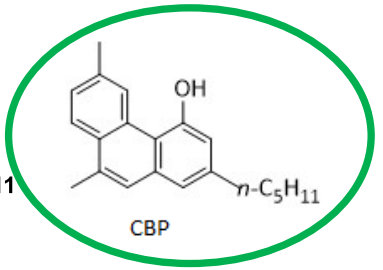




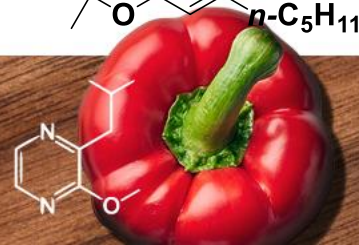








CBP



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VALIDATION OF GC-MS METHOD (ICH)

LOD, LOQ, LOL

0.5 mg/L; 1.5 mg/L; 2-100 mg/L

Spiking studies

Recovery: 85% - 115%

Retention indexes

Retention time correspondence (0.3 to 1.5%)

Spectral characteristics

- ✓ Mass spectral correspondence to pure compounds
- ✓ Ion ratio qualifier/quantifier must be constantly ± 20% of the average value.

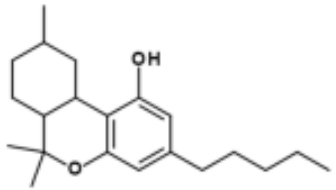
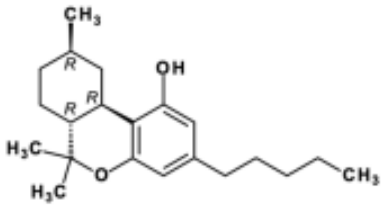
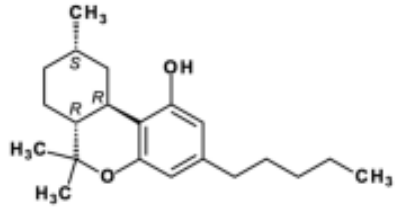
Recovery test – precision of the method

5 and 50 mg/L were used as calibrators (n= 3)

- ✓ Intraday precision: standard deviation 7% of the title concentration of the compound
- ✓ Interday precision: standard deviation 11%

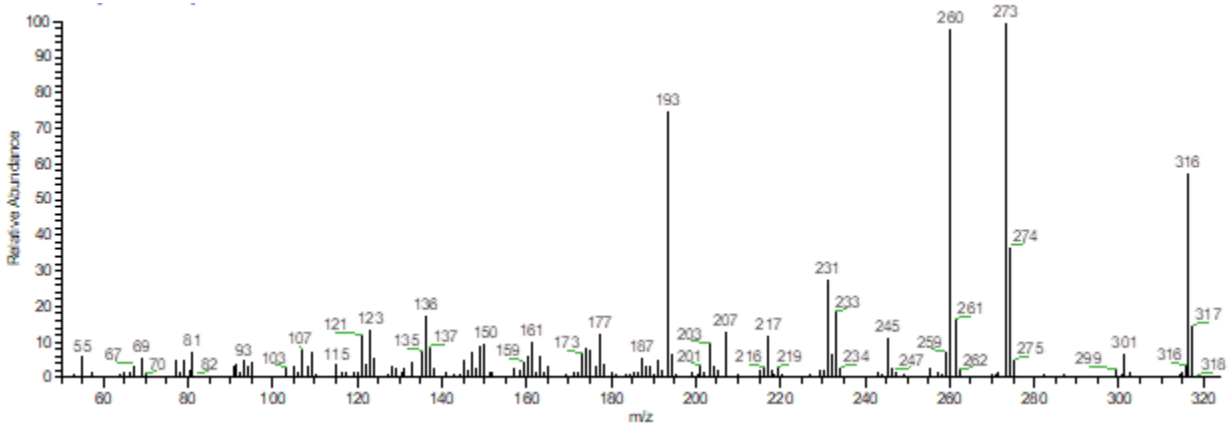
Compound	Retention time (min)	Retention index (found)	Retention index (literature)[a]	Mass (u.m.a.)
CBT	23.28	2276	N.A.	314
HCDN	24.23	2337	N.A.	312
CBND	24.67	2364	N.A.	310
CBL	24.75	2368	N.A.	314
CBD	25.5	2416	2393, 2383, 2385, 2366	314
CBC	25.71	2429	2417	314
Δ^8-iso-THC	25.77	2431	N.A.	314
iso-CBD	25.86	2438	N.A.	314
DHD	25.98	2444	N.A.	316
9-β-HHC	26.06	2449	2464	316
9-α-HHC	26.20	2458	2464	316
cis-THD	26.35	2468	2563	318
trans-THD	26.48	2476	2563	318
Δ^7-CBD	26.5	2477	N.A.	314
CBS	26.53	2479	N.A.	330
Δ^8-THC	26.73	2491	2475	314
CBE	26.73	2491	N.A.	330
Δ^9-THC	26.89	2467	2466	314

Molecular structure and molecular properties of HHC (unspecified stereochemistry), epimeric (*R*)-HHC and (*S*)-HHC (9β-HHC and 9α-HHC, respectively)

			
	HHC (unspecified stereochemistry)	9β-HHC or (9*R*)-HHC	9α-HHC or (9*S*)-HHC
Molecular formula	$C_{21}H_{32}O_2$	$C_{21}H_{32}O_2$	$C_{21}H_{32}O_2$
Molecular mass	316.48	316.48	316.48
Monoisotopic mass	316.2402	316.2402	316.2402

HHC: Mass fragmentation

m/z	273	260	193	316	274	231	233	136	261	317
relative abundance	999	979	748	609	368	278	184	177	164	146



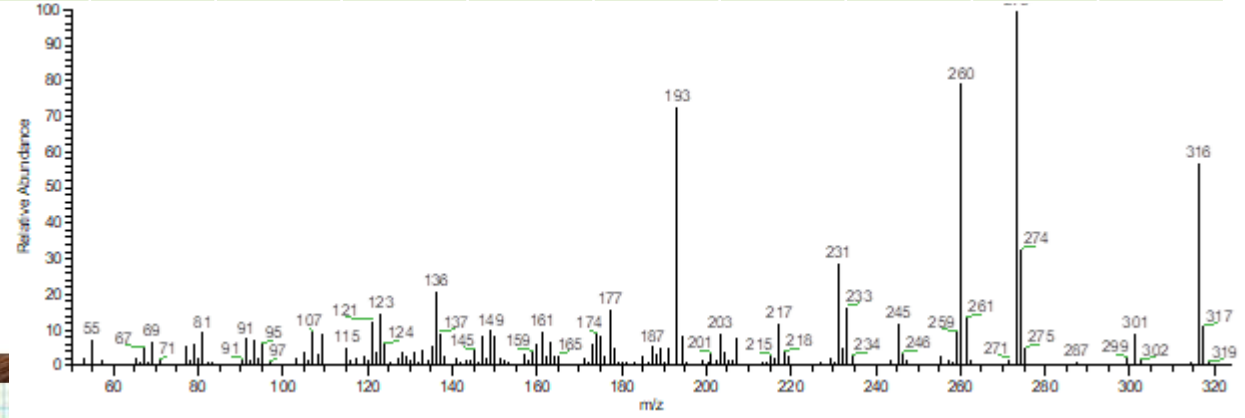
9-β

Retention time and index

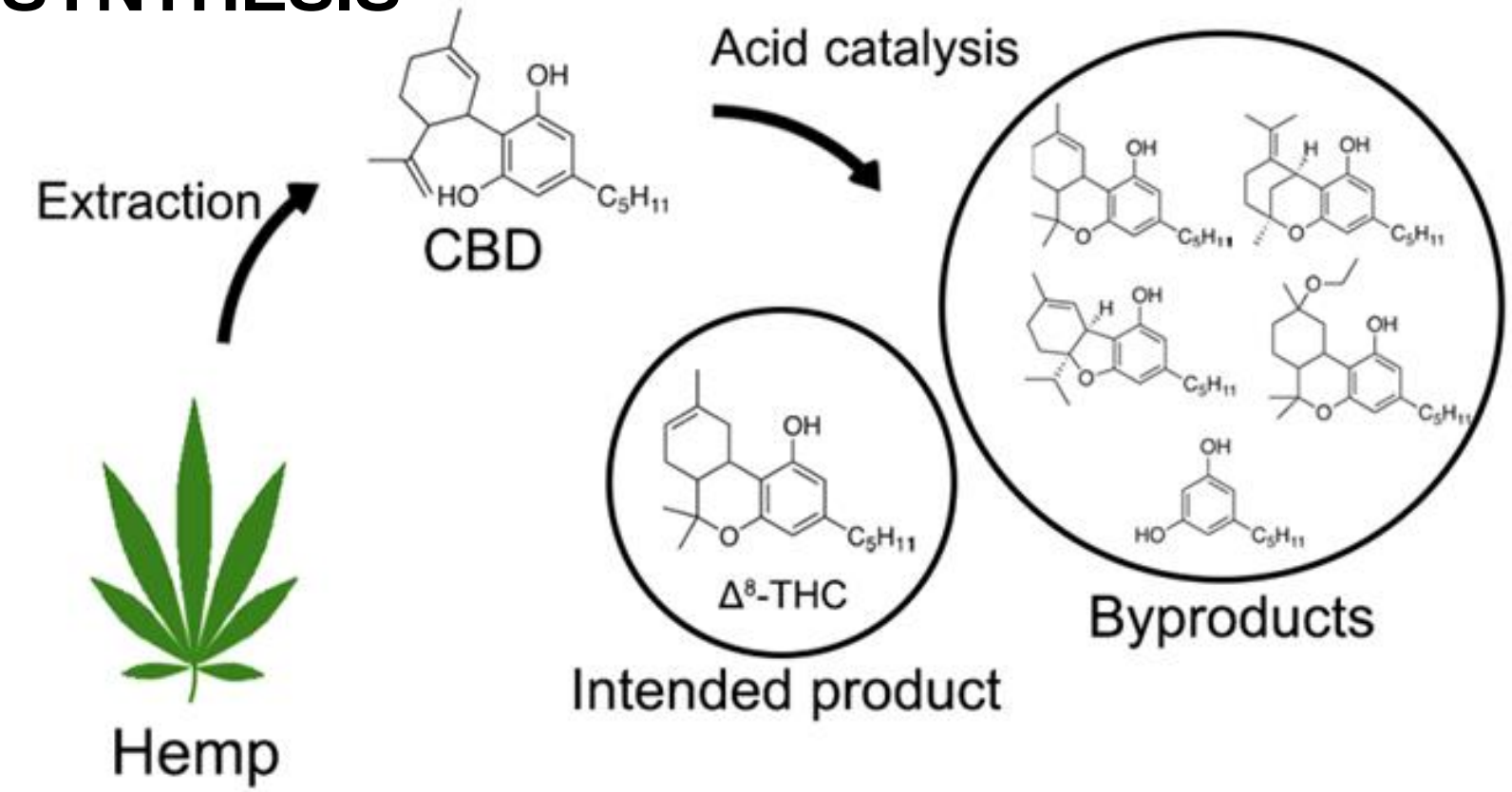
9β-HHC	26.06	2449
9α-HHC	26.20	2458

m/z	273	260	193	316	274	231	136	233	177	123
relative abundance	999	791	726	570	327	289	207	165	158	147

9-α

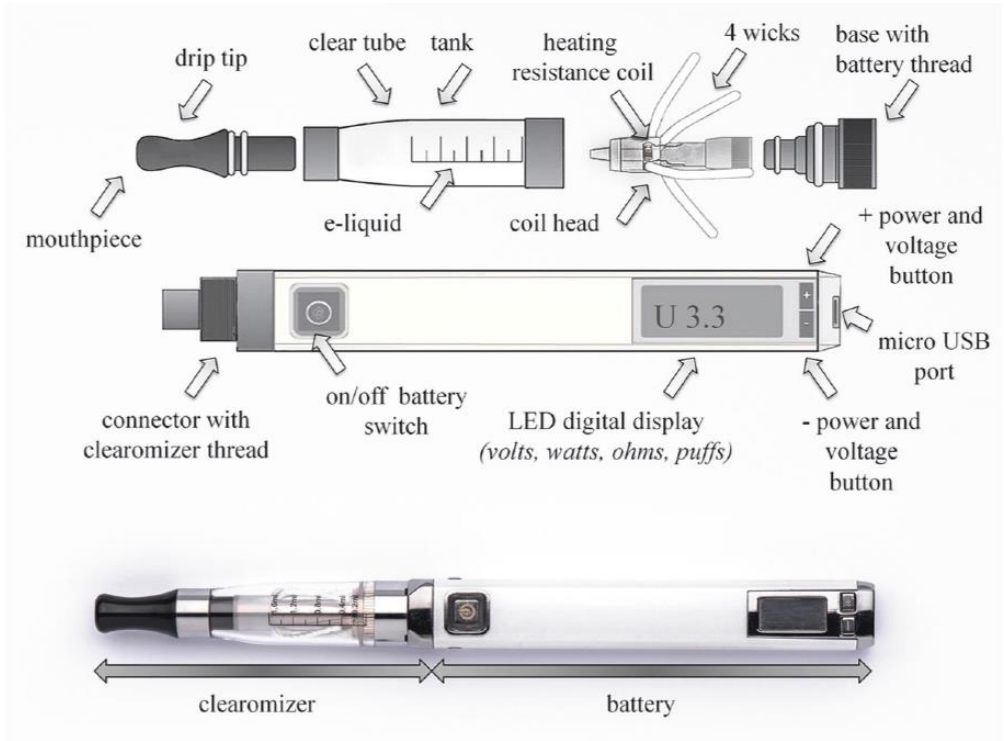


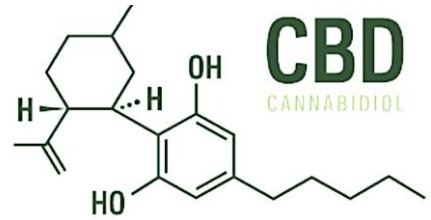
Δ⁸-THC & CBD VAPORIZERS: ADULTERANTS & BYPRODUCTS OF CHEMICAL SYNTHESIS



Meehan-Atrash, J., Rahman I., **Novel Δ8-Tetrahydrocannabinol Vaporizers Contain Unlabeled Adulterants, Unintended Byproducts of Chemical Synthesis, and Heavy Metals** *Chem Res Toxicol.* **2022**, *35(1),* 73–76. doi: 10.1021/acs.chemrestox.1c00388

ANALYSIS OF THE SMOKE PRODUCED BY ELECTRONIC CYGARETTES WITH CBD CONTAINIG E-LIQUID





Experimental set-up

CONDITIONS

- **Solvent:** glycerine, propylene glycol and mixtures
- **Power** of the coil
- **CBD concentration** (2 mg/L to 20 mg/L)
- 1 mL solution smoked

https://www.cbdworld.it/it/

OBJECTIVES

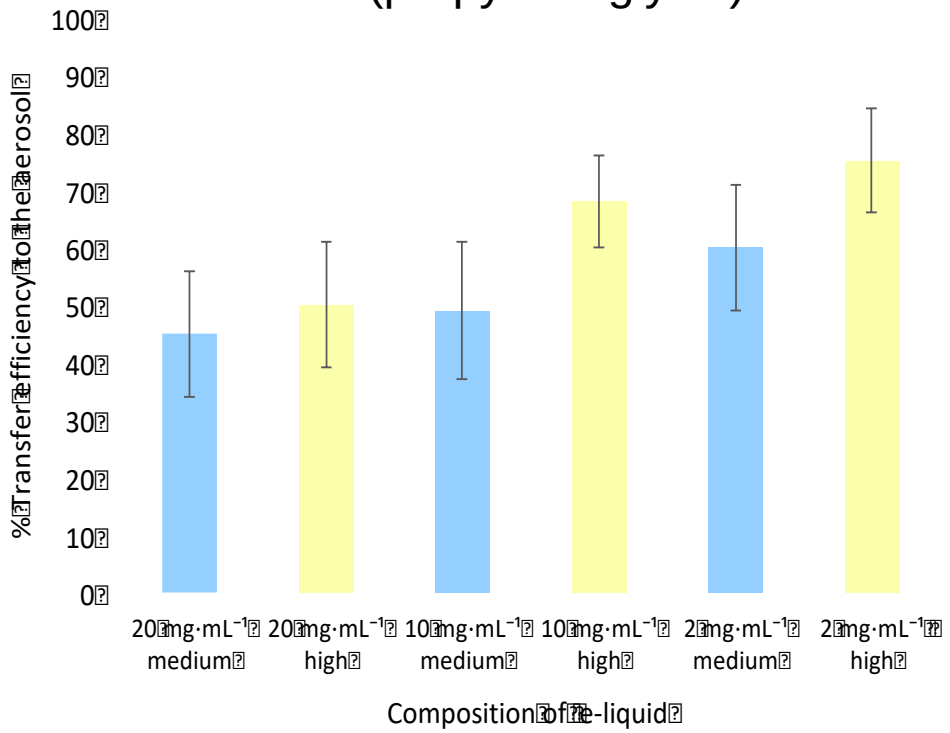
- Transfer efficiency
- Formation of other cannabinoids

Capucciati A *et al.* **CBD-Containing Liquids for e-Cigarettes: Formation of Psychotropic and Secondary Cannabinoids and Amount of CBD Surviving the Smoking Procedure**. *Forensic Sciences*. **2023**; *3(2)*, 258-272. https://doi.org/10.3390/forensicsci3020019

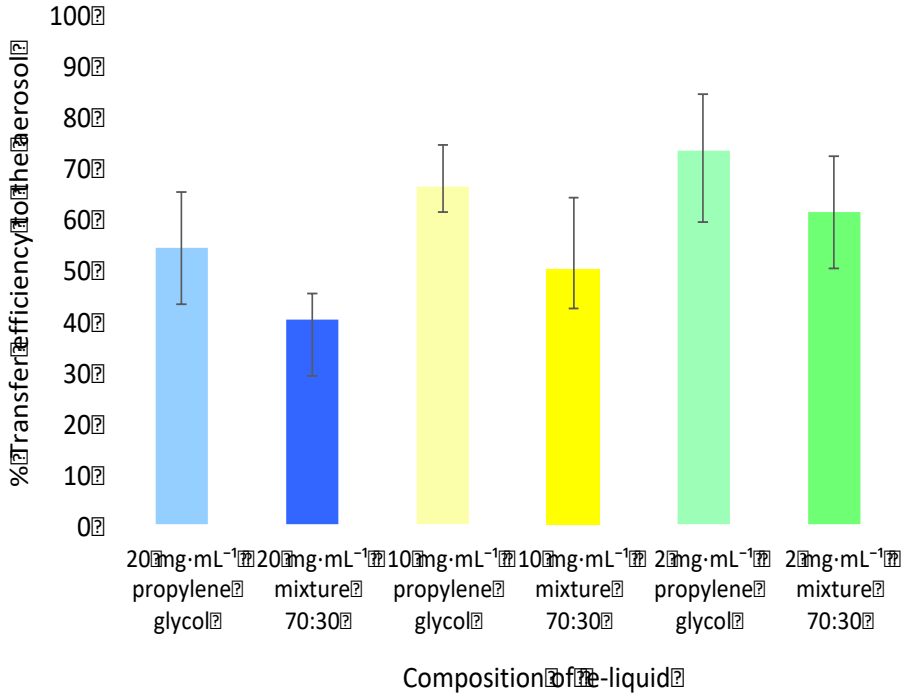
% OF TRANSFER EFFICIENCY OF CBD TO THE AEROSOL

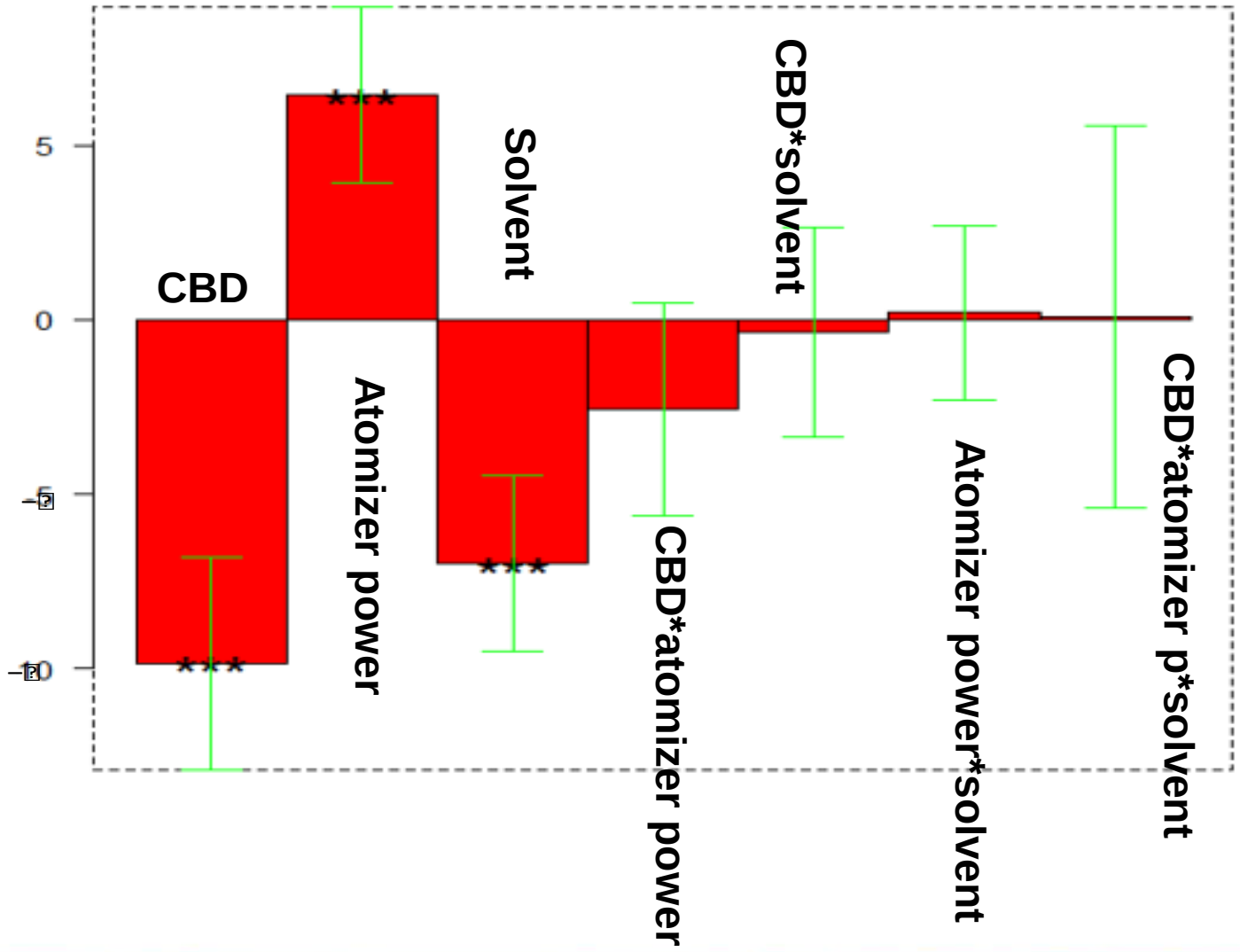
[CBD]: 20 mg·mL^{-1}, 10 mg·mL^{-1} , 2 mg·mL^{-1}

Medium and high atomizer power (propylene glycol)



Neat propylene glycol and 70:30 propylene glycol:glycerol mixture.




5
0
−5
−10
CBD
Atomizer power
Solvent
CBD*atomizer power
CBD*solvent
Atomizer power*solvent
CBD*atomizer p*solvent

- ✓ Transfer efficiency (t.e.) : 30% - 80%
- ✓ CBD concentration, solvent used, and atomizer power were statistically significant ($p < 0.001$). Each variable is independent from each other.
- ✓ Maximum t.e.: high atomizer power, propylene glycol as solvent, lowest CBD concentration
- ✓ Bioavailability CBD: (smoked) 11% - 30 %. Not useful for the systemic delivery of CBD
- ✓ No formation of THC-like compounds (Δ^8-iso-THC, Δ^8-THC, Δ^9-THC: each < 0.005 mg for each mg of vaped CBD)

PHOTOSTABILTY OF CANNABINOIDS

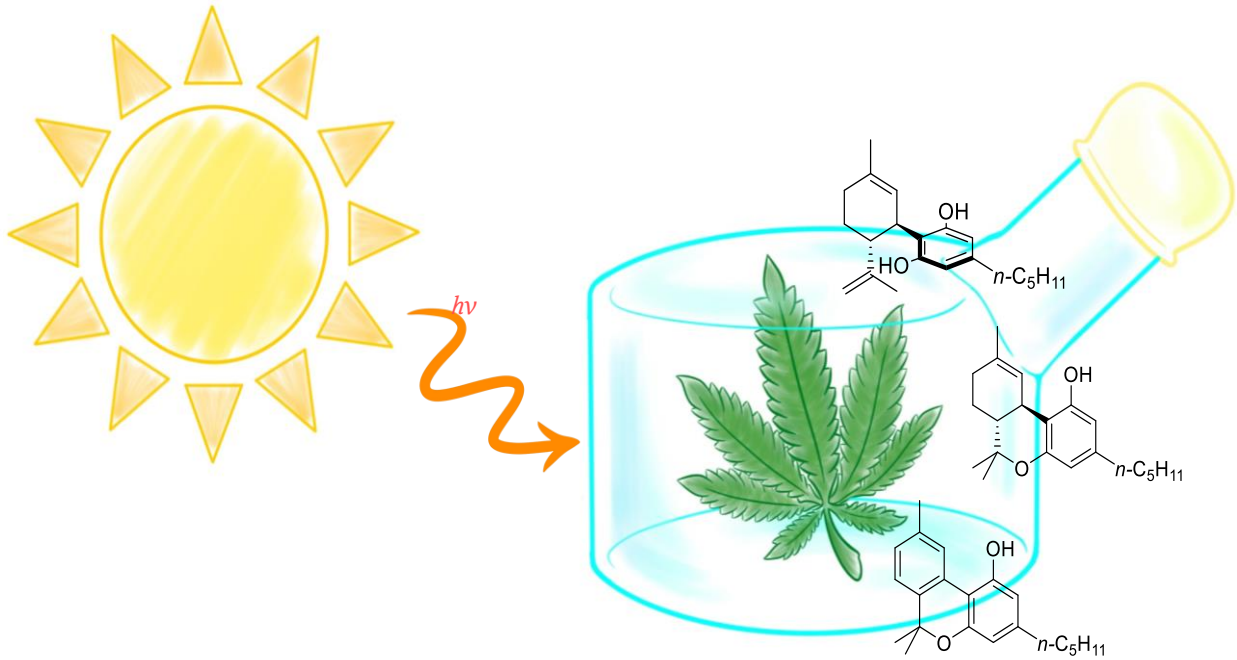
- Old studies (1970)
- Photostability studies lacking
- Stability of vegetal material/formulated drugs
- Possible interconversion of cannabinoids (CBD → THC)
- Minor cannabinoids neglected

Seccamani, P.; Franco, C.; Protti, S.; Porta, A.; Profumo, A.; Caprioglio, D.; Salamone, S.; Mannucci, B.; Merli, D. **Photochemistry of Cannabidiol (CBD) Revised. A Combined Preparative and Spectrometric Investigation.** *J. Nat. Prod.* 2021, 84, 2858–2865.

CANNABIS CHEMOTYPES STUDIED

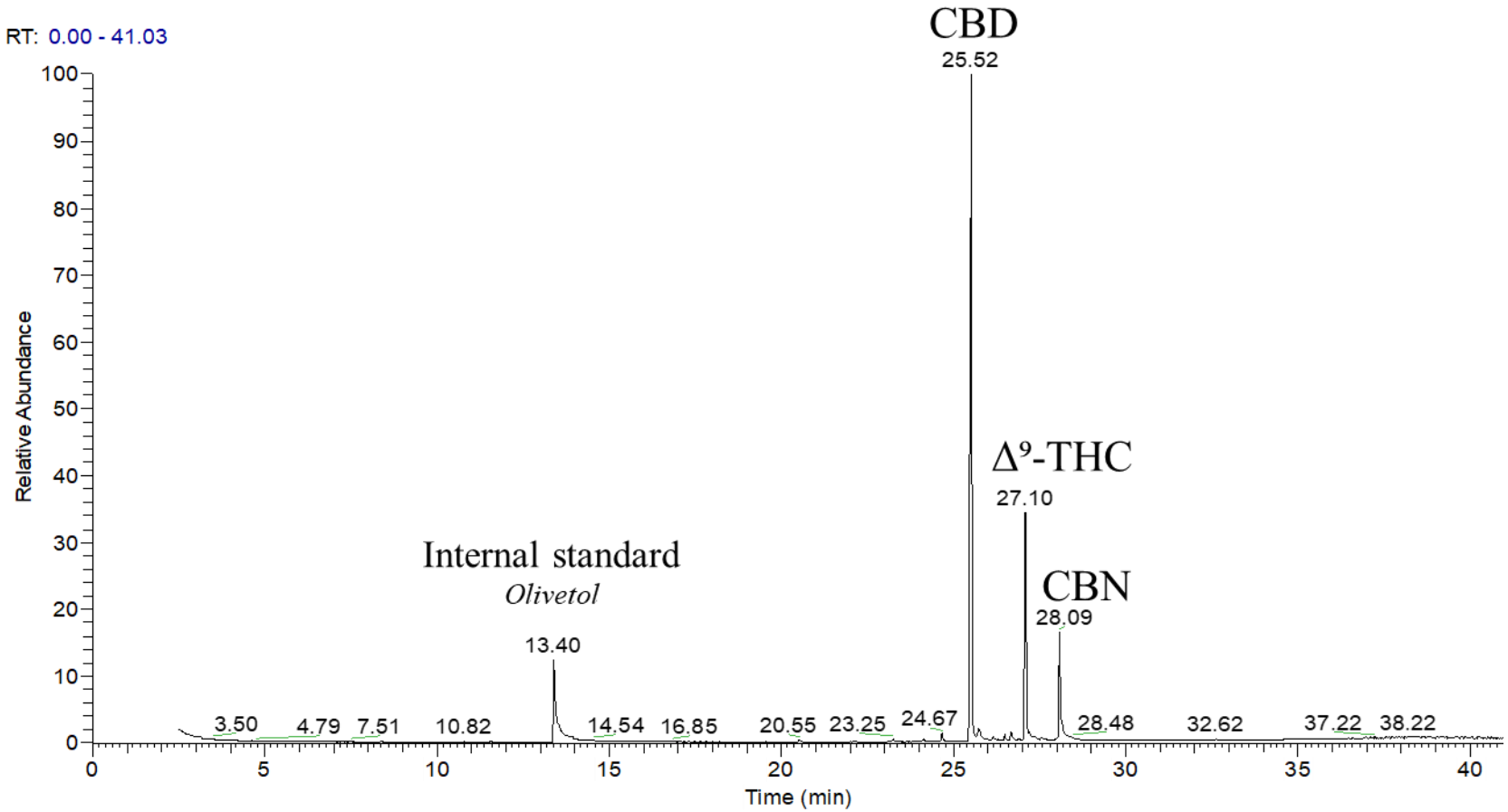
Cannabis var CBD (3%) (C1)

Cannabis var Δ^9-THC/CBD (3%/6%, CBN 2.3%) (C2)

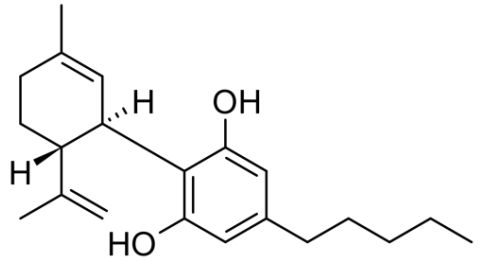


Cannabis var Δ^9-THC (2.9%, CBN 1%) (C3)

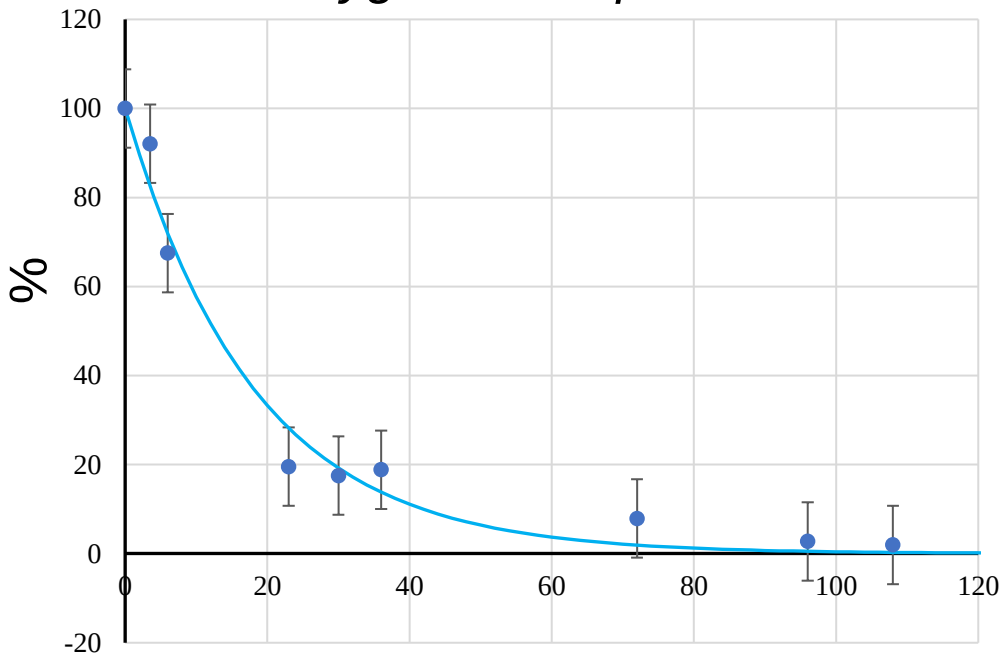
GC-MS: CBD, Δ⁹-THC and CBN in CB2



Cannabis var CBD C1

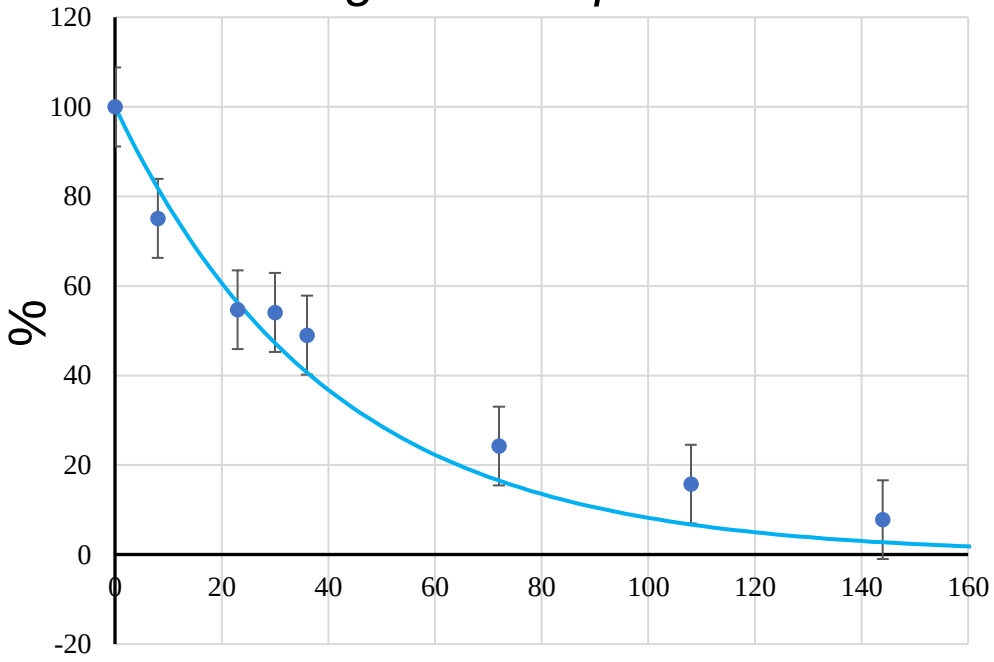


CBD/olivetol area ratio (%)
Oxygen atmosphere



Irradiation time (hours)

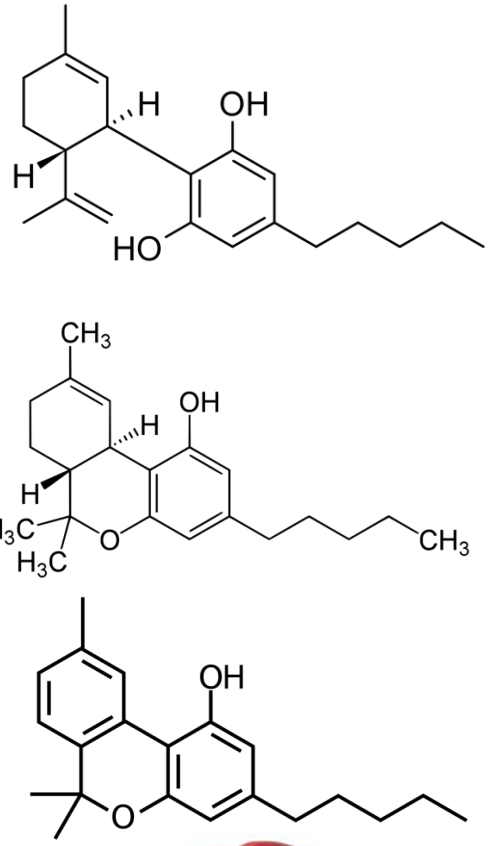
CBD/olivetol area ratio (%)
Nitrogen atmosphere



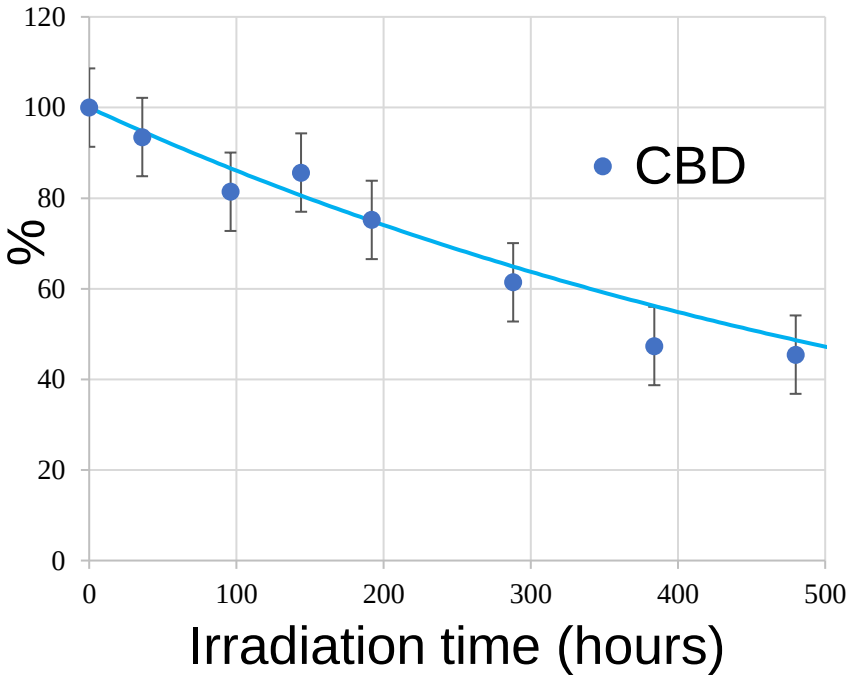
Irradiation time (hours)

Cannabis var Cannabis var Δ9-THC + CBD C2

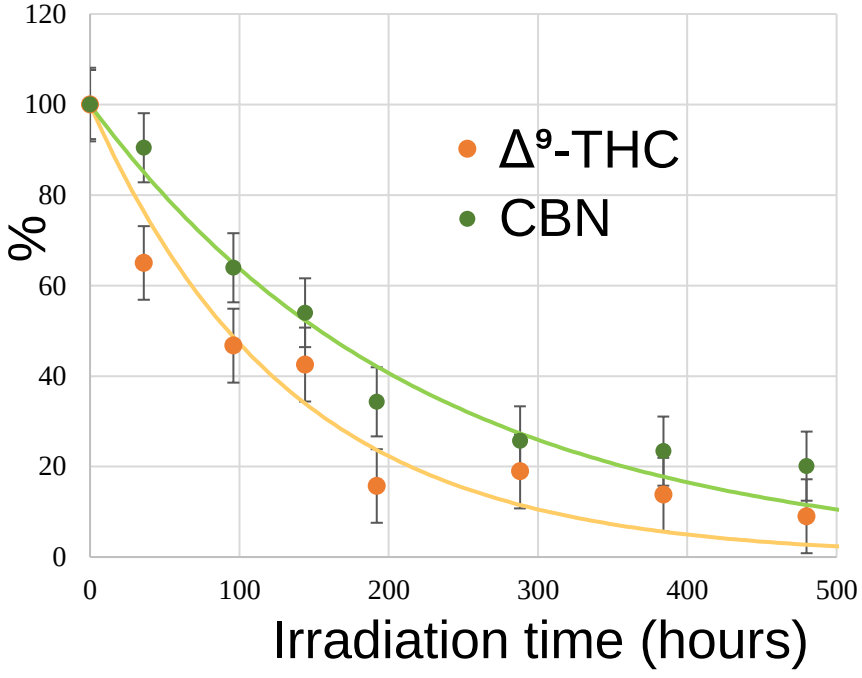
Nitrogen atmosphere



CBD/olivetol area ratio (%)

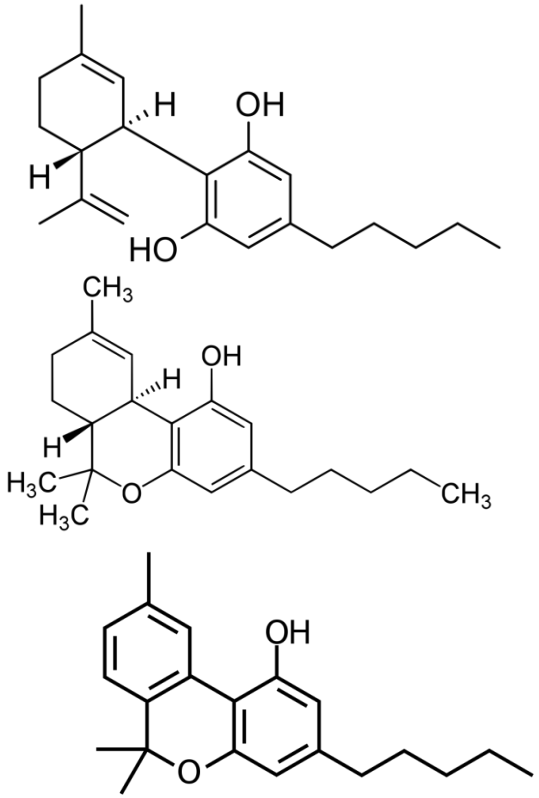


Cannabinoid/olivetol area ratio (%)

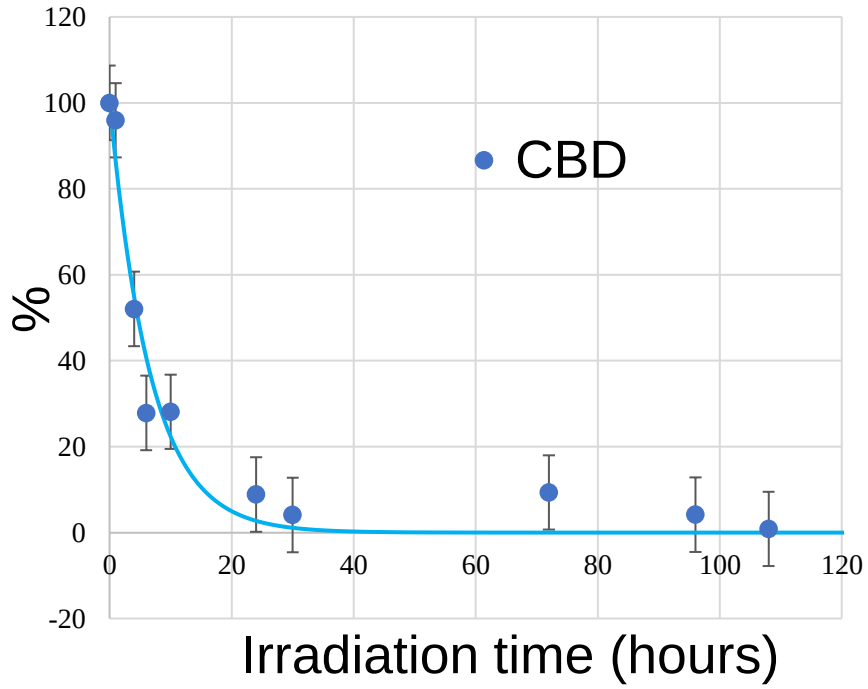


Cannabis var var Δ9-THC + CBD C2

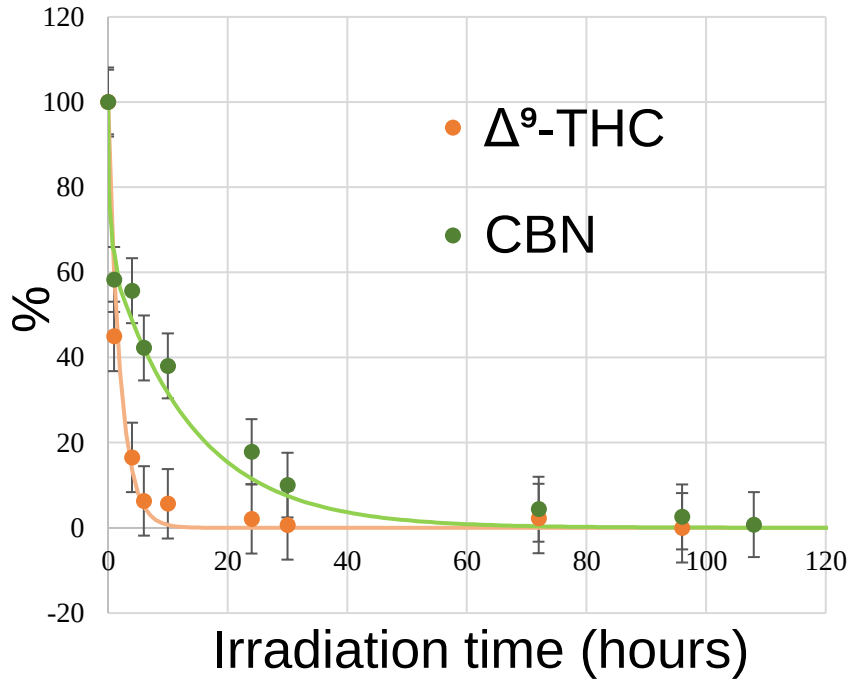
Oxygen atmosphere



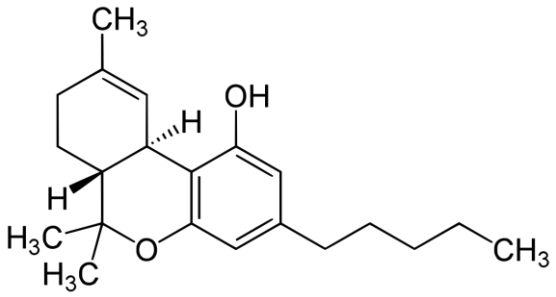
CBD/olivetol area ratio (%)

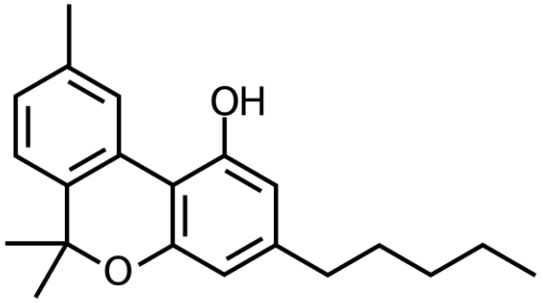


Cannabinoid/olivetol area ratio (%)

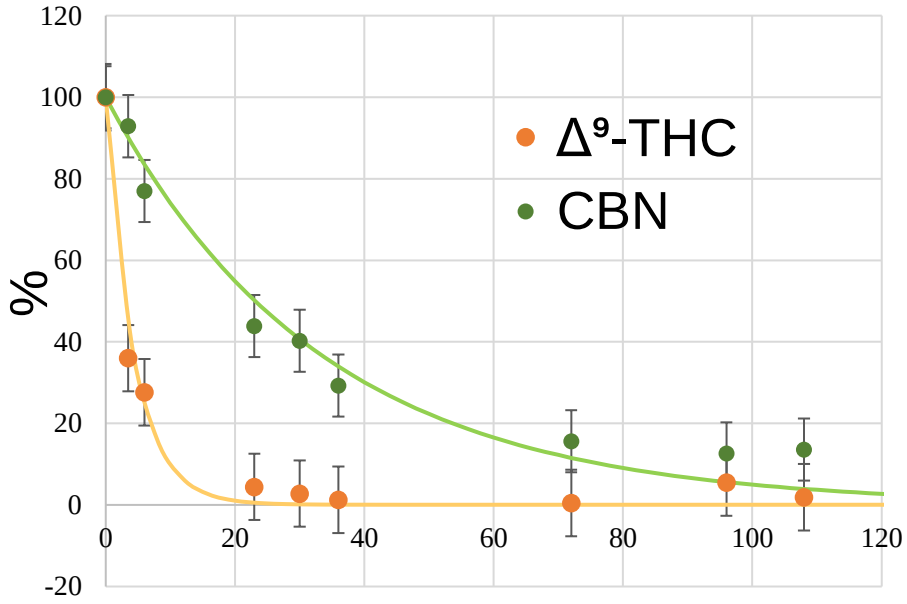


Cannabis var Δ9-THC C3

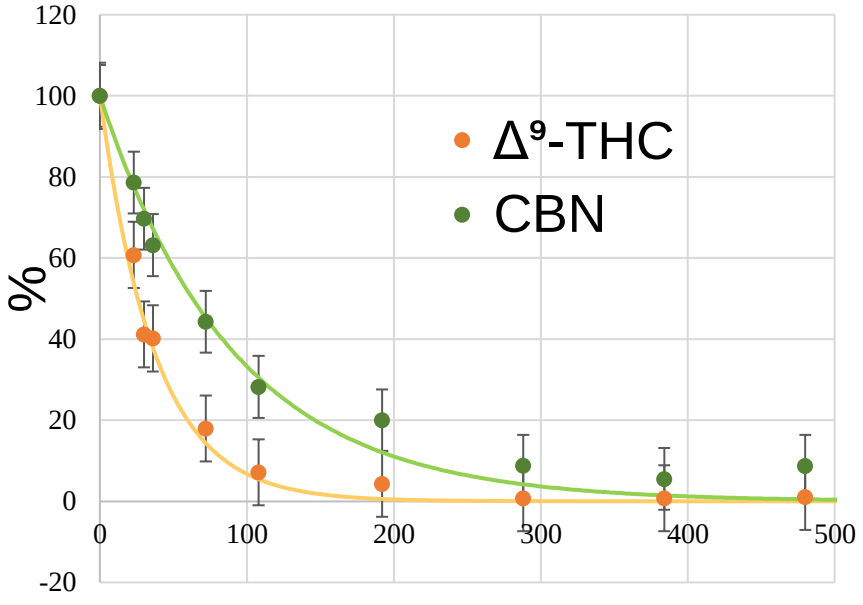




Cannabinoid/olivetol area ratio (%)
Oxygen atmosphere



Cannabinoid/olivetol area ratio (%)
Nitrogen atmosphere



PHOTODEGRADATION KINETIC

Cannabis type	Atmosphere	CBD		Δ^9-THC		CBN	
		k_d (h^{-1})	$t_{90\%}$ (h)	k_d (h^{-1})	$t_{90\%}$ (h)	k_d (h^{-1})	$t_{90\%}$ (h)
C1	Air	0.073(5)	1.44				
	Nitrogen	0.025(1)	4.21				
C2	Air	0.22(3)	0.48	0.43(4)	0.25	0.11(4)	0.96
	Nitrogen	0.0011(4)	95.78	0.007(1)	15.05	0.0045(4)	23.41
C3	Air			0.12(2)	0.88	0.036(3)	2.93
	Nitrogen			0.026(4)	4.05	0.012(1)	8.78

Pseudo-first order kinetic

CONCLUSIONS

- No interconversion between CBD and THCs
- Partial conversion of Δ^9-THC to CBN
- Oxygen greatly influence stability
- Minor cannabinoids not formed during irradiation

BEYOND THC-CONTAINIG CANNABIS

- Cannabis with <0.2-0.5% THC/THC-A is legal in many Countries
- Several “legal” Cannabis samples laced with (semi)synthetic cannabinoids or “legal” psychoactive cannabinoids

Δ^9-THC > HHC >Δ^8-THC >> CBN Δ^8-iso-THC (?)

1	0.9 – ½	½	¼ - 1/10	Psychotropic power

9β-HHC ≥≈ Δ^9- THC
9a-HHC: 1/10 9β-HHC

Grazie per l'attenzione!

Financial support: Finanziato dall'Unione europea - NextGenerationEU. I punti di vista e le opinioni espresse sono tuttavia solo quelli degli autori e non riflettono necessariamente quelli dell'Unione europea o della Commissione europea. Né l'Unione europea né la Commissione europea possono essere ritenute responsabili per essi - NOrCa - Not Ordinary Cannabis - Exploring the chemical space around hemp (Cannabis sativa L.) waste and by products from a circular economy perspective PRIN: PROGETTI DI RICERCA DI RILEVANTE INTERESSE NAZIONALE – Bando 2022 PNRR Prot. P2022TXJX8