Immunoassay screening in urine for synthetic cannabinoids – a feasible approach for forensic applications?

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Introduction and Aims

In therapeutic settings requiring abstinence, reliable screening methods for drugs of abuse and especially for new psychoactive substances like synthetic cannabinoids are needed. For economic reasons institutions of drug rehabilitation and forensic psychiatric hospitals often apply immunoassays to screen urine samples for synthetic cannabinoids. However, the wide structural diversity of this class of drugs makes it difficult to design suitable antibodies, and false negative results can impede the therapeutic process. This retrospective study was performed to check if two commercially available immunoassay kits are capable of detecting currently prevalent substances in authentic urine samples.

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
- Run time: 15 min

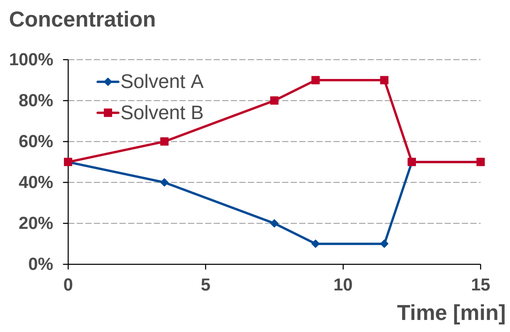


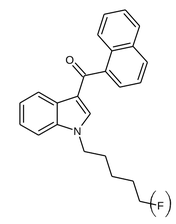
Figure: Gradient of the LC-Method

Mass spectrometry conditions:

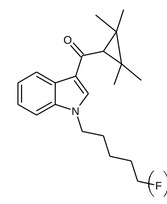
- SCIEX API 5000™
- sMRM – positive mode
- Min. 2 transitions per metabolite
- Metabolites of 43 synthetic cannabinoids
- Semi-quantitative determination (LLOQ = 0.05 – 0.1 ng/mL)

Immunoassay:

- Roche Cobas Integra® 800
- Homogeneous enzyme immunoassay (HEIA™)
- Kits from Immunalysis (Pomona, CA, USA):



Synthetic Cannabinoids-1®-Kit
JWH-018 / AM-2201
Cutoff: 20 ng/mL



Synthetic Cannabinoids-2®-Kit
UR-144 / XLR-11
Cutoff: 10 ng/mL

Results and discussion

Urine samples of 549 individuals from seven different forensic psychiatric hospitals located in the federal states of Bavaria and Baden-Württemberg were screened for synthetic cannabinoids by two immunoassays. Results were confirmed by an up-to-date LC-MS/MS method.

None of the patients was tested positive by either of the two immunoassays. In contrast, using LC-MS/MS analysis metabolites of synthetic cannabinoids were detected in 7.7 % of the samples.

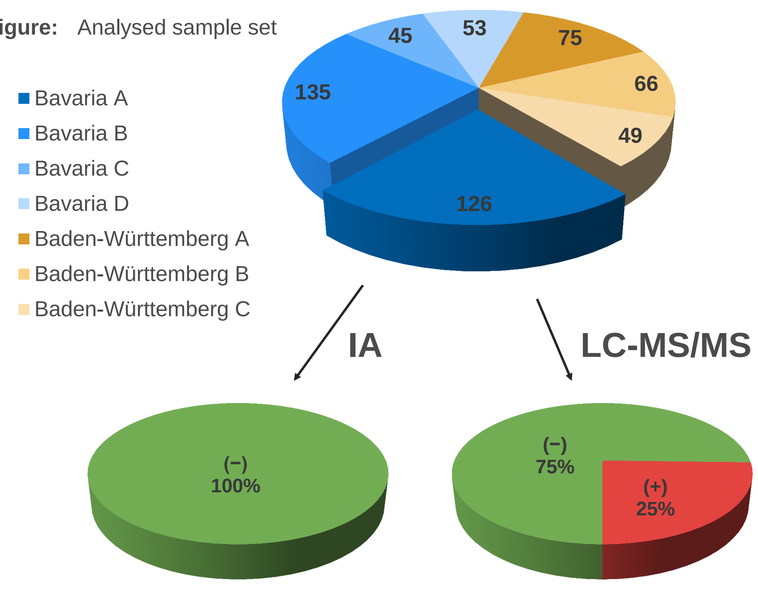


Figure: Difference in IA and LC-MS/MS analysis results of the collective „Bavaria A“

‚Sensitivity‘: 0% ‚Specificity‘: 100% ‚Accuracy‘: 92%		LC-MS/MS confirmation	
		negative	positive
IA	negative	92%	8%
	positive	0%	0%

Table: Fourfold table of the IA evaluation

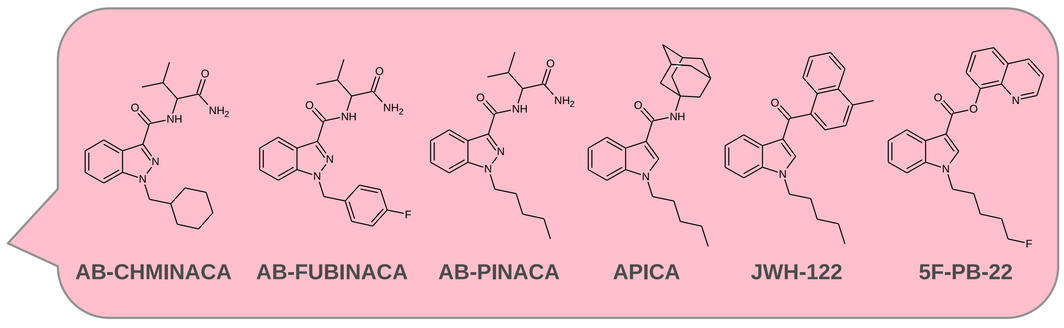


Figure: Consumed substances not detected by the immunoassay

The results can be explained by insufficient cross reactivity of the available antibodies for the ‘new generation’ synthetic cannabinoids. Another factor could be the generally low analyte concentrations in urine and an insufficient sensitivity of the immunoassay tests.

There were no marked differences regarding the positive rates across the two federal states or between hospitals applying immunoassay screening versus other means of abstinence control.

Conclusion

In the light of the very heterogeneuos groups of synthetic cannabinoids the use of immunoassays merits critical attention. It is strongly recommended not to rely on the evaluated IA 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 products.

	2012				2013				2014				2015	Cross reactivity[1,2]
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	
JWH-210														< 1%
JWH-122														25%
AM-2201														100%
JWH-018														100%
MAM-2201														50%
UR-144														100%
XLR-11														50%
EAM-2201														?
5F-PB-22														< 1%
AB-FUBINACA														?
AB-CHMINACA														?
MDMB-CHMICA														?

Table: Heat map of the most prevalent substances detected in serum samples since 2012 in the Institute of Forensic Medicine Freiburg.

0% 86%

Percent of positive samples in relation to all positive samples determined on a quarterly basis.

Acknowledgement

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References

[1] Datasheet “Synthetic Cannabinoids (HEIA™)” IMMUNALYSIS Corporation

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

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