Screening for synthetic cannabinoids in urine by immunoassay versus LC-MS/MS – an evaluation of the diagnostic efficiency

Florian Franz[1], Harald Ertl[2], Verena Angerer[1], Volker Auwärter[1]

[1]Institute of Forensic Medicine, Forensic Toxicology, Medical Center – University of Freiburg, Germany

[2]Drug Monitoring and Toxicology, Labor Lademannbogen, Hamburg, Germany

UNIVERSITÄTS KLINIKUM FREIBURG

Institute of Forensic Medicine
Forensic Toxicology

LABOR LADEMANNBOGEN

Introduction and Aims

Synthetic cannabinoids (SCs) have become an important family of designer drugs and are widely used as 'legal' alternative to cannabis. Therefore, there is a demand for reliable screening methods. For cost-efficient analysis immunoassays (IAs) targeting SC metabolites were introduced.[1,2] However, due to the structural diversity of this class of compounds and the rapidly changing range of available drugs it seems questionable if the applied antibodies show sufficient cross-reactivities. Two commercially available IA kits for urine were evaluated regarding their suitability for detecting the use of currently prevalent substances.

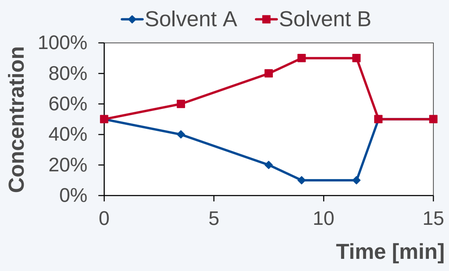
Methods

Liquid chromatography conditions:

- Luna® C18(2) column (150 mm × 2 mm, 5 µm)
- Solvent A: H_2O, 0.2% HCOOH, 2 mmol/L $NH_4^+HCOO^-$
- Solvent B: ACN

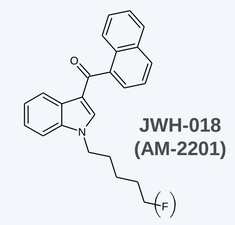
Mass spectrometry conditions:

- SCIEX API 5000™ – MRM(+) mode
- Metabolites of 45 SCs
- At least 2 transitions per metabolite
- Semi-quantitative for selected analytes (LLOQ = 0.05 - 0.1 ng/mL)



Immunoassay:

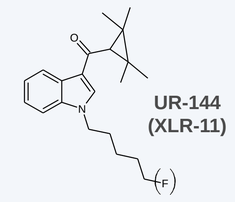
- Roche Cobas Integra® 400
- Homogeneous enzyme immunoassay (HEIA™)



JWH-018 (AM-2201)

Kits from IMMUNALYSIS Corp. (Pomona, CA, USA)

- Synthetic Cannabinoids-1® kit[1,2]:
  Calibrator: JWH-018 *N*-pentanoic acid (cut-off 20 ng/mL)
- Synthetic Cannabinoids-2® kit[1]:
  Calibrator: UR-144 *N*-pentanoic acid (cut-off 10 ng/mL)



UR-144 (XLR-11)

Results and Discussion

One hundred negative samples and one hundred samples positive for metabolites of only one SC (LC-MS/MS data) were selected consecutively from a pool of authentic urine samples collected from January to June 2015. The samples were blinded and re-analyzed using the two HEIAs.

Sensitivity: 2% Specificity: 99% Accuracy: 51%		LC-MS/MS confirmation	
		positive	negative
IA	positive	1.0%	0.5%
	negative	49.0%	49.5%

Using the cut-offs as recommended by the manufacturer[1], the combination of the two IAs led to a sensitivity of 2%, specificity of 99% and an accuracy (diagnostic efficiency) of 51%.

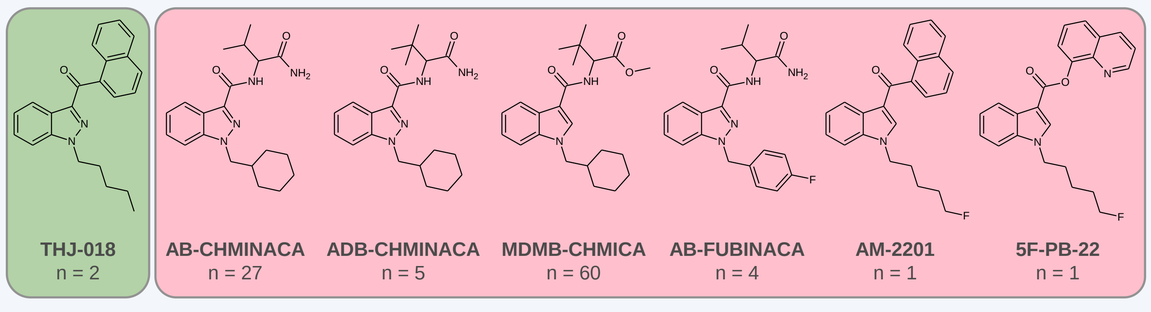


Fig. 1: Consumed SCs detected (green background) and not detected (red background) by the two IAs.

Lowering the cut-offs to half led to a sensitivity of 7% but did not improve the overall diagnostic efficiency. Plotting the IA data as Receiver Operating Characteristic (ROC) curves it is evident that the diagnostic efficiency can not be improved by changing the cut-off values.

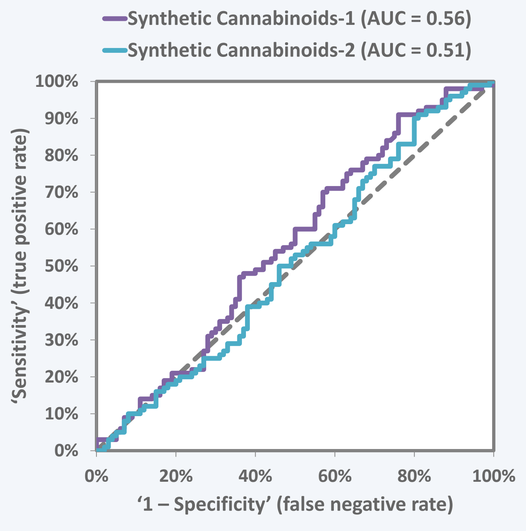


Fig. 2: ROC curves of the evaluated immunoassays showed an Area Under the Curve (AUC) slightly above 0.5 for both kits.

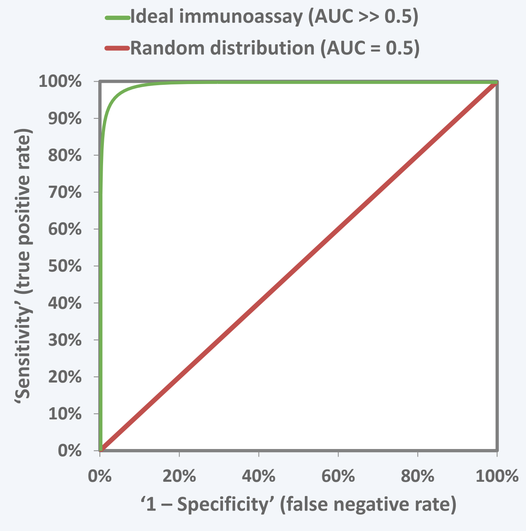


Fig. 3: Example of an ideal immunoassay with high sensitivity and high specificity (green) as well as a curve of random distribution (red).

The samples tested positive by the IA 'Synthetic Cannabinoids-1' were positive for THJ-018 metabolites (LC-MS/MS), which can be explained by the structural similarity of THJ-018 and JWH-018. Samples containing only metabolites of AB-CHMINACA, AB-FUBINACA, ADB-CHMINACA, AM-2201, MDMB-CHMICA or 5F-PB-22 were not detected by both IAs.

The results can be explained by an insufficient cross-reactivity of the available antibodies for the 'new generation' SCs (see also Fig. 4). Another factor could be the relatively low analyte concentrations in urine due to high potency of the drugs combined with an insufficient sensitivity of the immunochemical tests.

Conclusion

In the light of the structural inhomogeneity of synthetic cannabinoids the use of immunoassays merits critical attention. It is strongly recommended not to rely on the evaluated immunochemical tests for synthetic cannabinoids, neither in clinical nor in forensic settings. As the antibodies used for immunoassays of other providers probably show similar cross-reactivities, similar results can be expected for other commercially available immunoassay kits.

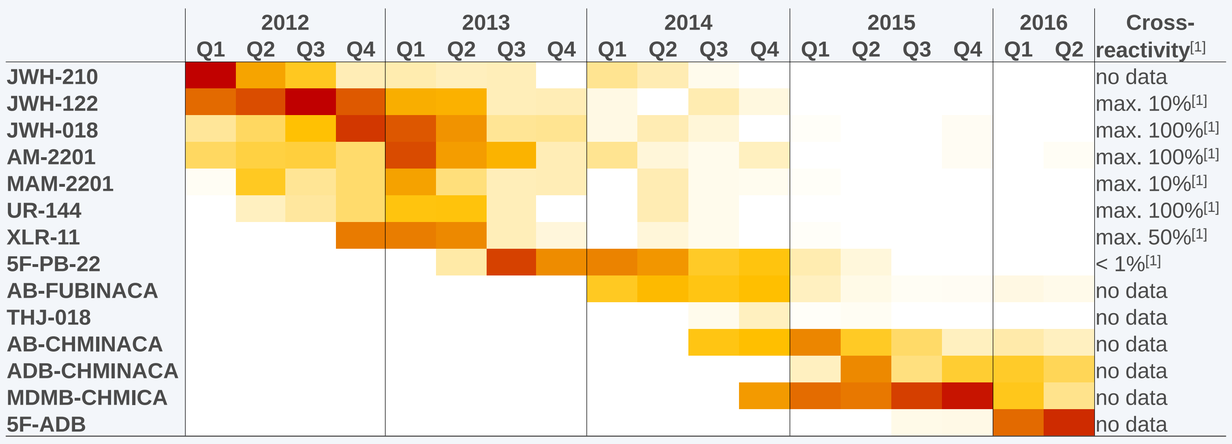


Fig. 4: Prevalence of selected substances detected in serum samples (n = 4551) since 2012 in the Institute of Forensic Medicine Freiburg and their cross-reactivity.

0% 76%

Percentage of positive samples in relation to all positive samples (n=973) determined on a quarterly basis.

Acknowledgement

This publication has been funded by the European Commission (JUST/2013/ISEC/DRUGS/AG/6421) and the Society of Hair Testing (SoHT) kindly granted a travelling scholarship.

References

[1] Datasheet of IMMUNALYSIS Corp. 'Synthetic Cannabinoids Homogenous Enzyme Immunoassay (HEIA™)'

[2] Barnes AJ et al., Forensic Sci Int. 2014 241:27–34

Contact

Florian Franz
Medical Center – University of Freiburg
Institute of Forensic Medicine
Albertstraße 9
79104 Freiburg, Germany
florian.franz@uniklinik-freiburg.de