Suspect and non-target screening workflows to investigate the *in vitro* and *in vivo* metabolism of the synthetic cannabinoid 5-Cl-THJ-108

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Use of synthetic cannabinoids cause similar effects as Δ^9-tetrahydrocannabinol. Long-term (ab)use can lead to health hazards and fatal intoxications. As most investigated synthetic cannabinoids undergo extensive biotransformation, almost no parent compound can be detected in urine which hampers forensic investigations.[1] Limited information about biotransformation products of new synthetic cannabinoids makes the detection of these drugs in various biological matrices challenging.

This study aimed to identify the main *in vitro* biotransformation products of 5-Cl-THJ-018 and compare the *in vitro* findings with authentic urine samples of 5-Cl-THJ-018 users. 5-Cl-THJ-018 was incubated with pooled human liver microsomes and cytosol to simulate Phase I and Phase II biotransformations. Samples were analyzed with liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (LC-QTOF-MS). Three different data-analysis workflows were applied for identifying the biotransformation products. The suspect screening workflow used a database built from literature and *in silico* biotransformation predictions by the Meteor Nexus software (Lhasa Limited). The two non-target screening methods used the MassProfiler Professional (Agilent) and the open-source software for mass spectrometry data processing MZmine 2.29 respectively. The obtained *m/z* features were further processed and visualized using open source R software.

A total of 23 biotransformation products were identified. Phase I biotransformation included hydroxylation, oxidative dechlorination and dihydrodiol formation pathways. Also, five glucuronidated and three sulphated phase II conjugates have been identified.

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References

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