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 (SC) have become an important family of designer drugs and are widely used in Europe. Therefore, the demand for reliable screening methods is constantly increasing. Different immunoassays (IA) targeting SC metabolites are available for cost-efficient analysis. However, due to the structural diversity of this class of substances and the highly dynamic changes on the drug market it seems questionable if the applied antibodies show sufficient cross reactivity for all relevant substrates. Hence, two commercially available IA kits for urine analysis 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: H₂O, 0.2 % HCOOH, 2 mmol/L NH₄HCOO
- Solvent B: ACN

Mass spectrometry conditions:
- SCIEX API 5000™ – MRM(+)/mode
- Metabolites of 45 SC
- At least 2 transitions per metabolite
- Semi-quantitative
  (LLOQ = 0.05 - 0.1 ng/mL)

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

Kits from IMMUNALYSIS Corp. (Pomona, CA, USA)
- Synthetic Cannabinoids-1®-kit:
  Calibrator: JWH-018 N-pentanoic acid (cut-off 10 ng/mL)
- Synthetic Cannabinoids-2®-kit:
  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 reanalysed using the two HEIA™.

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

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

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 to JWH-018. Samples containing only metabolites of AB-CHMINACA, ADB-FUBINACA, ADB-CHMINACA, AM-2201, MDBM-CHMINACA or SF-PC-22 were not detected by both IA.

The results can be explained by an insufficient cross reactivity of the available antibodies for the ‘new generation’ synthetic cannabinoids (see also Tab. 1). 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 immunological 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 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, analogical results can be expected for other commercially available immunoassay products.

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References

  Synthetic Cannabinoids Homogenous Enzyme Immunoassay (HEIA™)
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