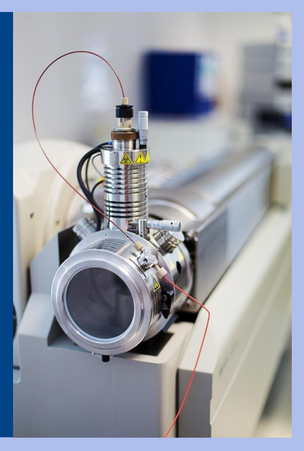
Validation of a New LC-MS/MS Assay for the Analysis of Drugs in Urine and Comparison with Established Analytical Methods (GC-MS and LC-MS/MS): Advantages for the Daily Laboratory Routine



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1 Background

Drugs of abuse and their preparations are psychotropic substances, that can cause consciousness and perception-altering effects in the central nervous system. In particular, habitual high-dose consumption of such drugs can damage the body, cause sequelae and lower life expectancy [1]. Several drugs can induce psychological or neurochemical dependence diseases under particular conditions. Depending on the neurochemical mode of action and the duration of administration, discontinuation or cancellation of certain drugs can lead to a mental or physical withdrawal syndrome [2]. Those affected are vulnerable to lose their social connections. Furthermore, dependency can potentially lead to procuring crime [3]. Drugs of abuse are classified into substance groups with similar chemical structures or the same mechanism of action. As part of an addiction therapy, of criminal proceedings or in context of occupational medicine, it is important to identify a drug abuse or to monitor the use of a substitution drug. Depending on the medical background, the search can be focused on a single active ingredient or on several groups of drugs.

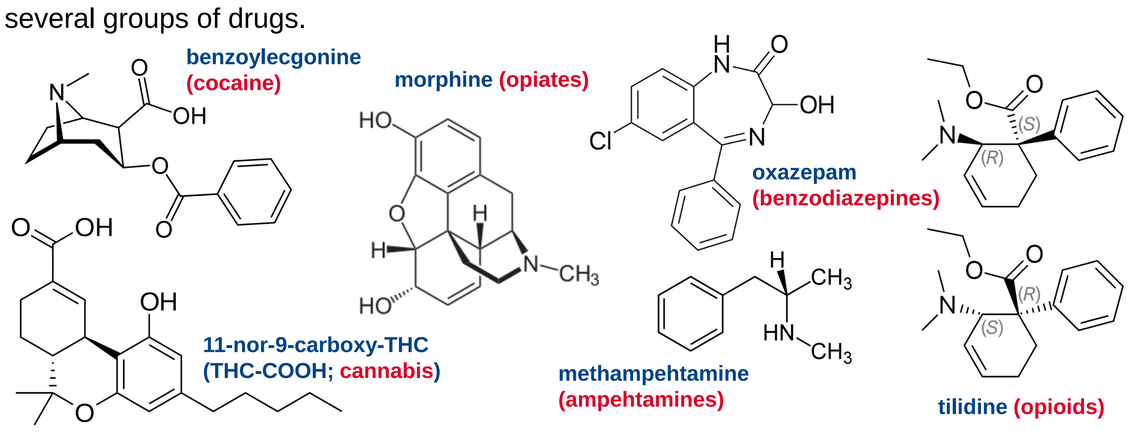

Fig. 1: Each substance group included in this study is represented by an example.

The first step of an urine drug test is usually a screening for the presence of a drug or its metabolites by an immunoassay. Subsequently, the identification and quantification of a specific substance is performed by a chromatographic and / or a mass spectrometric method [3]. For this purpose, a new LC-MS/MS assay (Chromsystems GmbH, Gräfelfing, Germany) was validated for the determination of 38 different drugs, which belong to frequently requested substance groups: Amphetamines, benzodiazepines, cannabis, cocaine, opiates and opioids (Fig.1). Overall, the kit allows detection of over 100 compounds.

2 Methods

The reagents, analytical column and mobile phases of the LC-MS/MS kit were provided by Chromsystems GmbH. Sample preparation was performed according to manufacturer`s instructions: 50 µl urine was mixed with an internal standard solution. An enzymatic hydrolysis was carried out for 2 hours and a precipitation reagent was added. After centrifugation, 100 µl supernatant was diluted with 150 µl dilution buffer and injected into the chromatographic system. The total run time of the analysis was 15 min using a Sciex QTRAP 5500 instrument (Darmstadt, Germany) combined with an Agilent 1260 HPLC system (Waldbronn, Germany).

Table 1: HPLC system settings for drugs of abuse *(Agilent 1260 Infinity).*

total run time	15 min
injection volume	10 µl
column oven	30°C
flow rate	0.45 µl/min

time (min)	mobile phase A (%)	mobile phase B (%)
0.00	100	0
0.20	100	0
10.2	0	100
12.0	0	100
12.1	100	0
15.0	100	0

3 Results

The evaluation of the assay was based on the GTFCh guideline for quality assurance [5]. Further, this LC-MS/MS assay was compared with established assays (GC-MS and LC-MS/MS).

Comparison of sample preparation in daily routine with established in-house GC-MS methods**:** The LC-MS/MS assay is based on a single sample preparation (see above), which takes about 2 hours per day (incubation time excluded). Regarding GC-MS, various optimized sample workups depending on the substance class are required. In all cases a hydrolysis step is necessary. Nevertheless, the GC-MS sample preparations, including additional extraction and derivatization steps, are considerably more extensive.

Table 2: Comparison of sample preparation in daily routine.

LC-MS/MS assay	In-house GC-MS methods
about 2 h per day (incubation time excluded)	about 6 h per day (incubation time excluded)
sample volume: 50 µl	sample volume: 1000 - 5000 µl
total run time: 15 min	total run time: 10 - 15 min

Validation results

Linearity and variance homogeneity (Mandel F-test / Cochran test): The ranges obtained cover clinically relevant concentrations for all analytes.

Precision and accuracy: Determination in series (intra-assay, VC <10%) showed acceptable values. It should be noted that the substances were found in different urine concentrations. This has an additional effect on the variation coefficents.

LLOD and LLOQ were determined with calibration lines and diluted matrix samples under realistic lab routine conditions (Tab. 2). The obtained sensitivity is comparable to the GC-MS methods. The LLOQ-requirements for forensic abstinence controls were met.

Table 2: LLOD ranges for included substance groups.

substance group	LLOD (µg/l)
amphetamines amphetmine, methamphetamine, MDA, MDMA, MDEA	1.0 - 2.9
benzodiazepines e. g. 3-OH-bromazepam, *a*-OH-alprazolam, nordiazepam, oxazepam, temazepam	1.3 - 6.6
cocaine-metabolites benzoylecgonine, norcocaine	0.7 - 0.9
opiates 6-monoacetylmorphine, codeine, dihydrocodeine, morphine	0.5 - 1.3
opioids e. g. EDDP, fentanyl, methadone, norbuprenorphine, norfentanyl, nortilidine, oxycodone, tilidine, tramadol	0.2 - 1.6
cannabis THC-COOH	3.6
z-drugs zaleplon, zopiclon, zolpidem	0.9 - 3.7

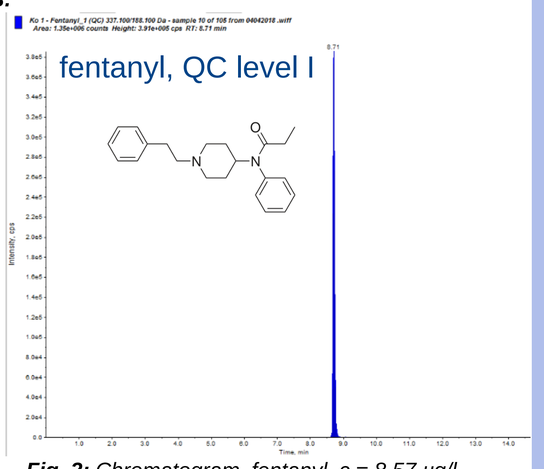

Fig. 2: *Chromatogram, fentanyl, c = 8.57 µg/l.*

Matrix effects, tested with 20 different spiked urine samples: Significant matrix effects were observed: 10 - 30%; small effects: Amphetamines, most benzodiazepines, opioids; relevant effects: 6-Monoacetylmorphine, morphine, benzoylecgonine; deuterated ISTD (for each analyte available) compensates matrix effects quite well.

Enzymatic hydrolysis was tested with four compounds: Codein-6-glucuronide, dihydrocodein-6-glucuronide, morphin-3-glucuronide and temazepam-glucuronide. The observed yields were in an acceptable range between 74 und 95 %.

Comparative analyses were carried out between an external accredited laboratory and the Drugs of Abuse LC-MS/MS-assay. Values correspond well to each other at concentrations within the linearity of the methods (Fig.3). Limits for the quantification were observed for high drug concentrations significantly above the calibration points.

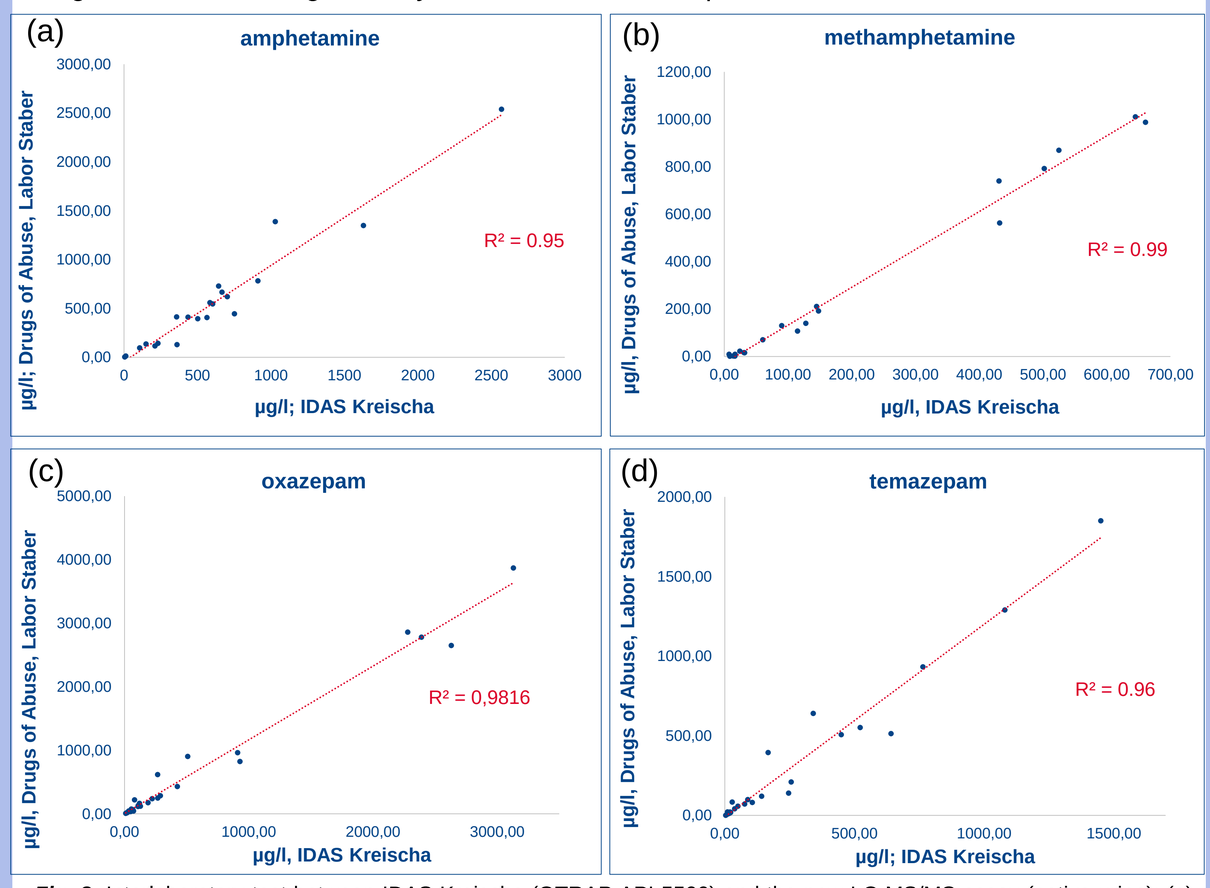

Fig. 3: Interlaboratory test between IDAS Kreischa (QTRAP API 5500) and the new LC-MS/MS assay (native urine): (a) amphetamine, 21 samples; (b) methamphetmine, 20 samples; (c) oxazepam, 31 samples; (d) temazepam, 26 samples.

Proficiency testing: Participation in three testing programs has been successful: SFD 1/18, GTFCh, DS 2/18 und 3/18, RfB (Tab. 4).

Table 4: Results SFD 1/18 proficiency testing, GTFCh.

group	analyt	target value (µg/l)	laboratory results (µg/l)	passed
amphetamines	MDMA	150	127	yes
z-drugs	zolpidem	300	266	yes
cannabinoids	THC-COOH	100	144	yes
cocaine / -metabolites	benzoylecgonine	150	156	yes
opiates	morphin (-3-glucuronid)	100	60	yes
opioids	tramadol	250	294	yes
buprenorphine	buprenorphine	40.0	25.2	yes

4 Conclusions

The presented LC-MS/MS assay is suitable and reliable for the determination of the substances included in this study. The outcome of the LC-MS/MS assay is comparable with results of reference methods. Therefore, the new LC-MS/MS assay can be applied for confirmation analyses for drugs of abuse. Due to the less labour-intensive sample preparation and reduced sample volume, the assay also offers advantages for the daily laboratory routine. This could also be proved by the fact that new method has already been used as a standard method with a high number of samples for almost a year. In addition, the LC-MS/MS kit has been forensically accredited as a toxicological screening procedure at the Staber laboratory for driving aptitude diagnostics.

5 Literature

[1] K. Behrendt, M. Backmund, J. Reimer, Drogenabhängigkeit, Suchtmedizinische Reihe Band 4, Deutsche Hauptstelle für Suchtfragen e. V., **2015**. [2] M. Madea, B. Brinkmann, *Handbuch gerichtliche Medizin, Springer Verlag,* **2003**. [3] Drogen und Suchtbericht, Bundesministerium für Gesundheit, **2017**. [4] A. M. Gressner, T. Arndt, *Lexikon der Medizinischen Laboratoriumsdiagnostik*, Band 1 Klinische Chemie, Springer Verlag, **2006**. [5] L. D. Paul, F. Mußhoff *et. al.*, *Richtlinie der GTFCh zur Qualitätssicherung bei forensisch-toxikologischen Untersuchungen, Toxichem Krimtech* **2009**;76*(3)*:142.

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