Stability test of selected synthetic cannabinoids and some of their human metabolites in sewage water and identification of transformation products

Petra Hehet Wiesbaden/D, Niklas Köke Idstein/D, Tobias Frömel Idstein/D, Daniel Zahn Idstein/D, Michael Pütz Wiesbaden/D, Thomas P. Knepper Idstein/D

Petra Hehet, Federal Criminal Police Office (BKA), Forensic Science institute, D-65203 Wiesbaden
Niklas Köke, Hochschule Fresenius, University of Applied Sciences, D-65510 Idstein

Corresponding e-mail address: knepper@hs-fresenius.de

Bundeskriminalamt

HOCHSCHULE FRESENIUS
UNIVERSITY OF APPLIED SCIENCES

Introduction

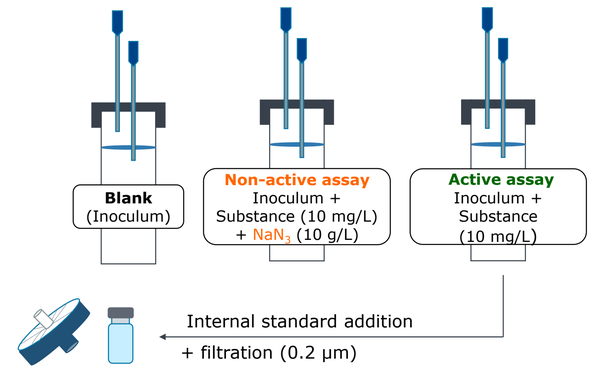
Wastewater-based epidemiology (WBE) is an indirect approach to estimate illicit drug consumption at the population level through the presence of drugs and their metabolites in the wastewater produced. This methodology, however, requires detailed knowledge about the fate of illicit drugs and drug metabolites during their residence time in the sewage system, specifically their stability and the transformation products that may eventually be formed from them[1, 2].

Synthetic cannabinoid receptor agonists (SCRAs), the most prevalent group of new psychoactive substances (NPS) [3], were selected as analytes for this biotransformation study. The stability of SCRAs and their relevant human metabolites in wastewater was investigated with HPLC-MS/MS. If a primary degradation was observed [4], the samples were analyzed further with HPLC-HRMS to identify possible **transformation products** (TPs).

Methods

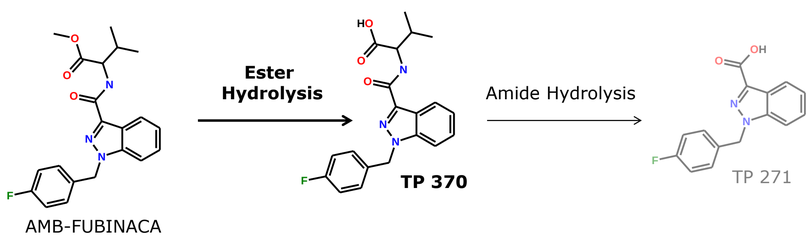
Sample preparation

- Inoculum: 95% wastewater treatment plant effluent and 5% activated sludge (w/w)
- Conditions: light exclusion, aerobic conditions, ambient temperature, pH (pH 7.47)

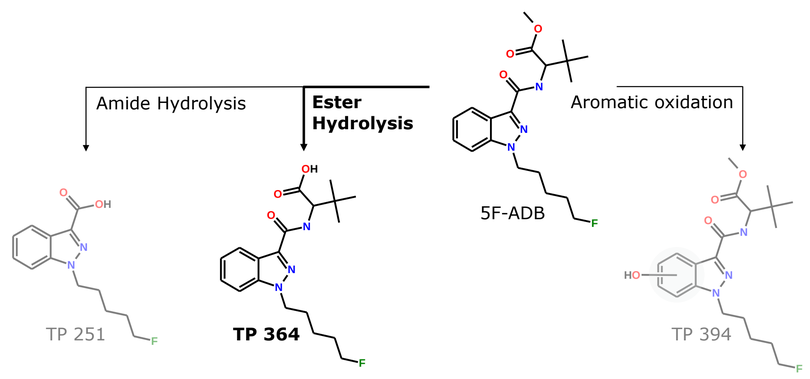


Transformation pathways

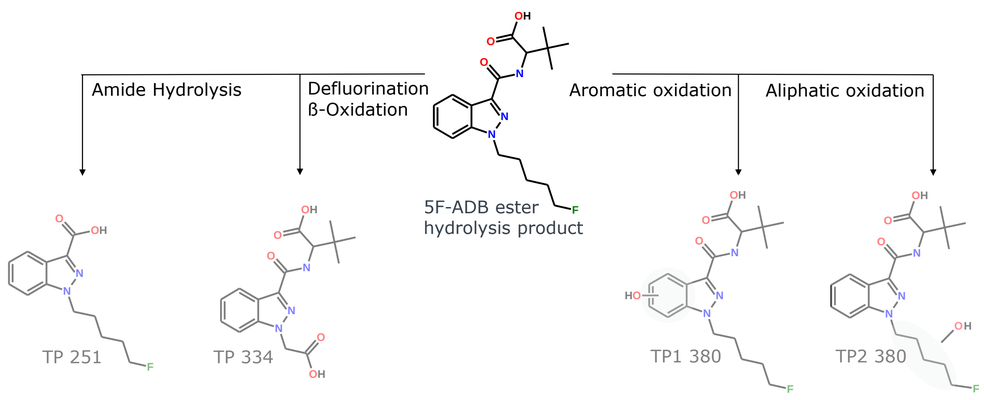
- TPs were tentatively identified based on their sum formula (mass accuracy ≤ 5 ppm, isotopic pattern fitting) and proposed fragmentation pathway
- Need of cross-quantification (quantification based on the response factors of the respective precursor compounds), due to a lack of reference standards



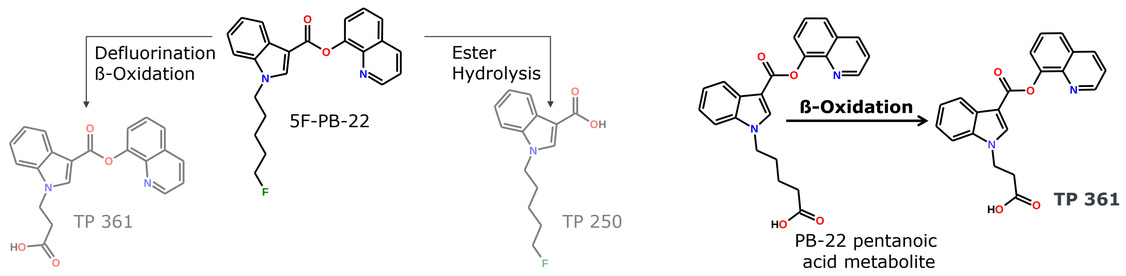
- TP 370: Approx. 20% formed after four days, not stable
- TP 271: Approx. 8% formed after eight days, stable



- TP 364: Approx. 12% formed after 29 days, stable
- TP 251: Approx. 0.4% formed after 23 days, stable
- TP 394: Approx. 1% formed after 17 days, not stable



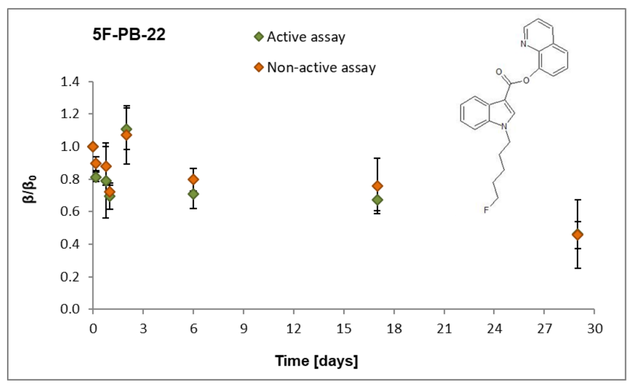
- TP 251: Approx. 3.3% formed after 29 days, stable
- TP 334: Approx. 2.6% formed after 13 days, stable
- TP1 380: Approx. 0.2% formed after 13 days, stable
- TP2 380: Approx. 0.4% formed after 13 days, stable

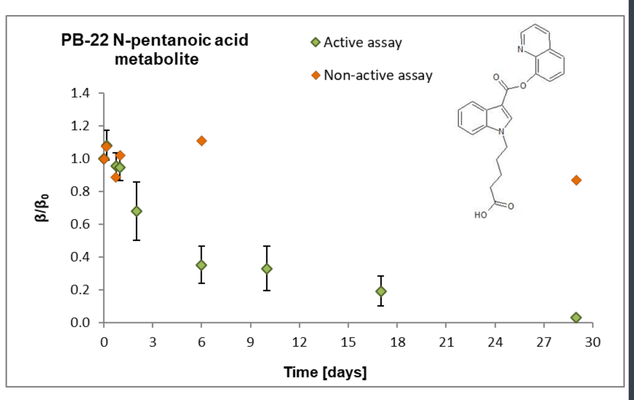


- TP 361: Approx. 1.2% formed after eight days, not stable
- TP 250: Approx. 0.5% formed after four days, stable
- TP 361: Approx. 55% formed after eight days, stable

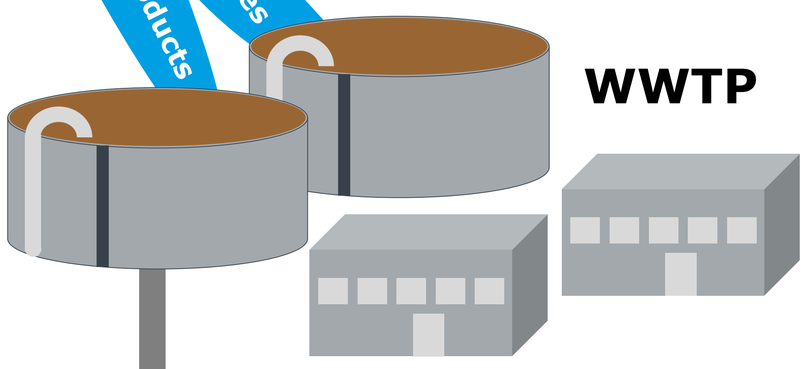
Stability profiles

Stability profiles of **5F-PB-22** and its metabolite **PB-22 N-pentanoic acid**

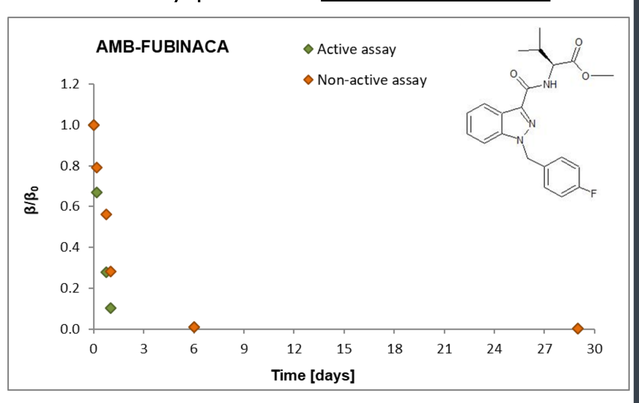




- Similar transformation processes (active/non-active; n=3) of 5F-PB-22 observed
- The human metabolite PB-22 N-pentanoic acid revealed a further biotransformation step, which was completed after 29 days (n=3)

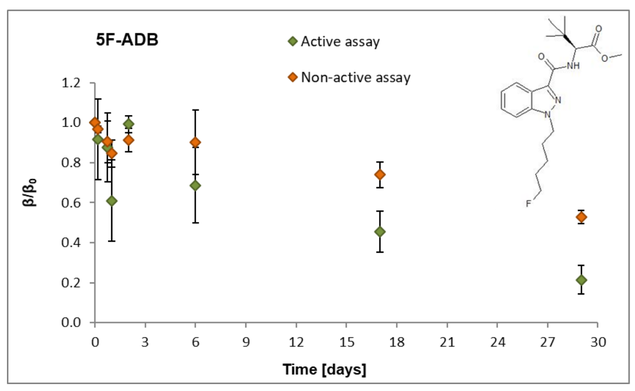


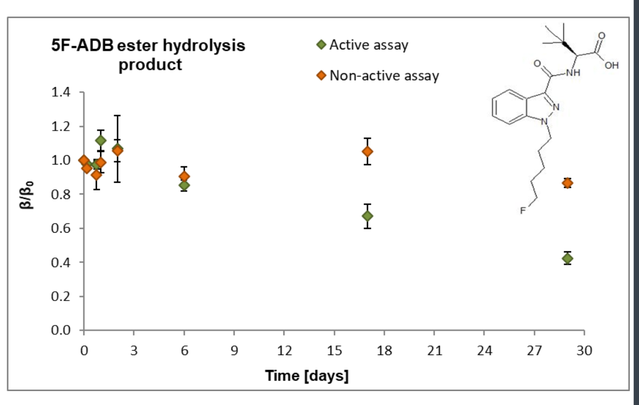
Stability profile of **AMB-FUBINACA**



- AMB-FUBINACA showed a rapid decrease in both assays (n=1) and primary transformation was completed after 6 d
- Concentration decrease by 90% was observed already after 24 h, results in the fastest degradation of a SCRA investigated in this study

Stability profiles of **5F-ADB** and its metabolite **5F-ADB ester hydrolysis product**





- Continuous decrease in both assays (n=3) with more pronounced microbial degradation of the human metabolite (5F-ADB ester hydrolysis product)

Conclusion

- The majority of the selected SCRAs can be considered as **stable** in sewage with typically 60 - 95% still present after 24 h
- Concentration deceases in active tests were mostly caused by hydrolysis or sorption
- Human **metabolites** of SCRAs are more prone to microbial degradation compared to their precursor compounds
- 10 TPs were identified via HPLC-HRMS
- **TP 361**, **TP 364** and **TP 370** (>10%) were determined with the most formation percentage and may be used in further WBE studies

stable SCRAs and TPs

TP 370
TP 361
MDMB-CHMCZCA
TP 364
EG-018
TP 394
TP2 380
TP 251
Cumyl-PeGaClone
TP 271
TP 334
TP1 380
TP 250

References:

[1] Bijlsma L, et al. *Chemosphere*, 2012;89(11):1399-1406.
[2] Postigo C, et al. *Anal Chem*, 2008;80(9):3123-3134.
[3] EMCDDA. Fentanils and synthetic cannabinoids: driving greater complexity into the drug situation. Luxembourg: Publications Office of the European Union; 2018.
[4] Hehet. P, et al. Stability test of selected synthetic cannabinoids and some of their human metabolites in sewage water and identification of transformation products. In preparation.

Acknowledgement:

This work has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 787128 (Project SYSTEM).
The contents of the poster are the sole responsibility of the authors and can in no way be taken to reflect the views of the European Commission.