Screening for psychotropic medical drugs in serum using ion trap MS - Customizing a screening approach to specific needs in the lab

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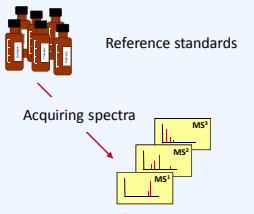
Introduction

Liquid chromatography-mass spectrometry has become a valuable tool for qualitative and quantitative analysis of biological specimen in clinical and forensic toxicology. Meanwhile, many different approaches using various types of instruments and platforms are used to develop an ultimate comprehensive screening method to detect and definitely identify as many compounds as possible in a single run. Nevertheless, in daily routine work, the question for the detection of a dedicated set of substances e.g. hypnotics in cases of DFSA, psychotherapeutics or benzodiazepines in DUID cases arise quite often. Additionally, robust methods and hardware as well as easy-to-use software solutions gain more and more importance when analyzing routine samples on an large scale. The aim of this project was to develop a spectral library of psychotropic medical drugs based on an open toxicology library concept recently developed with a comprehensive LC-MS^n screening approach (Toxtyper^{TM,} Bruker Daltonik).

Experimental

Spectra recording



New spectral library containing 105 psychotropic medical drugs

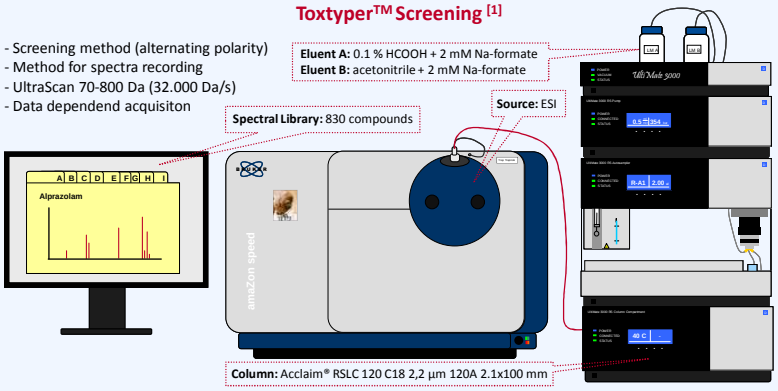
Toxtyper^{TM} Screening [1]

- Screening method (alternating polarity)
- Method for spectra recording
- UltraScan 70-800 Da (32.000 Da/s)
- Data dependend acquisiton

Eluent A: 0.1 % HCOOH + 2 mM Na-formate
Eluent B: acetonitrile + 2 mM Na-formate

Source: ESI

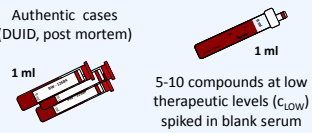
Spectral Library: 830 compounds



Column: Acclaim® RSLC 120 C18 2,2 µm 120A 2.1x100 mm

Serum samples

Authentic cases (DUID, post mortem)



5-10 compounds at low therapeutic levels (c_{LOW}) spiked in blank serum

+

IS: D4-Haloperidol, D4-Risperidone, D6-Paroxetine

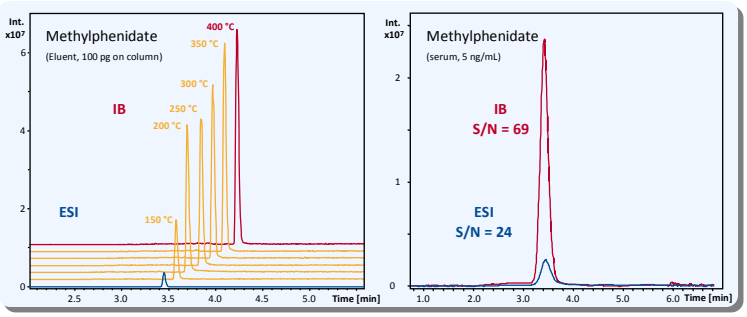
Alkaline liquid-liquid extraction

Spiked serum samples for evaluation of LODs and method comparsion

The new library was used to assemble a scheduled precursor list (SPL) of 105 compounds to trigger data dependent acquisition of spectra. For method evaluation, different drug-free human serum samples and blank serum fortified with different mixtures of psychotropic medical drugs, compiled according to the lower therapeutic concentration (c_{LOW}), were analysed. An ESI- and an ionBooster^{TM} source (IB)[2], respectively, were used for ionization. In addition, all samples were analyzed using the Toxtyper (TT) approach.

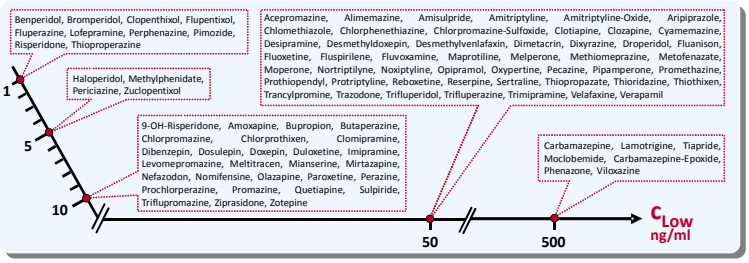
Results

Evaluating the ionBooster



All compounds tested during this evaluation show a signal increase of factor 3 to 20 when injected as eluent mixtures. Methylphenidate is an example for a compound with a significant signal increase when using IB and optimising the vaporizer gas temperature. Aside from signal intensity in spiked eluent (left), the signal-to-noise ratio in serum also increases (right), theoretically leading to lower detection limits or lower sample volume.

c_{LOW} of psychotropic medical drugs in serum



Evaluating detection limits in serum

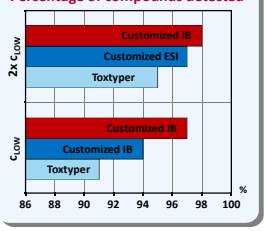
In blank serum samples and samples fortified with internal standards no compounds were identified and listed in the automatically generated reports. Two sets (c_{LOW} and 2x c_{LOW}) of 12 different mixtures containing a total of 96 compounds were analysed to evaluate the LLODs for the automatic identification in serum.

Using this approach and the ionBooster, 97 % of the analytes could be identified correctly at their respective c_{LOW}. The Toxtyper screening detected 91 % of the included analytes at their c_{LOW}.

Fluphenazine (1 ng/ml), imipramine (10 ng/ml) and fluoxetine (50 ng/ml) could only be detected with the presented screening method. Sulpiride and amitriptyline oxide could only be detected at their c_{LOW} (10 and 50 ng/ml) when using the ionBooster.

Perphenazine and thioproperazine were both not detected at c_{LOW} = 1 ng/ml. Perphenazine showed the least signal enhancement (factor 3) when using the ionBooster, while thioproperazine revealed undesirable chromatographic behaviour under the rather generic LC conditions used. The higher number of precursor masses in the SPL of the Toxtyper screening might explain the lower percentage of correct findings at low concentrations.

Percentage of compounds detected



Analysis of authentic cases

The results from our routine analysis of serum samples (QqQ) of several cases with known intake of psychotropic medical drugs were confirmed with positive findings, if the concentration range was above or around the assumed limit of detection from this evaluation study. Even in highly complex matrices like post mortem serum, analytes could be detected and identified correctly at sub-therapeutic levels along with toxic concentrations of other compounds (e.g. sample #3 and #4).

Sample # 3	Ion Trap	QqQ
Trimipramine	✓	2500 ng/ml
Promethazine	✓	13 ng/ml
Quetiapine	✓	14 ng/ml
Venlafaxine	✓	< 10 ng/ml
Desmethylvenlafaxine	✓	74 ng/ml

Sample # 4	Ion Trap	QqQ
Trimipramine	✓	>> 100 ng/ml
Chlorprothixene	✓	< 10 ng/ml

Conclusion

The open library concept of the Toxtyper enables fast and easy generation of new qualitative methods for the detection of xenobiotics in human specimens. The method generated in this project is a fast and robust tool for the detection and identification of 105 psychotropic medical drugs in serum. Evaluation in spiked human serum samples showed detection of low therapeutic levels for the majority of compounds, making the screening applicable for clinical and forensic samples (intoxication and post mortem cases). The restricted number of analytes enables a more dedicated sample preparation and potentially allows upgrading the method to a semi-quantitative approach after additional LC optimization.

The presented workflow can be transferred to other compound classes or subsets of analytes combined according to specific requirements in the lab.

References

[1] Huppertz et al.: Ein automatisiertes MSn-basiertes Screening Verfahren für die klinische und forensische Toxikologie, **Toxichem Krimtech 2013 (80): 299-303**

[2] Huppertz et al.: Evaluation of sensitivity enhancement using a high temperature ESI-sourece for various compounds of forensic interest (PE_{13}), **51st TIAFT Meeting, Funchal, Madeira, 2013**