LC-MS^n in clinical and forensic toxicology - An automated library-based screening approach

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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 MS²-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 and user-friendly data analysis and reporting are used to gain results in the shortest time possible.

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. Additionally, a blank human serum sample was prepared.

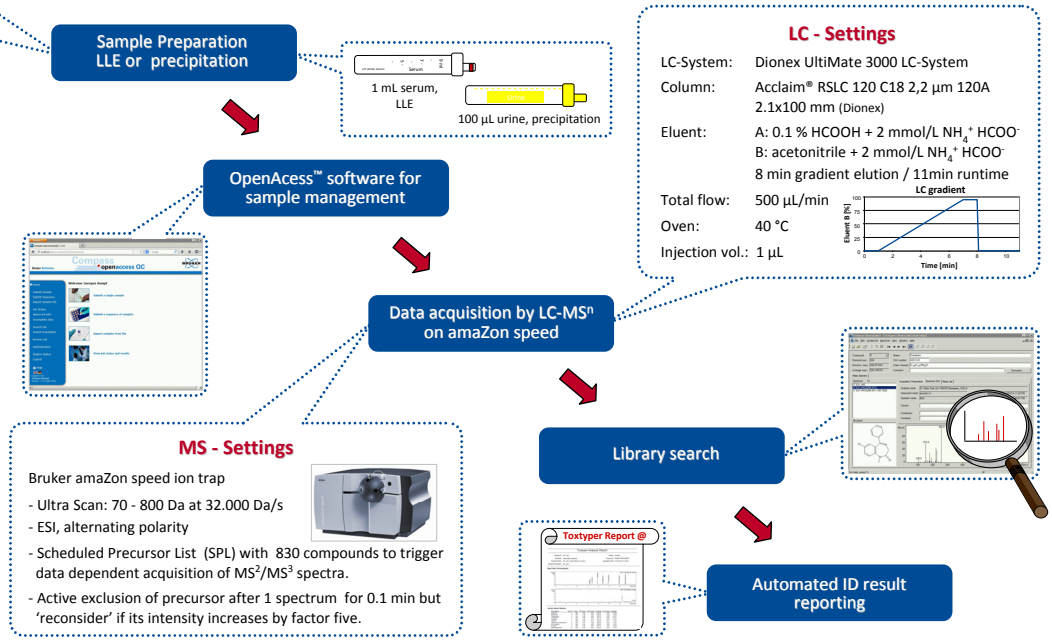
50 ng D5-Diazepam was added as internal standard. A routine alkaline liquid-liquid extraction (LLE) was used for sample preparation[1]. The extracts were aliquoted and analyzed in 5 different labs on 7 LC-MS systems equipped with the Toxtyper workflow (Bruker Daltonics). Identification and result reporting 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)

Toxtyper™ Workflow



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 MS² spectrum and therefore to a score value below the treshold for ID reporting. A potential adaption of the very conservative score value might be an option to achieve the identification of this compound.

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 MS² 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.

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)	✓	✓	✓	✓	✓	✓	✓

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 MS² 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.

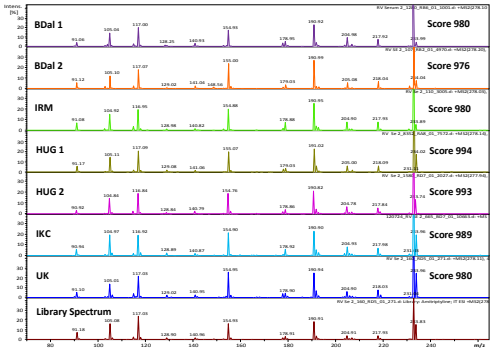


Fig. 1: MS² spectra of amitriptyline recorded from spiked serum samples on seven different amaZon speed ion traps

Conclusion

The Toxtyper workflow offers a fast and robust identification tool for clinical and forensic analysis. Combination of MS²/MS³ 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 reliable performance of this approach. However, the up-coming implementation of an optimized intensity threshold, set 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[2].

References

[1] Demme et al.: Systematic evaluation of 1-chlorobutane for LLE of drugs, 43rd International TIAFT Meeting, Seoul, Korea, **2005**

[2] Huppertz et. al.: 'Trapping Spice' - A comprehensive, automated LC-ion trap-MS screening approach for the detection of currently 38 synthetic cannabinoids in serum (WP133), 61st ASMS Conference, Minneapolis, MN, **2013**