A comprehensive routine LC-MS^n screening solution for clinical and forensic toxicology

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L.M. Huppertz[1], J. Kempf[1], S. Vogt[1], B. Schneider[2], S. Götz[2], M. Meyer[2]

[1] Institute of Legal Medicine, University Medical Center Freiburg, Germany
[2] Bruker Daltonik GmbH, Bremen, Germany

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 immunoassay solutions. Liquid chromatography-tandem mass spectrometry (LC-MS) combined with library searching is an emerging screening solution for toxicology. We describe a robust and easy-to-use solution – the Toxtyper®, workflow (see fig.1)– for the detection and identification of drugs and drugs of abuse in biological specimens. The workflow was tested with regard to method- and result-transferability from lab to lab. Therefore three spiked serum samples (and 1 blank serum) were sent to five different labs and analyzed using seven different LC-MS^n systems. A central feature of the Toxtyper® solution described here is the unique, patented SmartFrag™ technology that delivers complete and reproducible fragmentation for all analytes (see fig.2).

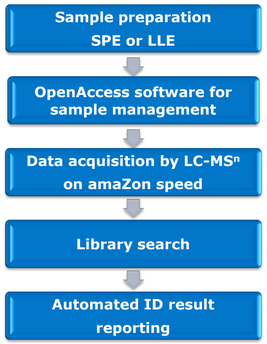

Fig.1 Toxtyper workflow

Methods

Sample preparation - LLE

Three mixtures of toxicologically relevant substances were spiked into blank human serum at different concentrations (Tab.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 0.5 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.

UHPLC conditions

The samples were redissolved with 25 µL of LC-eluent A:B, 50/50. 2 µl of these samples were separated on an Ultimate 3000 RSLC system using the settings described in Table 1.

LC settings	
LC system	Thermo Dionex Ultimate3000 RSLC
Eluent A	H_2O, 0.1% formic acid, 2 mM ammonium formiate, 1% acetonitrile
Eluent B	Acetonitrile, 0.1% formic acid, 2 mM ammonium formiate, 1% H_2O
Analytical column	Acclaim® RSLC 120 C18 2.2 µm, 120A, 2.1 x 100 mm
Flow rate	500 µl/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 UHPLC conditions of the Toxtyper.

LC-MS^n

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

MS settings	
Scan mode	UltraScan 32.500 *m/z* sec^{-1}
Scan range	70 - 800 *m/z*
Source	Electrospray ionisation (ESI)
Polarity	Zero Delay Alternating polarity
MS^n Acquisition	Data dependent using a Scheduled Precursor List with 830 compounds
	Active exclusion after 1 spectrum, „Reconsider" if intensity increase by factor 5
Target mass	300 m/z
ICC	200.000

Tab.2 MS^n conditions for the Toxtyper.

Library search and reporting

The data sets were post-processed using DataAnalysis (DA) 4.1 and then submitted to the DA 4.1 library search module. The automatically generated reports (see fig.3) from the different labs were evaluated and used for generation of the final result (see fig.4).

Interlaboratory Test Samples

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.3 Compounds spiked in human blank serum for the interlaboratory test.

Given in brackets are the respective spiked concentrations in ng/mL (spiked levels: no therapeutic level known, sub-therapeutic, therapeutic, toxic)

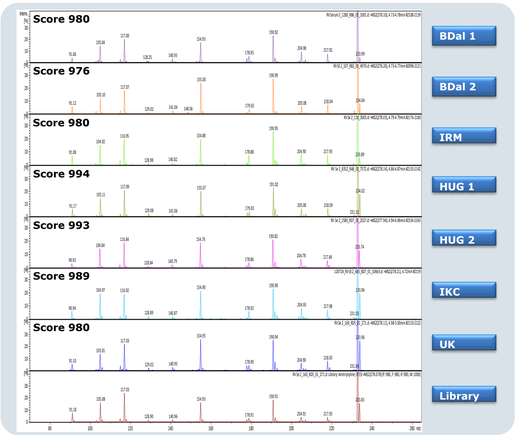

Fig.2 Transferability of MS/MS fragmentation results from lab to lab. Shown are the MS^2 spectra of Amitryptilin measured on 7 amaZon speed systems.

Results

- Spectral screening library of MS, MS^2, MS^3 spectra and retention times of over 830 compounds.
- Interlaboratory test of the Toxtyper screening on 7 LC-MS^n systems.
- 3 serum (and one blank) samples with spiked compounds in sub- and therapeutic and toxic concentration
- High positive identification rate of over 95%; only Trimipramine (1 system) and Metoprolol (2 systems) with failing ID due to coelution (see fig.4)
- Identified compounds with required MS^3 but no library hit for MS^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

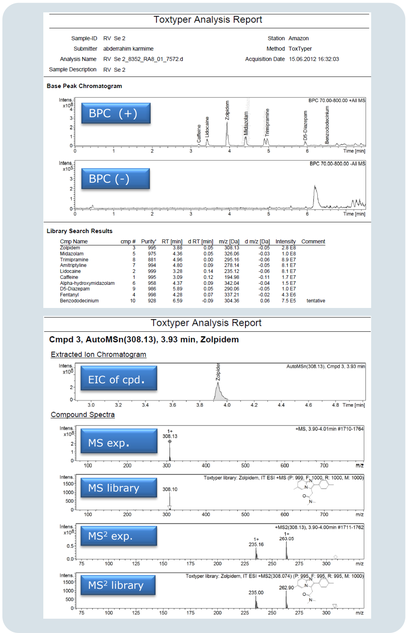

Fig.3 Result reporting of interlaboratory test serum 2.

Summary

The Toxtyper workflow offers a fast and robust identification tool for clinical and forensic analysis. The combination of MS^2/MS^3 spectral information and the respective retention time meets 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 labs. Basis for this transferability is the SmartFrag technology (see fig.2). The high rate of substances correctly identified in different laboratories reflects the superior performance of this approach.

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**
Amitryptyline	✓	✓	✓	✓	✓	✓	✓
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	-	✓	-	-	-	-	-

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

Conclusions

- Toxtyper ensures:
  - Fast results within 11 min
  - Transferability of results from lab-to-lab
  - High identification results
  - Confidence by retention time and MS^n library workflow

Ion Trap