Drug Screen Suite:
A Simplified but Comprehensive Solution for Toxicological Routine Screening Using Liquid Chromatography - High Resolution Mass Spectrometry

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Introduction

Due to the heavy workload in forensics, robust and easy-to-use solutions with a high degree of automation in data handling are essential, especially since evaluation of screening data can be a significant bottleneck considerably delaying case work.

Liquid chromatography - high resolution mass spectrometry (LC-HRMS) is one of the most comprehensive screening techniques in forensic toxicology. Retention time, the exact mass of the compound and its high-resolution MS/MS spectrum allows for a reliable identification of drugs and metabolites.

The Drug Screen Suite enables rapid identification of substances and automatic report generation including an easy-to-use workflow to extent existing spectral libraries and implement third-party libraries.

Objectives

- Evaluation of a comprehensive and highly automated UHPLC-HR-MS/MS spectral library screening method
- Proof of concept using various matrices like urine, serum, post-mortem blood, and vitreous humor

Methods

LC-System: Bruker Elute UHPLC
Eluent A: H_2O, 0.2% buffer mix, 1% eluent B
Eluent B: Methanol, 0.2% buffer mix
Gradient: 20 min gradient elution
Column: Intensity Solo 1.8 C18-2 100 x 2.1 mm

MS-System: Bruker impact II VIP or compact
Ion source: VIP HESI source, positive mode
Scan mode: AutoMS/MS @ 12 Hz
Scan range: *m/z* 30 - 1000

Fig. 1: UHPLC-QTOF system

Following the acquisition, the data is processed automatically, and the result is provided as PDF report.

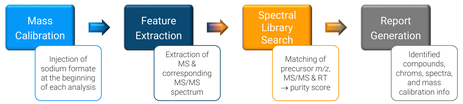

Fig. 2: Automatic data processing workflow

Different sorts of samples were used for evaluation of the screening approach. Sample preparation was carried out by liquid-liquid extraction (serum, blood), precipitation with cold acetonitrile (urine, serum) or solid-phase extraction (vitreous humor), respectively, according to the respective screening protocol.

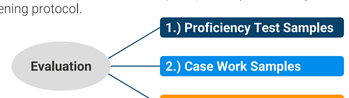

Fig. 3: Samples used for method evaluation of the Drug Screen Suite

Results

Proficiency Test Samples I

Several proficiency tests issued in 2024 by the Society of Toxicological and Forensic Chemistry (GTFCh) were analyzed, including the tests for urine screening (UF), abstinence monitoring (SFD), and general unknown analysis (QSA) as well as proficiency tests for the detection of benzodiazepines (BZF), neuroleptics (TDMA) and narcotics (BTMF) in serum.

UF 1/24

Substance	Conc. [µg/L]
Methamphetamine	1350
Nordazepam	500
Benzoylecgonine	400
Dihydrocodeine	450
Doxylamine	800
Norbuprenorphine	250
Oxycodone	250

UF 2/24

Substance	Conc. [µg/L]
MDMA	450
Nordazepam	500
THC-COOH	150
EDDP	350
Norbuprenorphine	200
GHB	30,000
O-Desmethyltramadol	400

UF 3/24

Substance	Conc. [µg/L]
Amphetamine	1100
Nitrazepam	600
Benzoylecgonine	250
Dihydrocodeine	750
Nortriptyline	800
LSD	6
Tramadol	700

SFD 1/24

Substance	Conc. [µg/L]
MDMA	200
Nordazepam	95
THC-COOH	40
Codeine	200
Norfentanyl	25
Ethylglucuronide	175
Norbuprenorphine	15

SFD 2/24

Substance	Conc. [µg/L]
Amphetamine	500
Benzoylecgonine	150
Dihydrocodeine	300
Nortilidine	125
Oxycodone	75
EDDP	200
O-Desmethyltramadol	250

SFD 3/24

Substance	Conc. [µg/L]
Methamphetamine	350
THC-COOH	85
Benzoylecgonine	350
Ethylglucuronide	300
Norbuprenorphine	75
Norfentanyl	125
7-Aminoflunitrazepam	100

QSA 1/24

Substance	Conc. [µg/L]
Fentanyl	40
Norfentanyl	25
Methadon	250
EDDP	200

QSA 2/24

Substance	Conc.
GHB	550 mg/L
Ethanol	1.5 g/L

QSA3/24

Substance	Conc. [µg/L]
Ketamine	7,500
Norketamine	1,500
MDMA	500
MDA	50
2C-B	200

Compounds in orange are not included in the used libraries, are either ESI negative compounds (e.g. Etg) or not suitable for ESI at all (e.g. EtOH). Therefore, QSA 2/24 was used to demonstrate that there are no false positive hits (Fig. 5).

Drugs included in the library could be identified with the Drug Screen Suite using autoMS/MS except for norbuprenorphine.

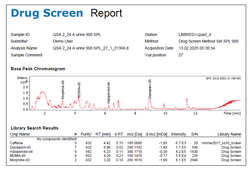

Fig. 5: Analysis Report of QSA 2/24

TDMA 1/24

Substance	Conc. [µg/L]
Clozapine	180
Desmethylclozapine	170
Olanzapine	50
Norolanzapine	30
Quetiapine	60
Norquetiapine	60
Amisulpride	125
Thioridazine	95
Chlorprothixene	95

For serum samples, both precipitation with 500 µL of ice-cold acetonitrile and alkaline liquid-liquid extraction with chlorobutane were used for sample preparation. The results demonstrate that the approach described is also suitable for serum samples and can be used to detect a wide range of medications and drugs.

Limiting factors here are mainly due to the rather simple sample preparation (e.g. not chlorobutane-compatible like THC-COOH).

Proficiency Test Samples II

BZF 1/24

Substance	Conc. [µg/L]
Clobazam	295
Norclobazam	1,2000
Flurazepam	45
Disalkylflurazepam	65
Triazolam	27
α-OH-Triazolam	22
Chlordiazepoxide	1,150
Demoxepam	925
3-OH-Bromazepam	95
7-Aminoclonazepam	28
7-Aminonitrazepam	120
α-OH-Alprazolam	32
Estazolam	260
Medazepam	235
Prazepam	470

BTMF 3/24 A

Substance	Conc. [µg/L]
THC	6
11-OH-THC	2.5
THC-COOH	120
Cocaine	30
Benzoylecgonine	185
Ecgoninemethylester	20
Cocaethylene	17
Morphine	40
6-Monoacetylmorphine	17
Codeine	85
Dihydrocodeine	200
Amphetamine	80
MDMA	55
MDA	25
MDEA	65
Methamphetamine	125

BTMF 3/24 B

Substance	Conc. [µg/L]
THC	10
11-OH-THC	5
THC-COOH	75
Cocaine	50
Benzoylecgonine	250
Ecgoninemethylester	37.5
Cocaethylene	37.5
Morphine	50
6-Monoacetylmorphine	25
Codeine	75
Dihydrocodeine	100
Amphetamine	75
MDMA	75
MDA	50
MDEA	75
Methamphetamine	75

Case Work Samples

To investigate performance under high matrix load, several extracts from post-mortem casework were reanalyzed. The tables below show, for example, the findings of a driver killed in a traffic accident. Routine screening analyses were performed by immunochemical assays and the Toxtyper® with subsequent quantification of relevant compounds using LC-MS/MS analysis.

Femoral Blood (A)

Substance	Routine Analyis	Drug Screen Suite
Amlodipine	60 µg/L	✓
Fentanyl	< 1.0 µg/L	✓
Ketamin	220 µg/L	✓
Metoprolol	230 µg/L	✓
Midazolam	16 µg/L	✓
Paroxetine	200 µg/L	✓
Ramipril	1,1 µg/L	✓
Ephedrine	350 µg/L	✓
Norephedrine	21 µg/L	✓
Tilidine	110 µg/L	✓
Nortilidine	160 µg/L	✓
Naloxone	detected	✓
4-Acetylaminoantipyrine	detected	✓
4-Formylaminoantipyrene	detected	✓
4-Methylaminoantipyrine	detected	✓
Aminoantipyrine	detected	✓
Ethyl glucuronide	detected	×
Ethyl sulfate	detected	×
Ondansetrone	detected	✓
Torasemide	detected	✓

Vitreous Humor (A)

Substance	Drug Screen Suite
4-Acetylaminoantipyrine	✓
4-Formylaminoantipyrene	✓
4-Methylaminoantipyrine	✓
Aminoantipyrine	✓
Amlodipine	✓
Clopidogrel	✓
Ephedrine	✓
Ketamine	✓
Metoprolol	✓
Midazolam	✓
Naloxone	✓
Nortilidine	✓
Ondansetrone	✓
Paroxetine	✓
Tilidine	✓

Urine (A)

Substance	Drug Screen Suite
4-Acetylaminoantipyrine	✓
4-Formylaminoantipyrene	✓
4-Methylaminoantipyrine	✓
Aminoantipyrine	✓
Amlodipine	✓
Clopidogrel	✓
Metoprolol	✓
Naloxone glucuronide	✓
Nortilidine	✓
Paroxetine	✓
Ramipril	✓
Tilidine	✓
Torasemide	✓

Apart from EtG and EtS, substances detected in the femoral blood, vitreous humor, and urine during toxicological analysis were successfully identified using the Drug Screen Suite.

The analysis of a post-mortem case without toxicological findings did not lead to any findings. Despite the high matrix load of post-mortem samples, no false positives were identified in the cardiac blood after precipitation or liquid-liquid extraction, in urine after precipitation, or in vitreous humor after two-step solid-phase extraction.

Spiked Urine Samples

To determine limits of identification (LOI), 100 µL of blank urine was spiked with 120 drugs and drugs of abuse most commonly detected in routine case at concentrations of 50, 10, 5.0, and 1.0 ng/mL.

Approximately 80% of the substances could be identified at concentrations of 10 ng/mL or lower. (Fig. 4).

Although some substances could still be detected at lower concentrations (e.g. fentanyl, see Fig. 5), review of the raw data also showed that, especially at low concentrations, low m/z interference signals may prevent successful library matching.

One possible workaround strategy here could be the substance-specific use of fit or purity value as identification criterion.

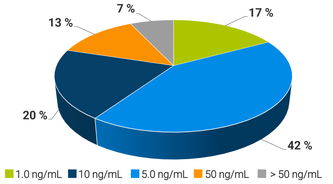

Fig. 4: Determination of Limits of Identification (LOI) in urine

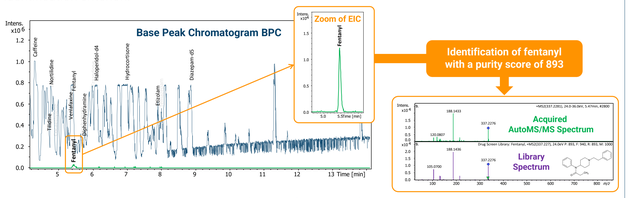

Fig. 5: Identification of 0.5 ng/mL fentanyl in urine

Conclusion

- Ready for use solution for rapid detection and identification of compounds in clinical or forensic toxicology
- The sensitivity allows for the identification of a wide range of active substances in forensic proficiency test samples
- Even in samples with high matrix load, the use of H MS (±5 mDa) leads to a very low number of false positives
- Open library concept helps to tackle the need of ongoing and timely method updates
- Automated data processing and reporting allows to reduce the turnaround time and facilitates the implementation of HRMS into the routine workflow
- Additional use of the instrument:
  - Identification of unknowns based on accurate mass, isotopic pattern and MS/MS fragmentation
  - Possibility of full quantitation or semi-quantification

Drug Screen Suite