Suspect and non-target screening workflows to investigate the *in vitro* and *in vivo* metabolism of the synthetic cannabinoid 5-Cl-THJ-018

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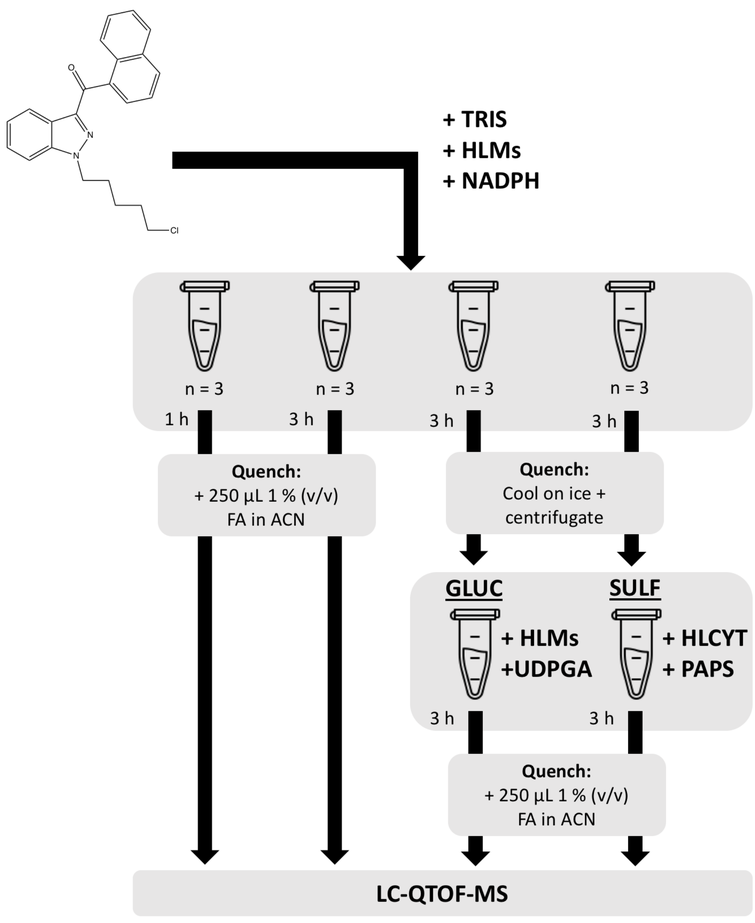
Introduction

Synthetic cannabinoids cause similar effects as Δ^9-tetrahydrocannabinol. (Ab)use can lead to health hazards and fatal intoxications. Most investigated synthetic cannabinoids undergo extensive biotransformation. As a result, parent compounds cannot be detected in urine and serum which hampers forensic investigations.[1] Limited information about biotransformation products of new synthetic cannabinoids makes detection of these compounds in biological matrices challenging.[2]

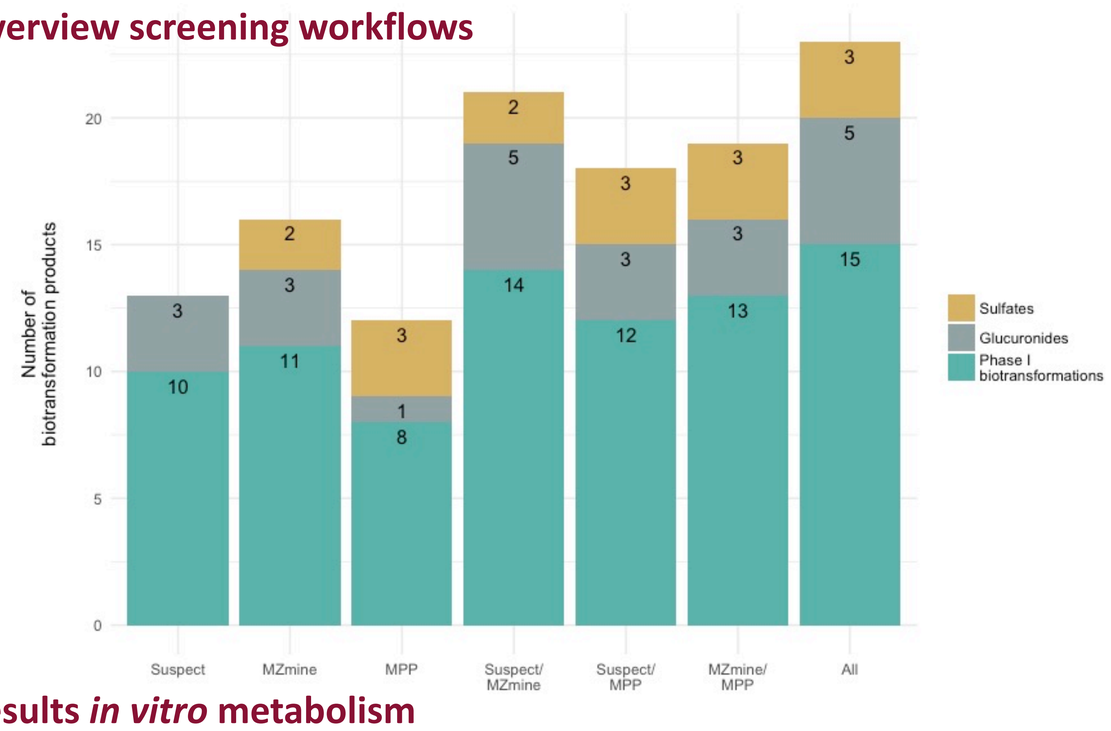
Objectives

- Identify ***in vitro* metabolites** of 5-Cl-THJ-018 using suspect and non-target screening workflows
- Evaluate added value of different non-target screening workflows
- Identify ***in vivo* metabolites** in an authentic urine sample of a 5-Cl-THJ-018 user using suspect screening

Experimental setup[3]



Overview screening workflows



Screening workflows

	Suspect screening	Mass Profiler Professional	MZmine + R
Data acquisition:	LC-QTOF-MS (ESI+ & ESI-) Data dependant acquisition		
Find Features:	MassHunter: Find By Formula	MassHunter: Molecular Feature Generator	MZmine
Prioritize features:	/	Mass Profiler Professional	R: invitRo - script
Identification:	In-house library	MassHunter: Generate formula	
Confirmation:	MS/MS information Literature & online databases Reference standard		

Results *in vitro* metabolism

ID	RT (min)	Workflow	Confirmation	Molecular Formula	Exact mass	Parent ion (m/z)	Δ mass (ppm)
Parent	15.03	All	Confirmed (MS/MS)	C23H21ClN2O	376.1342	377.1417 (ESI+)	-0.94
M01-A	7.48	MzMine	Confirmed (MS/MS)	C29H32N2O10	568.2057	569.2146 (ESI+)	1.81
M01-B	8.10	MzMine	Confirmed (MS/MS)	C29H32N2O10	568.2057	569.2142 (ESI+)	1.23
M02-A	8.43	All	Confirmed (MS/MS)	C23H20N2O4	388.1423	389.1511 (ESI+)	2.53
M02-B	8.77	All	Confirmed (MS/MS)	C23H20N2O4	388.1423	389.1529 (ESI+)	7.16
M02-C	10.46	All	Confirmed (MS/MS)	C23H20N2O4	388.1423	389.1533 (ESI+)	8.07
M02-D	10.73	All	Confirmed (MS/MS)	C23H20N2O4	388.1423	389.1513 (ESI+)	2.97
M03-A	8.43	Suspect / MzMine	Confirmed (MS/MS)	C23H22N2O5	406.1529	407.1619 (ESI+)	2.80
M03-B	8.77	Suspect / MzMine	Confirmed (MS/MS)	C23H22N2O5	406.1529	407.1615 (ESI+)	1.91
M04-A	8.50	All	Confirmed (MS/MS)	C23H22N2O3	374.1630	375.1723 (ESI+)	3.83
M04-B	8.86	All	Confirmed (MS/MS)	C23H22N2O3	374.1630	375.1706 (ESI+)	-0.63
M04-C	10.45	All	Confirmed (MS/MS)	C23H22N2O3	374.1630	375.1744 (ESI+)	9.4
M04-D	10.62	All	Confirmed (MS/MS)	C23H22N2O3	374.1630	375.1707 (ESI+)	-0.38
M04-E	10.86	All	Confirmed (MS/MS)	C23H22N2O3	374.1630	375.1711 (ESI+)	0.72
M05-A	8.50	Suspect / MPP	Confirmed (MS/MS)	C23H24N2O4	392.1736	393.1827 (ESI+)	3.35
M05-B	8.86	Suspect / MPP	Confirmed (MS/MS)	C23H24N2O4	392.1736	393.1820 (ESI+)	1.50
M06	8.99	All	Confirmed (MS/MS)	C29H30N2O10	566.1900	567.1970 (ESI+)	-1.51
M07-A	9.00	Suspect	Confirmed (MS/MS)	C29H28N2O9	548.1795	549.1920 (ESI+)	8.54
M07-B	10.10	Suspect	Confirmed (MS/MS)	C29H28N2O9	548.1795	549.1878 (ESI+)	0.85
M08	9.41	MzMine	Confirmed (MS/MS)	C29H29ClN2O10	600.1511	601.1592 (ESI+)	0.46
M09	10.39	All	Confirmed (MS/MS)	C23H22N2O4	390.1580	391.1664 (ESI+)	1.42
M10	10.84	MzMine / MPP	Confirmed (MS/MS)	C23H21ClN2O4	424.1190	425.1258 (ESI+)	-2.45
M11	11.00	Suspect	MS1 + Isotope	C29H29ClN2O9	584.1562	585.1655 (ESI+)	2.54
M12-A	11.47	MzMine	Confirmed (MS/MS)	C23H21ClN2O2	392.1292	393.1409 (ESI+)	9.9
M12-B	12.71	MzMine	Confirmed (MS/MS)	C23H21ClN2O2	392.1292	393.1364 (ESI+)	-1.47
M13	11.47	All	Confirmed (MS/MS)	C23H23ClN2O3	410.1397	411.1491 (ESI+)	3.81
M14	11.60	Suspect	Confirmed (MS/MS)	C18H12N2O	272.0950	273.1040 (ESI+)	4.29
M15	11.90	Suspect	Confirmed (MS/MS)	C23H20N2O3	372.1474	373.1552 (ESI+)	0.04
M16	12.12	All	Confirmed (MS/MS)	C23H22N2O2	358.1681	359.1762 (ESI+)	0.87
M17	12.82	Suspect / MzMine	Confirmed (MS/MS)	C23H21ClN2O3	408.1241	409.1326 (ESI+)	1.77
M18	12.89	MzMine	Confirmed (MS/MS)	C23H20N2O2	356.1525	357.1624 (ESI+)	5.69
M19	6.57	MzMine	MS1 + Isotope	C23H23ClN2O5	442.1295	441.1231 (ESI-)	3.15
M20	6.66	MzMine / MPP	Confirmed (MS/MS)	C23H20N2O8S	484.0940	483.0880 (ESI-)	2.58
M21	7.60	MPP	Confirmed (MS/MS)	C23H22N2O8S	486.1097	485.1030 (ESI-)	0.99
M22	8.60	MzMine / MPP	Confirmed (MS/MS)	C23H22N2O7S	470.1148	469.1077 (ESI-)	0.29
M23-A	9.32	MPP	MS1 + Isotope	C23H23ClN2O4	426.1346	425.1295 (ESI-)	6.43
M23-B	10.15	MPP	MS1 + Isotope	C23H23ClN2O4	426.1346	425.1281 (ESI-)	3.22

Conclusions

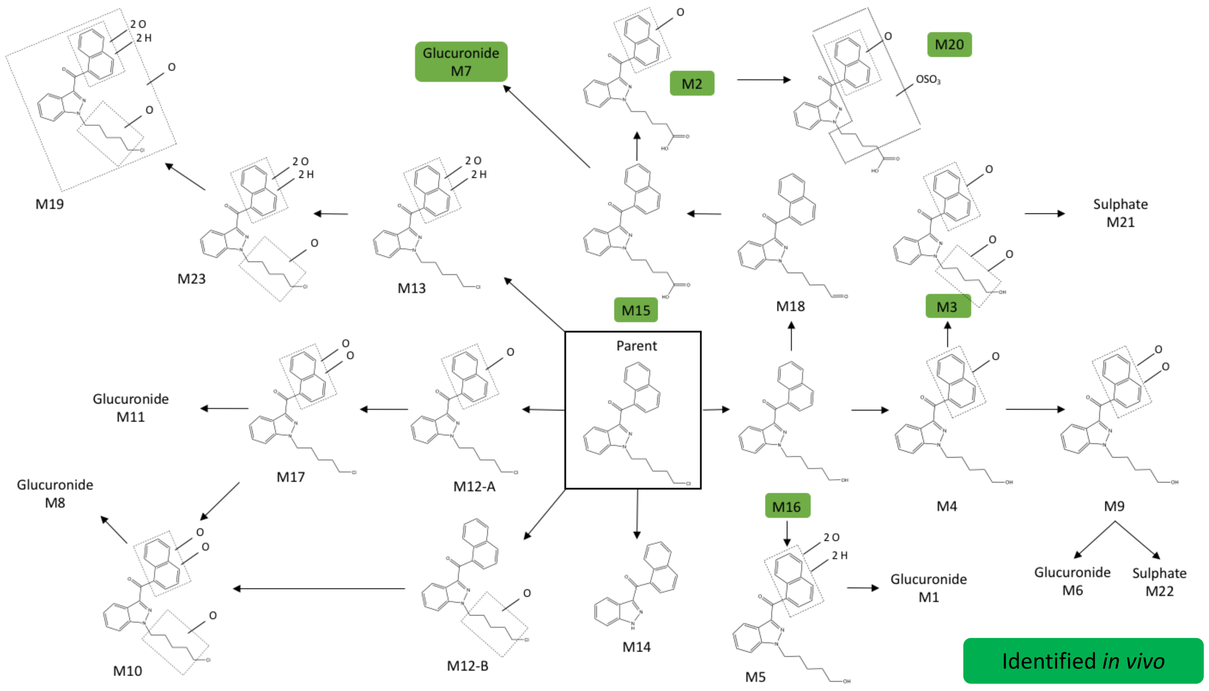
The synthetic cannabinoid 5-Cl-THJ-018 underwent extensive *in vitro* metabolism. Predominant Phase I pathways *in vitro* are **oxidative reactions** (with and without dechlorination) and subsequent Phase II **glucuronidation** and **sulfation**.

The application of **non-target screening** workflows in parallel to suspect screening **increased the number of metabolites being identified**.

Seven metabolites were also identified in the ***in vivo* urine sample**. All these metabolites started from the oxidative dechlorination of 5-Cl-THJ-018, followed by further oxidative reactions and/or Phase II biotransformation.

As only the dechlorinated metabolites were identified in this specific *in vivo* sample, no specific biomarkers could be proposed to distinguish 5-Cl-THJ-018 use from the use of the nonhalogenated or other similar halogenated analogues.

Suggested pathway



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[1]: Chimalakona KC, Seely KA, Bratton SM, *et al.* (2012); *Drug Metab Dispos*. 40: 2174-84
[2]: Favretto D., Pascali JP, Tagliaro F (2013); *J Chrom A.* 1287: 84-95
[3]: Mortelé O., Vervliet P., Gys C., *et al.* (2018); *J Pharm Biomed Anal.* 10; 158-167