"Psychotropics caught in a trap" - Adopting a screening approach to specific needs

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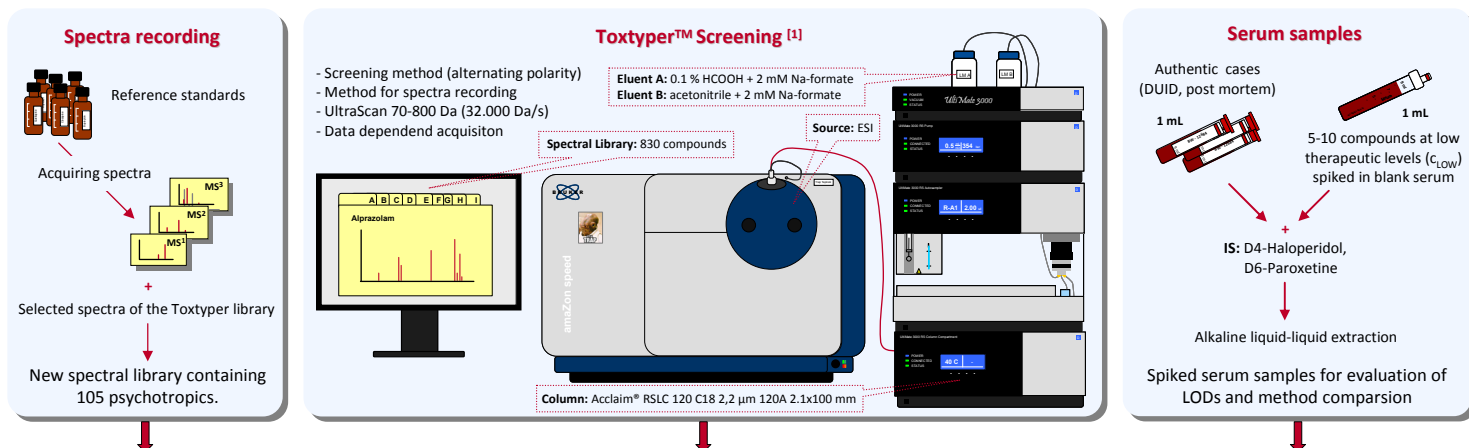
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Introduction

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 DUID 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 an 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-MS^n screening approach (Toxtyper^{TM}, Bruker Daltonik).

Experimental

Spectra recording

Reference standards

Acquiring spectra

MS^3
MS^2
MS^1

+

Selected spectra of the Toxtyper library

New spectral library containing 105 psychotropics.

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

A B C D E F G H I
Alprazolam

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

Serum samples

Authentic cases
(DUID, post mortem)

1 mL

1 mL

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

+

IS: D4-Haloperidol, D6-Paroxetine

Alkaline liquid-liquid extraction

Spiked serum samples for evaluation of LODs and method comparsion
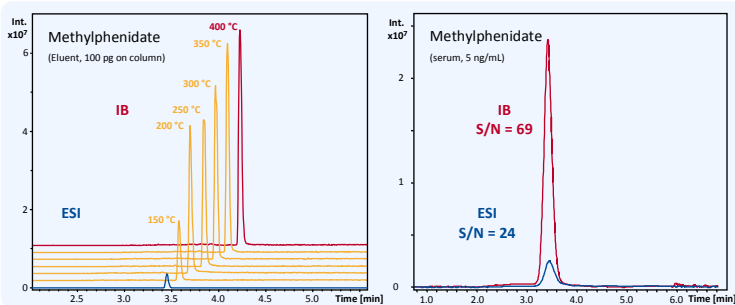
The new library was used to assemble a scheduled precursor list (SPL) of 105 compounds to trigger data dependent acquisition of spectra. For method evaluation, different drug-free human serum samples and blank serum fortified with different mixtures of psychotropics, compiled according to the lower therapeutic 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.
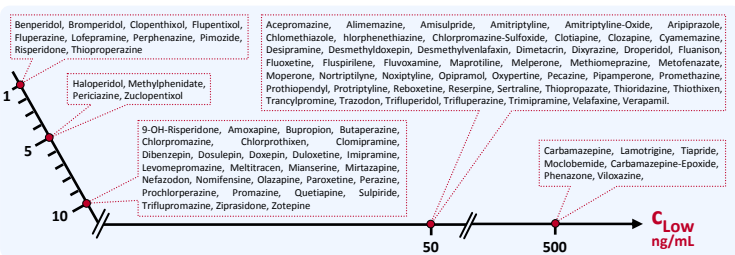
Results

Testing the ionBooster for psychotropics

Int. x10^7

Methylphenidate (Eluent, 100 pg on column)

400 °C
350 °C
300 °C
250 °C
200 °C
150 °C

IB
ESI

Int. x10^7

Methylphenidate (serum, 5 ng/mL)

IB S/N = 69

ESI S/N = 24

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 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.

c_{LOW} of psychotropics in serum

Benperidol, Bromperidol, Clopenthixol, Flupentixol, Fluperazine, Lofepramine, Perphenazine, Pimozide, Risperidone, Thioproperazine

Haloperidol, Methylphenidate, Periciazine, Zuclopentixol

9-OH-Risperidone, Amoxapine, Bupropion, Butaperazine, Chlorpromazine, Chlorprothixen, Clomipramine, Dibenzepin, Dosulepin, Doxepin, Duloxetine, Imipramine, Levomepromazine, Meltitracen, Mianserine, Mirtazapine, Nefazodon, Nomifensine, Olazapine, Paroxetine, Perazine, Prochlorperazine, Promazine, Quetiapine, Sulpiride, Triflupromazine, Ziprasidone, Zotepine

Acepromazine, Alimemazine, Amisulpride, Amitriptyline, Amitriptyline-Oxide, Aripiprazole, Chlomethiazole, hlorphenethiazine, Chlorpromazine-Sulfoxide, Clotiapine, Clozapine, Cyamemazine, Desipramine, Desmethyldoxepin, Desmethylvenlafaxin, Dimetacrin, Dixyrazine, Droperidol, Fluanison, Fluoxetine, Fluspirilene, Fluvoxamine, Maprotiline, Melperone, Methiomeprazine, Metofenazate, Moperone, Nortriptilyne, Noxiptyline, Opipramol, Oxypertine, Pecazine, Pipamperone, Promethazine, Prothiopendyl, Protriptyline, Reboxetine, Reserpine, Sertraline, Thiopropazate, Thioridazine, Thiothixen, Trancylpromine, Trazodon, Trifluperidol, Trifluperazine, Trimipramine, Velafaxine, Verapamil.

Carbamazepine, Lamotrigine, Tiapride, Moclobemide, Carbamazepine-Epoxide, Phenazone, Viloxazine.

c_{LOW} ng/mL

1
5
10
50
500

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 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 higher number of missed compounds at low concentrations.

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

Percentage of compounds detected

TT
ESI
IB

c_{LOW}
2x c_{LOW}

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

[1] Huppertz et al.: LC-MS^n in clinical and forensic toxicology An automated library-based screening approach (PE$_{14}$), **51st TIAFT Meeting, Funchal, Madeira, 2013**

[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**