Performance of LC-Ion Trap-MS Screening in Forensic Toxicology – A 7 Year Recap Using Proficiency Test Data of the Toxtyper®

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Objectives

The aims of this work are to evaluate the performance of the LC-ion trap-MS screening workflow in our lab and to identify unknown blind spots and possible improvements.

Introduction

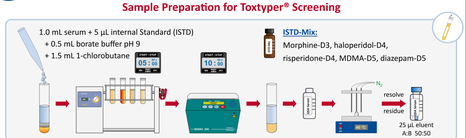
Liquid chromatography - mass spectrometry (LC-MS) is one of the most frequently used analytical techniques in forensic toxicology, either for qualitative or quantitative analysis. Comprehensive screening methods often include several hundred analytes, theoretically allowing the detection and identification of compounds of different drug classes and a wide range of physico-chemical properties.

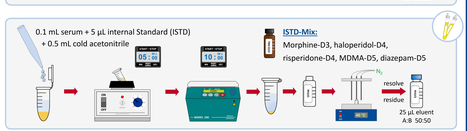
Besides the technical possibility of the instrument, the complete workflow - especially sample preparation - effects the potential detection of analytes in a given matrix. The high number of analytes makes it almost impossible to do a full validation as required for quantitative methods. So, besides the identification criteria for the respective MS method, the operator has to know the possibilities and - more important - limitations of the used screening workflow.

One piece of quality assurance is the use of certified external proficiency tests that represent possible case scenarios.

Analytical Method

Sample Preparation for Toxtyper® Screening





Toxtyper® LC-MSn Settings

LC-System: Dionex UltiMate 3000 LC-System

Eluent A:	Water, 2 mM ammonium formate, 0.1% formic acid, 1% acetonitrile
Eluent B:	Acetonitrile, 2 mM ammonium formate, 0.1% formic acid, 1% water
Gradient:	11 min gradient elution
Column:	Acclaim® RSLC 120 C18 2,2 µm 120A 2.1x100 mm

MS-System: Bruker amaZon speed™ ion trap

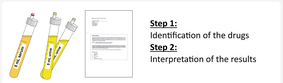
Ion source:	ESI source, alternating mode, Capillary: 4500 V, Dry Temp.: 160 °C
Scan mode:	UltraScan (70 - 800 Da at 32.500 Da/s)
MSn mode:	AutoMSn with Scheduled Precursor List of 950 compounds (DDA)

Proficiency Tests

Proficiency tests were acquired from Arvecon GmbH, Walldorf Germany, a company that organizes 27 different tests in the fields of Forensic Toxicology, Therapeutic Drug Monitoring, Blood Alcohol and Clinical Toxicology on behalf and in cooperation with the German Society of Toxicological and Forensic Chemistry (GTFCh). The scientific actuality of the tests, currently used in 34 countries, is guaranteed by the proficiency test coordinators, appointed by the GTFCh. Analytical data of two different drugs of abuse tests, both routinely carried out three times per year, were used for this evaluation. The first test just requires the identification of compounds in urine (UF), the second one (QSA) includes a short made-up case scenario and corresponding blood and urine specimens.

UF - Drugs in Urine

QSA - Qualitative Screening Analysis



Analytical Results Toxtyper Screening

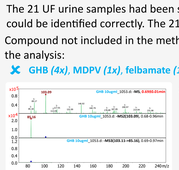
For this work proficiency test data from 2015 to 2021 was evaluated. There were three annual UF and QSA proficiency tests resulting in data of 21 serum and 42 urine samples. When simply comparing analytical findings from the Toxtyper® screening with the list of spiked compounds and categorizing the tests into **"Failed"** and "Passed" the over all results are very disillusioning, so a deeper look into results is necessary to gain useful information.

UF - Drugs in Urine

The 21 UF urine samples had been spiked with 56 different compounds leading to a total of 157 required findings, whereof 86.6 % could be identified correctly. The 21 missed identifications can be classified as followed:

Compound not included in the method at the time of the analysis:

✕ GHB *(4x)*, MDPV *(1x)*, felbamate *(1x)*



GHB:
- No retention
- Low intensity
- Unspecific MS^2 spectrum
- no MS^2 fragments > m/z 70

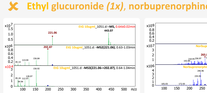
➡ Not Added to method

MDPV and felbamate:

➡ Added to method

Compounds included in the method but known not to be detectable with the used workflow:

✕ Ethyl glucuronide *(1x)*, norbuprenorphine *(6x)*



Ethyl glucuronide:
- No retention
- Low MS^1 intensity

➡ Test to add dimer

Norbuprenorphine:
- Low dose drug
- High fragmentation in MS^2

➡ Further tests needed

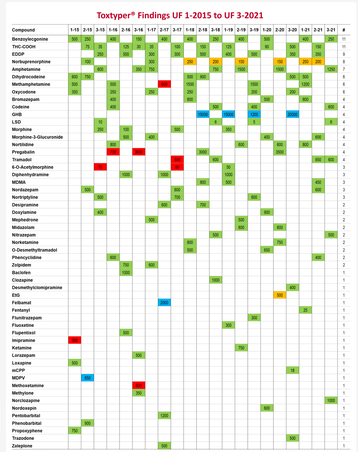
Compounds included in the method but not detectected:

✕ **Acetylmorphine *(2x)*, imipramine *(1x)*, tramadol *(1x)*, methamphetamine *(1x)*, methoxethamine *(1x)*, pregabalin *(2x)***

This leads to a false negative rate of **5.1 %**

Detailed results of the 21 UF proficiency tests are shown on the table on the right.

Toxtyper® Findings UF 1-2015 to UF 3-2021



QSA - Qualitative Screening Analysis

Twenty one serum and corresponding urine samples had been spiked with 50 different compounds to a total of 137 possible findings.

There was only a very low correct positive rate of 63.5 % when only comparing spiked compounds and analytical findings. This low number requires a more detailed evaluation of the data and missed compounds:

The majority of missed hits derives from compounds either not detectable by LC-MS screening[A] due to chemical properties or not included in the method[B]:

✕ Formic acid[A] *(2x)*, cyanide[A] *(2x)*, thiocyanate[A] *(1x)*, ethanol[A] *(10x)*, methanol[A] *(2x)*, GHB[B] *(2x)*, cathine[B] *(2x)*, demecolcine[B] *(2x)*, dehydronorketamine[B] *(2x)*, clomethiazole and metabolite[B] *(3x)*,

There are compounds included in the method but known not to be suitable for reliable identification with the used workflow:

✕ Ethyl glucuronide and ethyl sulphate *(6x)*

Compounds included in the method but not detected (false negatives):

✕ **morphine glucuronide *(3x)*, nordazepam *(2x)*, MDA *(1x)*, pregabalin *(3x)*, ritalinic acid *(1x)*, THC *(1x)*, THC-COOH *(3x)*, O-desmethyltramadol *(1x)*, tilidine *(1x)***

This still adds up to false negative rate of **15.1 %** in serum and **8.4 %** in urine. The QSA simulates a forensic case, so instead of simply checking compounds, the next level of data evaluation was carried out on the case level, with a focus on not detected compounds:

There are compounds spiked in serum and urine that could only be detected in the respective urine sample, because they are not extractable from serum with the used LLE:

morphine glue. *(3x)*, pregabalin *(1x)*, ritalinic acid *(1x)*, THC-COOH *(3x)* ➡ ✓

There are compounds spiked in serum and urine that could only be detected in the respective serum sample, probably because of concentrations issues with the used sample volume (100 µL) and extraction:

nordazepam *(2x)*, O-desmethyltramadol *(1x)*, tilidine *(1x)* ➡ ✓

Those seven compounds listed above could be successfully detected in the respective other matrix, leading to a correct interpretation of the case.

Flip chart QSA

Compound not detected in serum but its metabolite in urine:
THC *(1x)* ➡ ✓

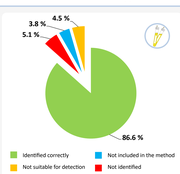
Metabolite not detected in urine but the drug in serum:
MDA *(1x)* ➡ ✓

Compound neither detected in serum nor in urine:
Pregabalin *(1x)* ➡ ✕

Pregabalin could not be detected in QSA 1-17 !

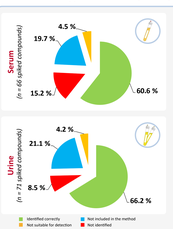
Result Summary

Toxtyper Results UF - Drugs in Urine (2015 - 2021)



- 21 samples with a total of 157 spiked analytes have been analysed
- False negative rate of **5.1 %** with no false negative of a detectable compound since 2018
- **Norbuprenorphine** and **EtG** are known issues
- GHB not detectable by this workflow because fragment ions are below the mass cut-off of the method
- Untargeted LC-MSn method available with focus on detection of metabolites (MWW library)

Toxtyper Results QSA - Qualitative Screening Analysis (2015 - 2021)



- 21 „cases" with a total of 137 spiked analytes have been analysed with a very low correct positive rate of **63.5 %** at first glance
- Thats mainly caused by compounds not detecable by LC-MSn (**12.4 %**), not included in the method (**8.0 %**), or only detected in one of the two matrices (**8.7 %**)
- EtG and EtS are known issues (**4.4 %**)

Conclusions

The Toxtyper® is one important tool for the systematic toxicological analysis in DUID and intoxication cases, especially when prescription drugs are involved.

- Toxtyper® screening led to a high correct positive rate of the UF proficiency tests. QSA proficiency tests area a bigger challenge, but - within its limitations - Toxtyper® screening and subsequent confirmation analyses would have led to correct interpretation of most of the given case examples.
- Although a wide range of analytes can be covered, there are known limitations - e.g. due to physico-chemical properties of compounds - requiring additional approaches like GC-MS and target LC-MS/MS analyses: No single method is perfect for a systematic toxicological analysis!
- Data of these proficiency tests lead to constant addition of analytes and improvement of detection criteria
- Besides analyzing fortified matrices and comparing screening results with the subsequent quantitative results, the analysis of certified proficiency tests is an effective and crucial way to assure the quality of a screening workflow

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