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Are NPS missed in routine forensic analysis of serum and hair?

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Aims: The number of new psychoactive substances (NPS) is increasing since 2005, whereby in 2014 101 new psychoactive substances were notified by the member States of the EU. In Luxembourg only data from laboratory results is available. The aim of the study was to assess whether NPS are missed in routine forensic analysis of serum and hair. **Methods:** Three different methods were developed using GC/MS for general unknown screening in serum and LC/ESI/MS/MS to perform targeted screening for NPS in hair and serum specimens. **Results:** A total of 465 forensic serum and hair samples were reanalysed. Only 2 positives were found in serum samples (JWH-081 and JWH-210) and no positive in hair specimens. **Conclusion:** The new developed methods have shown to be adequate to analyse NPS in serum and hair. The reanalysis of forensic specimens indicates that NPS use in Luxembourg seems to be still limited.

1. Introduction

Recently, new psychoactive substances (NPS) are enjoying growing popularity in the European Union. In 2014 101 new psychoactive substances were notified by the EU Early Warning System [1]. NPS are chemically modified derivatives of the classic drugs having similar effects. The aim of this study was the development of analytical methods for the screening and quantification of NPS in biological samples. 3 different methods were developed, a general unknown screening of serum by GC/MS and a targeted screening of NPS in serum or hair by LC/MS/MS. The new developed methods were used to reanalyse former forensic serum and hair samples.

2. Material and Methods

2.1. Chemicals and reagents

All chemicals and reagents were analytical grade. The following NPS were selected for this study: JWH-007, JWH-015, JWH-018, JWH-019, JWH-073, JWH-081, JWH-122, JWH-200, JWH-203, JWH-210, JWH-250, JWH-251, JWH-398, AM-694, AM-2201, AM-2233, RCS4, RCS8, CP47-497, WIN55-212, DMA, DMAA, PMA, PMMA, BZP, TFMPP, Mephedrone, Methedrone, Methylone, Butylone, Ethylone, 4-MTA, Cathine and Cathinone. They were purchased from LGC Standards or Lipomed.

2.2. Biological samples

Routine forensic serum and hair specimens were from a time period between 01.2012 and 06.2014. Origin of the serum samples was mostly driving under influence of drugs cases. Hair samples came from granting, maintaining or re-granting of driving license cases due to former drug abuse. Only persons up to an age to 30 years were selected for this study. Serum was

preferred to urine, as reference standards for metabolites are mostly not available and MS libraries are mostly only available for parent drugs.

2.3. Serum screening by GC/MS

To 1 ml serum was added a phosphate buffer at pH 6. Clean-up was performed by solid phase extraction using Chromabond C18ec columns (Macherey Nagel). Conditioning of the columns was done with methanol and water. After the sample was loaded, washing was done with carbonate buffer at pH 10 and elution with acetone/dichloromethane (2/1;v/v). The eluate was evaporated after adding 200 µl methanol/HCL 1% under a gentle nitrogen stream. The dry residue was acetylated and 1 µl was injected on a HP Ultra-2 GC column (Agilent technologies). The analysis was done by a 7890N GC (Agilent Technologies) coupled with a EI/MS detector (7977C Agilent technologies) in scan mode 50-650 amu. The following MS libraries were used: SWGDRUG MS Library 2.1, MPW 2011, Library for spice (Auwärter, Freiburg) (April 2014) and an in house library.

2.4. Targeted NPS screening in serum by LC/MS/MS

To 1 ml serum a phosphate buffer at pH 6 was added with methaqualone as internal standard. A liquid liquid extraction was performed using 1-chlorobutane. After centrifugation the upper phase is evaporated under a gentle nitrogen stream after adding 200 µl methanol/HCL 1%. The dry residue was reconstituted in 200 µl UPLC solution. 10 µl was injected on a BEH C18 column (Waters). The analysis was done by an Acquity UPLC (Waters) coupled with an ESI/MS/MS detector (Xevo Waters) in ESI^+ mode using MRM transitions.

2.5. Targeted NPS screening in hair by LC/MS/MS

After cleaning hair with water and acetone [2], pulverization and incubation with ethanol during 2 h in an ultrasonic bath, methaqualone as internal standard and 200 µl methanol/HCL 1% were added to the upper phase and evaporated under a gentle nitrogen stream. The dry residue was reconstituted in 200 µl UPLC solution. 10 µl was injected on a BEH C18 column (Waters). The analysis was done by an Acquity UPLC (Waters) coupled with a ESI/MS/MS detector (Xevo Waters) in ESI^+ mode using MRM transitions.

3. Results and Discussion

Regarding the GC/MS screening method LODs varied between 1 to 10 ng/mL serum.

For the targeted screening method in serum, following validation parameters were determined: mean recovery: 71 %, LLOQs: 0.002-1.860 ng/mL serum, mean intraday precision: 99.2 % and mean intraday accuracy: 5.9 %.

Regarding the targeted screening method in hair, following validation parameters were determined: mean recovery: 73 %, LLOQs: 0.02 – 6.44 pg/mg hair, mean intraday precision: 96.2 % and mean intraday accuracy: 10.7 %.

354 serum specimens were reanalysed by GC/MS and/or LC/MS/MS screening. Only 2 specimens showed to be positive (0.6 %). 2 different synthetic cannabinoids could be detected: JWH-081 at a concentration of 0.12 µg/L and JWH-200 at a concentration of 0.28 µg/L.

111 hair specimens were analysed by selected LC/MS/MS screening, however no positive hair specimen could be found.

4. Conclusions

The new developed methods for the detection of NPS in serum and hair have shown to be adequate to analyse NPS in serum and hair. The reanalysis of 465 forensic specimens indicates that NPS use in Luxembourg seems to be still limited.

However, in order to use these methods in routine work, it is mandatory to update these methods continuously by addition of new emerging NPS in the targeted screening methods and by updating the MS library.

5. References

[1] New psychoactive substances in Europe. An update from the EU Early Warning Systems EMCDDA, March 2015.

[2] Martins Ferreira L, Binz T, Yegles M. The influence of ethanol containing cosmetics on ethyl glucuronide concentration in hair. Forensic Sci Int. 2012;218:123-125.