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In vitro metabolic profiling of the synthetic cannabinoid receptor agonist CUMYL-THPINACA using high resolution mass spectrometry

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Background

- Synthetic cannabinoid receptor agonists (SC) remain popular drugs of abuse.[1]
- Currently, SC-adulterated CBD-hemp is sold as drug-hemp.
- Effects comparable to THC, but generally far more potent.
- Severe health risks for consumers due to high potency, reflected in several (lethal) overdose cases.[2]
- **Forensic and clinical analyses:** Knowledge on the *in vivo* metabolism of SC are a prerequisite for detection of an ingestion as metabolites are the main targets in urine screenings.[3]
- **Information on the metabolizing isoenzymes:** helps predicting metabolic drug-drug interactions.[4-5]

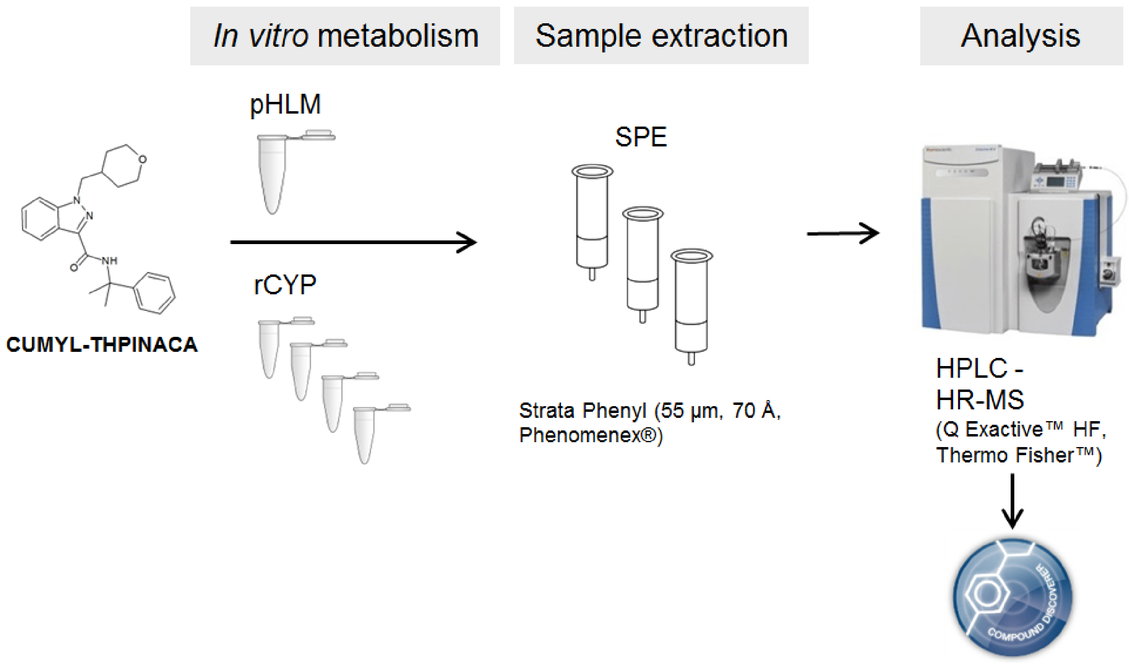

Figure 1: Summary of workflow

Material & Methods

***In vitro* metabolism:**
- Pooled human liver microsomes (pHLM)
- Recombinant Cytochrome P450 liver enzymes (rCYP: CYP3A4, CYP2C9, CYP2E1, CYP3A5, CYP2C8, CYP2B6, CYP2A6, CYP1A2, CYP2D6, CYP2C19, CYP3A5, CYP2B6)

Sample preparation (Figure 1):
1. Incubation with enzymatic model system for 0.5; 1; 1.5; and 2 h.
2. Solid phase extraction (SPE, Strata Phenyl cartridge) of incubation solution
3. Extract analysis with ThermoFisher™ LC-HRMS (LC: Ultimate 3000, MS: Q Exactive Orbitrap, equipped with Hypersil Gold™ analytical column) investigating diagnostic product ions using Compound Discoverer™ software.

Results & Discussion

Discovery: 28 Metabolites of CUMYL-THPINACA
- CUMYL-THPINACA was extensively hydroxylated, resulting in various **mono-, di-, and tri-hydroxylated metabolites** (Figure 2 and Table 1)
- Additional metabolites were formed by oxidation and subsequent dehydration at the oxacyclohexane moiety.
- Due to in-source dehydration being observed for M21* and M22*, further investigation is required of corresponding di- and tri-hydroxylated + dehydrated metabolites that are co-eluting with the respective di- and tri-hydroxylated metabolites.
- CUMYL-THPINACA was metabolized by **various CYP-isoforms**, mainly: CYP3A4, CYP3A5, CYP2C8, CYP2D6, and 2C19. **Relevant metabolic drug-drug interactions are, therefore, not expected.**

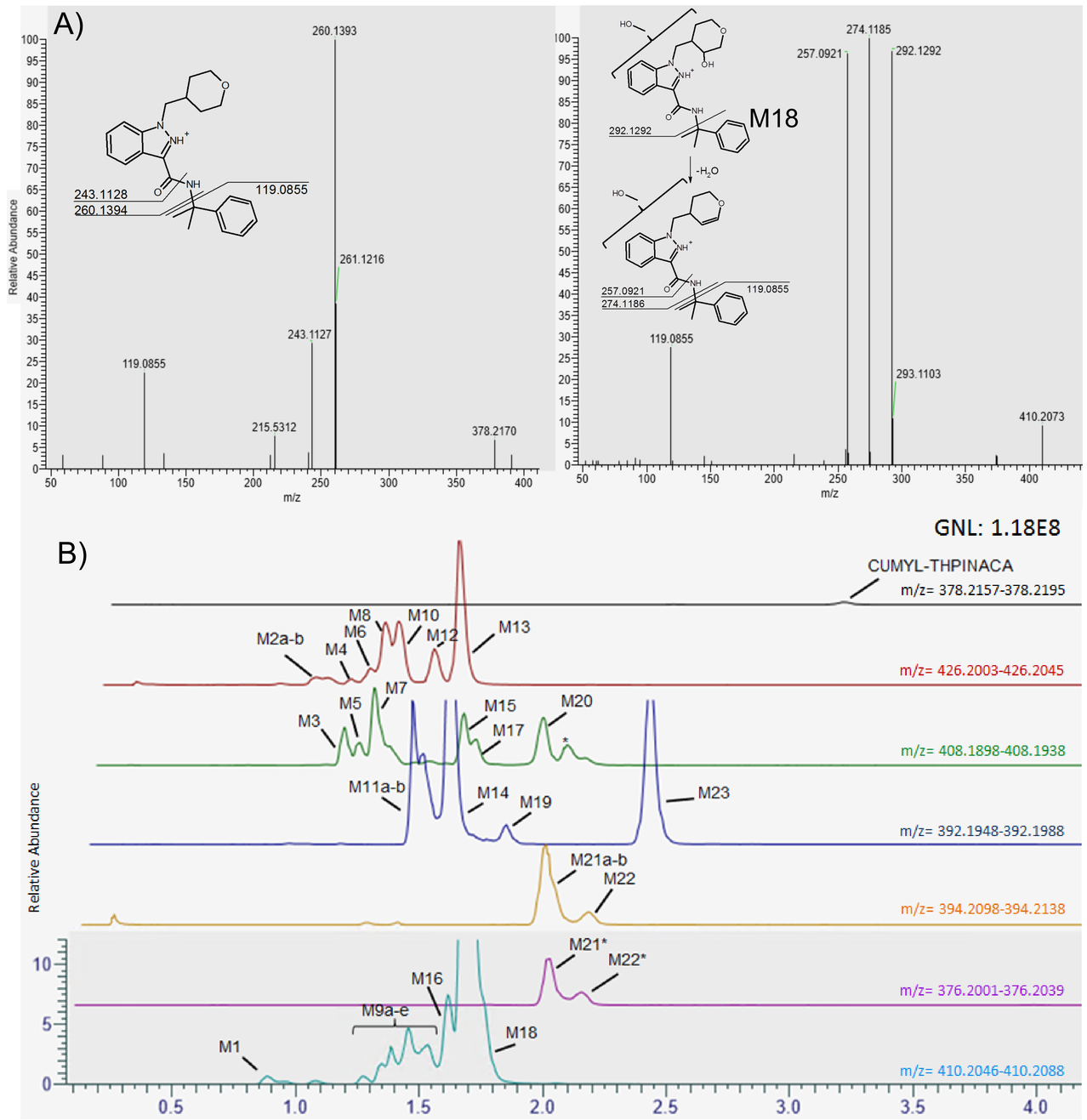

Figure 2:
A) Examples of MS^2 spectra of CUMYL-THPINACA and one major metabolite (M18)
B) Extracted ion chromatogram of a pHLM sample (2 h incubation)

Transformation	Formula	$[M+H]^+$	Mass change	Number of metabolites	Label	Retention time
monohydroxylation	C23H27N3O3	394.2118	+ O (16.9949 amu)	2	M21a-b, M22	1.99, 2.15
dihydroxylation	C23H27N3O4	410.2074	+ 2 x O (31.9898 amu)	8	M1, M9a-e, M16, M18	0.89, 1.23-1.54, 1.62, 1.69
dihydroxylation + dehydration	C23H25N3O3	392.1969	+ 2 x O - H2O (13.9792 amu)	5	M11a-b, M14, M19, M23	1.39-1.43, 1.54, 1.81, 2.39
trihydroxylation	C23H27N3O5	426.2023	+ 3 x O (47.9847 amu)	7	M2a-b, M4, M6, M8, M10, M12, M13	0.9-1.0, 1.08, 1.19, 1.23, 1.27, 1.42
trihydroxylation + dehydration	C23H27N3O5	408.1918	+ 3 x O - H2O (29.9741 amu)	6	M3, M5, M7, M15, M17, M20	1.08, 1.16, 1.20, 1.62, 1.88
CUMYL-THPINACA (parent)	C23H27N3O2	378.2176	-	-	-	3.07

Table 1: Summary of all detected metabolites

Conclusion & Outlook

- **CUMYL-THPINACA is extensively metabolized, as observed for other SCs.** Therefore, metabolites need to be considered when screening *urine samples.*
- To rule out in-source dehydration, further investigation, and potentially sample derivatization, should be conducted.
- **No drug-drug interactions are expected -** as several CYP isoforms contribute to the metabolism of CUMYL-THPINACA.
- ***In vitro* models are not able to fully mimic the *in vitro* situation:** *in vivo* analysis of authentic urine samples is required to elucidate the most relevant metabolites.
- Further investigations will include analysis of authentic urine samples (where available), activity studies of metabolites, and occurrence of in-source dehydration.

References:
1. **EMCDDA (2019), EU Drug Markets Report 2019, Publications of the European Union, Luxembourg.**
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4. **Gaunitz F*, et al.* Anal Bioanal Chem. 2019;411(16):3561-79.**
5. **Fantegrossi WE*, et al.* Life Sci. 2014;97(1):45-54.**

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