An automated MS-based screening procedure for clinical and forensic toxicology

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Introduction

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is an emerging screening technology in clinical and forensic toxicology. It is more specific than the widely-used immunoassays and provides more information than LC-UV detection, while covering a broader and in some ways a more complementary range of analytes when compared to GC-MS. Identification of substances is usually performed by retention time and using MS^2-spectra combined with library search or acquiring high resolution mass information.
We describe an automated and robust solution for the detection and identification of common drugs, drugs of abuse and metabolites in biological specimens by using the identification power of an LC-MS^n ion trap system. A fast LC-gradient for separation, the auto-MS^n capability of the amaZon speed™ ion trap for detection of analytes and a fully automated script for fast and user-friendly data analysis and reporting are used to gain results in the shortest time possible.

Toxtyper™ Workflow

Sample Preparation LLE or SPE
↓
OpenAcess™ software for sample management
↓
Data acquisition by LC-MSn on amaZon speed
↓
Library search
↓
Automated ID result reporting

Analytical Method

Sample Preparation

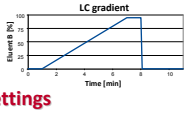
A standard alkaline liquid-liquid extraction (LLE) was used for sample preparation. It proved to be a suitable extraction method for a wide range of analytes like hypnotics, neuroleptics, antidepressants and many others.

1 mL serum
+ 50 ng D5-Diazepam (ISTD)
+ 0.5 mL borate buffer (pH 9)
+ 1.5 mL 1-chlorobutane

→ 3 min mixing and 5 min centrifugation at 4000 g
→ separate organic phase evaporate with N_2 at 40 °C
→ resolve residue in 25 µL solvent A/B (50:50) (v/v).

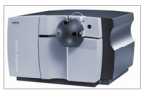
LC - Settings

LC-System: Dionex UltiMate 3000 LC-System
Column: Acclaim® RSLC 120 C18 2,2 µm 120A 2.1x100 mm (Dionex)
Eluent: A: 0.1 % HCOOH + 2 mmol/L NH_4^+ $HCOO^-$
B: acetonitrile + 2 mmol/L NH_4^+ $HCOO^-$
8 min gradient elution / 11min runtime
Total flow: 500 µL/min
Oven: 40 °C
Injection vol.: 2 µL



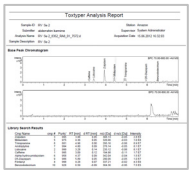
MS - Settings

Bruker amaZon speed ion trap



- Ultra Scan: 70 - 800 Da (32.500 Da/s)
- Alternating polarity
- Scheduled Precursor List (SPL) to trigger data dependent acquisition of MS^2/MS^3 spectra (830 compounds).
- Active exclusion of precursor after 1 spectrum for 0.1 min but 'reconsider' if it's intensity increases by factor five.

Data Evaluation and Reporting



DataAnalysis based automated data processing and result-reporting.
Pdf-reports can be accessed via web or automatically sent by e-mail.

Result overview of the Toxtyper report

Inter-laboratory Test

Three mixtures of toxicological relevant substances were spiked to blank human serum at different concentrations. The mixtures were compiled only in dependence on cases routinely found in forensic toxicology without considering retention time and molecular mass of the analytes. Sample preparation was performed according to the described LLE protocol. Additionally, a blank human serum sample was extracted. The extracts were aliquoted and analyzed in 5 different labs on 7 LC-MS systems equipped with the Toxtyper workflow (Bruker Daltonics). Identification and result reporting were carried out by an automated spectra library search algorithm as part of the DataAnalysis 4.1 software.

Table 1: Compounds spiked in human blank serum

Sample 1	Sample 2	Sample 3
Methadone (250)	Trimipramine (100)	Duloxetine (600)
EDDP (50)	Amitriptyline (100)	Nordoxepin (300)
Diazepam (100)	Zolpidem (500)	Mirtazapine (50)
Nordazepam (500)	Midazolam (150)	Metoprolol (200)
Oxazepam (200)	α-OH-midazolam (50)	
Temazepam (100)	Fentanyl (3)	
	Lidocaine (200)	

Given in brackets are the respective spiked concentrations in ng/mL (spiked levels: sub-therapeutic, therapeuthic, toxic)

Results

To prove the transferability of the Toxtyper workflow and to compare the overall performance of the different LC-MS ion trap systems, the automatically generated reports from the different labs were evaluated. If a substance was not identified, the respective raw data file was manually inspected to find a reasonable cause.

Sample 1: All compounds spiked in sample 1 could be identified by all participating labs.

Sample 2: The results of the automatic reports of sample 2 are summarized in the attached table. Trimipramine was not identified by 2 labs. Inspection of the the raw data revealed that in lab UK extensive coelution of matrix led to a mixed MS^2 spectrum and therefore to a score value below the cut-off for ID reporting. In lab HUG 2 trimipramine was not identified due to a software fault, but could be identified after the raw data was reprocessed using the newest DataAnalysis version.

Sample 3: Metoprolol was not identified by the systems at HUG 1 and HUG 2 due to coelution of mirtazapine which lead to a mixed MS^2 spectrum and subsequently to a score value below the cut-off for ID reporting.

Sample 4: In the blank sample as well as in other samples common false positives were identified but could mostly be excluded after manual inspection of the reports and the respective raw data file. A common 'false positive' for example is benzododecinium. This compound is used as skin disinfectant prior to blood withdrawal and is present in the sample as contamination.

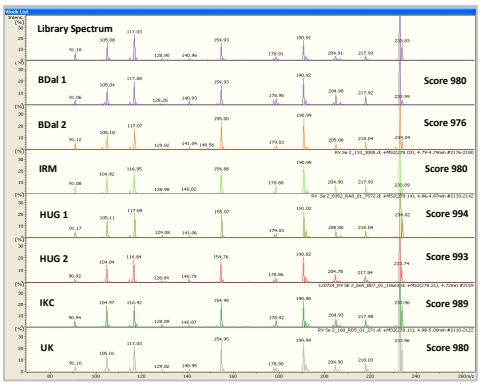


Fig. 1: MS^2 spectra of amitriptyline recorded from spiked serum samples on seven different amaZon speed ion traps

Table 2: Results from the interlaboratory test

Spiked Compounds	Participants						
Sample 2	IKC	IRM	HUG 1	HUG 2	UK	BDal 1	BDal 2
Amitriptyline	✓	✓	✓	✓	✓	✓	✓
α-OH-midazolam	✓	✓	✓	✓	✓	✓	✓
Fentanyl	✓	✓	✓	✓	✓	✓	✓
Lidocaine	✓	✓	✓	✓	✓	✓	✓
Midazolam	✓	✓	✓	✓	✓	✓	✓
Trimipramine	✓	✓	✓	(-)	(-)	✓	✓
Zolpidem	✓	✓	✓	✓	✓	✓	✓
D5-diazepam (IS)	✓	✓	✓	✓	✓	✓	✓
Ingredient of Serum							
Caffeine	✓	✓	✓	✓	✓	✓	✓
Theobromine	-	✓	-	✓	-	-	✓
Sample 3	**IKC**	**IRM**	**HUG 1**	**HUG 2**	**UK**	**BDal 1**	**BDal 2**
Duloxetine	✓	✓	✓	✓	✓	✓	✓
Metoprolol	✓	✓	(-)	(-)	✓	✓	✓
Mirtazepine	✓	✓	✓	✓	✓	✓	✓
Nordoxepin	✓	✓	✓	✓	✓	✓	✓
D5-diazepam (IS)	✓	✓	✓	✓	✓	✓	✓
Ingredient of Serum							
Caffeine	✓	✓	✓	✓	✓	✓	✓
Theobromine	-	✓	-	-	-	-	-

The Toxtyper analysis report (pdf-file) can be accessed via the web interface of Compass OpenAccess or sent automatically to the toxicologist in charge by e-mail. Nevertheless, data files can still be reviewed and processed manually using conventional DataAnalysis software tools.

The transferability and robustness of the fragmentation process of different amaZon speed ion traps can be demonstrated by comparing the fragmentation reproducibility of spiked compounds. Figure 1 shows the MS^2 spectra of amitriptyline recorded from spiked serum extracts of all participants and the respective library spectrum.
Due to the SmartFrag™ technology - an algorithm ensuring a complete fragmentation by amplitude ramping - most variation and tuning can be removed from the MS/MS process resulting in highly reproducible and transferable fragmentation patterns from lab to lab.

Conclusion

The Toxtyper workflow offers a fast and robust identification tool for clinical and forensic analysis. Combination of MS^2/MS^3 spectral information together with the respective retention time meets common criteria for identification of analytes. The presented data of this inter-laboratory test proved the efficiency and transferability of the complete workflow on 7 independent systems in different clinical or research labs. The high rate of correctly identified substances in different laboratories reveals the high performance of this approach. However, the up-coming implementation of an optimized intensity threshold, individually for each compound may help to reduce false positive findings.

The high degree of automation offered by Compass OpenAccess is ideally suited for the transfer of this solution to routine laboratories. The use of additional libraries adopted to solve specific questions such as the detection of illicit drugs, offers further screening possibilities e.g. for high-throughput screenings of certain substance classes [1].

References

[1] A comprehensive automated screening method for synthetic cannabinoids in serum using an LC-MS ion trap(P55), 91st Annual Meeting of the German Society of Legal Medicine, Freiburg, September 18th - 22nd, **2012**