# Forensic drug screening in urine using liquidchromatography-time-of-flight mass spectrometry: A qualitative/quantitative approach



**Forensic Toxicology** 

**Institute of Forensic Medicine** 

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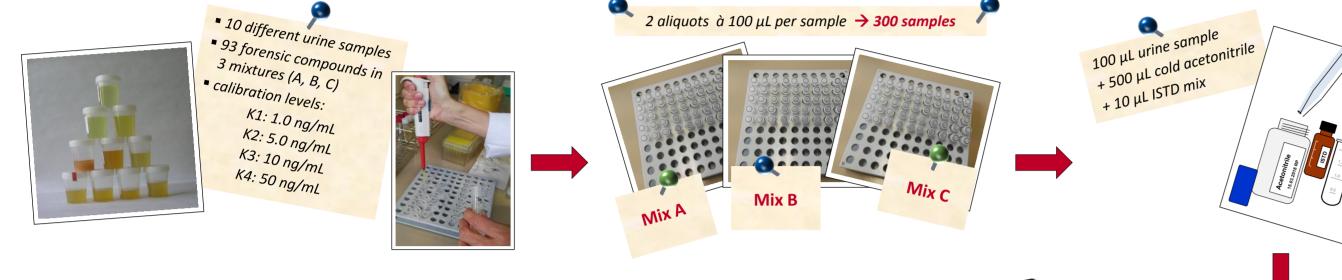
### Introduction

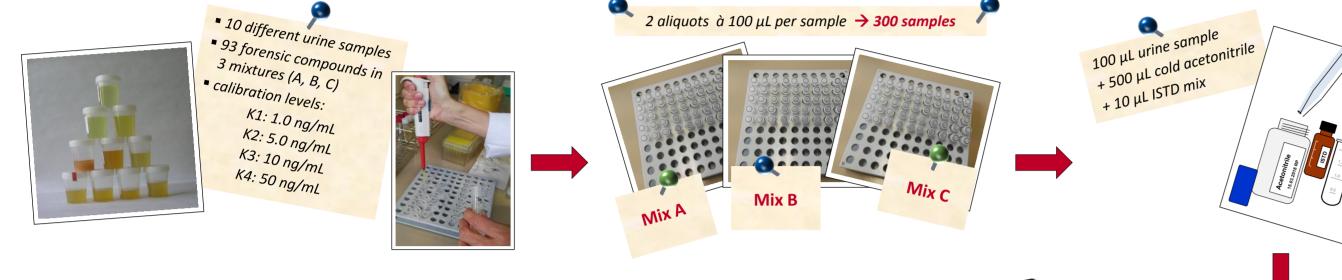
Full scan based screening methods using LC-QTOF-MS are a valuable tool for forensic analysis due to the possibility of qualitative/quantitative and retrospective data evaluation in a single run. In this study a previously developed LC-QTOF-MS screening workflow was validated for qualitative and drugs of abuse in human urine. To assess the methods' limitations regarding its applicability to urine screening in post-mortem toxicology, workplace drug testing, drug facilitated crime (DFC), and intoxication cases as well as to prove that cut-off values for sobriety and fitness-to-drive testing are met, a basic validation including limits of detection, limits of quantitation, linearity, accuracy, selectivity, and precision was carried out.

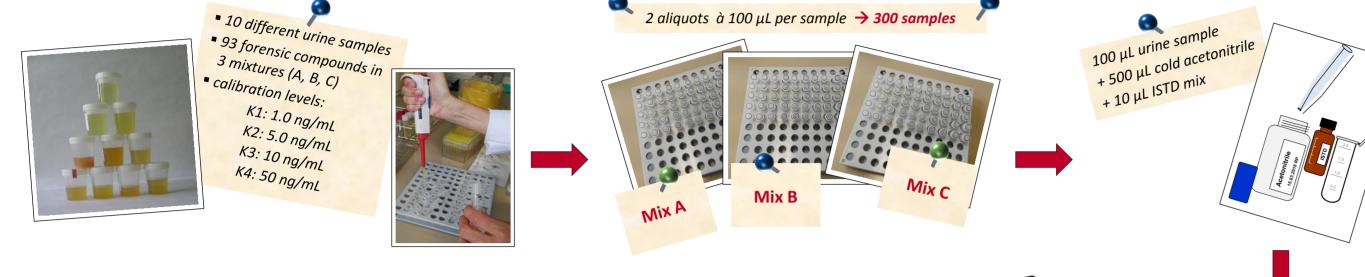
## **Methods**

#### **Sample Preparation**

Ninety three substances of forensic relevance were spiked into ten different urine samples at the concentrations 1.0, 5.0, 10, and 50 ng/mL.







### **Compounds of Interest**

For this evaluation 93 of the most common drugs, drugs of abuse, and their metabolites detected in routine case work at our institute were chosen.

6-Monoacetylmorphine (MAM)	Clomipramine	MDEA	Quetiapine	
7-Aminoclonazepam	Codeine	Midazolam	Remifentanyl	
7-Aminoflunitrazepam	Desmethyldoxepin	Mirtazapine	Risperidone	
alpha-Hydroxymidazolam	Doxepin	Norclomipramine	Triazolam	
Amitriptyline	Doxylamine	Nordazepam	Zolpidem	MIX A
Amphetamine	Ecgoninemethylester	O-Desmethylvenlafaxine		W
Atomoxetine	Flubromazepam	Opipramol		
Bromazepam	Gabapentin	Paracetamol		
Citalopram	Ketamine	Pregabaline		
Carbamazepine	Levetiracetam	Nortilidine	Tilidine	
Clonazepam	MDA	Nortriptyline	Trazodone	
Cocaethylene	MDMA	Oxazepam	Trimipramine	
Desipramine	Melperone	Oxycodone	Venlafaxine	MIX B
Diphenhydramine	Methadone	Piritramide	Etizolam	
Fentanyl	Methylphenidate	Promazine		
Flunitrazepam	Morphine	Ritalinic acid		
Haloperidol	Norfentanyl	Temazepam		
9-Hydroxyrisperidone	Dihydrocodeine	Norcitalopram	Sertraline	
Alprazolam	EDDP	Noroxycodone	Sufentanil	
Amisulpride	Fluoxetine	Nortrimipramine	THC-COOH	
Benzoylecgonine	Lamotrigine	(Imipramine)	Tramadol	
Buprenorphine	Lorazepam	O-Desmethyltramadol	Zopiclone	l v
Carbamazepine-epoxide	mCPP	Olanzapine		XIW
Clozapine	Methamphetamine	Paroxetine		
Cocaine	Metoclopramide	Pethidine		
Diazepam	Norbuprenorphine	Promethazine		

Samples were precipitated with cold acetonitrile addition of seven isotope labeled after compounds as internal standards (ISTD). The dried residues were reconstituted and analyzed in duplicate on two LC-QTOF-MS systems in two different labs (Freiburg and Bremen).

## 0.5 mL o Reconstitute dry residue in 25 µL LC eluent nject 2 µL to LC-QTOF-MS

#### **Analytical Methods**

HPLC:

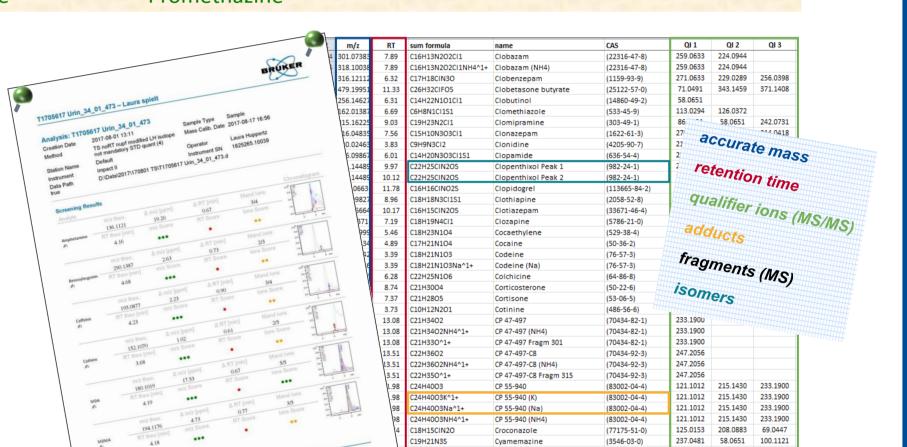
Separation was performed on a Bruker Intensity Solo C18 column using a 14 min gradient elution. The MS (Bruker impact II) was operated in positive electrospray ionization mode generating a full scan and broad band CID spectra (bbCID) using collision energy spread.

Column: Bruker Intensity Solo 1.8 C18-2, 2.1\*100 mm with pre-column Mobile phase A:  $H_2O/MeOH 99/1$ , 5 mM  $NH_4$  formate / 0.01% HCOOH Mobile phase B: MeOH, 5 mM NH<sub>4</sub> formate / 0.01% HCOOH multistep gradient 5 - 99.9% in 15 min (20 min cycle) Gradient: flow gradient 0.2 - 0.48 mL/min, Flow rate: Injection vol.: 2 μL 40°C Column temp.: Bruker impact II QTOF mass spectrometer MS: lonization: ESI(+), 2500 V



#### **Data Analysis**

Data evaluation was performed with TASQ 1.4 software using the TargetScreener HR 3.0 accurate mass database containing mass spectrometric and chromatographic information of 2184 drugs, drugs of abuse, new psychoactive substances (NPS), metabolites, and pesticides.



C19H21N3S C18H22N2 C13H11^1+ C20H21N

Cyamemazine

Cyclizine Cyclizine Fragm 167 Cyclobenzaprine

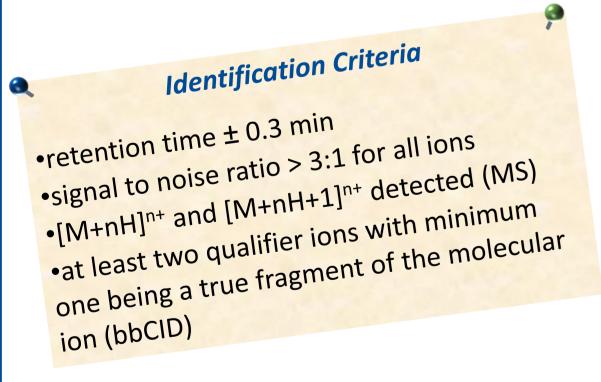
Full scan rapidly alternating TOF MS (4 eV) with bbCID (30 eV +/- 6 V) @ 2Hz

### Results

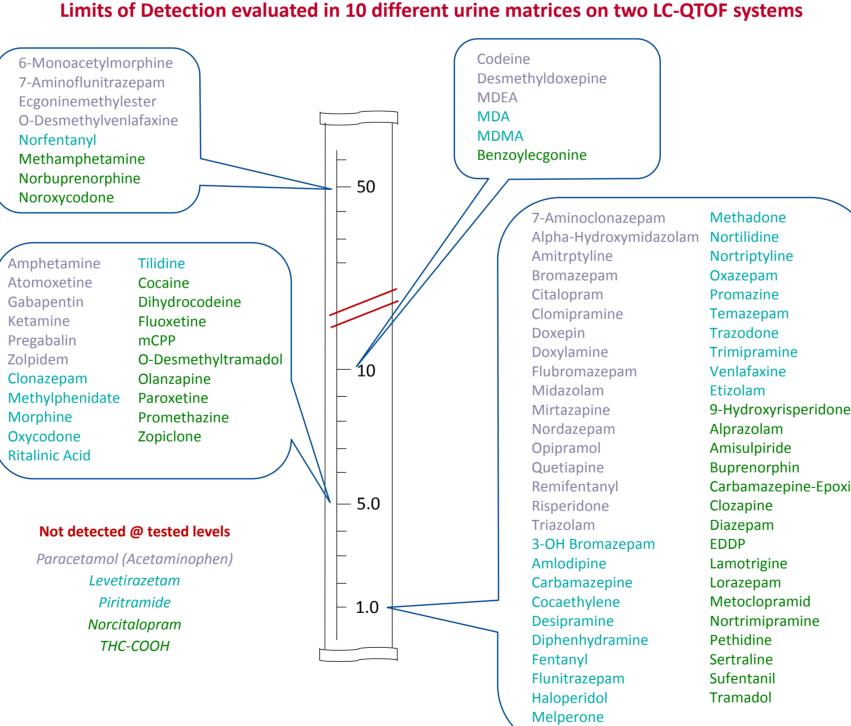
#### Limits of Detection (LOD) and Selectivity

LOD was set to the concentration at which a substance was detected in 95% of all measurements (n = 40, due to duplicate determination) according to the identification criteria on the right.

Identification at the lowest concentration (1.0 ng/mL) was achieved for 60% of the tested compounds.



five compounds Only could not be detected in samples at the all investigated concentrations, probably due to matrix effects and/or ionization low yields.



	Most substances with legal cut- offs according to German regulations for abstinence screening in fitness-to-drive assessment (CTU3 criteria), were detected well below the required cut-off concentrations.	Comp Amphetami Amphetami MDA MDEA MDMA Methamphe Cocaine: Benzoylecge Cocaine Opiates: Codeine Dihydrocod Morphine
e kide	Typical 'date rape drugs' showed LODs sufficient for detection of a recent uptake of these drugs.	Methadone EDDP Methadone Benzodiaze Diazepam Nordazepam Alprazolam Hydroxyalp
	Designer benzodiazepines and fentanyl derivatives were detected with high sensitivity.	Bromazepan Flunitrazepa 7-Aminoflur Lorazepam Cannabinoid

#### Minimum LOQ requirements according to German

(3546-03-0)

(82-92-8) (82-92-8) (303-53-7) (512-15-2)

237.0481 58.0651 100.1121

 257,0451
 55,0521

 167,0855
 152,0621

 167,0855
 152,0621

 216,0934
 231,1168
 84,0808

 72,0808
 129,0699
 157,1012

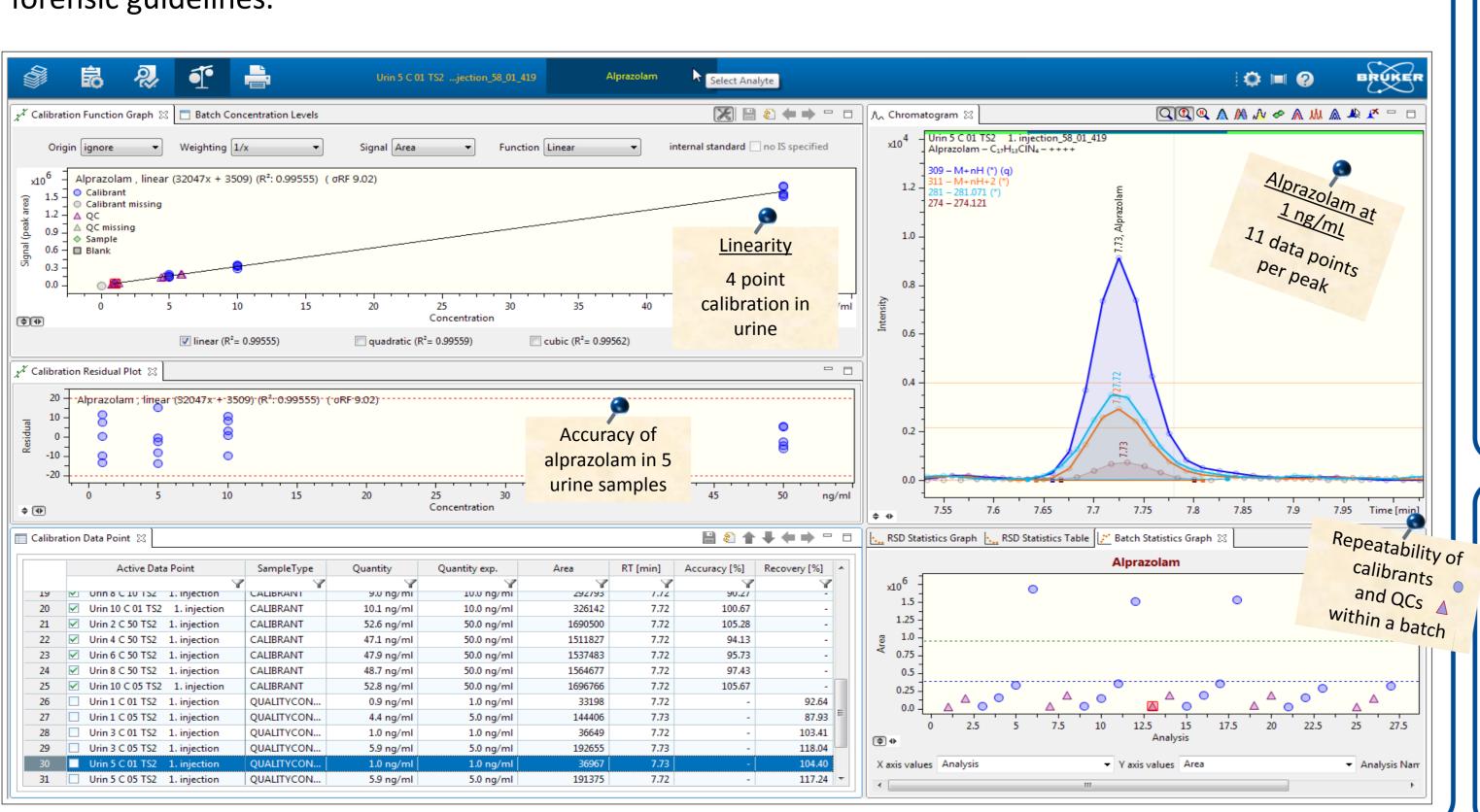
CTU3 Criteria							
Compounds	CTU3 LOQ	LC-QTOF LOQ					
Amphetamines:							
Amphetamine	50 ng/mL	5.0 ng/mL					
MDA	50 ng/mL	10 ng/mL					
MDEA	50 ng/mL	10 ng/mL					
MDMA	50 ng/mL	10 ng/mL					
Methamphetamine	50 ng/mL	50 ng/mL					
Cocaine:	-	-					
Benzoylecgonine	30 ng/mL	10 ng/mL					
Cocaine	30 ng/mL	5.0 ng/mL					
Opiates:							
Codeine	25 ng/mL	10 ng/mL					
Dihydrocodeine	25 ng/mL	5.0 ng/mL					
Morphine	25 ng/mL	5.0 ng/mL					
Methadone:							
EDDP	50 ng/mL	1.0 ng/mL					
Methadone	50 ng/mL	1.0 ng/mL					
Benzodiazepines:							
Diazepam	50 ng/mL	1.0 ng/mL					
Nordazepam	50 ng/mL	1.0 ng/mL					
Oxazepam	50 ng/mL	1.0 ng/mL					
Alprazolam	50 ng/mL	1.0 ng/mL					
Hydroxyalprazolam	50 ng/mL	not investigated					
Bromazepam	50 ng/mL	1.0 ng/mL					
Flunitrazepam	50 ng/mL	1.0 ng/mL					
7-Aminoflunitrazepan	-	50 ng/mL					
Lorazepam	50 ng/mL	1.0 ng/mL					
Cannabinoids:							
ТНС-СООН	10 ng/mL	> 50 ng/mL					
Alcohol:							
Ethylglucuronide	100 ng/mL	not investigated					

#### **Quantitative Results**

The linear dynamic ranges were four magnitudes or greater. LOQ was set to the lowest LOD. The precision ranged from 8 % to 30 %. The method showed good selectivity/specificity fulfilling the requirements, and overall accuracy met the criteria for bioanalytical method validation according to forensic guidelines.

### Conclusion

The analytical possibilities and limitations of an LC-QTOF approach for screening urine samples were evaluated for 93 forensically relevant compounds with high prevalence in our everyday case work.



For a screening method, selectivity and LODs are the most important analytical parameters. Evaluated LODs were comparable with those of standard triple quadrupole (QqQ) methods for the majority of compounds investigated. Although, high end QqQ may reach lower LODs, considering the high number of analytes due to full scan analysis, LC-QTOF is a valuable tool for toxicological analysis and the presented LODs are sufficient for most analytical problems in everyday case work.

Given the high frequency of new psychoactive substances emerging on web-based drug markets and related fatalities, this is of particular interest to the forensic field due to the possibility of retrospective data evaluation. Extrapolating the here presented urine analysis results, application to blood and hair samples seems promising and will be evaluated in a subsequent study.

## Acknowledgements

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### Contact

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