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Prevalence of Synthetic Cannabinoids in Abstinence Control Urine Samples Analyzed at the Zurich Institute of Forensic Medicine

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1. Introduction

Screening procedures for abstinence control usually cover classical legal and illegal drugs of abuse but do not include new psychoactive substances (NPSs) such as synthetic cannabinoids (SCs). The detection of their consumption would cause high costs, as screening for NPSs requires sophisticated LC-MS techniques. Therefore, the assessment of the prevalence of SCs in abstinence control samples sent to the Zurich Institute of Forensic Medicine should finally provide data on the need to include SCs in screening procedures for abstinence control.

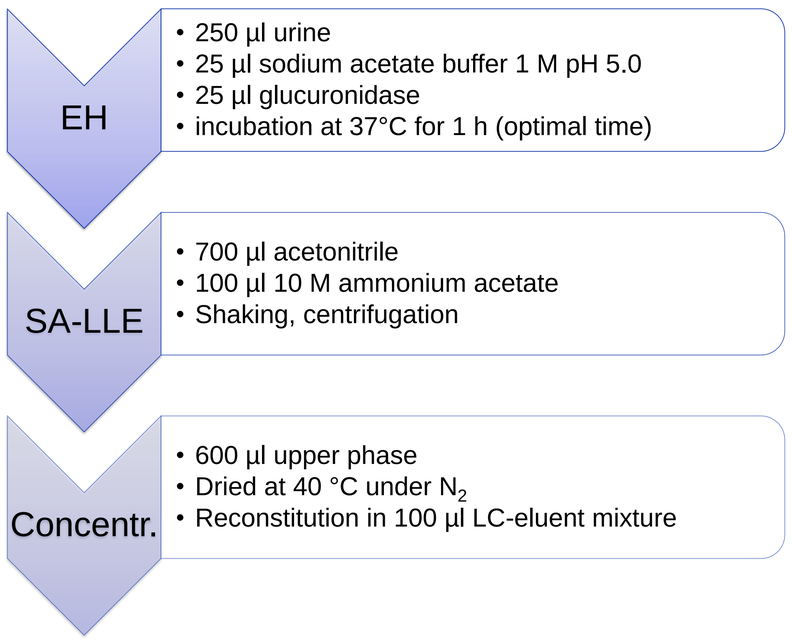

Fig.1:. Schematic depiction of the sample preparation including enzymatic hydrolysis (EH), salting-out with liquid-liquid extraction (SA-LLE), and concentration

2. Sample preparation

Urine sample preparation was performed using salting-out liquid-liquid extraction after enzymatic hydrolysis (**Fig. 1**).

Briefly, 250 µl urine was mixed with 25 µl of 1 M sodium acetate buffer pH 5.0, 25 µl glucuronidase, and 10 µl IS-mixture. After incubation at 37°C for 60 min, 700 µl acetonitrile and 100 µl 10 M ammonium acetate were added. 600 µl upper layer was dried and reconstituted in 100 µl eluent. After concentration, 10 µl were injected into the LC-MS system.

3. LC-MS/MS-EPI analysis

- The samples were analyzed on a Thermo Dionex U3000 coupled to a Sciex 5500 QTrap system with information dependent acquisition of MS^2 mass spectra (MRM-IDA-EPI mode)
- Gradient elution was performed on a Synergi PolarRP 100x2.0 mm, 2.5 µm using 10 mM NH_4 formate and acetonitrile with 0.1% formic acid each
- For each analyte, a single transition was used with a retention time window of 60 s.
- Identification criteria were defined to include retention time ±0.2 min, signal-to-noise-ratio >3:1 and the (subjective) fit to the reference spectrum.
- For each synthetic cannabinoid at least two metabolites were included.
- To date, the method includes 417 MRM (75 Cannabinoids and 339 metabolites.

4. Method validation

- Ten blank authentic urine samples from different sources were analyzed for interfering peaks.
- Selectivity regarding other drugs was investigated injecting a mixture of drugs of abuse
- Limit of detection (LOD) was investigated for selected metabolites
- Matrix effects and recovery was investigated for selected metabolites
- For prospective validation, authentic urine samples were analyzed and the results were compared to the results from the Institute of Forensic Medicine, Freiburg, Germany

Results

- No interfering peaks with matching MS2 mass spectra were detected
- LODs were reached down to 0.05 ng/mL
- Recoveries were above 80% and matrix effects within ± 25%.

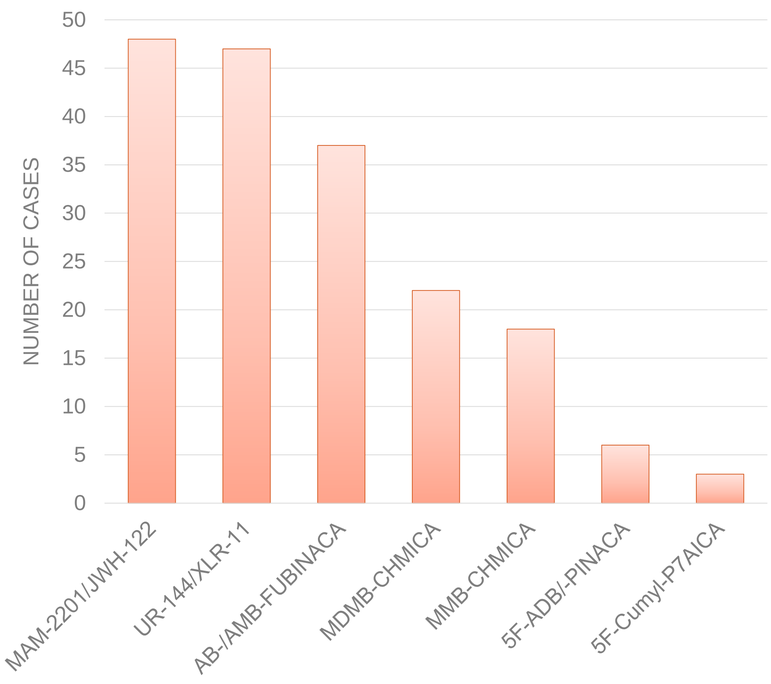

Fig. 3 Number of cases in which metabolites of a certain synthetic cannabinoid were detected.

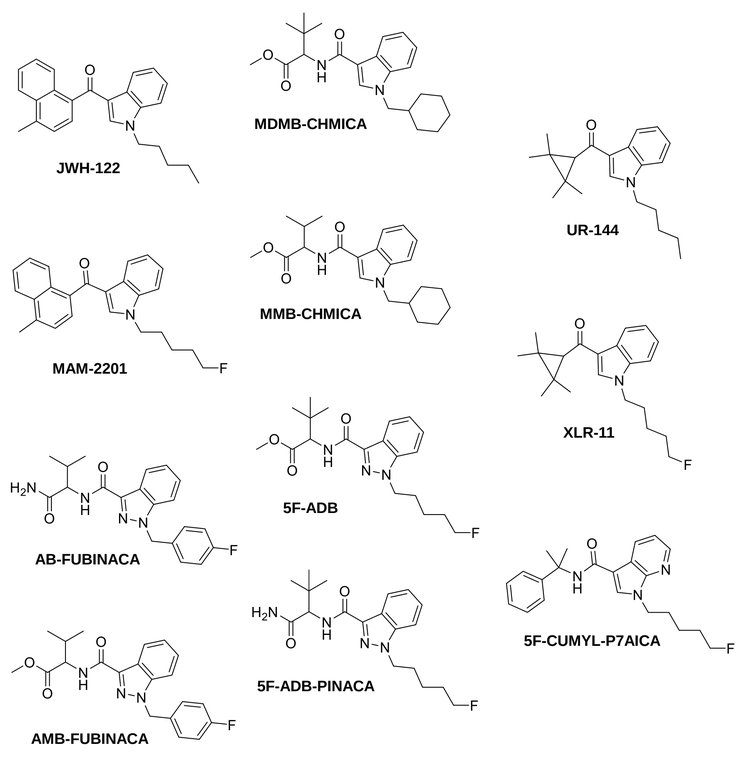

Fig. 4 Chemical structures of MAM-2201, JWH-122, UR-144, XLR-11, AB-FUBINACA, AMB-FUBINACA, MDMB-CHMICA, MMB-CHMICA, 5F-ADB, 5F-ADB-PINACA and 5F-CUMYL-P7AICA

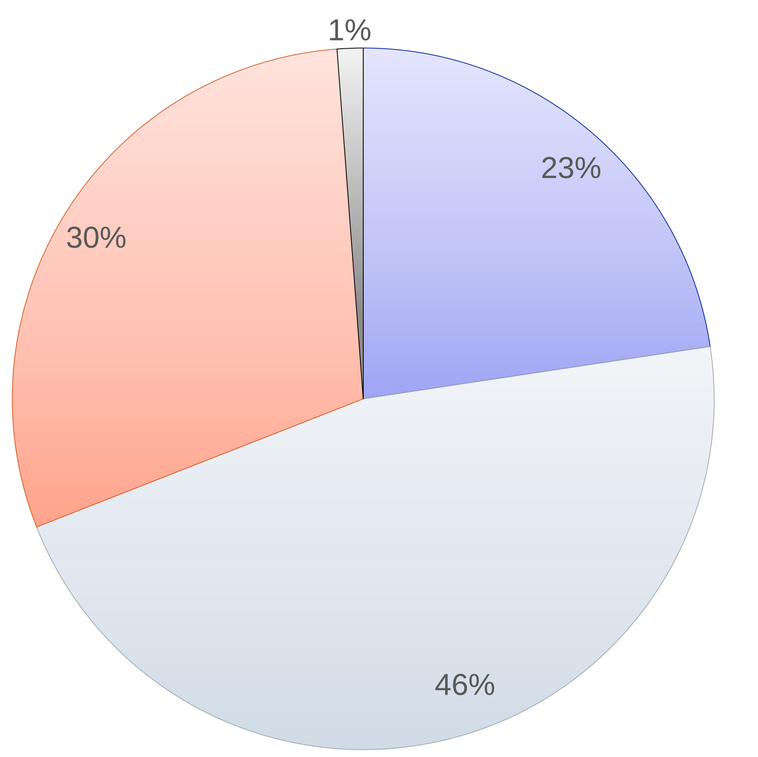

Fig. 2 Number of consumed synthetic cannabinoids detected per sample within the positive cases

4. Results

- In total, 487 urine samples from people under abstinence control were screened for 75 different SCs and their metabolites.
- In 17% of the samples, consumption of SCs was detected.
- Among the positive samples, 80% contained metabolites of 2 or more different SCs, and 33% contained metabolites of 3 or more SCs. In one sample, metabolites of even 5 different SCs were identified (**Fig. 2**).
- As some SCs share the same metabolites, the parent compound was not always unambiguously deductible.
- Metabolites of the following SCs were identified: 5F-ADB and/or 5F-ADB-PINACA, 5F-MDMB-P7AICA, AB-FUBINACA and/or AMB-FUBINACA, JWH-122 and/or MAM-2201, MDMB-CHMICA, MMB-CHMICA, and UR-144 and/or XLR-11. (**Fig. 3** and **4**).

Conclusion

Despite low prevalence for NPSs in the general population, this study proves that the consumption of synthetic cannabinoids in abstinence control cases in Switzerland is frighteningly frequent. Standard screening procedures do no longer cover the complexity of the drug market in Switzerland. New methods are necessary which are able to detect NPSs in abstinence control.

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