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UNIVERSITÀ  
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# Missione 4 Istruzione e Ricerca

**Applicazione della GC-MS  
per l'analisi di caramelle  
gommose contenenti  
cannabinoidi e per  
l'identificazione del  
processo sintetico utilizzato  
nella loro produzione.**

27/06/2025



# Applicazione della GC-MS per l'analisi di caramelle gommosi contenenti cannabinoidi e per l'identificazione del processo sintetico utilizzato nella loro produzione.

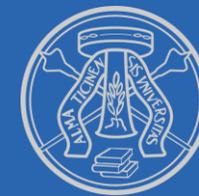
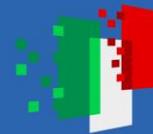
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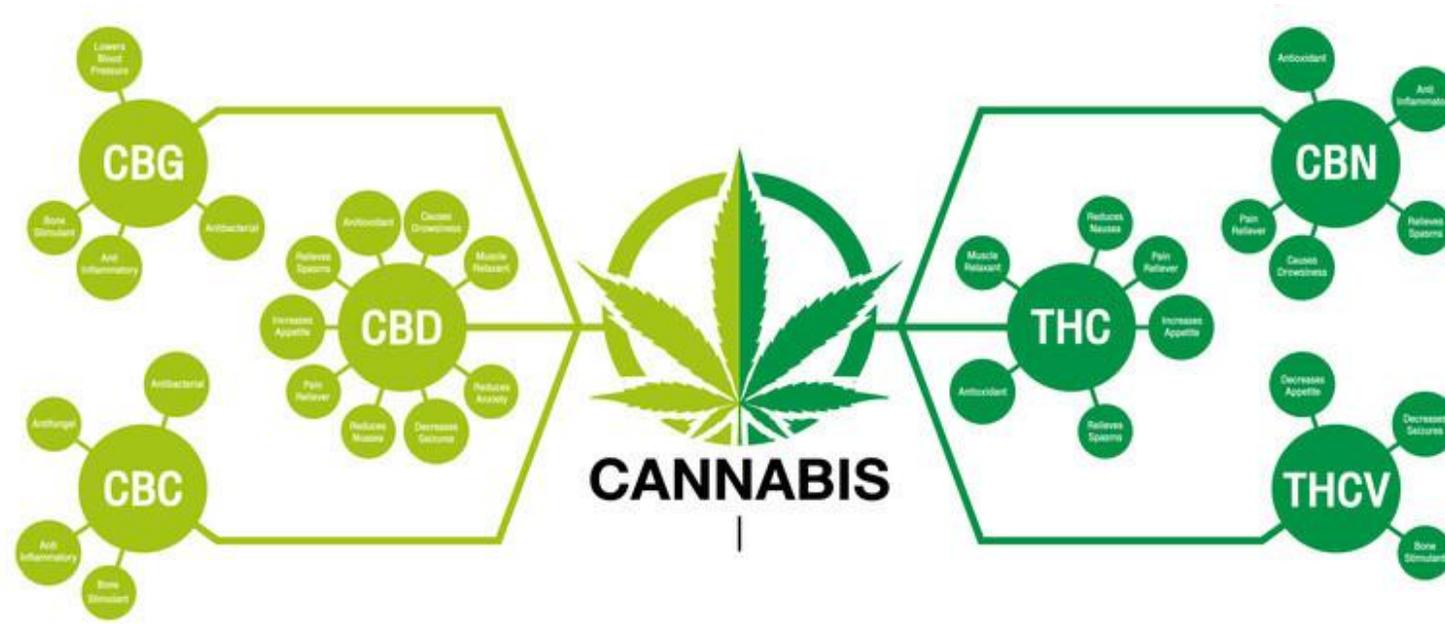
## OVERVIEW

- ✓ 1: *Validation of a GC MS method for the analysis of cannabinoids-containing gummies*
- ✓ 2: *application to real samples*
- ✓ 3: *identification of a NPS, HHC-C9*



## BIOLOGICAL EFFECTS OF CANNABINOIDS

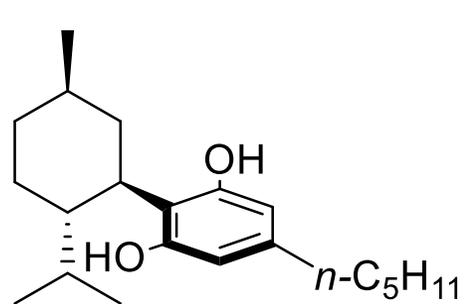
- CBD (cannabidiol)
  - Analgesic/anti-inflammatory
  - Anxiolytic
  - Antiepileptic
- $\Delta^9$ -THC ( $\Delta^9$ -tetrahydrocannabinol)
  - **Psychotropic**
  - Antiemetic
  - Appetite stimulant
  - Immunomodulatory
  - Biphasic effects: possible anxiety and paranoia



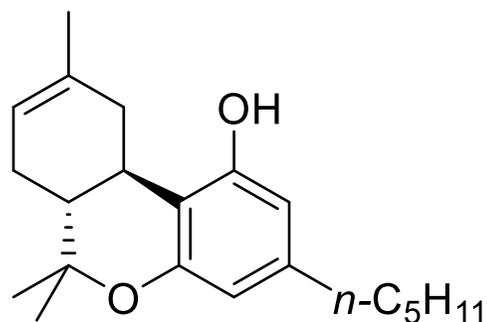
Different Chemotypes

## Psychotropic cannabinoids

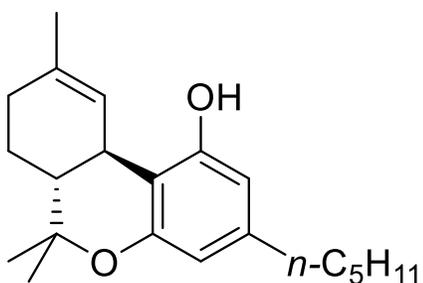
➤  $\Delta^9$ -THC,  $\Delta^8$ -THC, HHC, THD (?)



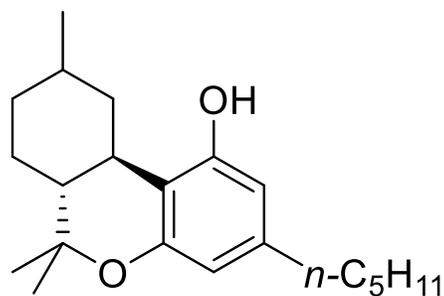
THD



$\Delta^8$ -THC



$\Delta^9$ -THC



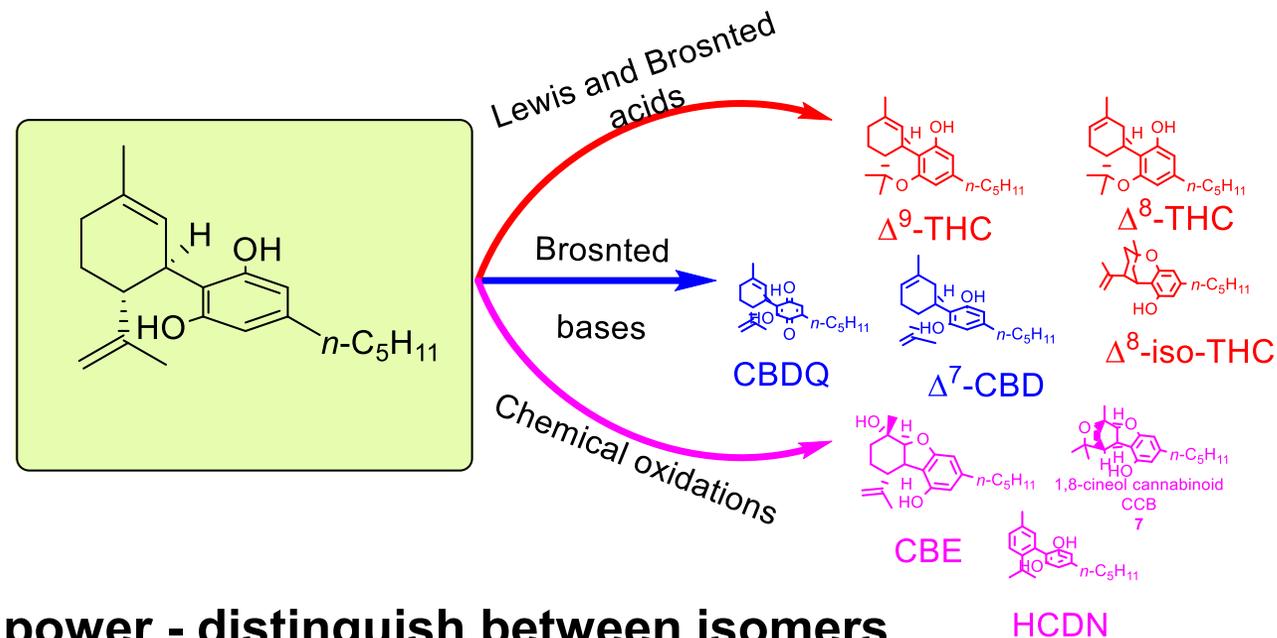
HHC



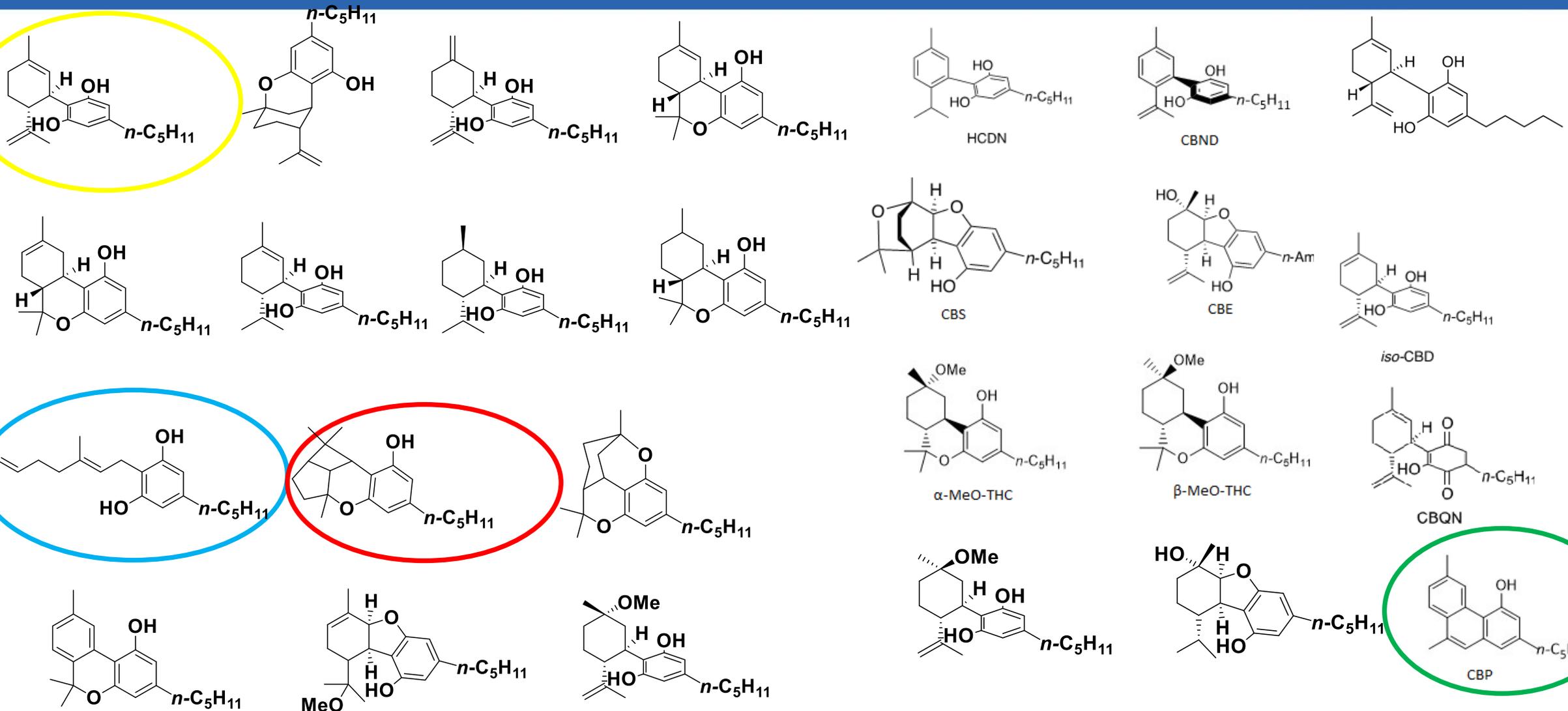
## ANALYTICAL APPROACH- STANDARD SYNTHESSES

➤ Common analytical techniques:

- **GC-MS**
- **HPLC-MS**



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





## VALIDATION OF GC-MS METHOD (ICH)

Spiking of commercial, non-containing cannabinoids gummies with CBD,  $\Delta^8$ -THC, HHC

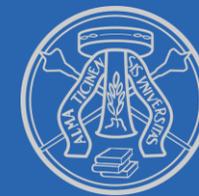
*I: Freezing and extraction with hexane*

### Extraction procedure



*II: water/ hexane extraction*

*III: water/ethanol extraction*



Cannabinoid	Extraction Method	Spiking (mg cannabinoid ·g of gummy <sup>-1</sup> )	Recovery (%)
CBD	<i>III</i>	2.8	110±8
		5.6	106±1
		11.2	114±14
Δ <sup>8</sup> -THC	<i>III</i>	2.0	120±2
		4.0	101±10
		8.0	94±11
HHC	<i>III</i>	2.0	114±4
		4.0	108±6
		8.0	97±10



## VALIDATION OF GC-MS METHOD (ICH)

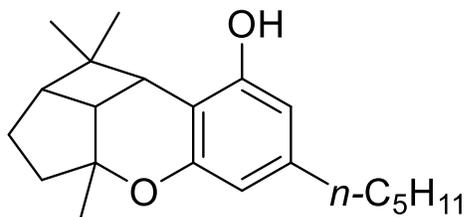
LOD, LOQ



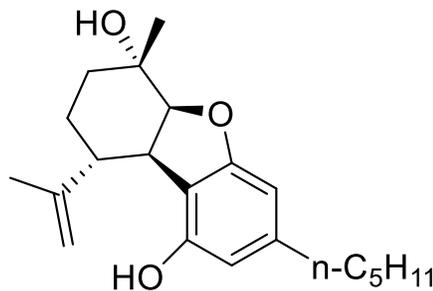
0.06 mg·g<sup>-1</sup>, 0.19 mg·g<sup>-1</sup>

Calibration factor:  $1/x^2$  (quadratic increase in variance vs. concentration).

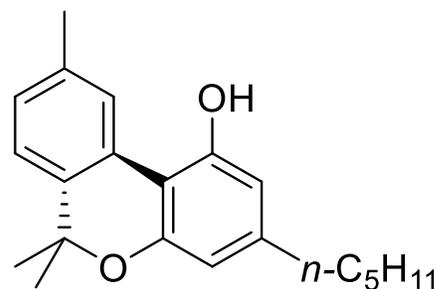
A **quadratic model** to build the calibration curve (Mandel test).



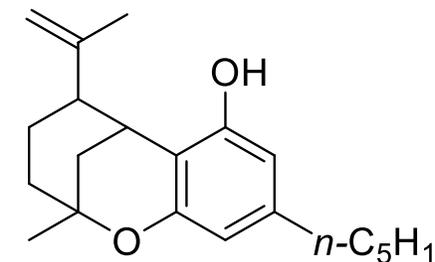
CBL



CBE



CBN



$\Delta^8$ -iso-THC



## Retention indexes

Retention time correspondence (0.3% to 1.5%)

## Spectral characteristics



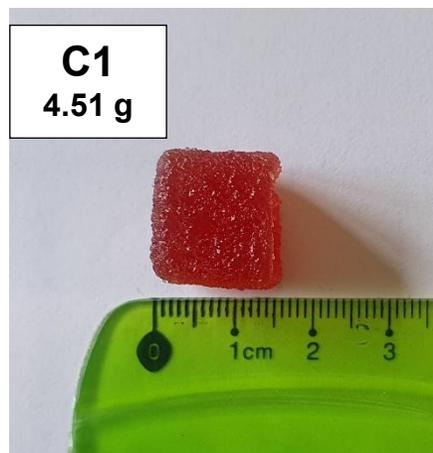
- ✓ Mass spectral correspondence to pure compounds
- ✓ Ion ratio qualifier/quantifier must be constantly  $\pm$  20% of the average value.



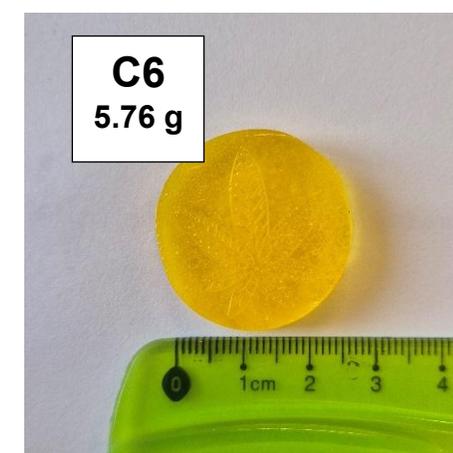
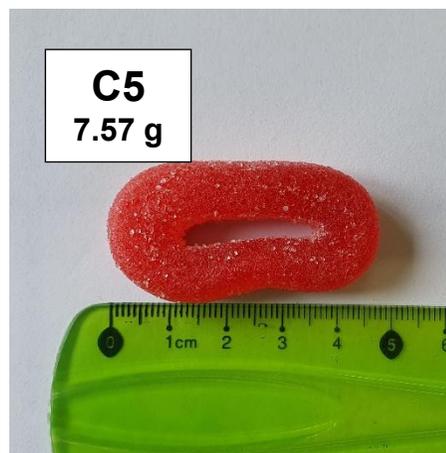
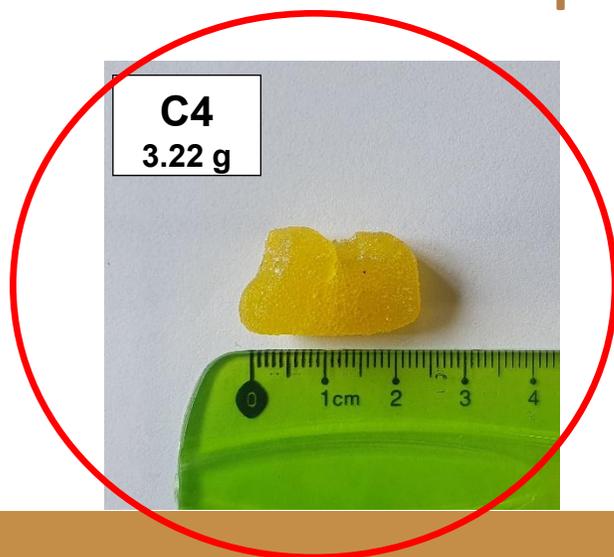
5 and 50 mg/L were used as calibrators (n= 3)



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

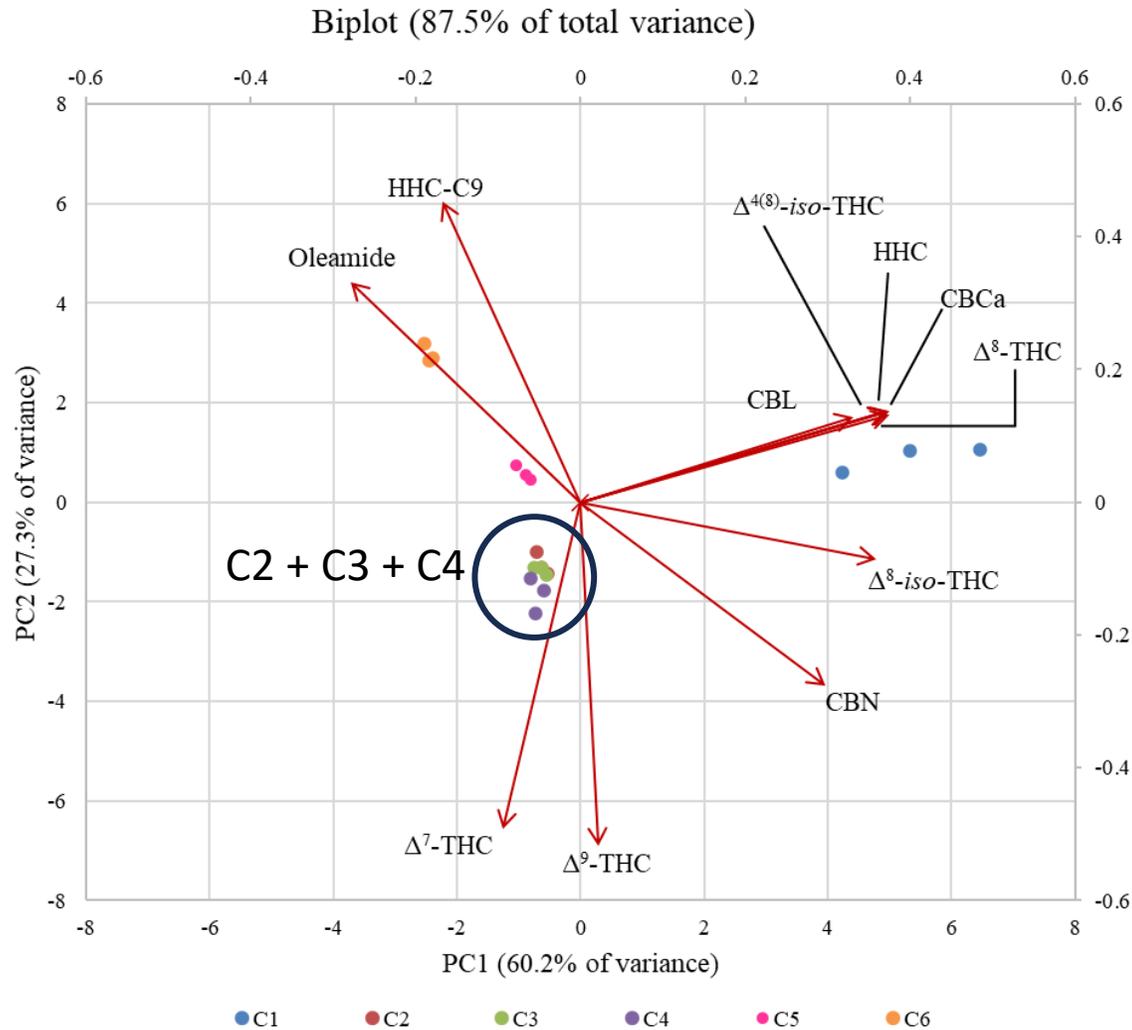


## Real samples: Cannabinoids-containing gummies from NEWS Project





Cannabinoid	mg of compound·g of gummy <sup>-1</sup>					
	C1	C2	C3	C4	C5	C6
THCV	+	-	-	-	-	-
CBL	0.40 ± 0.08	-	-	-	-	-
CBD	-	-	-	+	-	-
$\Delta^{4(8)}$ - <i>iso</i> -THC	1.4 ± 0.1	-	-	-	-	-
$\Delta^8$ - <i>iso</i> -THC	0.39 ± 0.03	+	+	0.19 ± 0.05	-	-
$\Delta^7$ -THC	-	+	+	0.29 ± 0.05	-	-
<b><i>cis + trans</i>-HHC</b>	<b>10 ± 1</b>	-	-	-	-	-
CBCa	0.37 ± 0.05	-	-	-	-	-
CBE	+	-	-	-	-	-
<b><math>\Delta^8</math>-THC</b>	<b>17.1 ± 1</b>	0.21 ± 0.04	+	0.20 ± 0.02	-	-
<b><math>\Delta^9</math>-THC</b>	3.7 ± 0.2	<b>9.0 ± 0.8</b>	<b>10 ± 1</b>	<b>9.8 ± 0.9</b>	0.61 ± 0.04	-
CBN	0.29 ± 0.01	+	0.21 ± 0.03	0.20 ± 0.06	+	-
<b>HHC-C9</b>	-	-	-	-	-	<b>2.5 ± 0.4</b>
Oleamide	-	1.0 ± 0.3	1.4 ± 0.5	1.0 ± 0.3	1.2 ± 0.5	3.4 ± 0.3



*Biplot of the PCA model on the first two principal components, with red arrows representing the vectors of the loading values of the variables (compounds reported in Table 2 minus THCV, CBD and CBE). The colored dots are the scores of the gummies*



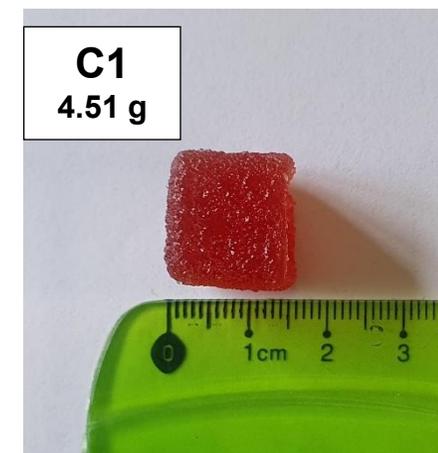
C1: psychotropic: *cis* + *trans*-HHC +  $\Delta^8$ -THC

THCV, a minor natural cannabinoid

CBCa from the reaction of cannabigerol and acids

Acid catalyzed cyclization of natural CBD, leading to  $\Delta^8$ -THC, followed by incomplete catalytic hydrogenation. The presence of CBL, CBE,  $\Delta^8$ -*iso*-THC and  $\Delta^{4(8)}$ -*iso*-THC confirms these assumptions

CBN: aromatization of THC<sub>s</sub> during the reactions or during storage

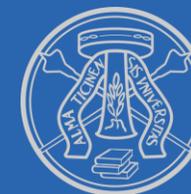
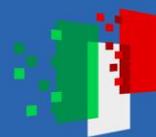




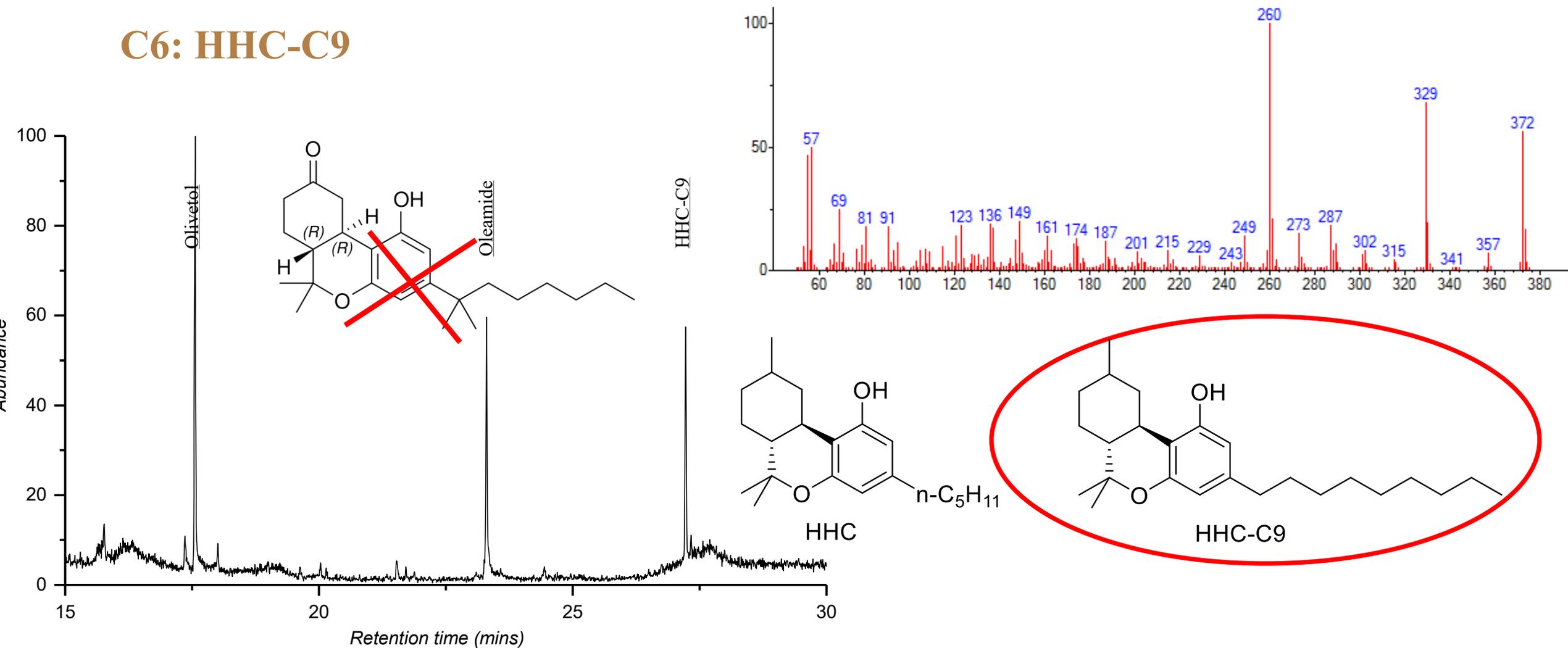
- C6: hexahydrocannabinonol ((9-R)-HHC-C9)
- oleamide
- HHC-C9 is completely synthetic (unprecedented case)
- HHC-C9: similar biological effects to HHC
- HHC-C9 was identified by extracting it from the gummy and performing GC-MS, HRMS,  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR analyses.
- In the e-cig liquid sized jointly with C6: 50% solution of HHC-C9 in propylene glycol/glycerin

**C6**  
5.76 g





## C6: HHC-C9





## Conclusions:

- Identification and quantification of cannabinoids in gummies
- Minor cannabinoids formed during synthesis
- Identification of synthetic pathways
- Identification of a new cannabinoid
- No standard: need to purify and use the compound as a standard



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*Grazie per l'attenzione!*



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