Verification and Quantification of 30 drugs from different drug classes in urine using high-resolution accurate-mass spectrometry for forensic toxicology

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Background

After forensic toxicology screening, a verification is normally performed. A verification method should be able to properly quantify target analytes as well as unambiguously verify the identity of the compound. A number of different methods are normally required to cover the most common groups of compounds for verification of screening results. To create a streamlined workflow, a method for verification of 30 common drugs of abuse from different drug classes, e.g. Amphetamines, Benzodiazepines, Opioides and Cannabis was developed.

Methods

Urine was mixed with ammonium acetate buffer, internal standards (deuterated analogs for all compounds) and β-glucuronidase enzyme to hydrolyze conjugates. After 2h incubation at 60°C, methanol was added and the sample was centrifuged at 20000xg for 10 minutes. The supernatant was diluted and injected onto a 2.1x100 mm Thermo Scientific™ AccuCore™ Phenyl Hexyl Column. The compounds were eluted using a gradient from 2-98 % Methanol and water, both containing 0.1% formic acid. The compounds were detected on a Thermo Scientific™ Q Exactive™ Orbitrap™ mass spectrometer equipped with a Heated Electrospray Ion Source run in Parallel Reaction Monitoring (PRM) mode. The results were evaluated using Thermo Scientific™ TraceFinder™ 4.1 software. For quantification, area ratio of the response compared to the response of the internal standard was used. In each batch calibration curves from Lower Limit of Quantification (2 ng/mL for compounds with lowest cutoff) to 1000 ng/mL were used. For identification, retention time, m/z, ion ratio of two qualifier ions and matching against a spectral library was used.

Results

Quantification of all compounds could be performed with bias and CVs better than ±20% for all compounds down to LLOQ. Positive identification using 2 confirming ions and library match could be done at the cutoff level (Buprenorphine, THC-COOH, Fentanyl and 6-MAM, 5 ng/mL, Benzodiazepines and opioids, 50 ng/mL, Methadone and EDDP, 100 ng/mL, Amphetamines, 200 ng/mL).

Conclusions

The implemented method proved that the Q Exactive high-resolution accurate-mass spectrometer is suitable for both robust verification and quantitation. To use only one verification method that covers multiple drug classes simplifies the workflow for the forensic toxicology laboratory compared to using one method per compound group.