Comprehensive clinical toxicology screening by a novel ion trap MS^n workflow

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Introduction

In the field of clinical and forensic toxicology there is a high demand for specific, and comprehensive techniques overcoming the well-known limitations of current technologies such as GC-MS, LC-UV/DAD and immunoassays (IAs). Liquid chromatography-tandem mass spectrometry (LC-MS/MS) combined with library searching is a powerful screening solution for toxicology. On the basis of the Toxtyper® workflow we describe a robust and easy-to-use solution for the detection and identification of drugs and drugs of abuse in biological specimens (see fig.1). This workflow was evaluated with regard to method- and result-transferability from lab to lab. Three spiked serum samples and one blank serum were sent to five different labs and analyzed on seven different Toxtyper LC-MS^n systems in total. A central and unique feature of the described workflow is the automated ramping of the excitation voltage during fragmentation (SmartFrag™), which enhances the reproducibility of fragmentation patterns between instruments.

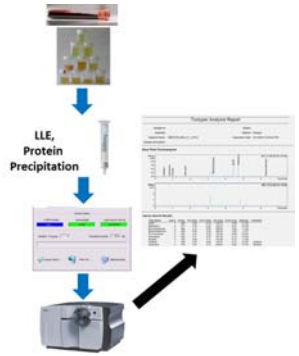

Fig. 1 Workflow of the toxicology screening by LC-MS^n

Methods

Sample preparation for serum

Mixtures of toxicologically relevant substances were spiked into three blank human serum samples at different concentrations (Tab. 1). Sample preparation was carried out using a liquid-liquid extraction (LLE) protocol: 1 mL of serum was spiked with 50 ng of D5-diazepam as an internal standard and then mixed with 0.5 mL borate buffer (pH 9) and 1.5 mL 1-chloro-butane. After a 3 min mixing step, the solution was centrifuged at 4000 × g for 5 min. The organic phase was separated, aliquoted, and evaporated at 40°C with N_2.
Two microliters of the redissolved samples (in 25 µl) were separated on an Ultimate3000 RSLC system using a 11 min LC-MS method (9 min gradient) on an Acclaim RSLC C18 column at a flow rate of 500 µl/min.

LC-MS^n

amaZon speed ion trap systems (Bruker Daltonics) were used for generation of MS and MSn spectra in alternating polarity mode. Data were acquired using a data-dependent scheduled precursor list approach.

Library search and reporting

The data sets were processed by the DataAnalysis™ (4.1) library search module. The automatically generated reports (fig. 3) from the different labs were evaluated and used for generation of the final result (fig. 4).

Sample 1	Sample 2	Sample 3
Methadone (250)	Trimipramine (100)	Duloxetine (600)
EDDP (50)	Amitryptiline (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)	

Tab. 1 Seventeen compounds were spiked in 3 human serum samples. In brackets spiked concentrations in ng/mL: no therapeutic level known, sub-therapeutic, therapeutic, toxic

Results

On the basis of a spectral MS^n library that holds currently 839 toxins, drugs and drugs of abuse we carried out an interlaboratory test with 7 ion trap LC-MS^n systems (Toxtyper) to prove the robustness, transferability and reproducibility of this screening workflow. The fragmentation results on all systems revealed a high degree of reproducibility (see fig. 2) which is the basis for transferability of library search results. Over 97% of the compounds were correctly identified. Only Trimipramine (1 system) and Metoprolol (2 systems) were not identified due to co-elution effects. Two compounds were identified as False Positives and two as *tentative*. Compounds are labeled as *tentative* if the intensity is below a user defined intensity threshold or if a required MS^3 could not be matched with the library spectrum. In the blank serum we were able to detect caffeine and the internal standard, but no False Positives. The fully automated user management and result reporting was conducted in Compass OpenAccess (result report, fig. 3). The complete list of identifications is summarized in Tab. 2.

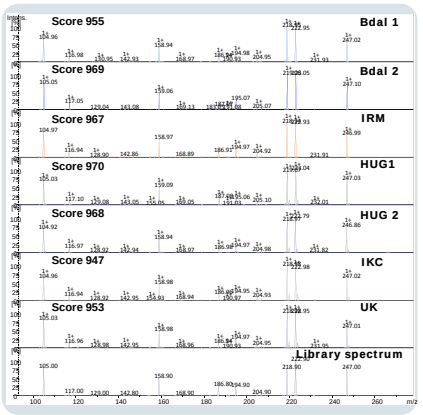

Fig. 2 Lab-to-lab transferability of MS/MS fragmentation results based on SmartFrag. Shown are the MS^3 spectra of Methadone measured on 7 amaZon speed systems.

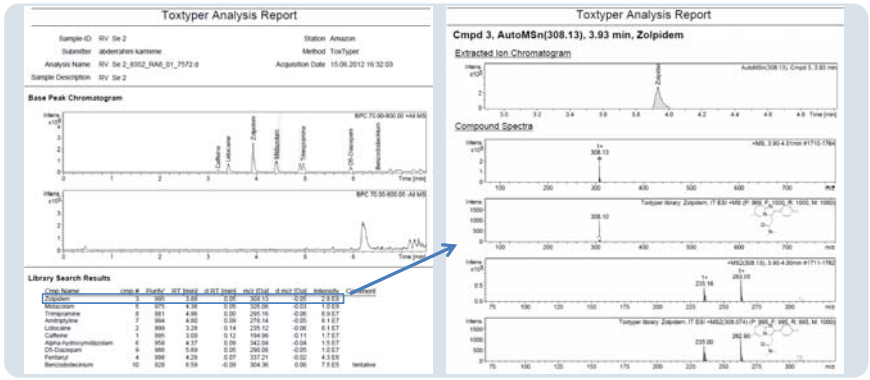

Fig. 3 Example of result reporting of test serum 2 from the interlaboratory test.

Spiked Compounds	Participants						
Sample 1	**IKC**	**IRM**	**HUG 1**	**HUG 2**	**UK**	**BDal 1**	**BDal 2**
Methadone	✔	✔	✔	✔	✔	✔	✔
EDDP	✔	✔	✔	✔	✔	✔	✔
Diazepam	✔	✔	✔	✔	✔	✔	✔
Nordazepam	✔	✔	✔	✔	✔	✔	✔
Oxazepam	✔	✔	✔	✔	✔	✔	✔
Temazepam	✔	✔	✔	✔	✔	✔	✔
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**
Duloxetin	✔	✔	✔	✔	✔	✔	✔
Metoprolol	✔	✔	-	-	✔	✔	✔
Mirtazepine	✔	✔	✔	✔	✔	✔	✔
Nordoxepine	✔	✔	✔	✔	✔	✔	✔
D5-diazepam (IS)	✔	✔	✔	✔	✔	✔	✔
Ingredient of Serum							
Caffeine	✔	✔	✔	✔	✔	✔	✔
Theobromine	-	✔	-	-	-	-	-

Tab. 2: Results from the interlaboratory test. Three spiked serum and one blank serum sample were measured in 5 different laboratories on 7 amaZon speed Toxtyper LC-MS^n systems.

Summary

An interlaboratory test with 7 LC-MS^n systems (Toxtyper) revealed a positive identification rate of over 97% for the spiked serum samples with only 2 False Positives and 2 tentative hits. Due to the automated ramping of the excitation voltage (SmartFrag™), reproducible and complete fragmentation was achieved for different compounds on all LC-MS systems.

Conclusions

- The presented workflow ensures fast and robust screening results within 11 min
- A high level of transferability of the results from lab-to-lab was achieved due to the automated ramping of the excitation voltage (SmartFrag™)
- The workflow results in reliable identification results with a very low level of false positives
- A high level of confidence is provided by the qualified MS^n library including MS^2/MS^3 spectra as well as retention time information

Toxicology