

Screening for psychotropics using LC-MSn: Adapting a comprehensive screening approach to specific needs

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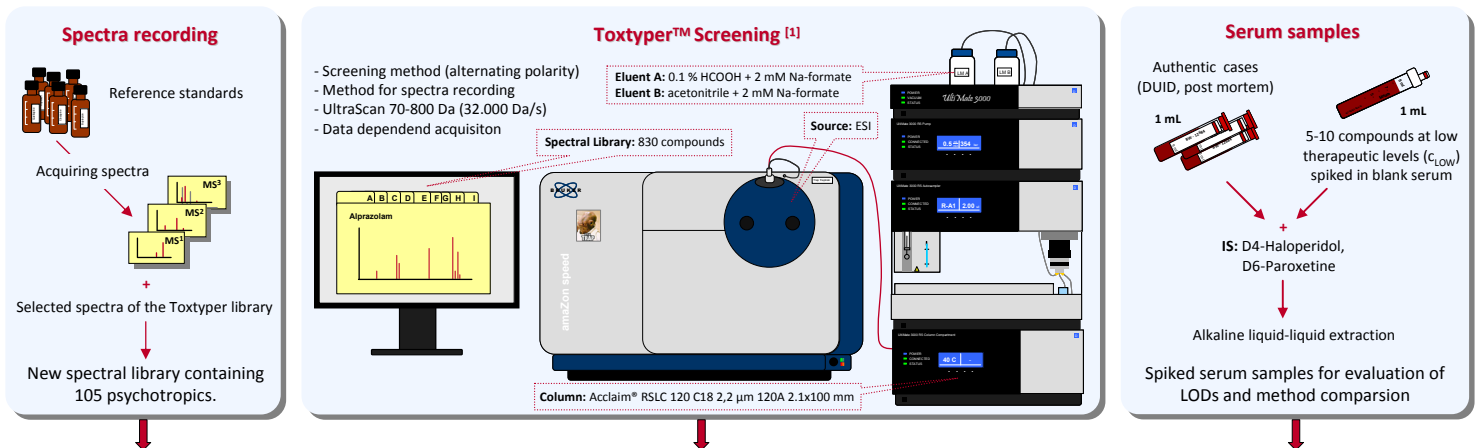
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Introduction

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, psychotropics or benzodiazepines in DUI cases arise quite often. Additionally, robust methods and hardware as well as easy-to-use software solutions gain more and importance when analysing routine samples on a large scale. The aim of this project was to develop a spectral library of psychotropic drugs based on an open toxicology library concept recently developed with a comprehensive LC-MSⁿ screening approach (ToxtyperTM, Bruker Daltonik).

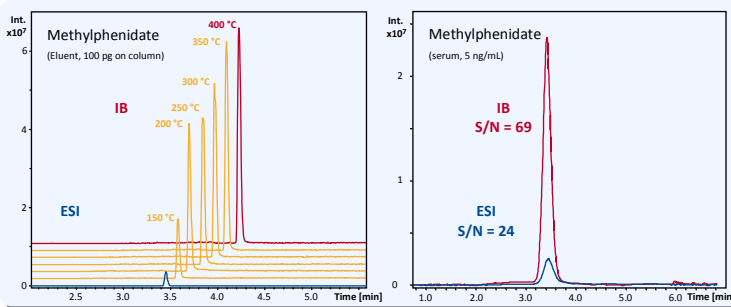
Experimental



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 psychotropics, compiled according to the lower therapeutic concentration (c_{LOW}), were analysed. An ESI- and an ionBoosterTM source (IB)^[2], respectively, were used for ionization. In addition, all samples were analyzed using the Toxtyper (TT) approach.

Results

Testing the ionBooster for psychotropics



All compounds tested during this evaluation show a signal increase of factor 3 to 20 when injected as eluent mixtures. Methyphenidate is an example for a compound with a significant signal increase when using IB and optimising the vapor 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.

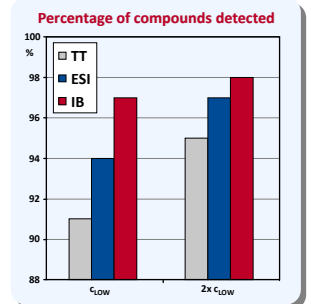
Evaluating detection limits in serum

In blank serum samples and samples fortified with two 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.

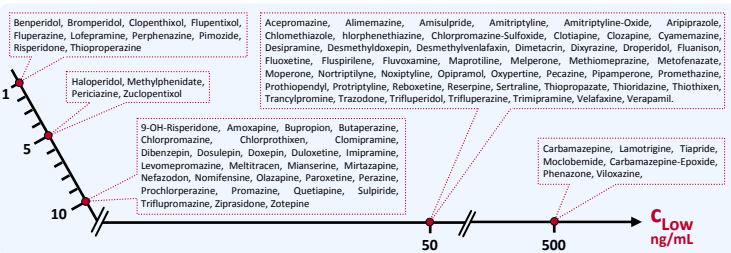
Using this screening approach and the ionBooster, 97 % of the analytes could be identified correctly at their respective c_{LOW} . The Toxtyper screening detected 91 % of the included analytes at their c_{LOW} . Fluphenazine (1 ng/mL), imipramine (10 ng/mL) and fluoxetine (50 ng/mL) could only be detected 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 thiothixene were both not detected at $c_{LOW} = 1$ ng/mL. Perphenazine showed the least signal enhancement (factor 3) when using the ionBooster, while thiothixene 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 higher number of missed compounds at low concentrations.

For further improvement of the detection and identification and potentially upgrading to a semi-quantitative approach, the LC gradient should be optimized to distribute the analytes more equally over the runtime.



c_{LOW} of psychotropics in serum



Real samples

The results from our routine analysis of serum and urine samples of several cases with known intake of medical psychotropic drugs were confirmed with positive findings, if the concentration range was above or around the assumed limit of detection from this evaluation study.

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 medical psychotropic 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

- Huppertz et al.: LC-MSⁿ in clinical and forensic toxicology. An automated library-based screening approach (PE₁₄), 51st TIAFT Meeting, Funchal, Madeira, 2013
- Huppertz et al.: Evaluation of sensitivity enhancement using a high temperature ESI-source for various compounds of forensic interest (PE₁₃), 51st TIAFT Meeting, Funchal, Madeira, 2013