Targeted forensic screening and semi-quantitation of drugs in plasma using high-resolution accurate-mass detection and online sample preparation

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Background

The opportunity to screen a very large panel of compounds on a single injection of a low volume of sample is of high importance in forensic toxicology; improving the throughput by reducing the runtime of the method plays an important role as well. Moreover, the use of multiple parameters for identification and confirmation provides additional confidence in the outcome of the screening.

Methods

A spectral library and compound database for the screening and semi-quantitation of more than 1500 compounds in plasma and other biological matrices, were developed. For each compound, the database includes the exact mass, isotopic pattern, retention time, and exact masses of its main fragments. A Thermo Scientific™ Transcend™ II TLX-1 system was used with two different analytical approaches, one based on high-performance liquid chromatography (HPLC), and the other based on online sample extraction using Thermo Scientific™ TurboFlow™ technology prior to HPLC separation. Runtimes were 15.5 minutes for the LC-only approach and 16.75 minutes when using TurboFlow. Detection was performed using a Thermo Scientific™ Q Exactive™ Focus Orbitrap™ high-resolution, accurate-mass spectrometer with heated electrospray ionization with polarity switching. Detection was performed by FullMS in data-dependent MS2 acquisition mode with an inclusion list. Full Scan data were acquired with a resolution of 35,000 FWHM at m/z 200, and the MS2 spectra for confirmation were acquired with a resolution of 17,500 FWHM at m/z 200. Thermo Scientific™ TraceFinder™ 4.1 software was used for data processing. A panel of 41 compounds covering different compound classes, retention times, and polarities was selected to evaluate the sensitivity of the online extraction method in plasma.

Results

A database containing compound-related information was created for both methods. For the quantitation method, sensitivity was evaluated for the 41 selected compounds using the TurboFlow approach. Results proved that it is possible not only to perform a screening workflow with identification and confirmation of compounds, but also a quantification with LOD down to 0.5 ng/mL.

Conclusions

The implemented method proved that the Q Exactive Focus high-resolution accurate-mass spectrometer is suitable for both target screening with multi-parameter confirmation. Moreover, the same approach was successfully applied to the quantification of 41 compounds in plasma with a simplified sample pre-treatment.