AAFS, February 2024

FSF FORENSIC SCIENCES FOUNDATION
EMERGING FORENSIC SCIENTIST AWARD

Metabolism of THC-O-Acetate (THCO) – An Emerging Drug Threat

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- There are no real or apparent conflicts of interest related to the content of this presentation.
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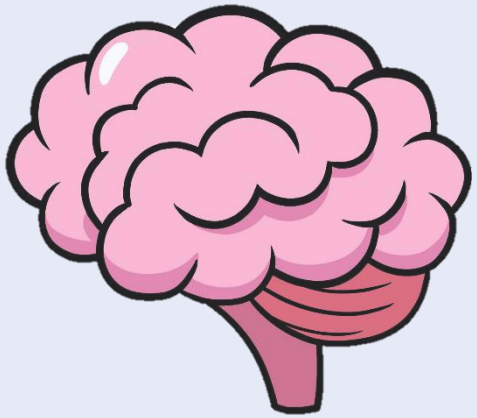
Cannabinoids

Cannabinoid: C21 or C22 terpene phenolic skeleton

Endocannabinoids

- Brain-derived



e.g. Anandamide, 2-arachidonoylglycerol

Phytocannabinoids

- Plant-derived, naturally occurred

e.g. Δ9-THC, Δ8-THC, CBD, CBG, CBN…

Semi-Synthetic Cannabinoids

- Plant-derived cannabinoids followed by chemical modification

e.g. HCO

Synthetic Cannabinoids

- Chemically synthesized

e.g. JWH-018, JWH-024, K2, AB-FUBINACA….

Statement of the Problem

Past → **Controlled Substances Act**

Cannabis sativa
(Marijuana and Hemp)→ Illegal

2018 → **2018 farm bill (P.L. 115-334)**

Hemp→ Legal in U.S.

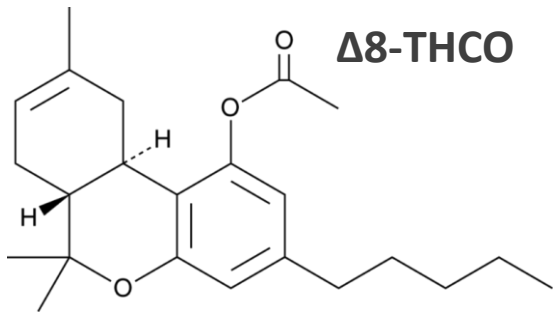
Marijuana→ Schedule I CS

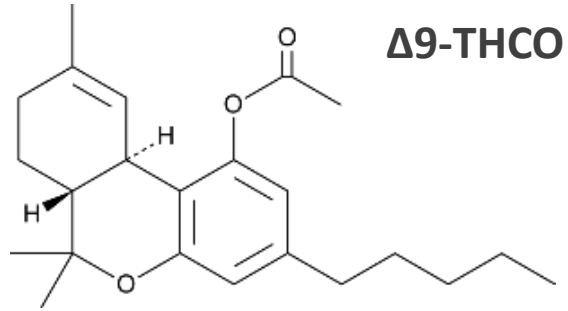
Now → **Rapid growth of market**

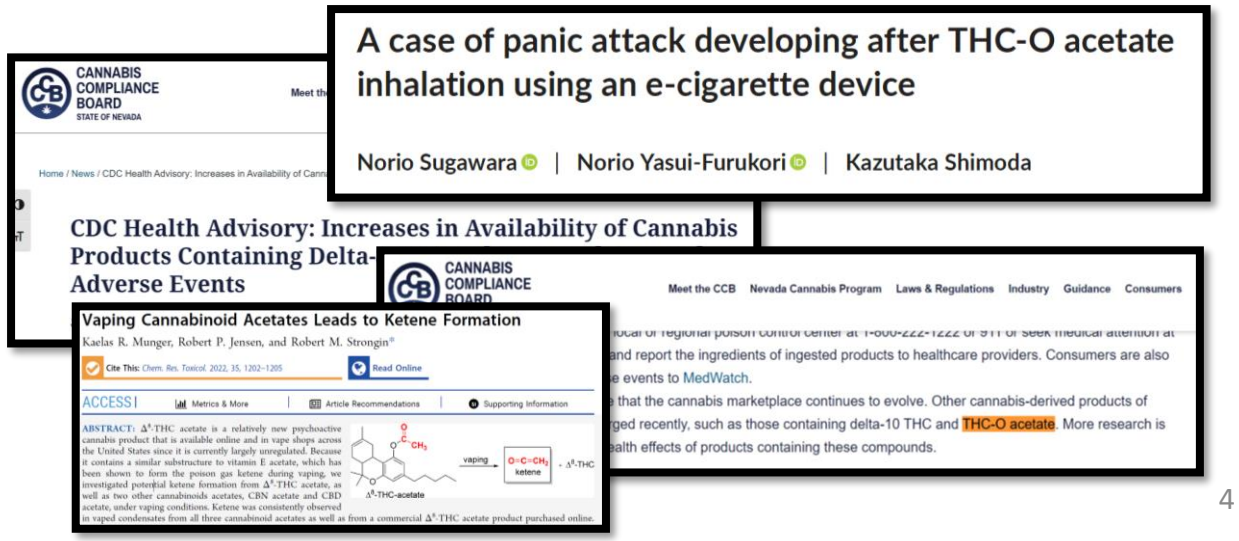
Hemp or CBD derivatives semi-synthetic cannabinoids lead to public health concerns

Challenge: Numerous hemp derivatives semi-synthetic cannabinoids remain underexplored in scientific research

THC-O Acetate (THCO)



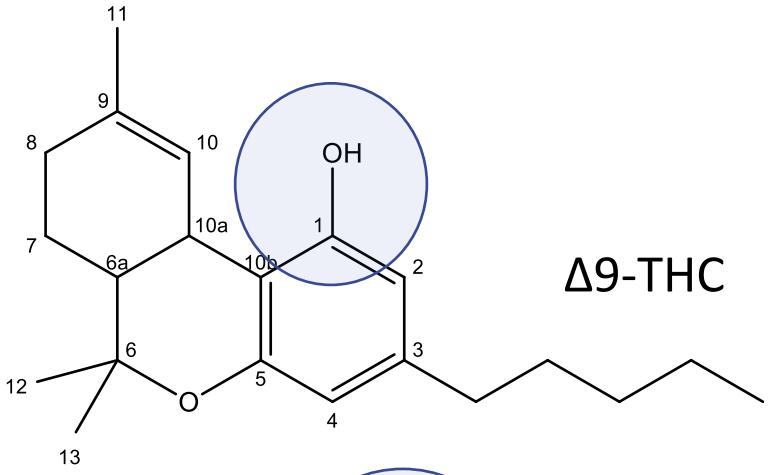




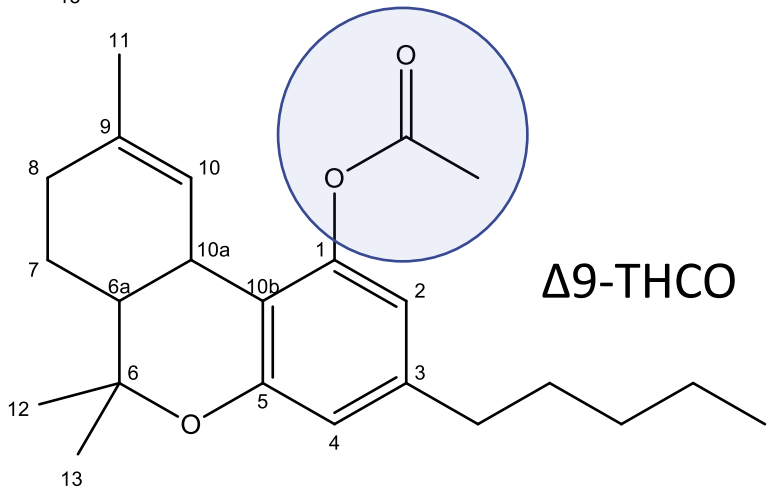
THC-O-Acetate

Semi-synthetic cannabinoid

The acetylation of THC

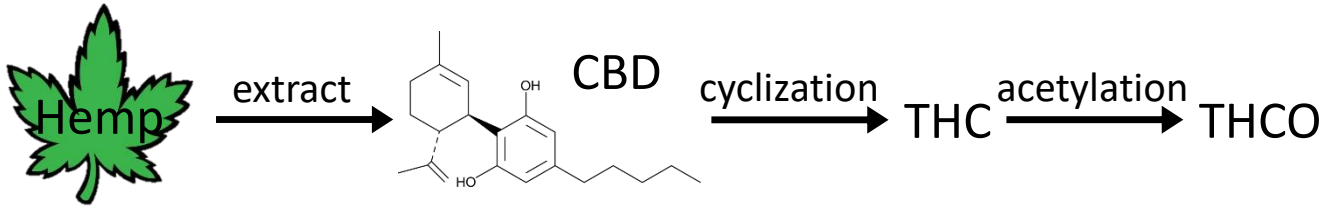


Δ9-THC



Δ9-THCO

- First reported in 1942 (Wollner et al)
  - Greater lipophilicity→2x potency of THC
- Semi-synthetic cannabinoid from hemp



- Increasing in popularity

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Advance Access Publication Date: 8 June 2022
Article

OXFORD

Δ8-THC, THC-O Acetates and CBD-di-O Acetate: Emerging Synthetic Cannabinoids Found in Commercially Sold Plant Material and Gummy Edibles

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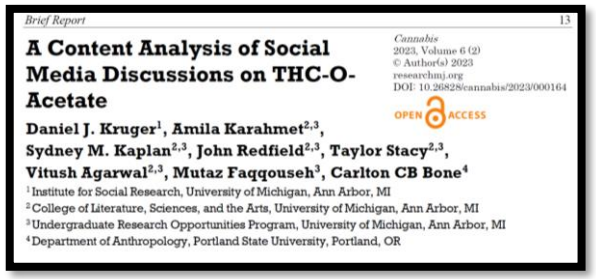

Brief Report 13

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OPEN ACCESS

A Content Analysis of Social Media Discussions on THC-O-Acetate

Daniel J. Kruger[1], Amila Karahmet[2,3], Sydney M. Kaplan[2,3], John Redfield[2,3], Taylor Stacy[2,3], Vitush Agarwal[2,3], Mutaz Faqqouseh[3], Carlton CB Bone[4]

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[3]Undergraduate Research Opportunities Program, University of Michigan, Ann Arbor, MI
[4]Department of Anthropology, Portland State University, Portland, OR

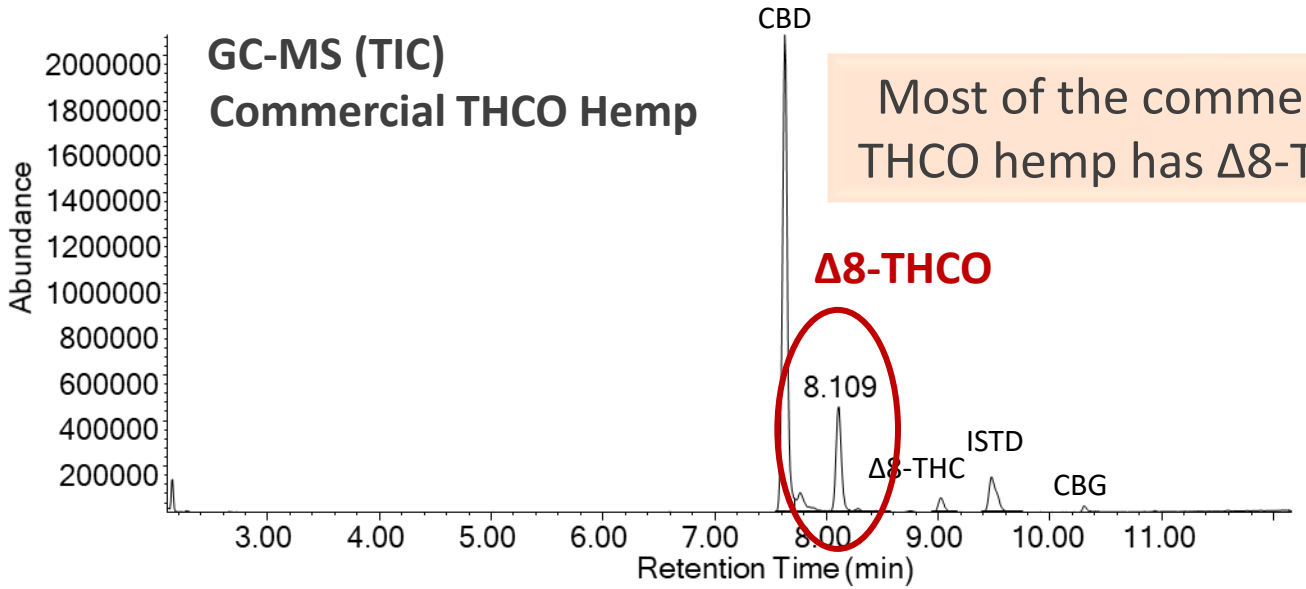
- No known information regarding:
  - Metabolism
  - Pharmacodynamics
  - Stability

Delta-8 THCO and Delta-9 THCO Classified as Schedule I Drugs: This Week in Cannabis Investing

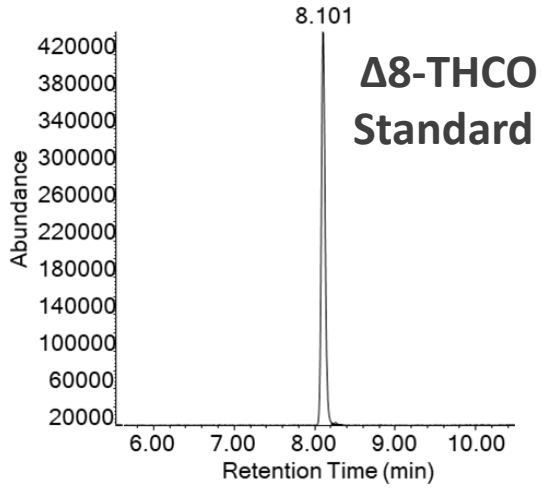
The Drug Enforcement Agency said delta cannabinoids, including Delta-8 THCO and Delta-9 THCO, are synthetically derived from hemp, and, therefore, are not considered hemp.

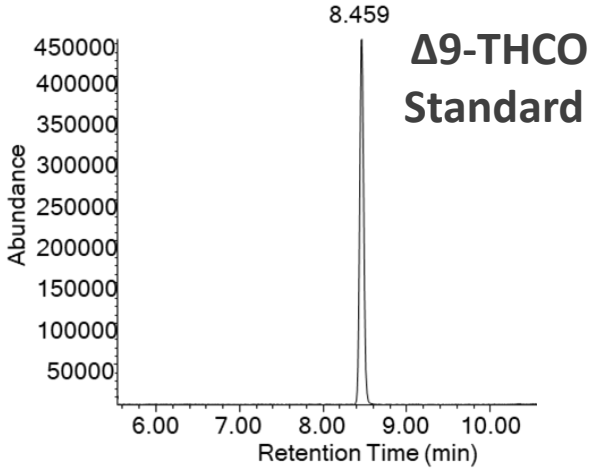
Commercial THCO Hemp

- **Commercially available THCO Hemp** (Before THCO was schedule I)



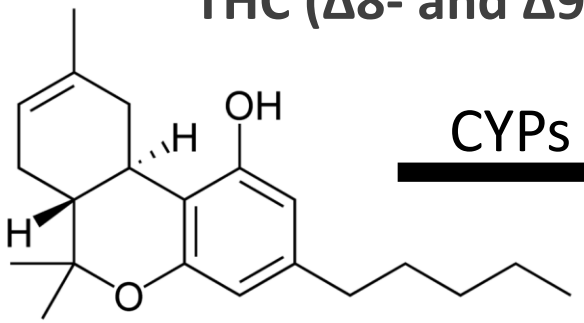
Most of the commercial THCO hemp has Δ8-THCO





Drug Metabolism

THC (Δ8- and Δ9-THC)

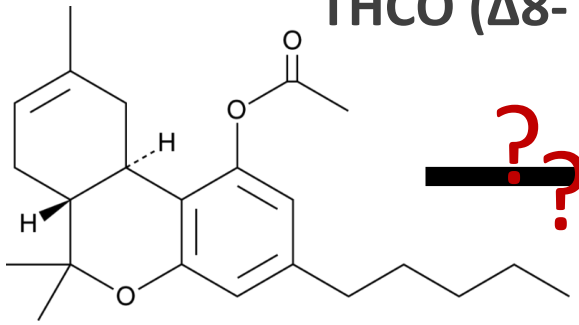


CYPs →

11-OH-THC
7-OH-THC
1'-OH-THC
2'-OH-THC

3'-OH-THC
4'-OH-THC
Many others

THCO (Δ8- and Δ9-THCO)



→ ? ? ? ? ? ?

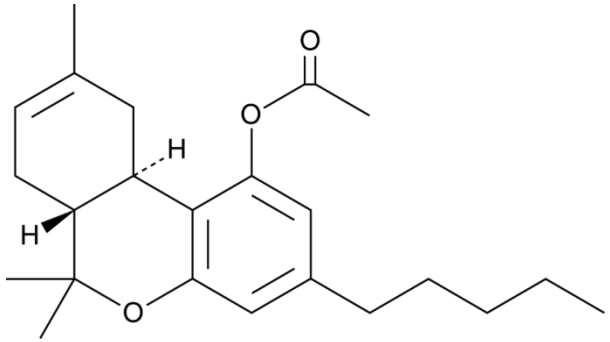
Investigate the metabolism pathway of Δ8-THCO and Δ9-THCO

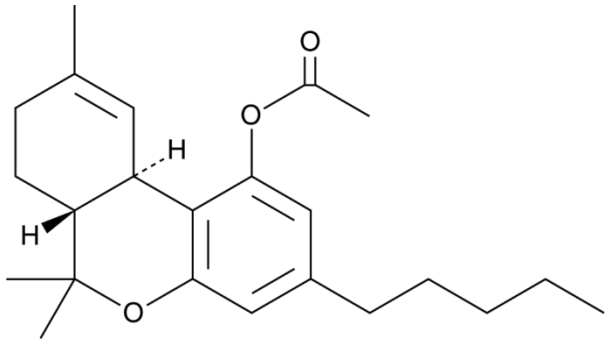
Metabolism Studies

- In vivo
  → Comprehensive, expensive, difficult
- **In vitro**
  → Controlled, inexpensive, easy, lacks endogenous factors
  - Human Liver Models → Human Liver Microsome (**HLMs)**
    - Phase I Enzymes ← cytochrome P450s (CYPs), carboxylesterases, epoxide hydrolase, UDP glucuronyl transferase, etc.
    - Phase II Enzymes
  - Human Plasma
    - Esterase → proteases, lipases, and esterases
  - …. Many others

Experimental Design

Investigate of the metabolism pathway of Δ8-THCO and Δ9-THCO with LC-QTOF-MS



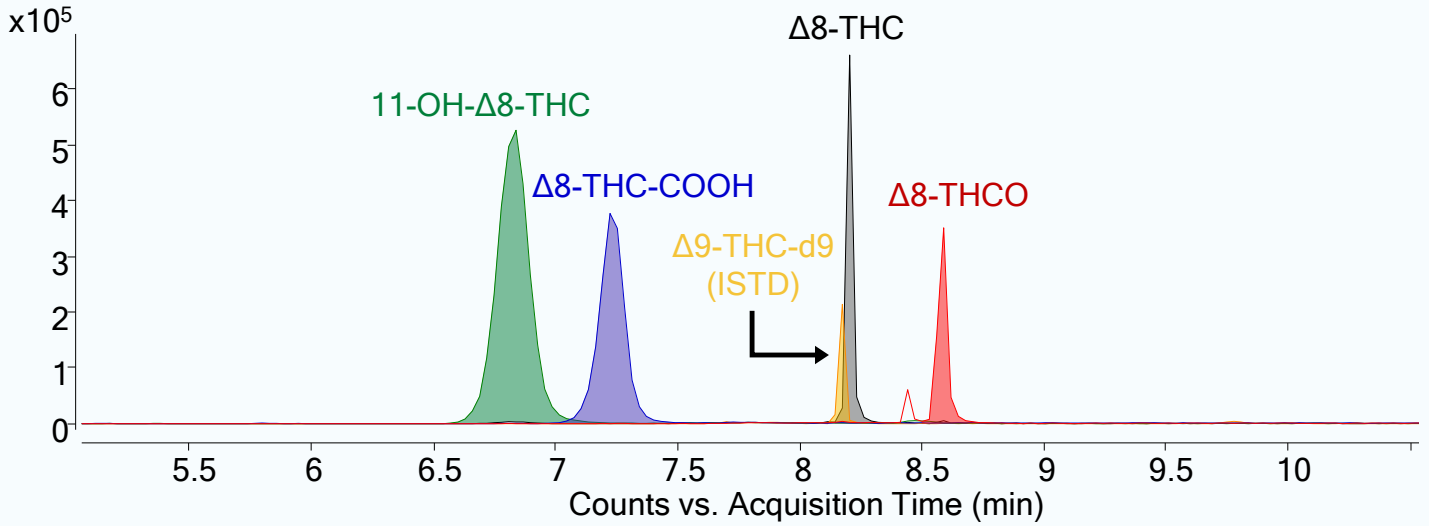


Δ8-THCO & Δ9-THCO Metabolism

- Method development and optimization
  - Compound optimization
  - Source optimization
  - LC optimization
  - Sample prep optimization
- Metabolism studies
  - Human liver microsome (HLM)
    - Cytochromes P450 (CYPs)
    - Carboxylesterase (CES)
  - Potential metabolites screening
  - Human plasma pool

Δ8-THCO

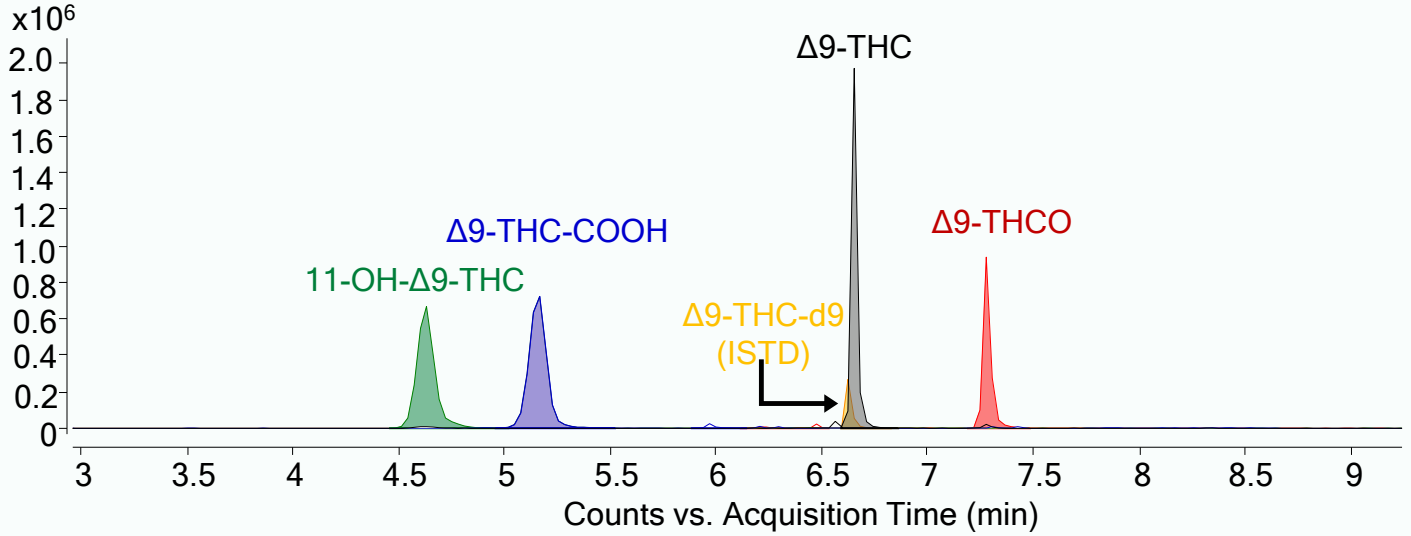
50% B (6.5 min) —0.2 min→ 57% B —1.3 min→ 98% B (1 min) —1 min→ 50% B



Parameters	Optimized Conditions
Column	Poroshell 120 EC-C18
Mobile A/B	H_2O+0.1%FA/ACN+0.1%FA
Injection/ESI	5µL/positive
Drying Gas	350 °C, 13 L/min
Sheath Gas	200 °C, 12 L/mi
Nebulizer	20 psi
Cap/Noz V	4500/2000 V

Δ9-THCO

50% B (1 min) —0.5 min→ 55% B (3 min) —1.5 min→ 95% B (4 min) —1 min→ 50% B



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Injection/ESI	5µL/positive
Drying Gas	350 °C, 13 L/min
Sheath Gas	200 °C, 12 L/mi
Nebulizer	20 psi
Cap/Noz V	4500/2000 V

Sample Preparation Optimization

Initial procedure

THCO in glass conical tube

↓ Air-dry

Reconcentrate with phosphate buffer (pH7.4)

↓

Transfer to 1.5 µL plastic tube

↓

Enzyme addition and Incubation

↓

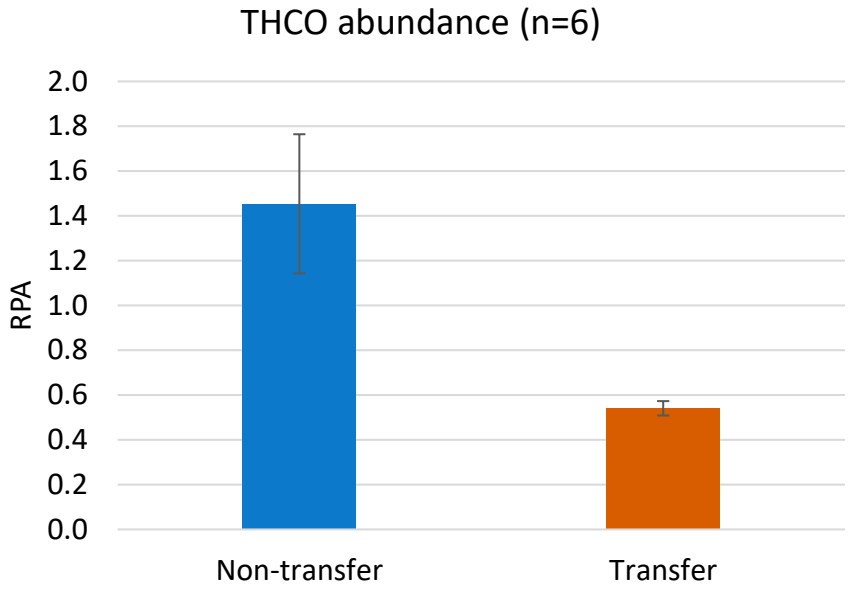
1:1 mix with Stop Solution

↓ Centrifuge

1:5 dilution with Mobile A/B

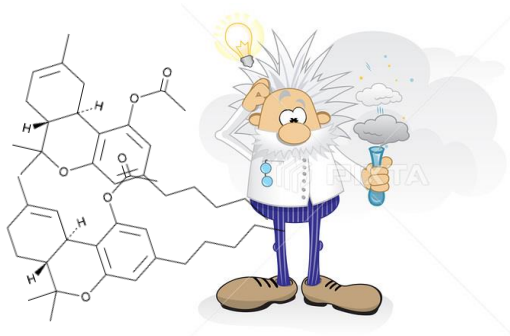
↓

LC/Q-TOF/MS



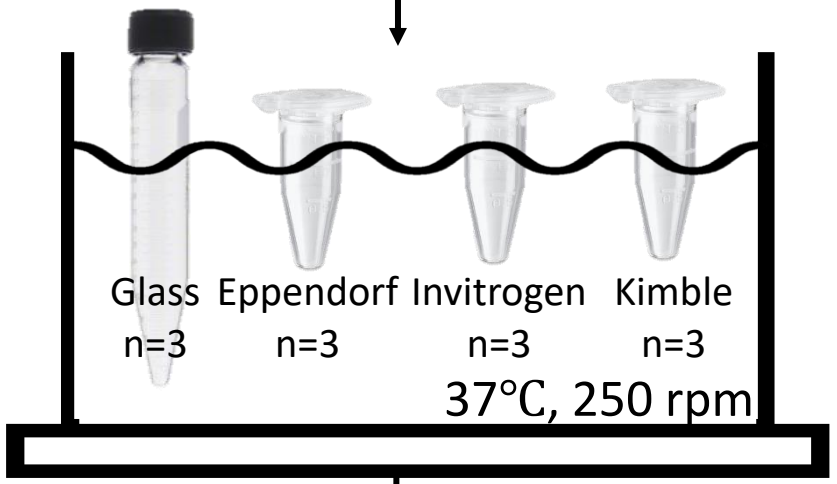
Surface absorption test

Stop Solution:
ice cold Δ9-THCO-d9 (5µM)
in mobile phase B (ACN)



Surface Absorption Test

50μM THCO in phosphate buffer (pH7.4)



Collect sample at T0, T30, T60, T120

1:1 mix with Stop Solution

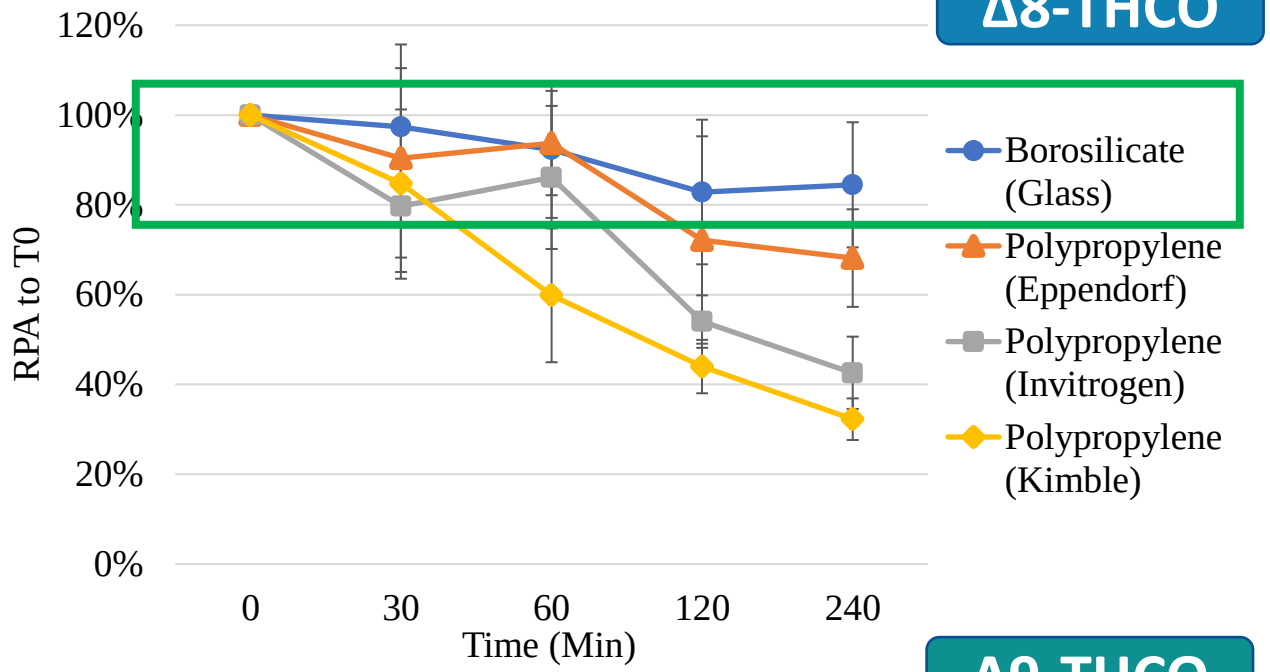
Centrifuge

1:5 dilution with Mobile A/B

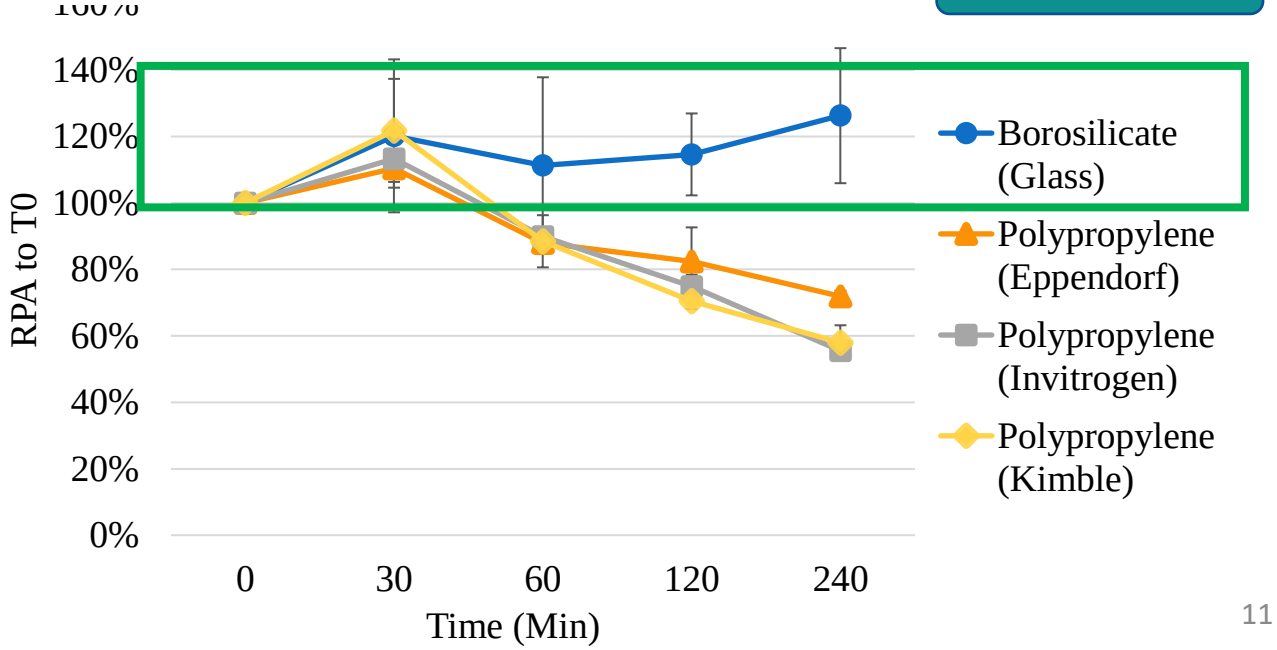
LC/Q-TOF/MS

All incubations to be done in glass tube

Δ8-THCO

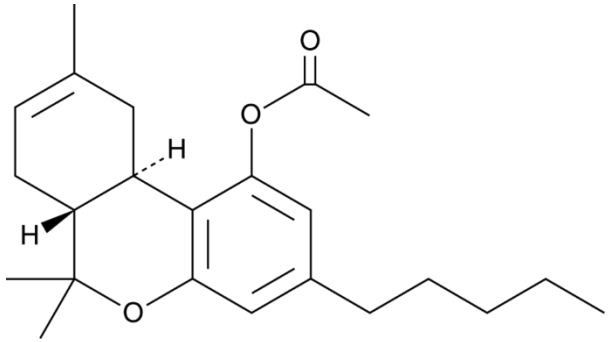


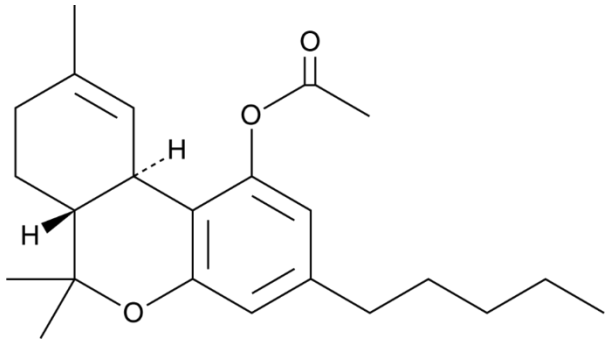
Δ9-THCO



Experimental Design

Investigate of the metabolism pathway of Δ8-THCO and Δ9-THCO with LC-QTOF-MS





Δ8-THCO & Δ9-THCO Metabolism

- Method development and optimization
  - Compound optimization ✓
  - Source optimization ✓
  - LC optimization ✓
  - Sample prep optimization ✓
- Metabolism studies
  - Human liver microsome (HLM)
    - Cytochromes P450 (CYPs)
    - Carboxylesterase (CES)
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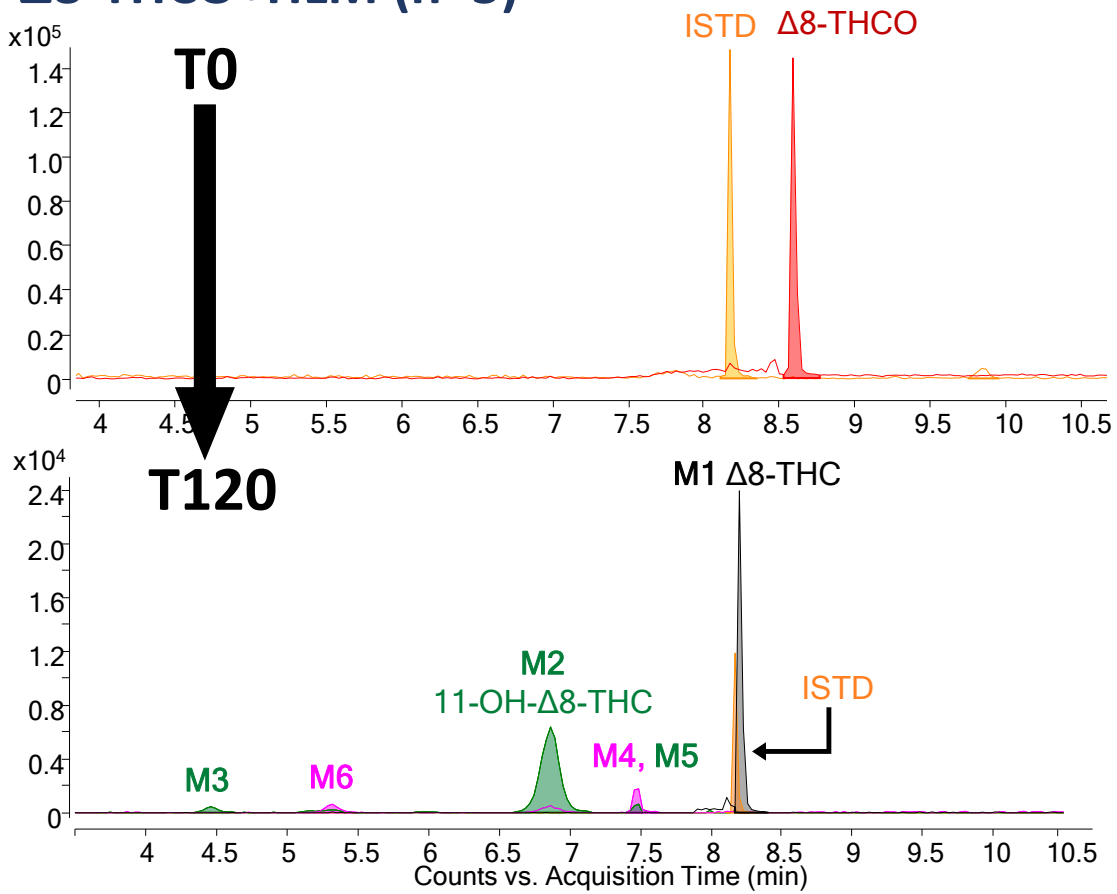
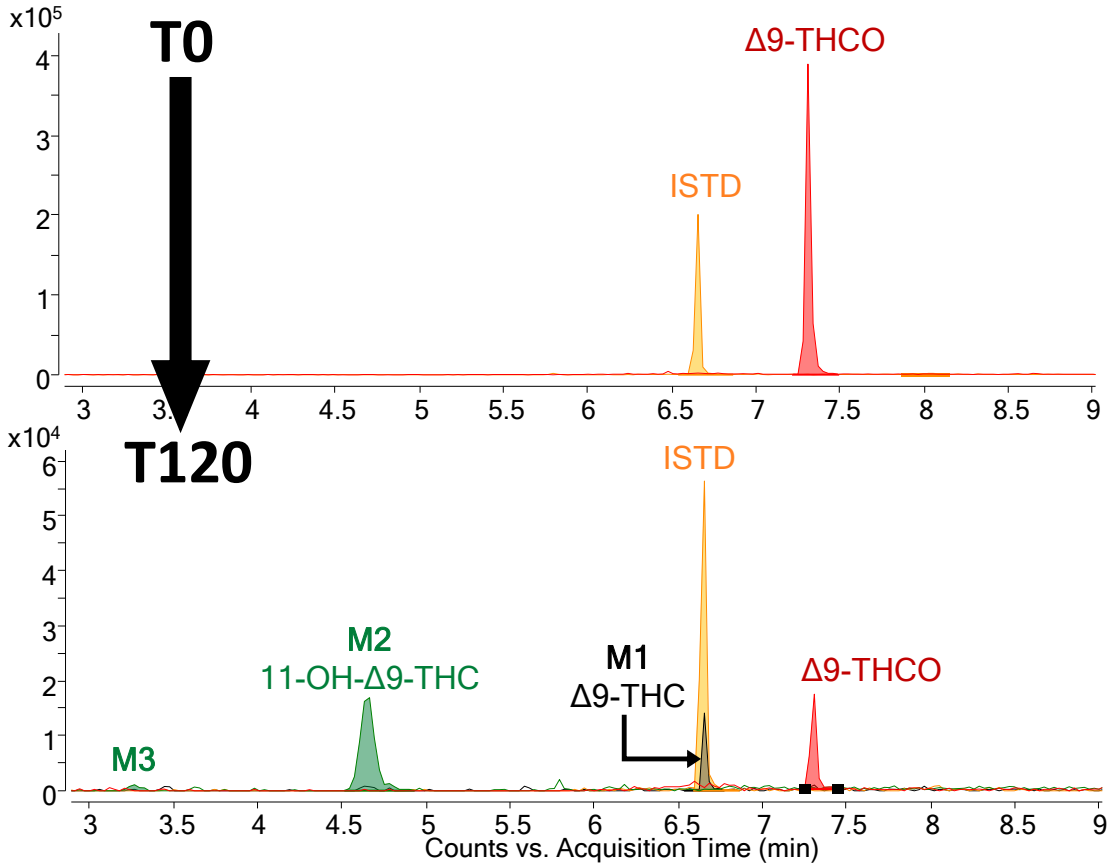
THCO Metabolites Screening: HLM

➢ Δ8-THCO+HLM (n=3)



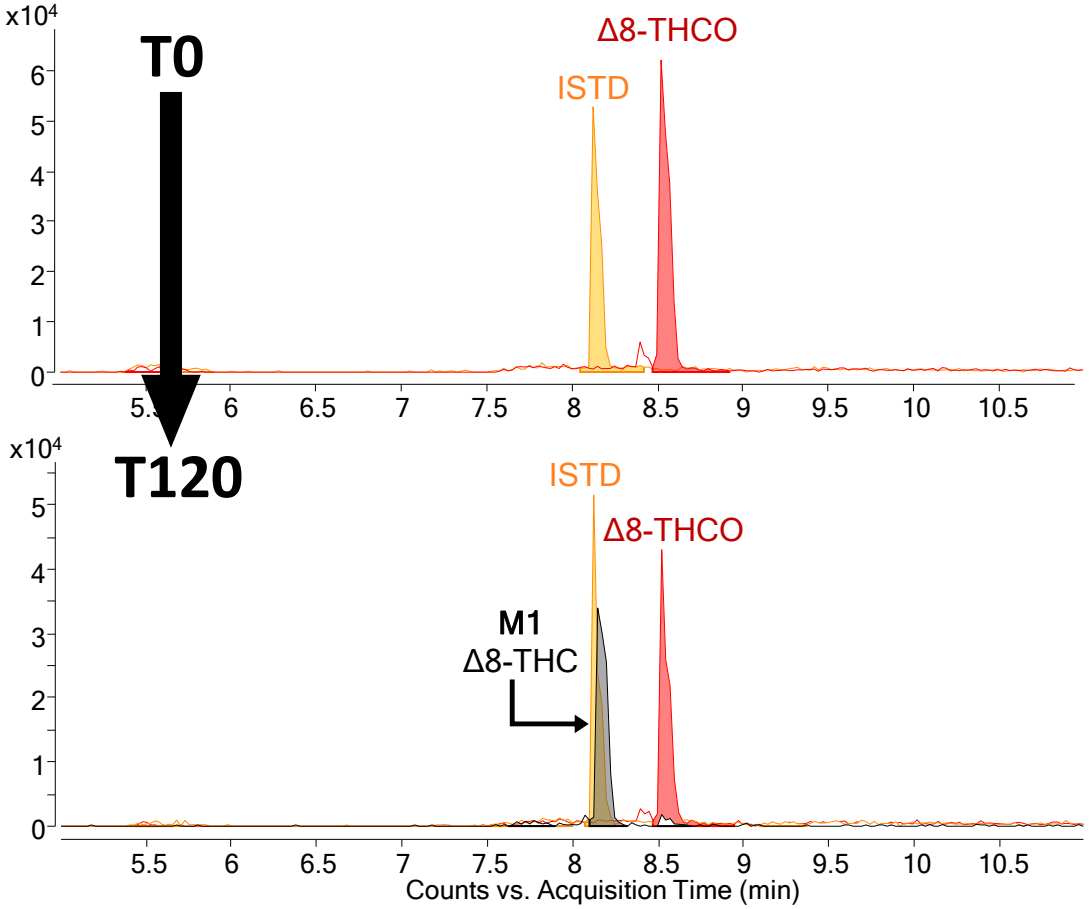
➢ Δ9-THCO+HLM (n=3)



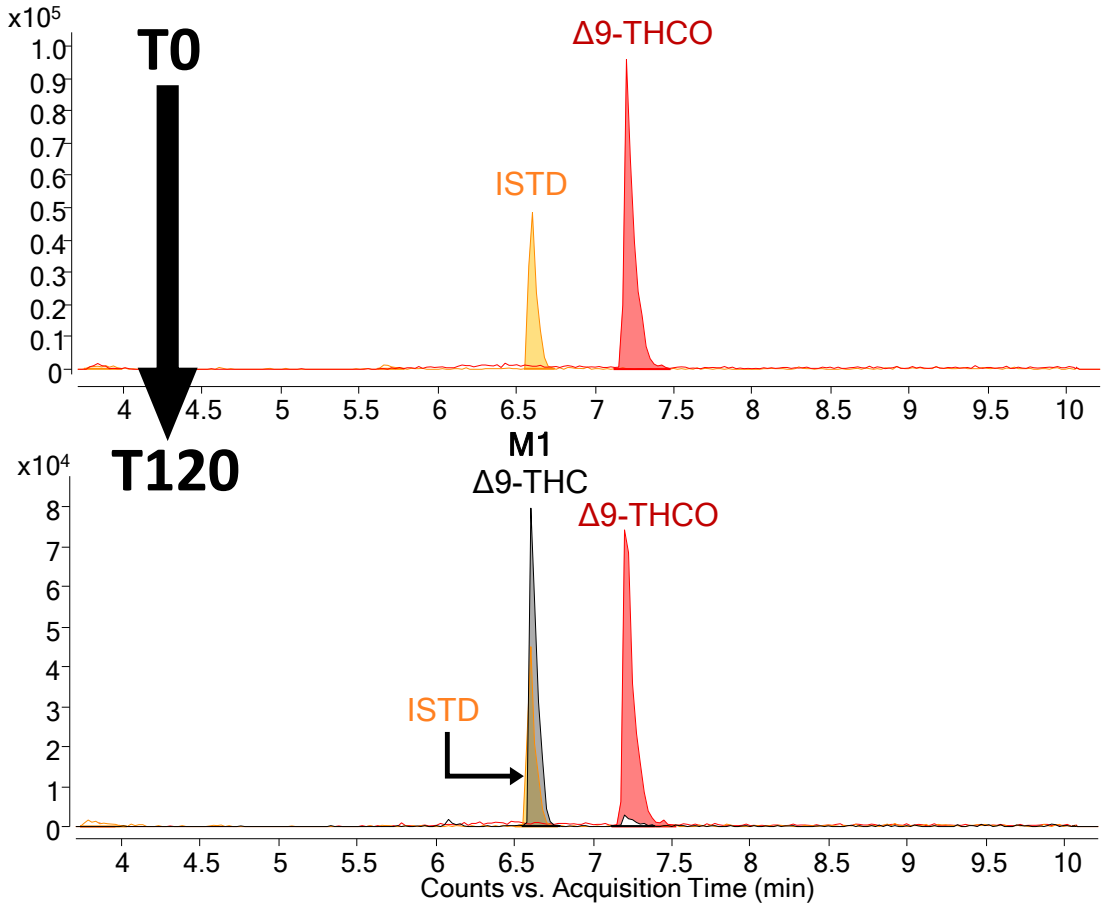
Number	Accurate Mass $[M+H]^+$	Molecular Formula	Mass Error	Identification
M1	315.2324	$C_{21}H_{30}O_3$	Within ± 5ppm	THC
M2	331.2273	$C_{21}H_{28}O_2$	Within ± 5ppm	11-OH-THC
M3, M5	331.2268	$C_{21}H_{30}O_3$	Within ± 5ppm	THC+O
M4, M6	313.2165	$C_{21}H_{28}O_3$	Within ± 5ppm	THC-2H

THCO Metabolites Screening: Plasma

➢ Δ8-THCO+Human Plasma (n=3)



➢ Δ9-THCO+Human Plasma (n=3)



Number	Accurate Mass $[M+H]^+$	Molecular Formula	Mass Error	Identification
M1	331.2273	$C_{21}H_{30}O_3$	Within ± 5ppm	THC

THCO Metabolites Screening Summary

➢ In vitro incubation with human plasma pool

Δ8-THCO: Δ8-THC formation
Δ9-THCO: Δ9-THC formation

Attributed to the esterase within human plasma (BChE, PON1, albumin esterase, and AChE).

➢ THCO → THC in HLM

In HLM: cytochrome P450s, flavin monooxygenases, carboxylesterases, epoxide hydrolase…etc

Several CYPs have been reported to be involved in the metabolism of cannabinoids

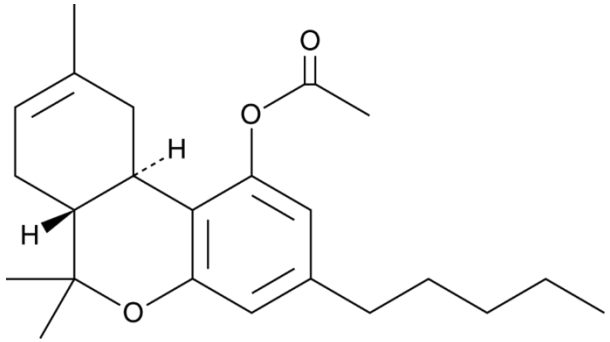
Reported in literatures:

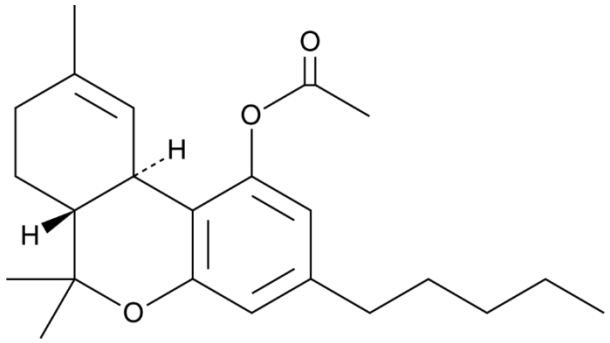
Cannabinoid	CYPs
Δ9-THC	2C9, 2C19, 2D6, 3A4
11-OH-Δ9THC	2C19
Δ8-THC	2C9, 3A4
CBD	1A1, 1A1, 2C9, 2C19, 2D6, 3A4, 3A5
CBN	2C9, 3A4
CBG	2C8, 2C9, 2D6, 3A4, 2J2

12 CYPs is chosen to be tested in this study: **CYP1A1, 1A2, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, 2E1, 2J2, 3A4, 3A5**

Experimental Design

Investigate of the metabolism pathway of Δ8-THCO and Δ9-THCO with LC-QTOF-MS





Δ8-THCO & Δ9-THCO Metabolism

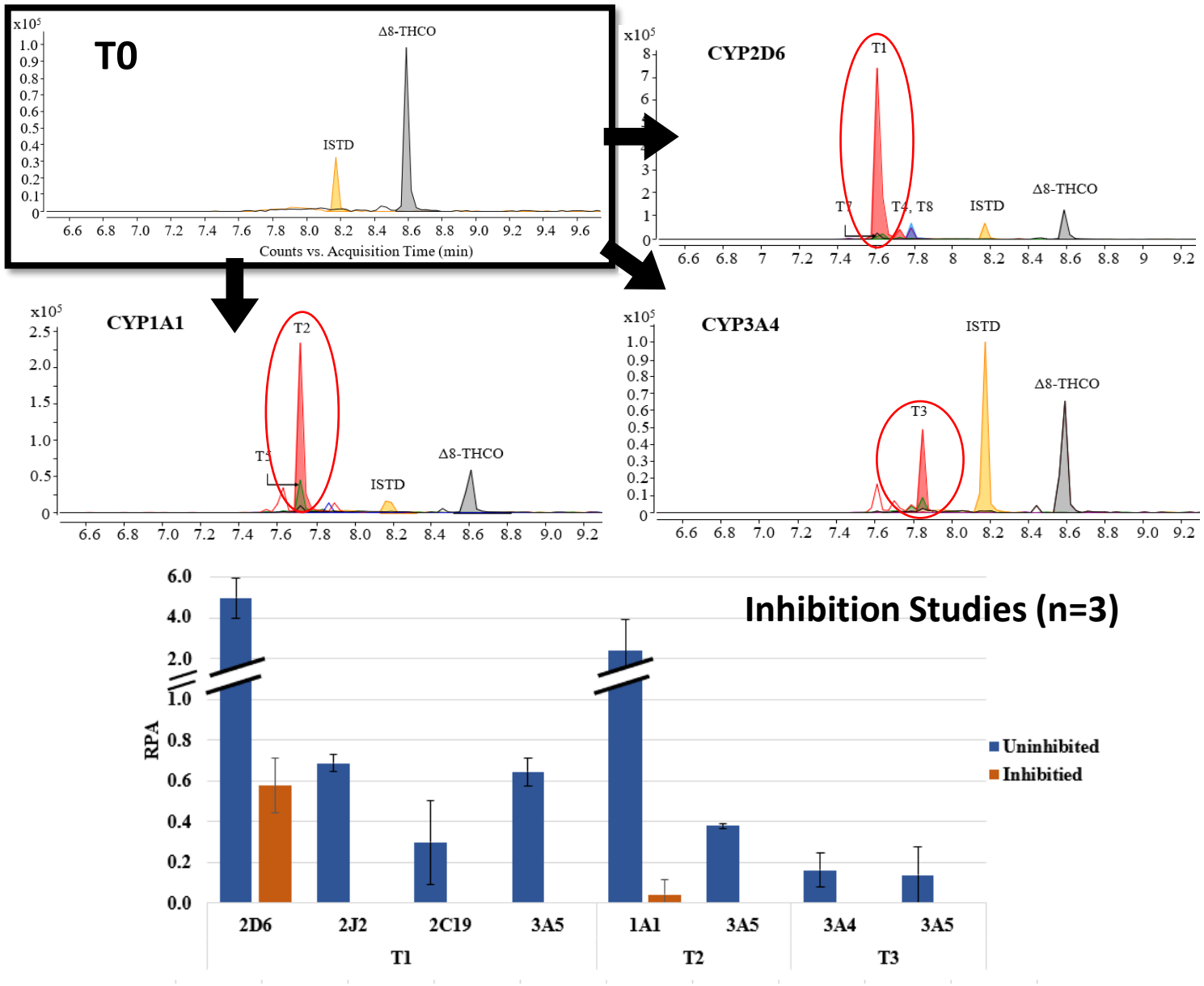
- Method development and optimization
  - Compound optimization ✓
  - Source optimization ✓
  - LC optimization ✓
  - Sample prep optimization ✓
- Metabolism studies
  - Human liver microsome (HLM)
    - Cytochromes P450 (CYPs)
      - CYP1A1, 1A2, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, 2E1, 2J2, 3A4, 3A5
    - Carboxylesterase (CES)
  - **THCO→THC**
  - Human plasma pool

Δ8-THCO and CYPs

None of the twelve tested rCYPs were involved in Δ8-THCO→Δ8-THC

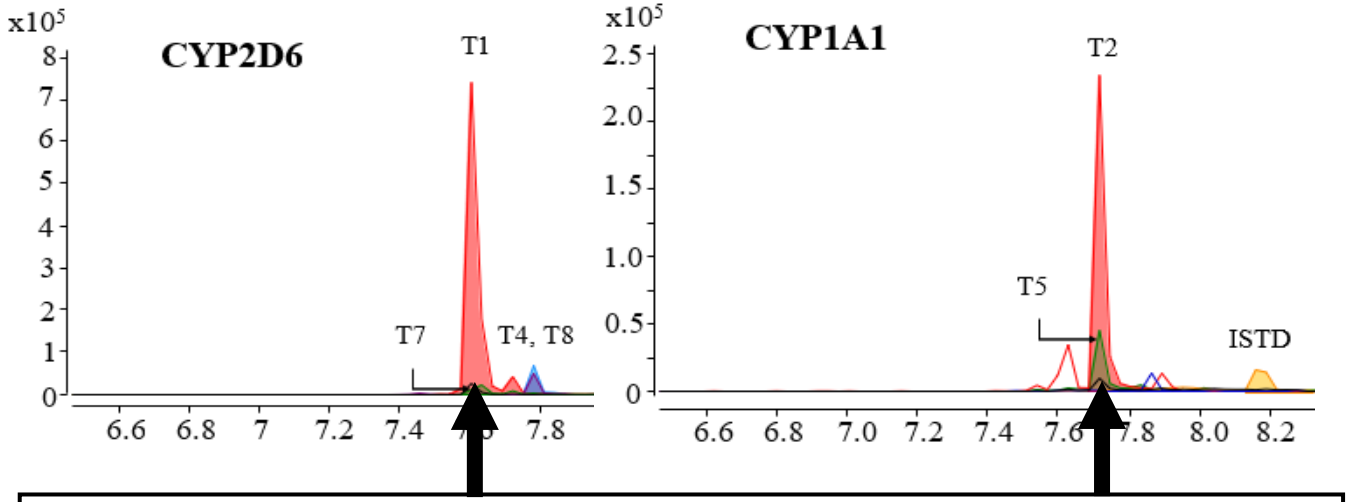
Metabolite	RT (min)	Mass Error (ppm)	Proposed Metabolite	rCYP
T1	7.60	0.5	THCO-2H	2C8
	7.61	1.7	THCO-2H	2C9
	7.60	0.1	THCO-2H	2C18
	7.63	**-0.5**	**THCO-2H**	**2C19**
	7.61	**1.9**	**THCO-2H**	**2D6**
	7.61	**1.2**	**THCO-2H**	**2J2**
	7.61	**1.1**	**THCO-2H**	**3A5**
T2	**7.72**	**-0.6**	**THCO-2H**	**1A1**
	7.73	**0.0**	**THCO-2H**	**3A5**
T3	**7.85**	**0.2**	**THCO-2H**	**3A4**
	7.84	**1.0**	**THCO-2H**	**3A5**
T4	7.78	-0.4	THCO-4H	2D6
	7.78	-0.8	THCO-4H	2J2
	7.79	1.0	THCO-4H	3A5
T5	7.72	0.1	THCO+O	1A1
T6	7.84	2.1	THCO+O	2C18
	7.83	2.4	THCO+O	2C19
	7.85	0.1	THCO+O	3A4
	7.85	0.3	THCO+O	3A5
T7	7.64	0.3	THCO+O	2D6
	7.64	1.5	THCO+O	2J2
T8	7.78	-1.3	THCO+O-2H	2D6

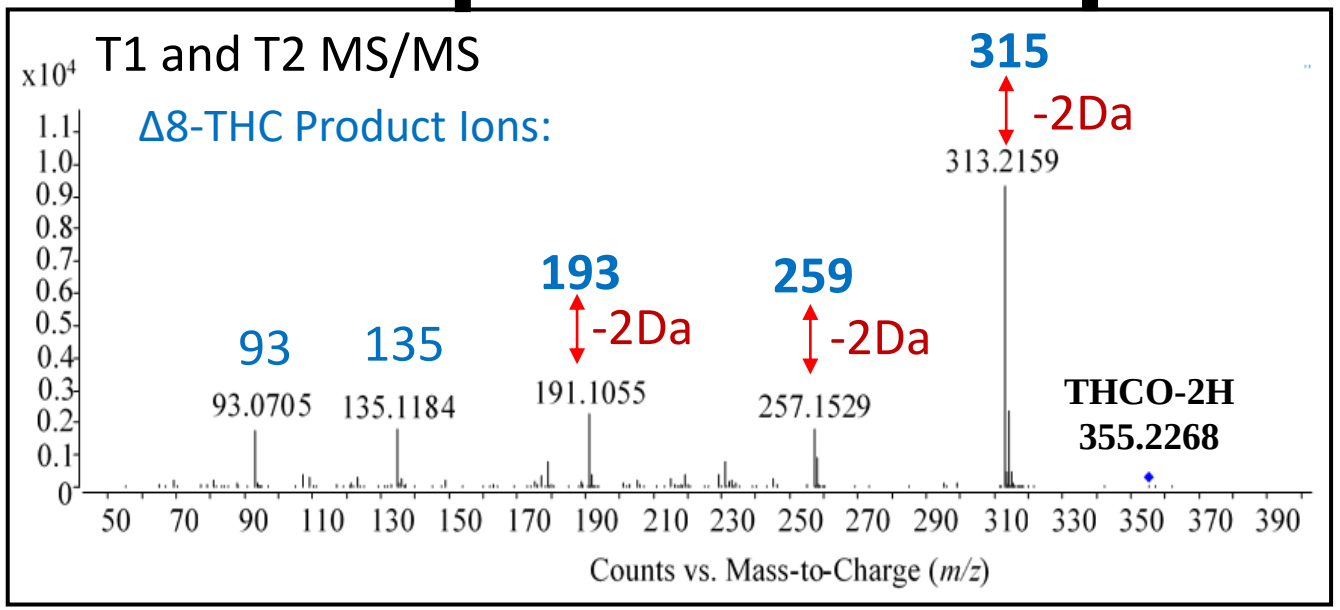
T1, T2, T3 showed high intensity in metabolite profiles

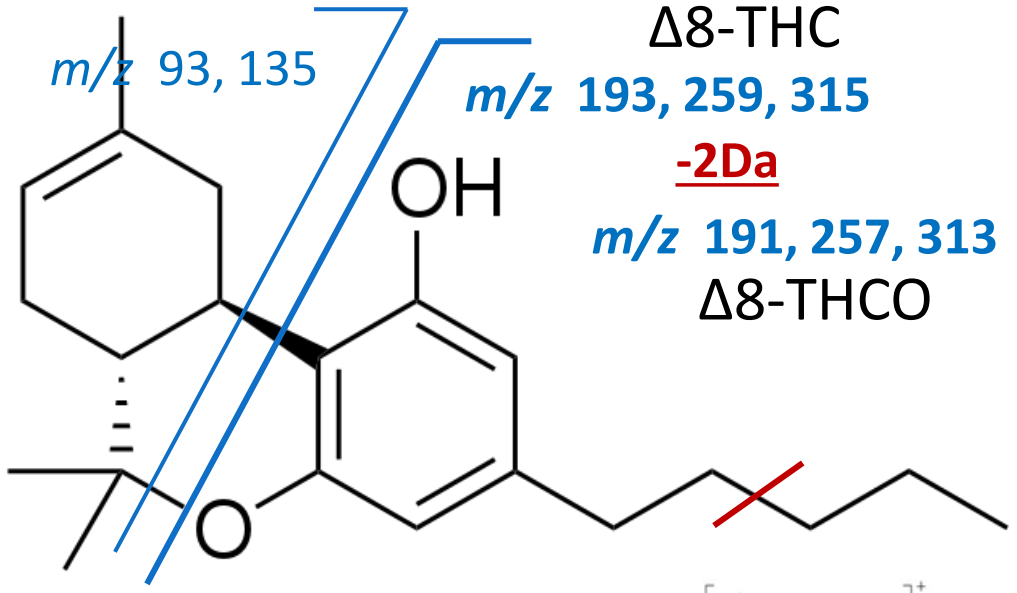


Desaturation Metabolites of Δ8-THCO

- **T1 and T2 metabolite**







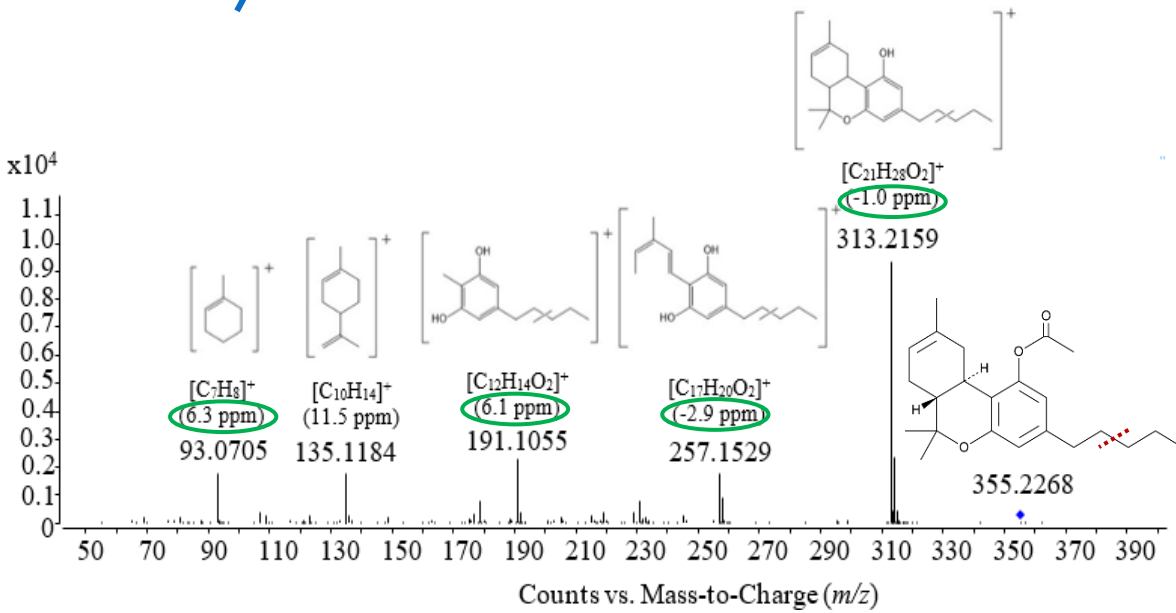
Δ8-THC

***m/z* 193, 259, 315**

-2Da

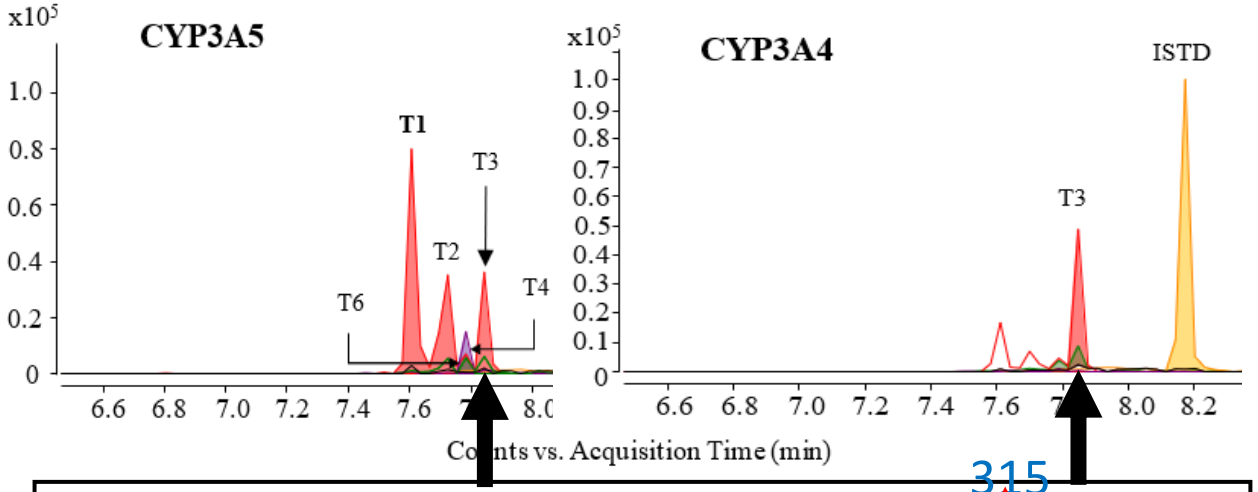
***m/z* 191, 257, 313**

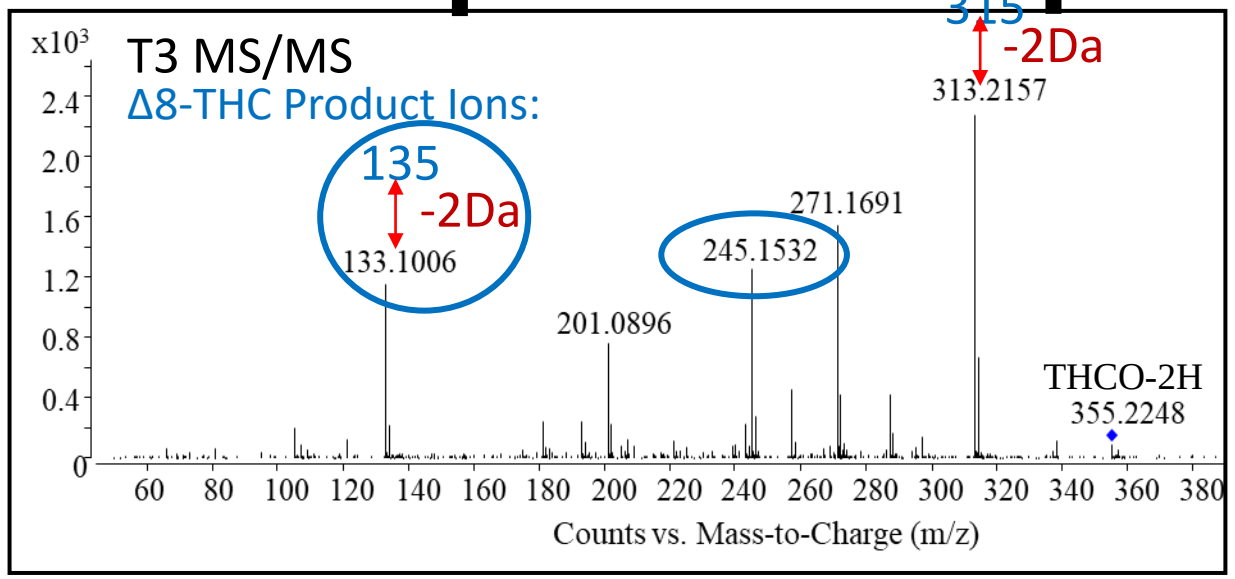
Δ8-THCO

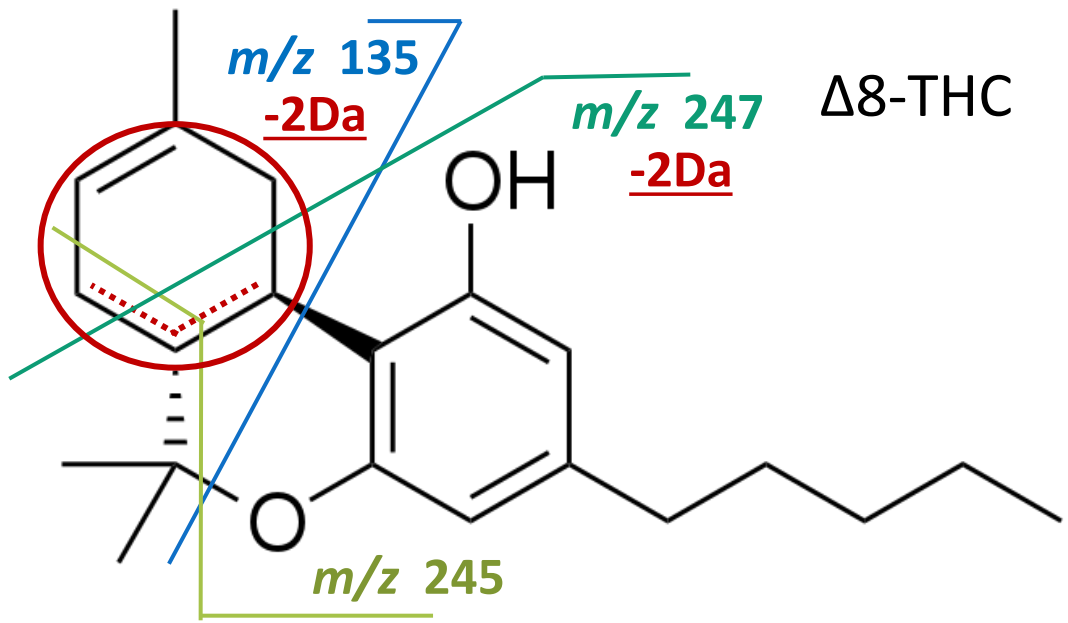


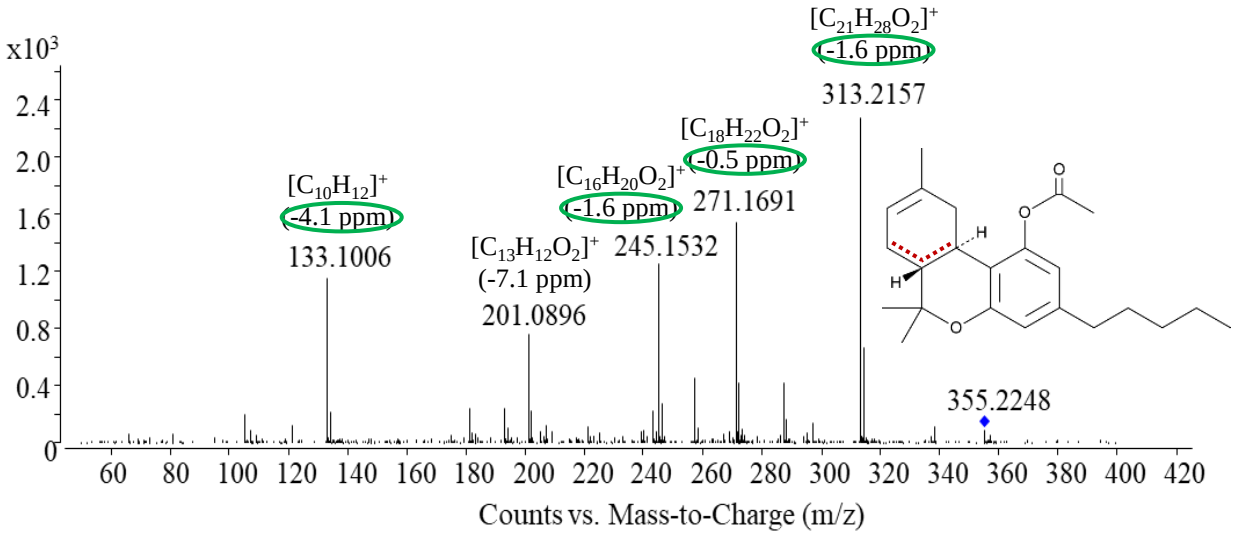
Desaturation Metabolites of Δ8-THCO

➢ T3 metabolite







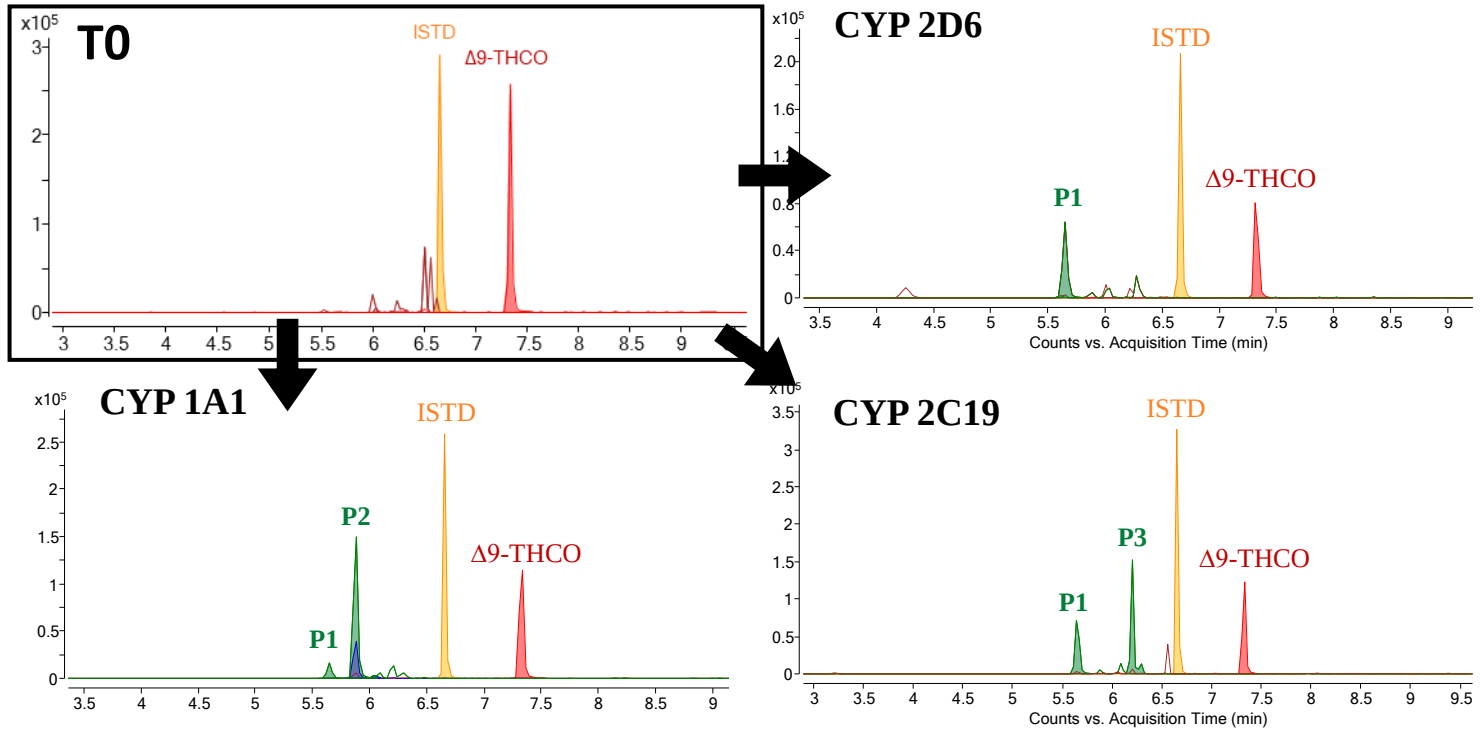


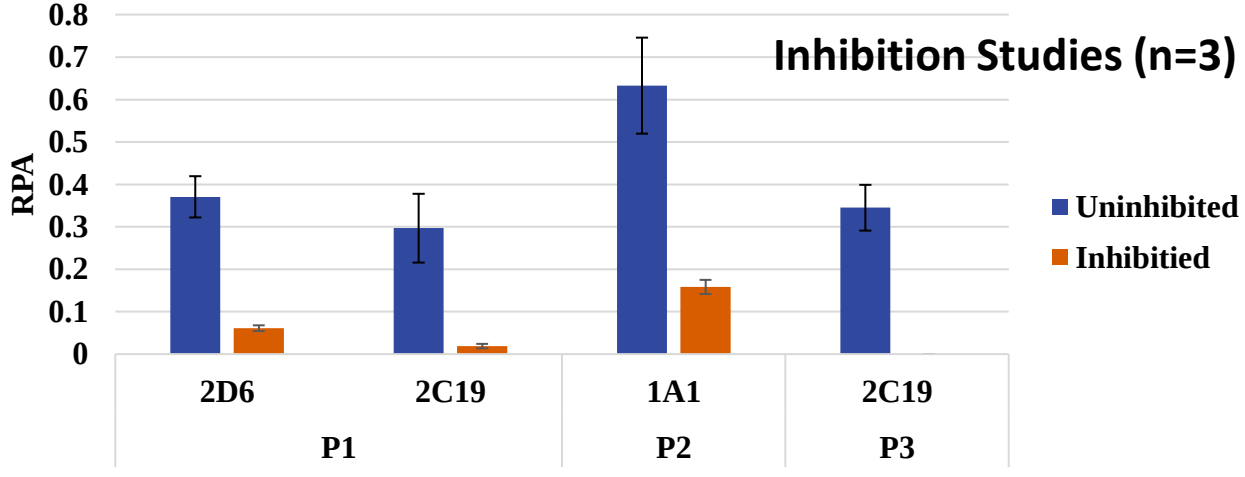
Δ9-THCO and CYPs

None of the twelve tested rCYPs were involved in Δ9-THCO→Δ9-THC

Metabolite	RT (min)	Mass Error (ppm)	Proposed Metabolite	rCYP
P1	5.65	-1.1	THCO-2H	1A1
	5.65	-0.1	THCO-2H	2C8
	5.64	1.1	THCO-2H	2C18
	5.64	**1.6**	**THCO-2H**	**2C19**
	5.65	**-0.1**	**THCO-2H**	**2D6**
	5.64	-1.3	THCO-2H	2J2
	5.65	-3.1	THCO-2H	3A4
	5.65	**-1.3**	**THCO-2H**	**3A5**
P2	**5.89**	**1.2**	**THCO-2H**	**1A1**
	5.89	1.4	THCO-2H	1A2
	5.89	4.3	THCO-2H	3A4
	5.87	**-1.3**	**THCO-2H**	**3A5**
P3	**6.21**	**-0.1**	**THCO-2H**	**2C19**
P4	6.05	2.7	THCO-4H	3A5
P5	5.89	1.3	THCO+O	1A1
	5.87	-1.1	THCO+O	3A5

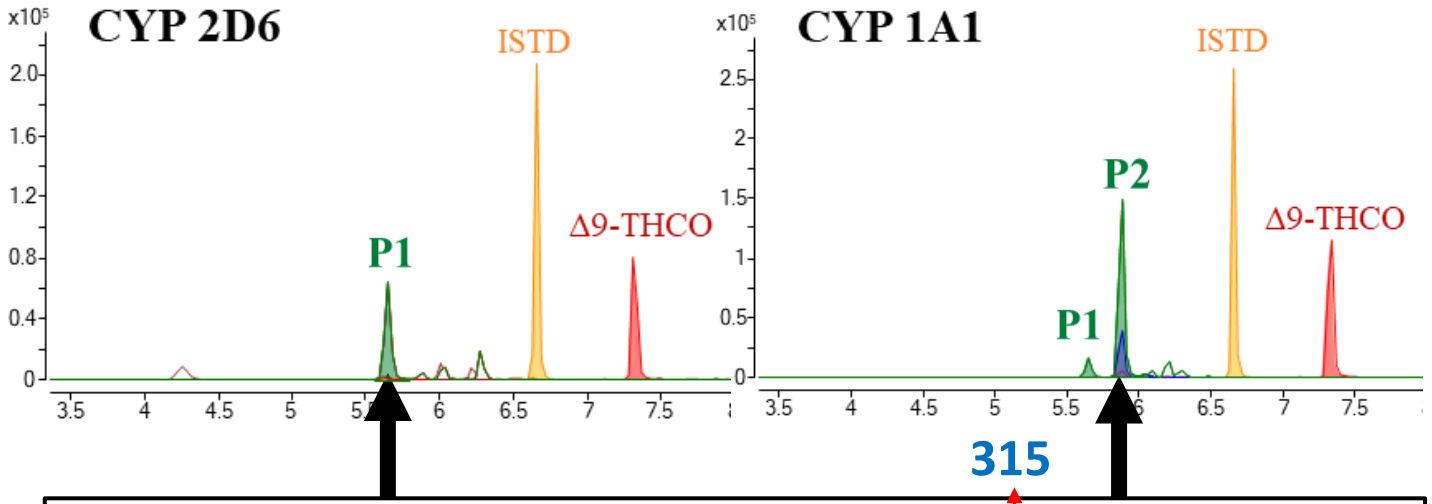
P1, P2, P3 showed high intensity in metabolite profiles

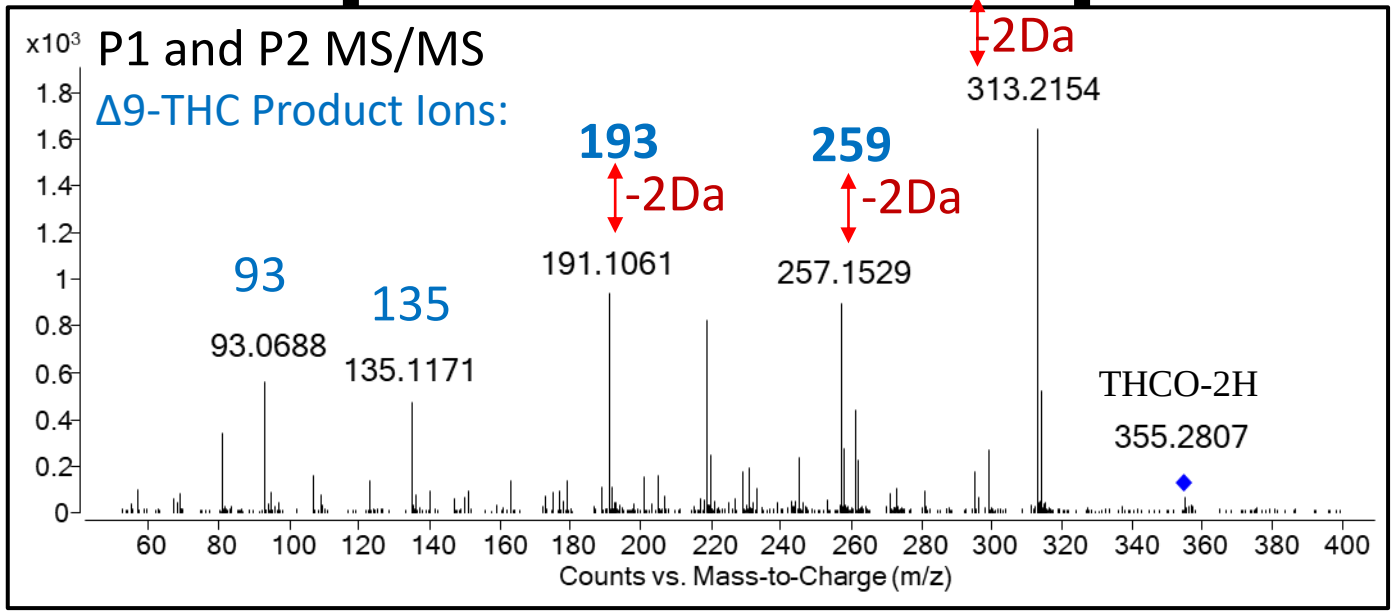


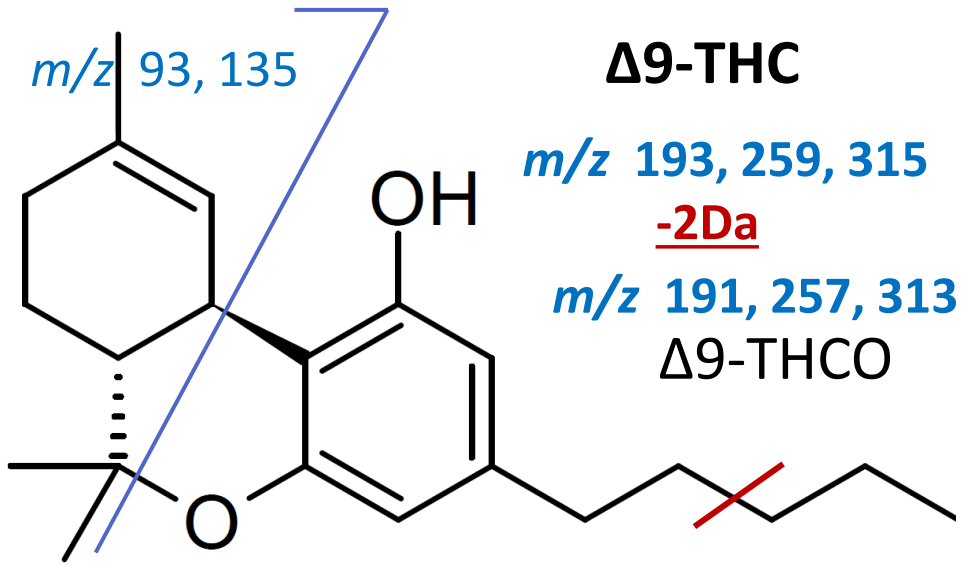


Desaturation Metabolites of Δ9-THCO

- **P1 and P2 metabolite** (similar to T1 and T2)







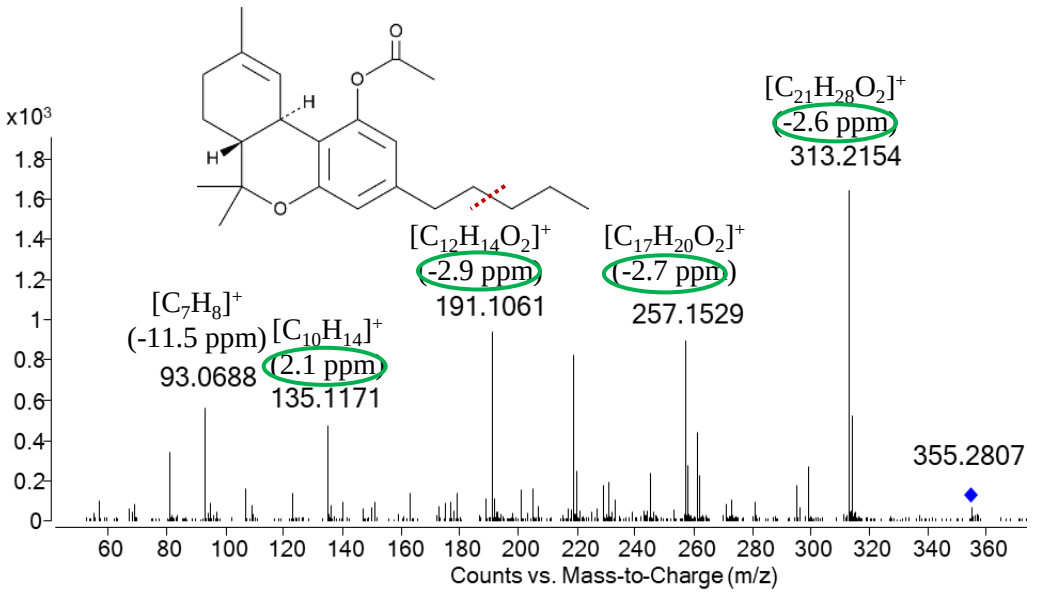
Δ9-THC

***m/z* 193, 259, 315**

-2Da

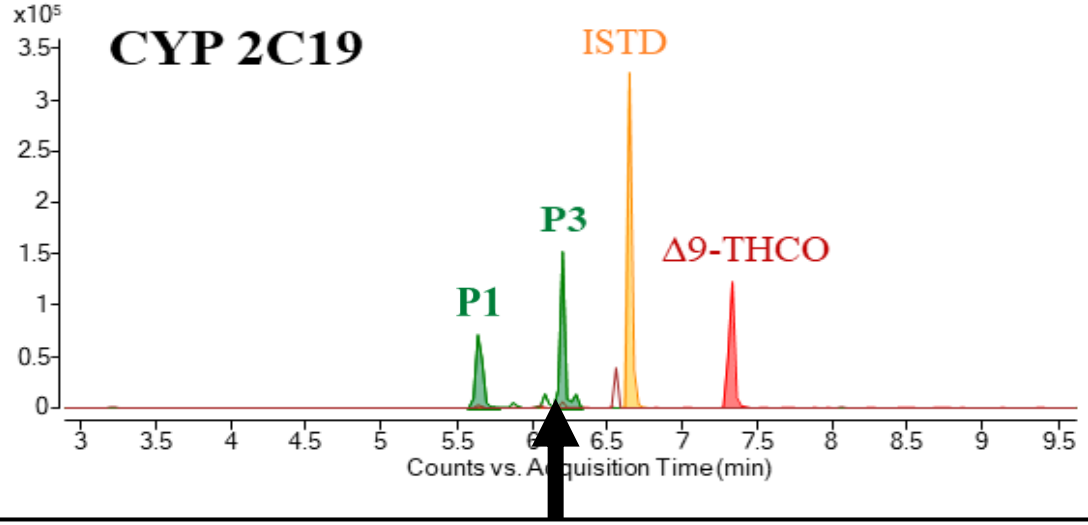
***m/z* 191, 257, 313**

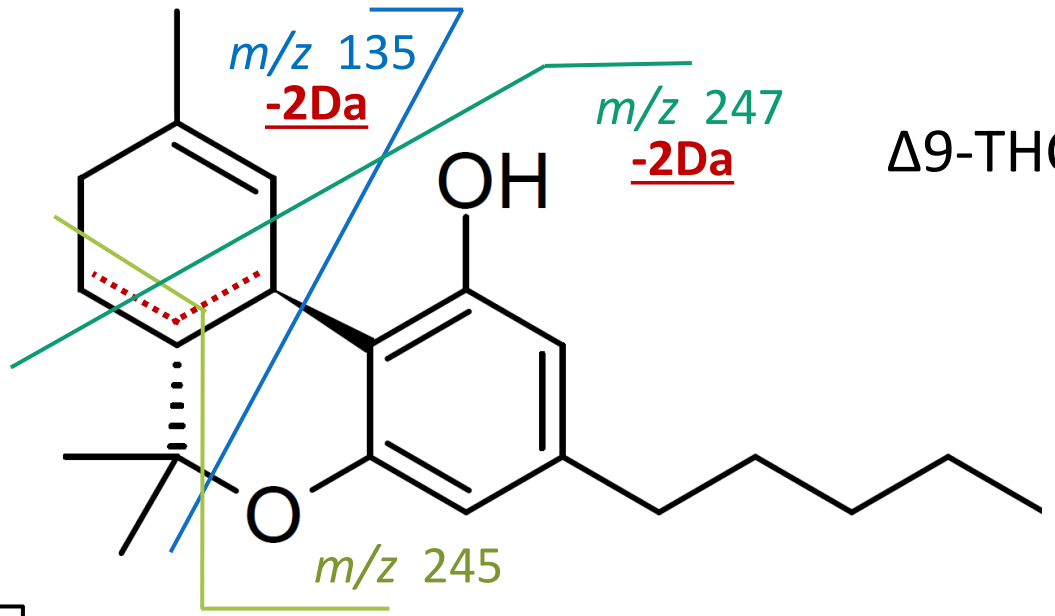
Δ9-THCO

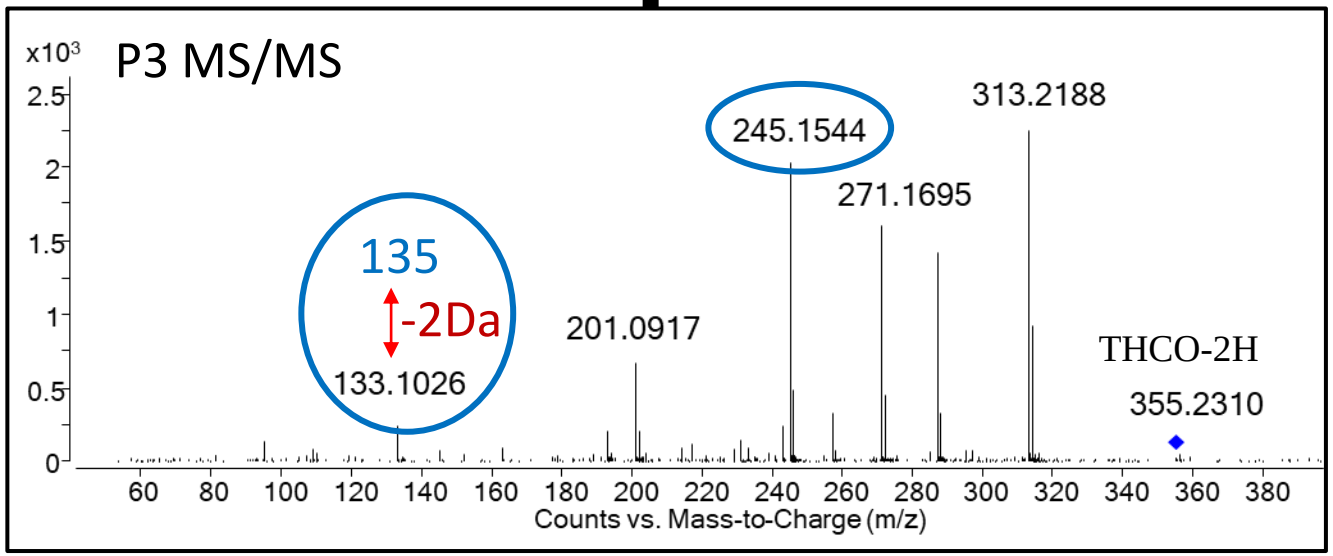


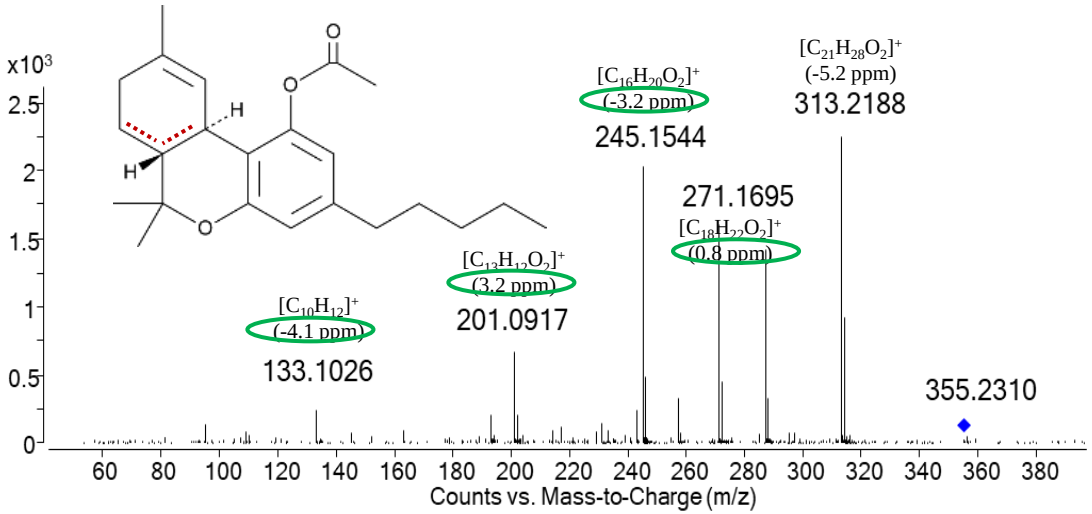
Desaturation Metabolites of Δ9-THCO

- **P3 metabolite** (similar to T3)

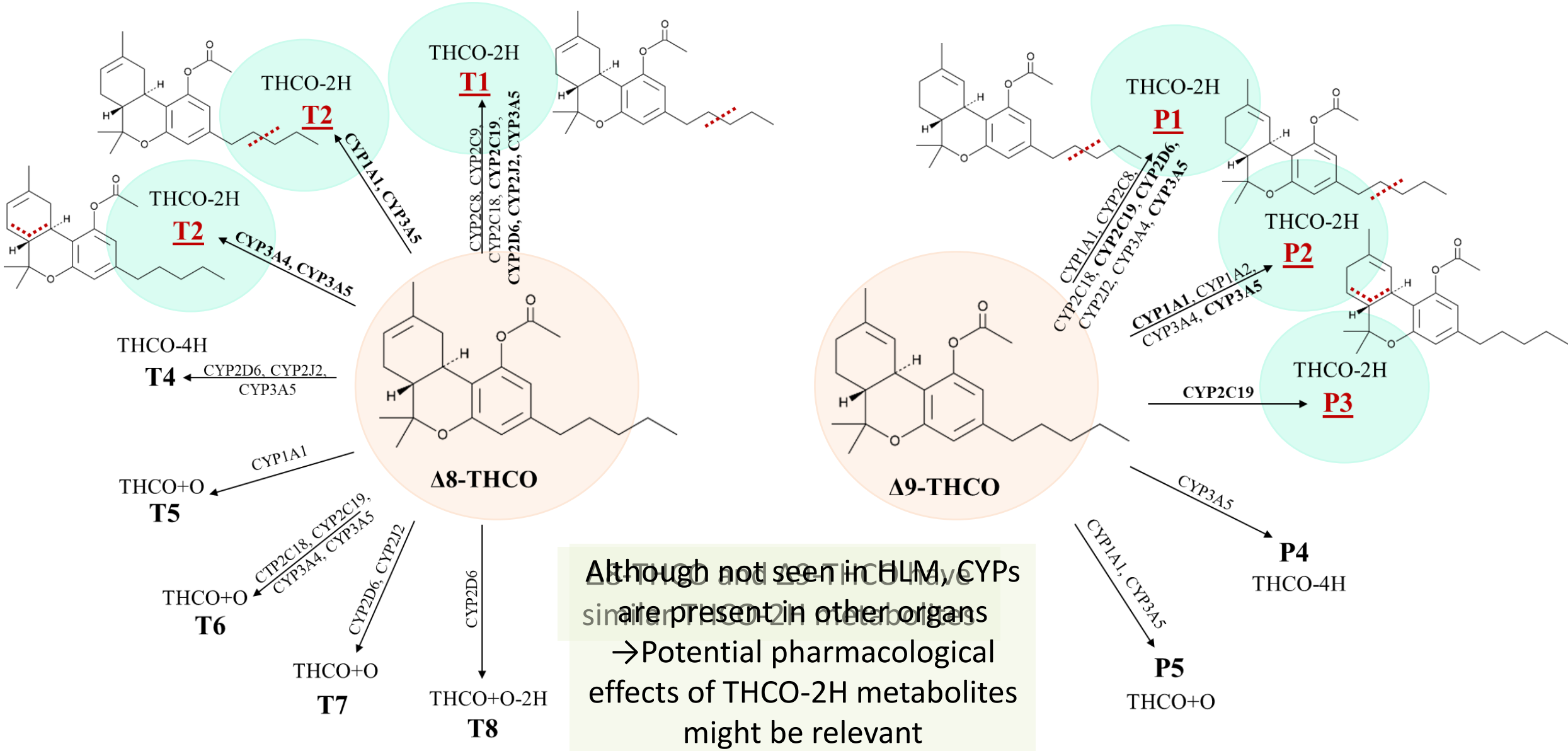








Summarized THCO Metabolites

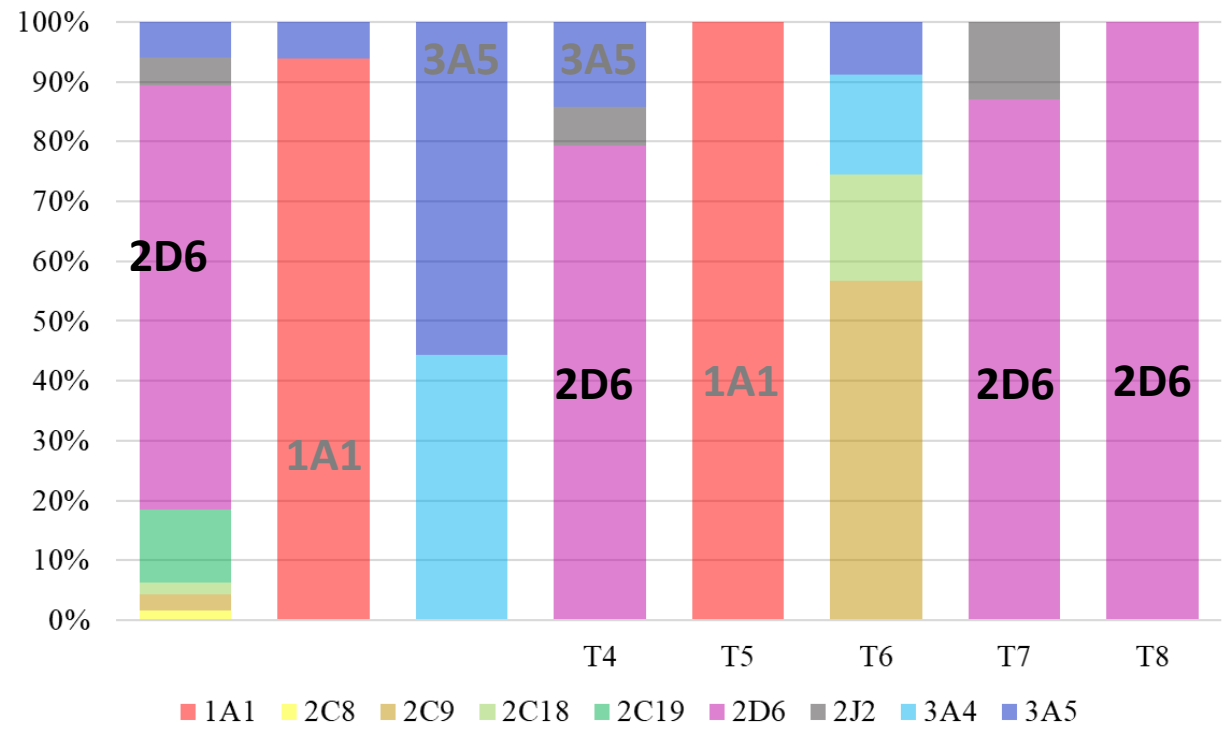


Although not seen in HLM, CYPs are present in other organs

→Potential pharmacological effects of THCO-2H metabolites might be relevant

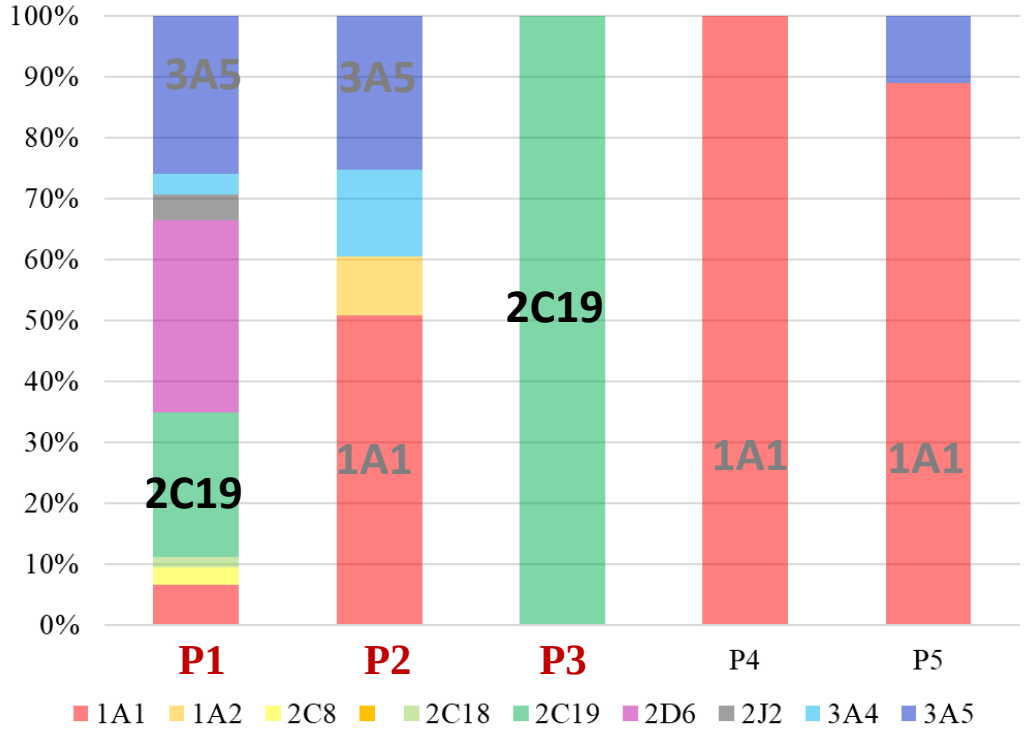
CYPs Contribution to THCO Metabolites

➢ Δ8-THCO with CYPs



CYP1A1, CYP2C8, CYP2C9, CYP2C19, CYP2C18, **CYP2D6**, CYP2J2, CYP3A4, **CYP3A5**

➢ Δ9-THCO with CYPs

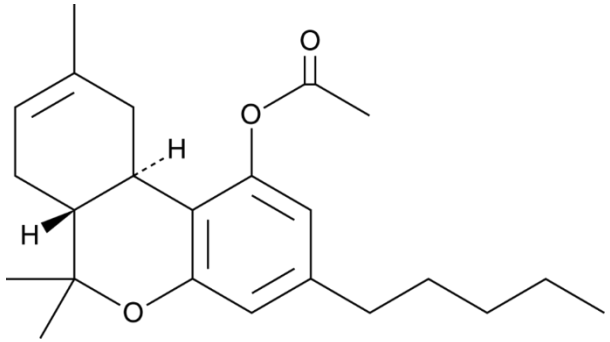


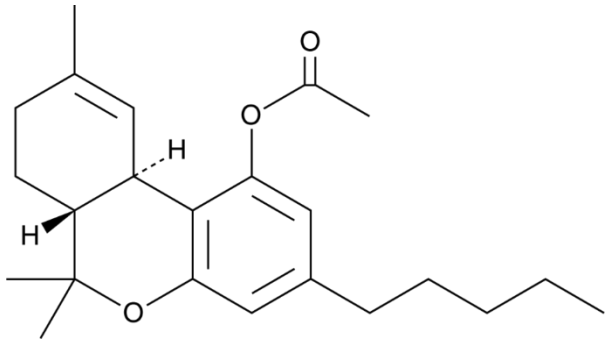
CYP1A1, CYP1A2, CYP2C8, **CYP2C19** CYP2C18, CYP2D6, CYP2J2, CYP3A4, **CYP3A5**

- CYPs involved in the formation of major and minor metabolites of Δ8- and Δ9-THCO are highly similar
- Although Δ8-THCO and Δ9-THCO have similar THCO-2H metabolites, some different CYPs in charge

Experimental Design

Investigate of the metabolism pathway of Δ8-THCO and Δ9-THCO with LC-QTOF-MS





Δ8-THCO & Δ9-THCO Metabolism

- Method development and optimization
  - Compound optimization
  - Source optimization
  - LC optimization
  - Sample prep optimization
- Metabolism studies
  - Human liver microsome (HLM)
    - **THCO→THC**
    - Cytochromes P450 (CYPs) **→THCO-2H**
      - CYP1A1, 1A2, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, 2E1, 2J2, 3A4, 3A5
    - Carboxylesterase (CES) **THCO→THC?**
      - CES1b, CES1c, CES2
  - Human plasma pool

Metabolism: Carboxylesterases

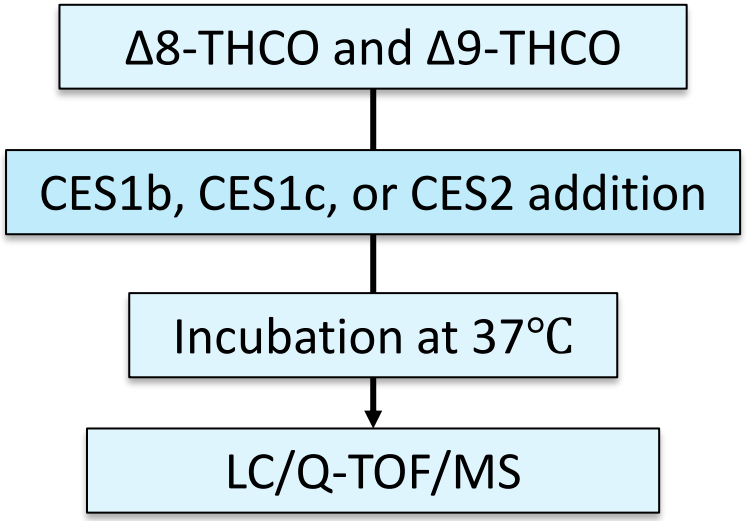
- **THCO→ THC in HLM**

  In HLM: ~~cytochrome P450s~~, flavin monooxygenases, carboxylesterases, epoxide hydrolase, and UDP glucuronyl transferases

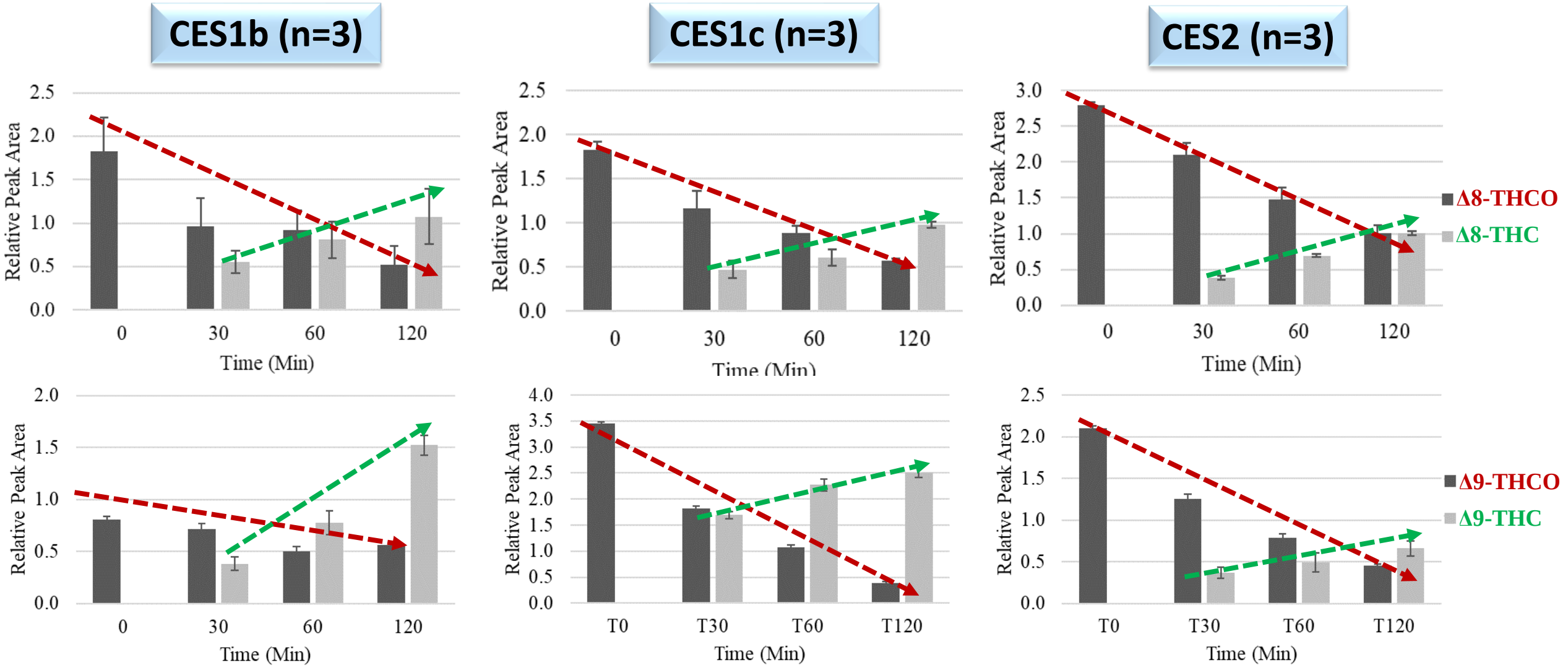
- **Carboxylesterases → CES1 and CES2 are most studied**

  - CES1:
    80%–95% of hydrolytic activity in liver
    (major variants: CES1b and hCES1c)

  - CES2:
    Expressed in the gastrointestinal tract and kidney



Metabolism: THCO and Carboxylesterases



All three CES are involved in the transformation of THCO to THC

Conclusions

Δ8-THCO

Metabolite Formation	CYP
High Intensities	**1A1**, 2C19, **2D6**, 2J2, **3A5**
Low Intensities	2C8, 2C9, 2C18, 3A4
None	1A2, 2B6, 2E1

Δ9-THCO

Metabolite Formation	CYP
High Intensities	**1A1**, **2C19**, 2D6, **3A5**
Low Intensities	1A2, 2C8, 2C18, 2J2, 3A4
None	2C9, 2B6, 2E1

- CYPs resulted in the formation of prominent **desaturated metabolites** of Δ8-THCO and Δ9-THCO
- CYPs were **NOT** involved in the transformation of THCO to THC
- THCO to THC transformation was attributed to **carboxylesterases** (1b, 1c, and 2), and other **plasma esterase**
- Further research is need to identified observed metabolites chemistry and its impact on public health and safety

Acknowledgements

- Department of Forensic Science
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Sam Houston State University™

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THANK YOU FOR YOUR ATTENTION

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