A comprehensive routine LC-MS\textsuperscript{n} screening solution for clinical and forensic toxicology

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Introduction

There is high demand in clinical and forensic toxicology for specific, comprehensive, and transferable techniques that overcome the well-known limitations of current GC-MS, LC-UV/DAD, and immunoassays solutions. Liquid chromatography-tandem mass spectrometry (LC-MS\textsuperscript{2}) combined with library searching is an emerging screening solution for toxicology. We describe a robust and easy-to-use solution - the Toxtyper\textsuperscript{R} workflow (see Fig.1) - for the detection and identification of drugs and drugs of abuse in biological samples. The workflow was tested with regard to methodological requirements, the success rate of spiked samples, and validation of the respective workflow results. All results were compared to the respective workflow results. The high rate of substances correctly identified in different laboratories reflects the superior performance of this approach.

Methods

Sample preparation - LLSE

Three mixtures of toxicologically relevant substances were spiked into blank human serum at different concentrations (Table 3). Sample preparation was carried out using a liquid-liquid extraction (LLE) protocol: serum (1 mL) was spiked with 50 ng of D5-diazepam as an internal standard and then mixed with 0.1 g of sodium sulfate (pH 9) and 1.5 mL of chloroform. After a 3 min shaking step, the solution was centrifuged at 4000 \times g for 5 min. The organic phase was separated, aliquoted, and evaporated at 40\degree C with N\textsubscript{2}.

<table>
<thead>
<tr>
<th>Sample preparation</th>
<th>SPE or LLE</th>
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Data acquisition by LC-MS\textsuperscript{n} on amaZon speed

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<th>Library search</th>
<th>Automated ID result reporting</th>
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Fig.1 Toxtyper workflow

<table>
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<tr>
<th>Methods</th>
<th>Sample preparation - LLSE</th>
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Table 3: Toxtyper workflow

<table>
<thead>
<tr>
<th>Sample</th>
<th>1</th>
<th>2</th>
<th>3</th>
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For research use only. Not for use in diagnostic procedures.

URPLC conditions

The samples were re-injected with 25 \mu L of LC-eluato AS. 25 \%.

<table>
<thead>
<tr>
<th>LC system</th>
<th>Thermo Dions 900 Ultimate3000 RSLC</th>
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Eluent A

| Formic acid 0.1% | Ammonium formate 2 mM |

Eluent B

| Acetonitrile 100% | Ammonium formate 2 mM |

Analytical column

| Acclaim T3 RSLC 120 C18 2.2 \mu m, 100A, 2.1 x 100 mm |

Flow rate

500 \mu L/min

Gradient:

- 0.0 to 1.0 min: 1% B
- 1.0 to 8.0 min: 1% B to 95% B, linear
- 8.0 to 9.0 min: 95% B
- 9.0 to 9.06 min: 95% B to 1% B, linear
- 9.06 to 11 min: 1% B

Tab. 1: URPLC conditions of the Toxtyper

LC-MS\textsuperscript{n}

Seven different amaZon speed ion trap systems were used for generation of MS and MS\textsuperscript{2} spectra in continuous polarity switching mode (see Table 2). Data were acquired using a data-dependent scheduled precursor ion approach.

<table>
<thead>
<tr>
<th>MS settings</th>
<th>Scan mode</th>
<th>Scan range</th>
<th>Source</th>
<th>BCDP</th>
<th>Polarity</th>
<th>Acquisition</th>
<th>Data dependent scheduling</th>
<th>BPC</th>
</tr>
</thead>
</table>

Tab. 2: MS settings for the Toxtyper

Library search and reporting

The data sets were post-processed using DataAnalysis (DA) 4.1 and then submitted to the Ava 4.1 library (Score 70). The automatically generated reports (see Fig.2) from the different labs were evaluated and used for generation of the final result (see Fig.3).

Results

- Spectral screening library of MS, MS\textsuperscript{2}, MS\textsuperscript{3} spectra and retention times of over 830 compounds.
- Interlaboratory test of the Toxtyper screening on 7 LC-MS\textsuperscript{n} systems.
- 3 (and one blank) samples with spiked compounds in sub- and therapeutic and toxic concentrations.
- High positive identification rate of over 95%; only Trimipramine (1 system) and Metoprolol (2 systems) with 100 to 500 \mu L to solution (see Fig.4).
- Identified compounds with required MS\textsuperscript{2} library hit for MS\textsuperscript{3} are marked as tentative (see Fig.3) to minimize false positives.
- Automated sample management and result reporting by OpenAccess software.
- The workflow was checked frequently by running Quality Control samples.

Summary

The Toxtyper workflow offers a fast and robust identification for clinical and forensic analysis. The combination of MS\textsuperscript{2}/MS\textsuperscript{3} spectral information and the respective retention time meet common criteria for identification of analytes. The results of the interlaboratory test demonstrated the efficiency and transferability of the complete workflow over seven independent systems in different clinical and research laboratories. Basis for this transferability is the SmartPep technology (see Fig.2). The high rate of substances correctly identified in different laboratories reflects the superior performance of this approach.

Conclusions

- Toxtyper ensures:
  - Fast results within 11 min
  - Transferability of results from lab-to-lab
- High identification rates
- Confidence by retention time and MS\textsuperscript{2} library workflow

Fig.4 Results from the interlaboratory test. Spiked serum samples were measured on 7 Toxtyper amaZon speed LC-MS\textsuperscript{n} systems in 5 different laboratories.

Fig.2 Transferability of MS/MS5 fragmentation results from lab to lab. Shown are the MS\textsuperscript{3} spectra of Aminepymtrol measured on 7 amaZon speed systems.

Fig.3 Result reporting of interlaboratory test serum 2.