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

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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. 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 the detection of currently prevalent substances.

Methods

Liquid chromatography conditions:
- Luna® C18(2) column (150 mm x 2 mm, 5 μm)
- Solvent A: H₂O, 0.2% HCOOH, 2 mmol/L NH₄HCOO
- 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)

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

Kits from IMMULYSIS Corp. (Pomona, CA, USA)
- Synthetic Cannabinoids-1® kit[1]; Calibrator: JWH-018 N-pentanoic acid (cut-off 20 ng/mL)
- Synthetic Cannabinoids-2® kit[2]; Calibrator: UR-144 N-pentanoic acid (cut-off 10 ng/mL)

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.

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, MDBM-CHIMICA or 5F-PB-22 were not detected by both IAs.

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%.

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 cannot be improved by changing the cut-off values.

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.

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References

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

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