

# 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 substructures. Hence, two commercially available IA kits for urine analysis were evaluated regarding their suitability for detecting the use of currently prevalent substances.

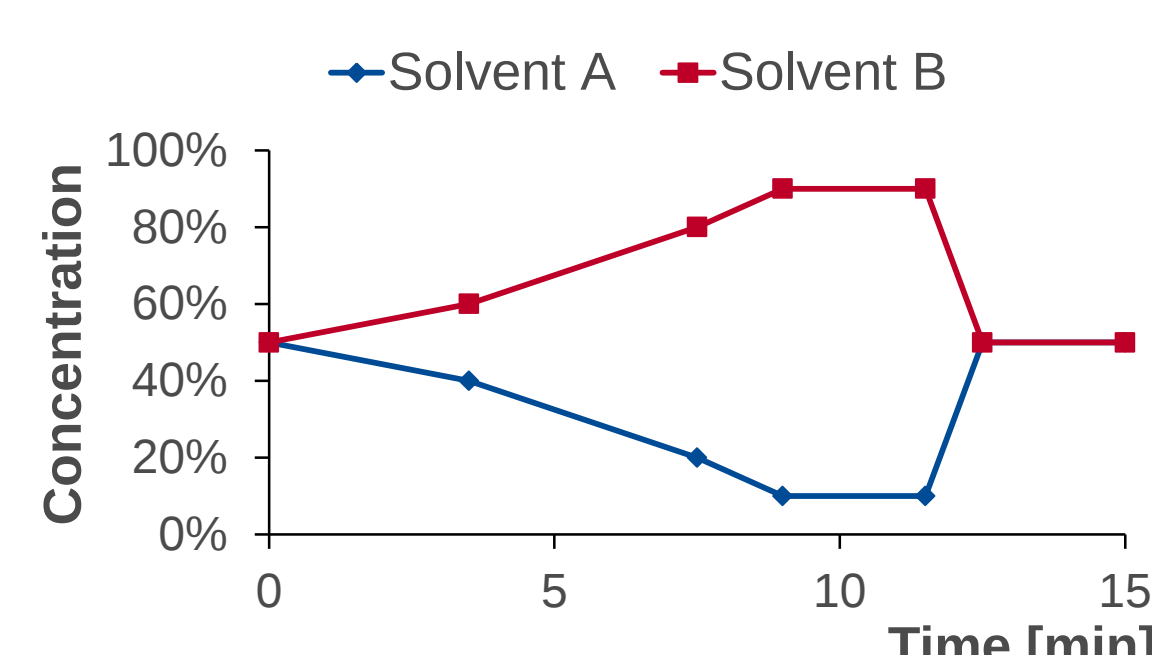
## Methods

### Liquid chromatography conditions:

- Luna® C18(2) column (150 mm × 2 mm, 5 µm)
- Solvent A: H<sub>2</sub>O, 0.2 % HCOOH, 2 mmol/L NH<sub>4</sub><sup>+</sup>HCOO<sup>-</sup>
- 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)

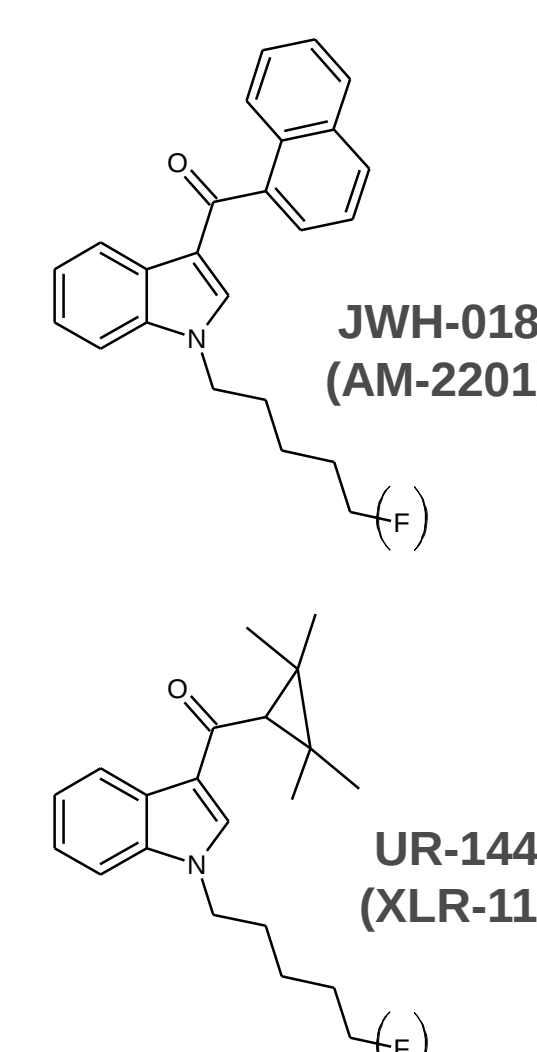


### Immunoassay:

- 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™.

| IA       | LC-MS/MS confirmation |          |
|----------|-----------------------|----------|
|          | positive              | negative |
| positive | 1 %                   | 0.5 %    |
| negative | 49 %                  | 49.5 %   |

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 %.

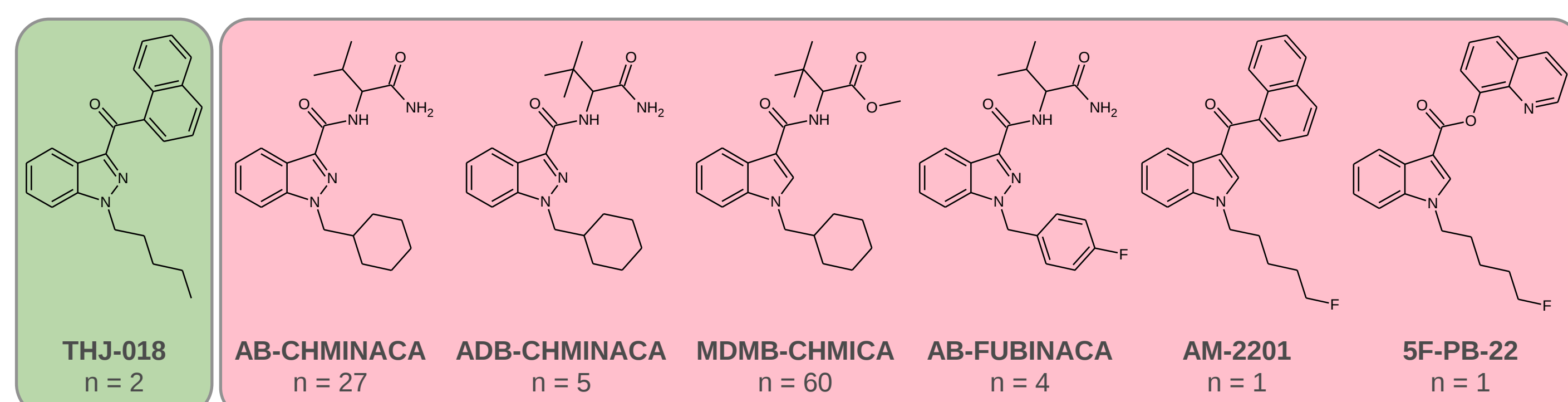


Fig. 1: Consumed SC detected (green background) and not detected (red background) by the two immunoassays.

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, AB-FUBINACA, ADB-CHMINACA, AM-2201, MDMB-CHMICA or 5F-PB-22 were not detected by both IA.

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 it is evident that the diagnostic efficiency can not be improved by changing the cut-off value.

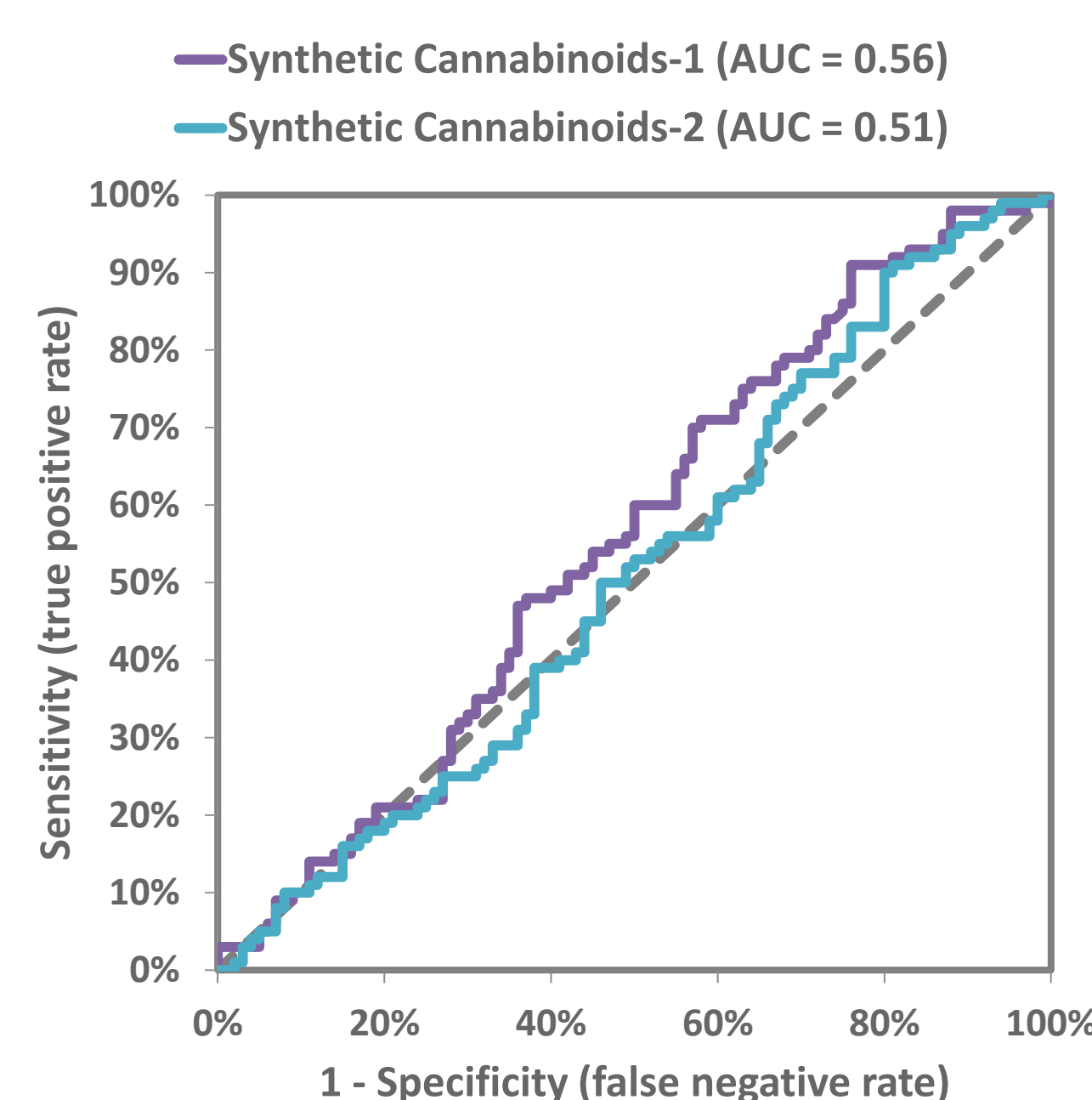


Fig. 2: ROC curves of the evaluated immunoassays showed an Area Under the Curve (AUC) slightly above 0.5 for both kits.

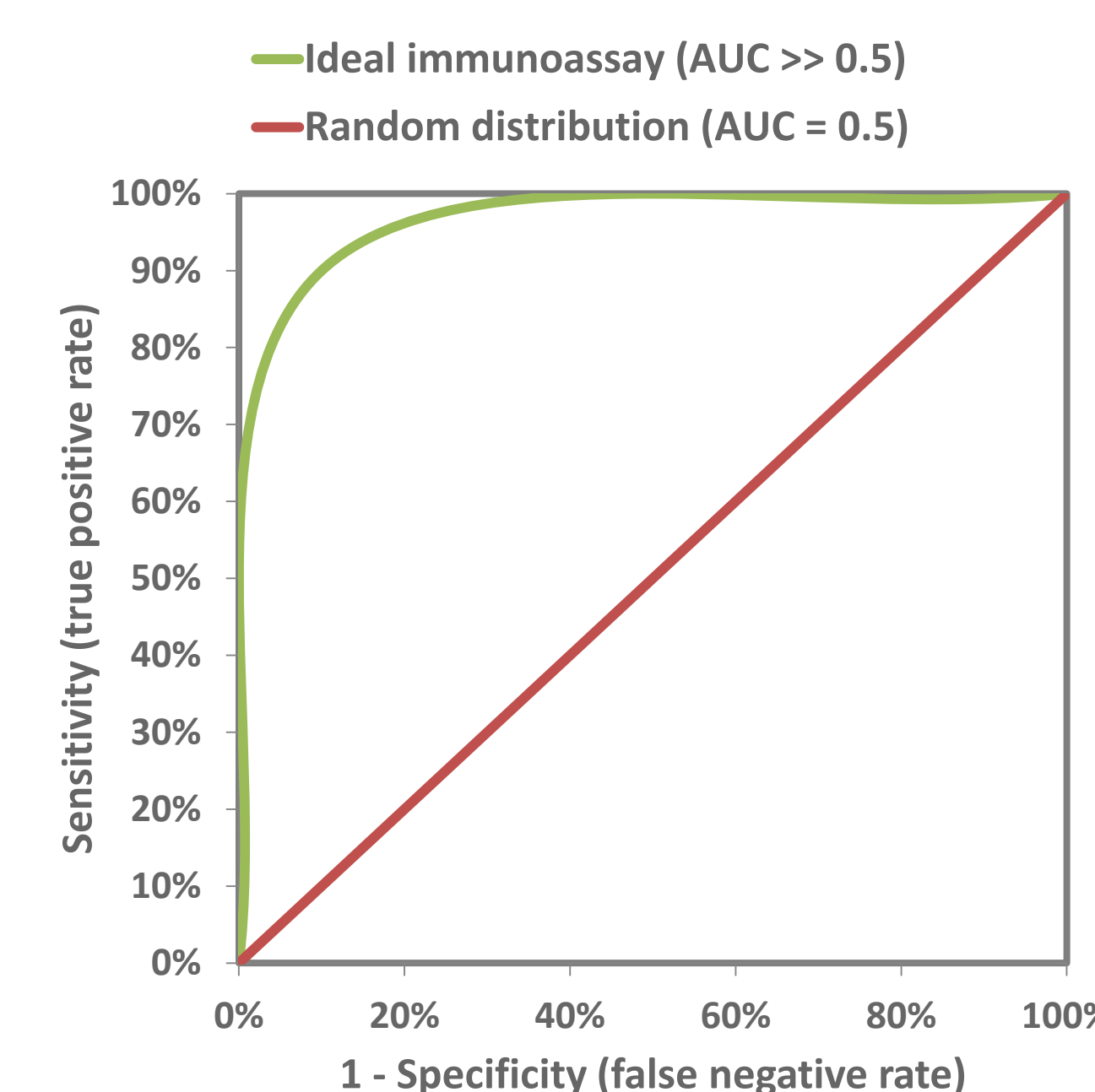


Fig. 3: Example of an ideal immunoassay with high sensitivity and high specificity (green) as well as a curve of random distribution (red).

The 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 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 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, analogous results can be expected for other commercially available immunoassay products.

|              | 2012 |    |    |    | 2013 |    |    |    | 2014 |    |    |    | 2015 | Cross reactivity <sup>1</sup> |
|--------------|------|----|----|----|------|----|----|----|------|----|----|----|------|-------------------------------|
|              | Q1   | Q2 | Q3 | Q4 | Q1   | Q2 | Q3 | Q4 | Q1   | Q2 | Q3 | Q4 | Q1   |                               |
| JWH-122      |      |    |    |    |      |    |    |    |      |    |    |    |      | max. 10 % <sup>1</sup>        |
| JWH-018      |      |    |    |    |      |    |    |    |      |    |    |    |      | max. 100 % <sup>1</sup>       |
| AM-2201      |      |    |    |    |      |    |    |    |      |    |    |    |      | max. 100 % <sup>1</sup>       |
| MAM-2201     |      |    |    |    |      |    |    |    |      |    |    |    |      | max. 10 % <sup>1</sup>        |
| UR-144       |      |    |    |    |      |    |    |    |      |    |    |    |      | max. 100 % <sup>1</sup>       |
| XLR-11       |      |    |    |    |      |    |    |    |      |    |    |    |      | max. 50 % <sup>1</sup>        |
| 5F-PB-22     |      |    |    |    |      |    |    |    |      |    |    |    |      | < 1 % <sup>1</sup>            |
| AB-FUBINACA  |      |    |    |    |      |    |    |    |      |    |    |    |      | no data                       |
| AB-CHMINACA  |      |    |    |    |      |    |    |    |      |    |    |    |      | no data                       |
| MDMB-CHMICA  |      |    |    |    |      |    |    |    |      |    |    |    |      | no data                       |
| ADB-CHMINACA |      |    |    |    |      |    |    |    |      |    |    |    |      | no data                       |

Tab. 1: Prevalence of selected substances detected in serum samples since 2012 in the Institute of Forensic Medicine Freiburg and their cross reactivity.

0 % 86 %  
Percentage of positive samples in relation to all positive samples determined on a quarterly basis.

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## References

- [1] Datasheet of IMMUNALYSIS Corp. 'Synthetic Cannabinoids Homogenous Enzyme Immunoassay (HEIA™)'
- [2] Barnes AJ et al., Forensic Sci Int. 2014 241:27–34

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