

# **Fast and confident identification of drugs and their metabolites using ion trap LC-MSn analysis and a library of >4,500 compounds**

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## **Introduction**

Comprehensive screening of urine samples in forensic toxicology and clinical research is focused on the unambiguous identification of parent drugs and their corresponding metabolites. GC-MS is currently the gold standard technology in toxicology screening due to the availability of large and well annotated spectral libraries. Here, we present the evaluation of a comprehensive and robust forensic toxicology ion trap based LC-MSn spectral library screening to detect and confirm both parent drugs and metabolites in urine as alternative or complementary technology to GC-MS.

## **Methods**

Eleven urine samples were worked-up by acid hydrolysis, liquid-liquid extraction, acetylation, and analyzed after GC separation by full scan EI-MS according to the GC-MS standard urine screening approach (SUSA) as published by Maurer et al. For the LC-MSn analysis, the urine samples were prepared by protein precipitation according to the LC-MSn standard urine screening approach (SUSA). Acquired data (full scan MS, MS2 and MS3) were searched against the Toxtyper library (900 compounds) and the recently published Maurer/Wissenbach/Weber (MWW, Wiley-VCH, Weinheim, Germany, 2014) LC-MSn library which contains > 4500 compound entries including 3000 metabolites.

## **Preliminary Data**

A combined library search approach using Toxtyper and MWW library was evaluated. In the first round spectra were searched against the Toxtyper library resulting in highly reliable

identification of mainly the parent drugs. In the second step non-identified compound spectra were searched against the MWL library providing additional detection of metabolites and thereby increased confidence for drug identification. Most compounds could be identified with both approaches, GC-EI-MS and LC-MSn. The GC-MS approach identified 50 different drugs in the 11 urine samples, whereas the LC-MSn approach revealed 60 drug identifications.

Compounds such as primidone and THC carboxylic acid were not identified by LC-MSn due to analysis in positive ionization mode only. On the other hand several hypertension drugs, antibiotics and neuroleptics such as pipamperone could be identified solely by LC-MSn. The detection of metabolites fortifies correct LC-MSn identifications of multiple parent compounds, e.g. butylscopolamine was confirmed by the presence of 5 corresponding metabolites.

Identification of drugs with fast metabolic rate such as propranolol (4-5 hours half-life period) is only possible through detection of their metabolites. Three metabolites of propranolol could be identified via the MWL library whereas the parent compound had been already completely metabolized. The presented LC-MSn screening workflow using combined spectral library searching of both, the Toxtyper and Maurer/Wissenbach/Weber library represents a valuable tool for comprehensive and reliable identification of toxicologically relevant compounds and their metabolites in urine, blood and other body fluid samples.