Validated LC-MS/MS method for qualitative and quantitative analysis of 75 synthetic cannabinoids in serum

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Introduction:

Besides synthetic cathinones, synthetic cannabinoids are among the most common new psychoactive substances reported to the EMCDDA in the last few years[1]. Since 2011, the number of new synthetic cannabinoids reported to the EMCDDA was relatively stable and amounted about 30 compounds per year. Therefore, it is necessary to update analytical methods regularly. For use in forensic cases, a validation of the methods is mandatory.

Method

Sample preparation:

1 ml serum
10 µl internal standard (c = 25 ng/ml)
0.5 ml carbonate buffer (pH 10)
↓
1.5 ml extraction mixture 1 (hexane/ethyl acetate, 99/1, v/v)
↓
Gentle mixing 5 min
centrifugation 2860xg, 20 min → 1.5 ml extraction mixture 2 (hexane/ethyl acetate, 80/20, v/v)
↓
Transfer of 1 ml of each organic supernatant into one HPLC vial
↓
Evaporation to dryness of combined extracts under gentle stream of nitrogen at 40 °C
↓
Reconstitution in 100 µl mobile phase (A/B, 50/50, v/v)
↓

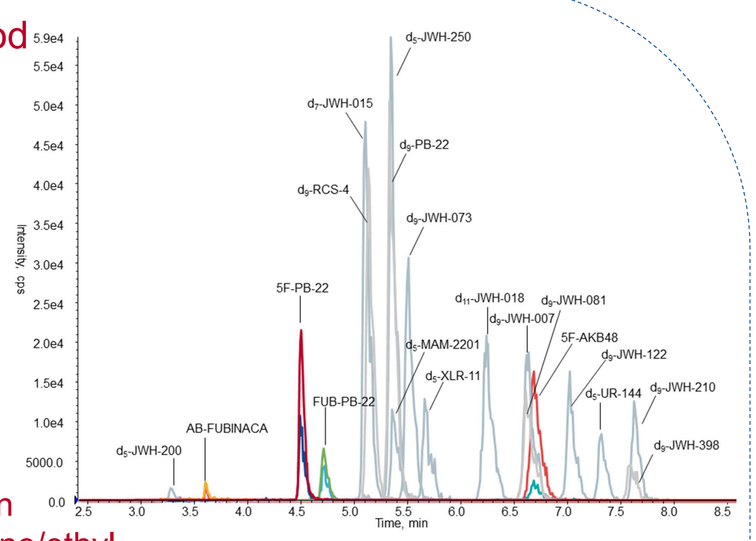
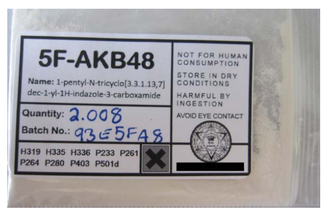


Fig. 1: Total ion chromatogram of an authentic serum sample.
AB-FUBINACA 0.27 ng/ml;
5F-AKB48 0.19 ng/ml;
FUB-PB-22 0.44 ng/ml;
5F-PB-22 0.27 ng/ml



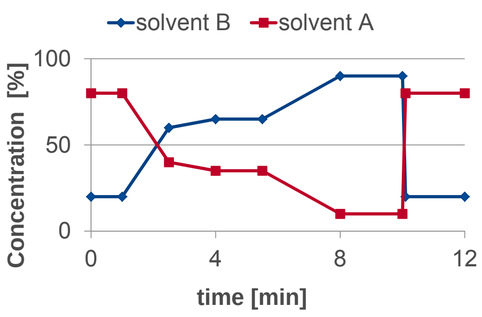


Fig. 2: Gradient

LC-ESI-MS/MS:

- Mass spectrometer:
  - QTrap® 4000 triple quadrupole linear ion trap mass-spectrometer with a TurbolonSpray interface (AB Sciex, Darmstadt, Germany)
- HPLC:
  - Shimadzu Prominence HPLC system (3 LC-20ADsp isocratic pumps, Duisburg, Germany)
  - Temperature of autosampler: 10 °C
  - Temperature of column oven: 40 °C
- Column:
  - Kinetex C18, 100 Å (100 x 2.1 mm, 2.6 µm) with equivalent guard column (Phenomenex, Aschaffenburg, Germany)
- Solvents:
  - Solvent A: water with 1 % acetonitrile, 2 mmol/L ammonium formate, 0.1% formic acid
  - Solvent B: acetontrile with 2 mmol/L ammonium formate and 0.1 % formic acid
  - Gradient elution (gradient see Figure 2)

Results

Selectivity and specifity were sufficient for all analytes. 59 of the compounds met the requirements of the GTFCh guidelines regarding linearity and accuracy and can therefore be accurately quantified with limits of quantification (LOQs) ranging from 0.1 to 2.0 ng/ml. 14 of the compounds can be analysed semiquantitatively, because accuracy was outside the acceptable range of ±20 % (but lower than ±30 %). Two of the compounds can only be analysed qualitatively because accuracy and linearity were not sufficient. All compounds included in the method are listed in Flipbook 1. The calibration ranges and the limits of detection and quantification, as well as the optimised MS parameters are listed in flipbook 2.

Flipbook 1: Synthetic cannabinoids included for validation and new compounds (not yet validated)

Flipbook 2: Calibrations ranges, limits of detection (LOD) and MRM transitions of all analytes

Conclusion

The method was validated for 75 compounds, 59 of them can be quantified precisely, 14 are determined semiquantitatively and two qualitatively. The group of compounds carrying a valineamide moiety (e.g. AB-FUBINACA; AB-CHMINACA, etc.) showed relatively high ion suppression. To compensate for matrix effects, the use of a deuterated internal standard is advised, and for some of these analytes deuterated analogues are available now.

Since the validation was completed, 35 new substances were added to the method (see flipbook 1).

The method was succesfully applied to authentic serum samples. An example of a positive serum sample is shown in figure 1.

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References:

[1] European Monitoring Centre for Drugs and Drug Addiction (2015), *New psychoactive substances in Europe. An update from the EU Early Warning System (March 2015)*, Publications Office of the European Union, Luxembourg

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