Phase I Metabolism of the New Synthetic Cannabinoid Cumyl-4CN-BINACA and Detection in Human Urine Samples

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Introduction

Synthetic cannabinoids (SCs) are a class of new psychoactive substances (NPS) commonly sold as 'legal highs' via online shops. The SC Cumyl-4CN-BINACA (1-(4-cyanobutyl)-*N*-(1-methyl-1-phenyl-ethyl)-1*H*-indazole-3-carboxamide) was originally described in a patent application of Bowden and Williamson from 2014 (compound SGT-78).[1] The compound emerged in January 2016 on the European drug market and was first identified by the Hungarian police in a herbal mixture.[2] Other cumyl derivatives covered in the patent, like Cumyl-PINACA or Cumyl-5F-PINACA, were detected on the European drug market before.[2] In contrast to other prevalent SCs, Cumyl-4CN-BINACA has an aliphatic nitrile function which is a rarely observed feature within this class of drugs. In the Institute of Forensic Medicine in Freiburg (Germany), the substance was detected in 20 'legal high' products purchased in 2017 (total number of purchased products 188). This sharp increase in prevalence necessitated the development of analytical methods for the detection of this drug. Since urine samples are usually the preferred matrix for drug abstinence testing and SCs are known to be extensively metabolized prior to renal excretion, the main *in vivo* metabolites have to be identified for a reliable detection in urine.

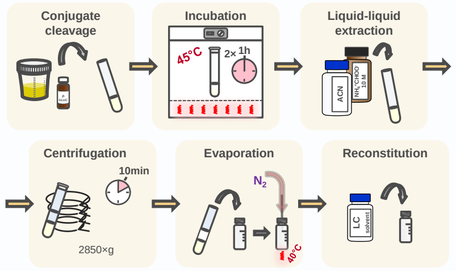
Fig. 1. Different products containing Cumyl-4CN-BINACA

Objective

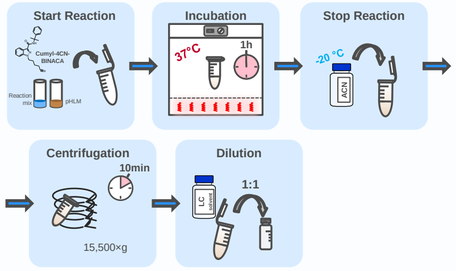
Aim of this study was to identify reliable Cumyl-4CN-BINACA consumption markers for urine analysis and the application to authentic case samples.

Methods

Extraction of Cumyl-4CN-BINACA *in vivo* phase-I metabolites from authentic human urine samples (n=16):



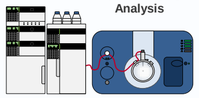
Preparation of *in vitro* phase-I metabolites with pooled human liver microsomes (pHLM)[3]:



Analysis of urine and pHLM samples:

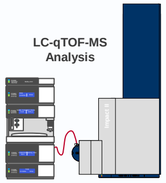
General Liquid Chromatography Conditions:
- Kinetex C18 column (100 mm × 2.1 mm, 100 Å, 2.6 µm)
- Solvent A (1% ACN, 0.1% HCOOH, 2 mM $NH_4^+HCOO^-$)
- Solvent B (ACN with 0.1% HCOOH, 2 mM $NH_4^+HCOO^-$)

LC-ESI-MS/MS Analysis



Nexera X2 (Shimazu) + QTRAP 5500 (Sciex)

LC-qTOF-MS Analysis



UltiMate 3000 (Thermo) + impact II (Bruker)

QTRAP 5500 Experiments:
- Precursor scan (Prec)
- Enhanced product ion scan (EPI)
- Multiple reaction monitoring (MRM)

Impact II Experiments:
- Full scan MS / bbCID
- Full scan MS / Auto-MS/MS

Results

Placeholder Flip-Chart

Fig. 2. Product ion spectra of Cumyl-4CN-BINACA and of the detected metabolites.

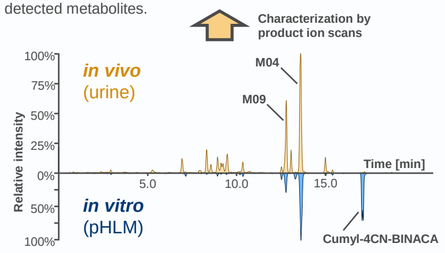


Fig. 3. Total ion chromatograms of the *in vivo* and *in vitro* phase-I metabolic profiles.

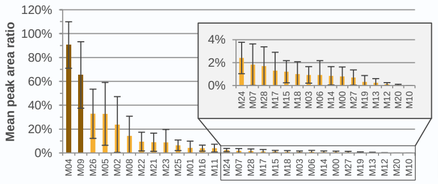


Fig. 4. Evaluation of *in vivo* phase-I metabolites as consumption markers for urine analysis. Error bars show the absolute standard deviations as an indicator for the variation of the rank position within the investigated collective.

The butanoic acid metabolite (M04) and a metabolite mono-hydroxylated at the cumyl moiety (M09) were the most abundant phase I metabolites *in vivo*.

In total, 28 phase-I metabolites of Cumyl-4CN-BINACA were detected *in vivo*. The postulated metabolic pathway included *N*-dealkylation, hydroxylation, formation of dihydrodiols, formation of the 4-hydroxybutyl metabolite and further oxidation to the butanoic acid metabolite.

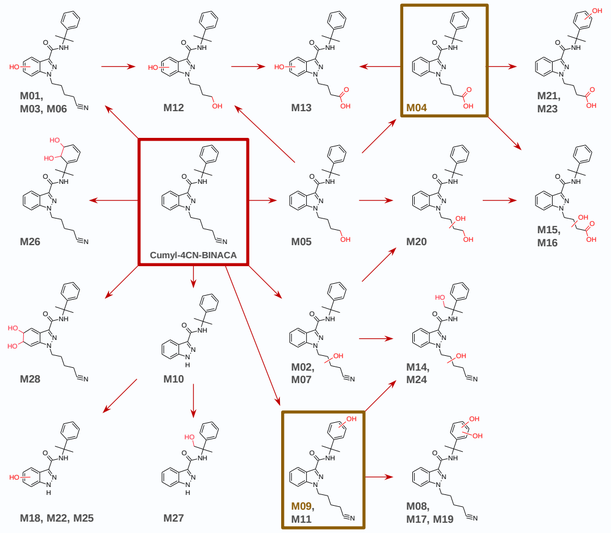
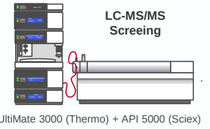


Fig. 5. Postulated phase-I biotransformation of Cumyl-4CN-BINACA.

The metabolites M04 and M09 were included into an LC-MS/MS routine screening method.



UltiMate 3000 (Thermo) + API 5000 (Sciex)

API 5000 Scan Mode:
- Positive electrospray ionization
- Scheduled MRM
- 2 transitions per compound

LC Conditions:
- Luna C18(2) column
- Solvent A (1% ACN, 0.1% HCOOH, 2 mM $NH_4^+HCOO^-$)
- Solvent B (ACN with 0.1% HCOOH, 2 mM $NH_4^+HCOO^-$)

Prevalence in authentic samples

Among the urine samples tested positive for SC in 2017 (n=474), 72 were positive for metabolites of Cumyl-4CN-BINACA.

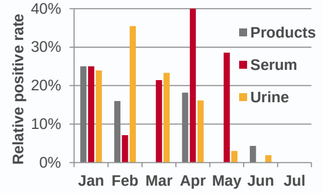


Fig. 6. Cumyl-4CN-BINACA positive samples in relation to all SC positive samples per month.

Discussion & Conclusions

The nitrile function of Cumyl-4CN-BINACA is subject to extensive metabolism (formation of the 4-hydroxybutyl metabolite (M05) and further oxidation) leading to metabolites highly abundant in human urine samples. This might also apply to other substances with a terminal nitrile function. It seems likely that this reaction is comparable to the hydrolytic defluorination of compounds with a terminal fluorine atom at the *N*-alkyl side chain. An alternative mechanism might be the hydrolysis of the nitrile function with subsequent decarboxylation combined with hydroxylation in position 4 of the *N*-alkyl chain. The butanoic acid metabolite (M04) was the most abundant phase-I metabolite in human urine among the investigated sample collective and should be targeted for maximum sensitivity (drug abstinence testing). The most abundant *in vivo* phase I metabolite with intact nitrile function, and therefore a highly specific Cumyl-4CN-BINACA marker, was a metabolite mono-hydroxylated at the cumyl moiety (M09). Due to the rapid spreading of Cumyl-4CN-BINACA, it is strongly recommended to add this compound to screening methods for SCs.

Acknowledgment

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References

[1] Bowden and Williamson, WO 2014/167530 A1
[2] EDND of the EMCDDA
[3] 'Mammalian Liver Microsomes, Guidelines for Use' TF000017 Rev 1.0 (BD Biosciences)

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