Toxtyper for automated and semi-quantitative screening of drugs consumed in drug consumption rooms

Drug consumption rooms are seen as an important element to minimize drug-related health problems (e.g. infection risk) and promote contact of drug users with employees of drug help programs.

Introduction

The first drug consumption room in Frankfurt am Main was established in 1995 in an attempt to deal with the precarious situation in Germany's largest open drug scene near Frankfurt with about 200 deaths in public places at that time. The intention was to relocate drug consumption from public areas to a controlled, hygienic and safe environment. Since 2000, the 3rd Amendment of the German Narcotics Law serves as a legal basis for drug consumption rooms, legalizing already existing institutions and enabling the start of new drug help projects. The six federal states where drug consumption

Keywords: drug screening, amazon speed, semi-quantitative determination

Author: Ronja Peter[1,2], Markus Meyer[3], Volker Auwärter[1], Jürgen Kempf[1]
[1] Institute of Forensic Medicine, Medical Center - University of Freiburg, Faculty of Medicine, University of Freiburg, Germany; [2] Offenburg University of Applied Sciences, Offenburg, Germany; [3] Bruker Daltonik GmbH, Bremen, Germany

rooms are established - Berlin, Hamburg, Hesse, Saarland, Lower Saxony and North Rhine-Westphalia - passed additional regulations for establishing and operating such institutions. While the German Narcotics Law explicitly prohibits the analysis of drugs from/for users ("Drug Checking"), authorities agreed on anonymous analysis of drugs consumed in three consumption rooms around Frankfurt main station and a scientific evaluation of the findings in cooperation with the drug department of the city of Frankfurt. The main objective of this project was to gather detailed information on the type and quality of the drugs used by these clients with a special focus on the prevalence of New Psychoactive Substances (NPS) in street drugs.

Methods

Sample preparation

Weighable amounts of powder were dissolved in acetonitrile (c = 1 mg/mL) and subsequent dilutions in LC eluent A (c = 2.5 µg/mL) were used for quantitative analysis. Packings with trace amounts of powder were rinsed with acetonitrile, diluted in eluent after reweighing of the dried residue and analyzed qualitatively, see Figure 1.

Quantitative Screening

The standard Toxtyper™ 2.0 approach was modified by adding about 200 new compounds - mostly designer stimulants and synthetic opioids - and using the ion source in ESI positive mode only to obtain more data points over the chromatographic peak. In contrast to the smartMRM approach of the amazon speed, the semi-quantification approach of the Toxtyper 2.0 is based on MS^1 full scan data.

To set up the quantitative part of the screening the linear range of each analyte has been evaluated first. Using

Table 1: UHPLC-MS^n method parameters

	LC-Conditions
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
Analytical column	Acclaim® RSLC 120 C18 2,2 µm 120A 2.1x100 mm
Flow rate	500 µL/min
Injection volume	2 µL
Gradient:	0.0 to 0.2 min: 1% B
	0.2 to 0.5 min: 1% B to 35% B, linear
	0.5 to 6.0 min: 35% B to 40% B, linear
	6.0 to 8.5 min: 40% B to 95% B, linear
	8.5 to 11.0 min: 95% B
	11.0 to 11.1 min: 95% B to 1% B, linear
	11.1 to 13.0 min: 1% B
	MS- Conditions
MS-System	amaZon speed™ ion trap
Ion source	ESI source, positive mode
Scan mode	UltraScan: 70 - 800 Da at 32,500 Da/s
	Auto MS^n mode: n = 3
	Scheduled Precursor List to trigger MS^2 and MS^3 spectra

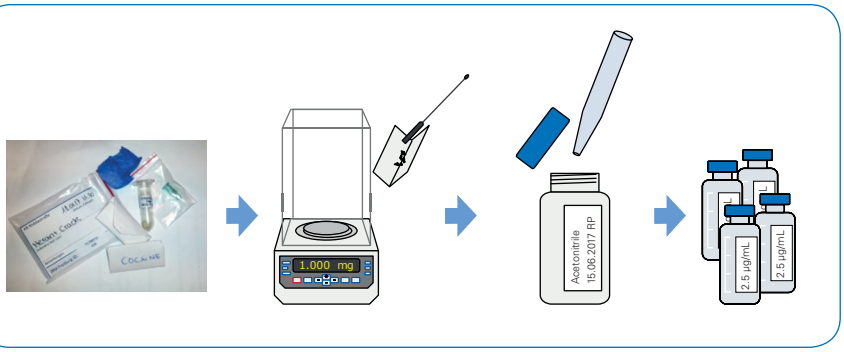

Figure 1: Sample preparation workflow for different drug specimens

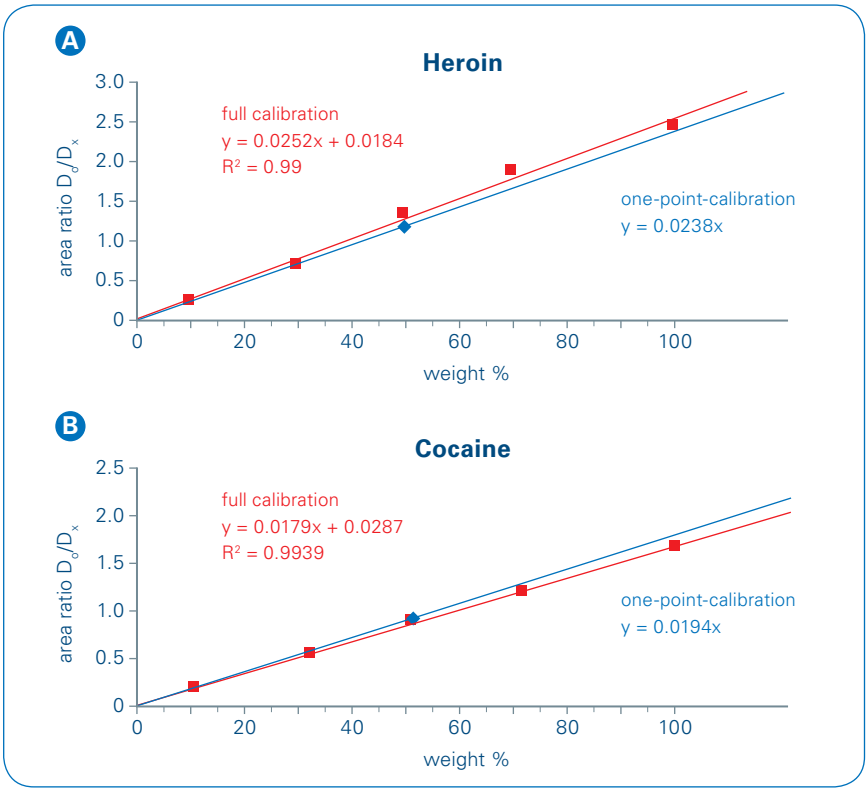

Figure 2: Comparison of full calibration and one-point-calibration (semi-quantitative) results exemplified for heroin and cocaine

the peak area ratio of the molecular ion of the compound and an assigned deuterated internal standard (ISTD), upper (ULOQ), and lower limits (LLOQ) of the linear range, as well as the concentration of the calibration sample were added to a .csv-file that is linked to the Toxtyper 2.0 software.

LC-MS conditions

With the exception of the ionization mode, the LC-MS conditions were identical to the standard Toxtyper approach. Zero delay polarity switching was turned off and the ion source was operated in positive ESI mode only. The LC-MS conditions are shown in Table 1.

Data evaluation

Automated data processing and reporting was carried out using the DataAnalysis software package according to the Toxtyper workflow. Finally, qualitative and semi-quantitative results can be reviewed by the Toxtyper graphical user interface or/and by a simple pdf report.

Results

Development and evaluation of the method

Heroin and cocaine were supposed to be the most common drugs among this user group. So, linearity and limits of detection (LOD) for these drugs, poppy alkaloids, common extenders and degradation products were determined first. Regression coefficients (R^2) of calibration curves (1 to 120 wt.%) ranged from 0.9777 to 0.9993. R^2 of the main drug analytes with corresponding isotope labeled standards were found to be higher than 0.99 and were in good agreement with data from respective one-point-calibrations as shown by two examples in Figure 2 for heroin (A) and for cocaine (B).

Qualitative analysis of drug samples

A total of 409 different drug samples were sent in for analysis. Samples consisted of powder residues (P), syringe filters (F) or packing material (M) only, or varying combinations of the latter. Taking into account samples with multiple specimens, we analyzed 468 different samples. As expected, heroin and cocaine were the drugs found most in this user group and the analytical findings of the powder samples were in good agreement with the information given by the user. Few samples labeled as cocaine or heroin only, were found to be vice versa or a mixture of both.

Among a total of 2415 hits, 24 different substances could be identified: Three major active agents (amphetamine, heroin and cocaine), 10 typical by-products and 11 other compounds like commonly used extenders.

Heroin could be detected in 213 samples (P: n=158, F: n=24, M: n=31), cocaine in 166 samples (P: n=83, F: n=34, M: n=49), and cocaine plus heroin in 61 samples (P: n=17, F: n=25, M: n=19). There was one single amphetamine finding and 27 samples where no drugs could be found at all.

Up to now, no NPS, like designer fentanyls or stimulants could be detected in quantifiable amounts, although two samples indicated the presence of fentanyl (see below).

The analgesic phenacetin could be detected in 63 % of the cocaine specimen analyzed, 25 % of them containing levamisole as additional extender. Heroin samples were regularly extended with acetaminophen and caffeine. In addition, the opium alkaloids noscapine and papaverine as well as 6-MAM and 6-Acetylcodeine (6-AC) were found in these samples.

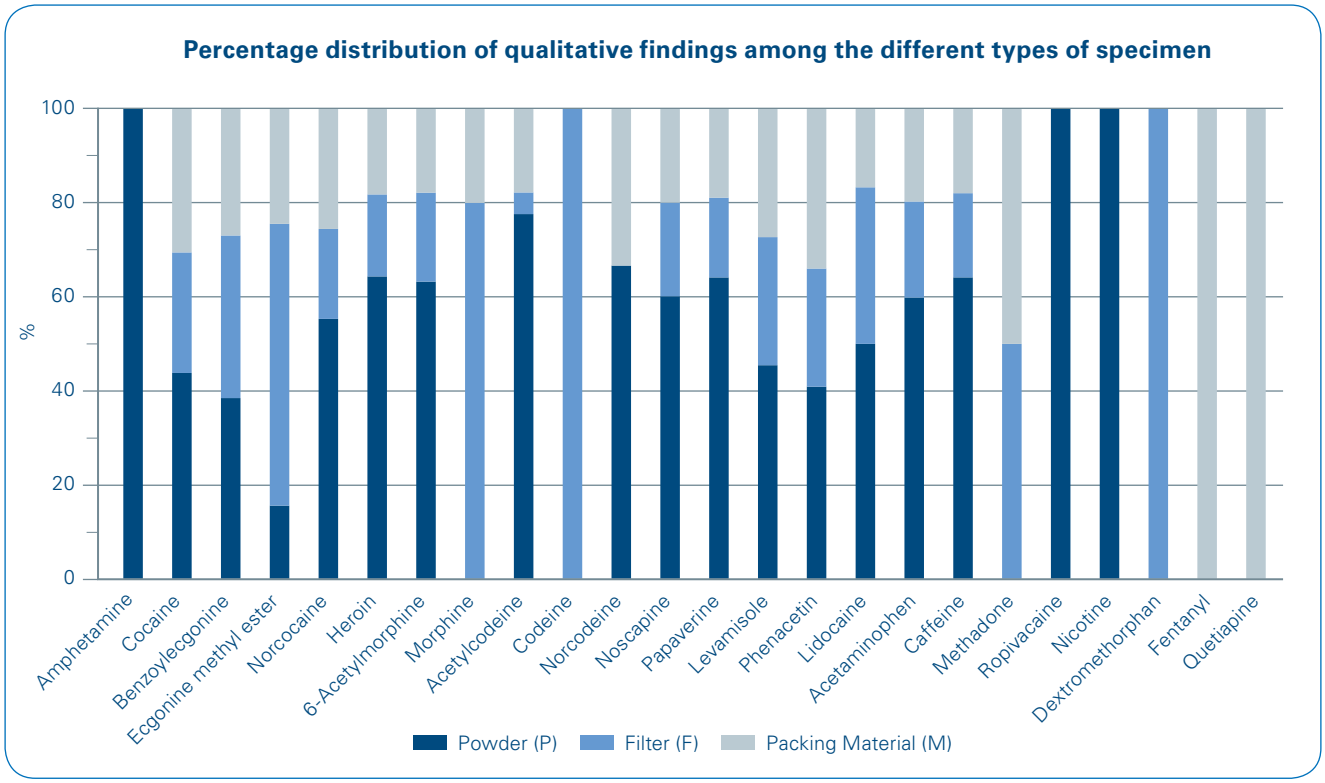

Figure 3: Distribution of drugs among different specimen

Quantitative analysis of drug samples

From 265 specimens with weighable amounts of powder we performed a quantitative analysis, in summary: 165 heroin samples, 83 cocaine samples, and 17 cocaine/heroin samples, respectively. Cocaine concentrations ranged from 1 to 100 wt.%, with 50% of the findings between 49 and 96 wt.%. Heroin concentrations ranged from 1 to 58 wt.%, with 50 % of the findings between 3 and 13 wt.% (see Figure 4 shown in gray). For comparison, the purity of seized cocaine and heroin according to data from the European Drug Report 2017 is shown in blue compared to our findings in gray (see Figure 4).

According to the annual report of the German Monitoring Centre for Drugs and Drug Addiction (GMCCDA) of 2016, medium heroin content of seized drugs was 45.1%, or 22.6% and 19.3% at medium and lower distribution levels, respectively. The average heroin content found in samples of this study was around 9%. For cocaine the range of active

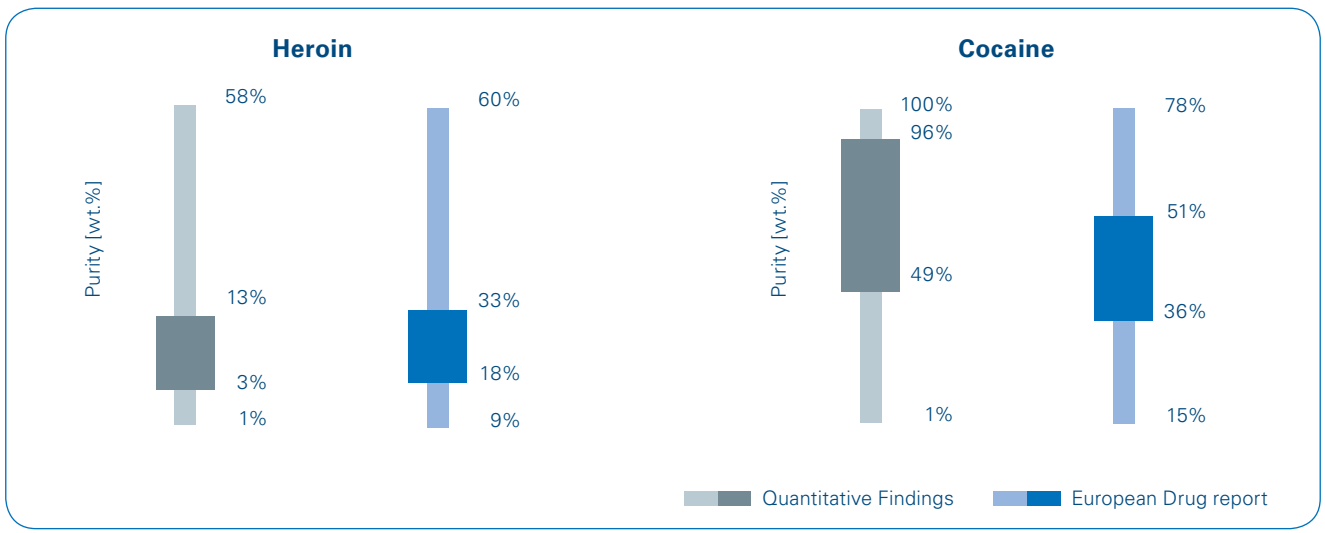

Figure 4: Comparison of quantitative findings for heroin and cocaine between the European Drug Report (blue color) and this study (grey color)

ingredient content meets the average cocaine levels of 74.1% and 70.4% for low distribution levels reported for Germany in 2016, whereby it should be noticed that most of the cocaine in this user group is consumed as crack.

After detection of the local anesthetic drug ropivacaine in a heroin specimen, the linear range for ropivacaine was evaluated and a ropivacaine content of 19 wt.% could be quantified in a second analysis of the sample, subsequently.

In two packing materials the opioid fentanyl could be detected besides cocaine, phenacetin and levamisole. Unfortunately, there were no weighable amounts of powder preservable, for quantitative analysis.

Limits of detection

The presented LC-MS^n approach enables automated identification and quantitative determination of the active ingredients and cutting agents of drug preparations with active ingredient contents down to 1% by weight (based on the 2.5 µg/mL solution). If lower levels are expected and quantification is of interest, the dilution step during sample preparation can easily be adjusted to match the linear range of the calibration. LODs are typically in the range from 1.25 - 200 ng/mL, which corresponds to 0.05 - 0.5 wt.% of the analyzed powder (see Figure 5), which is of particular interest for detecting highly potent opioids like fentanyl derivatives potentially added to heroin preparations.

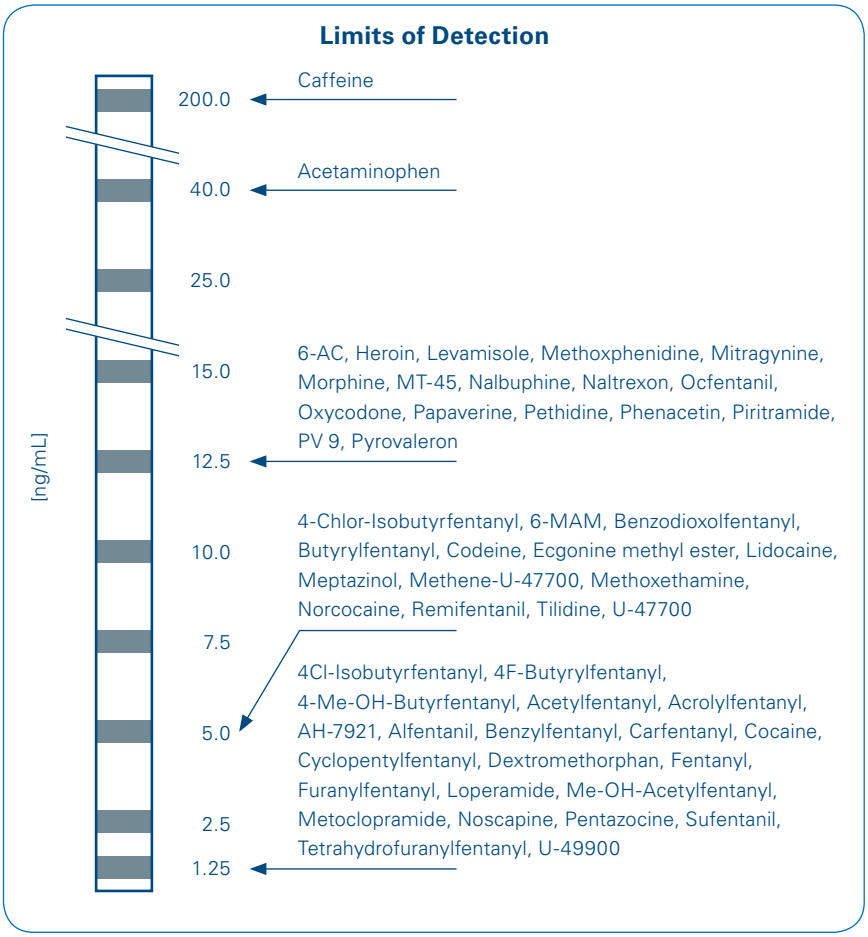

Figure 5: Limits of detection of all drugs found during this study

Conclusion

- The Toxtyper has been shown to be a valuable tool for qualitative, quantitative and semi-quantitative analysis of diverse drug related specimens.
- As expected, cocaine and heroin are the most common drugs consumed in the three consumption rooms in this area of Frankfurt. Up to now, there were no unusual analytical findings apart from the detection of fentanyl in cocaine and ropivacaine in heroin samples.
- The approach is not yet validated for legal cases dealing with exact quantification of drug amounts but it's an easy-to-use method for qualitative and semi-quantitative analysis of all kinds of powders and materials and can serve as a valuable and fast tool to assess the potential harm of street drugs.
- In addition to this study, the Toxtyper workflow has also been used in forensic casework to identify drugs of abuse and/or NPS in seized materials e.g. pills or powders containing designer stimulants and synthetic cannabinoids.

Learn More

You are looking for further Information?
Check out the link or scan the QR code for more details.

www.bruker.com/toxtyper-applications

References

[1] **Automated semi-quantitative screening of drugs consumed in drug consumption rooms in Frankfurt, Germany using LC-ion trap-MS**, Ronja Peter; Louis Maljers; Markus Meyer; Volker Auwärter; Jürgen Kempf, 292477, TP 169, *Proceedings of the 66th ASMS Conference on Mass Spectrometry and Allied Topics*, San Diego, California, June 3-7, 2018.

For research use only. Not for use in diagnostic procedures.

Bruker Daltonics GmbH & Co. KG

Bremen · Germany
Phone +49 (0)421-2205-0

Bruker Scientific LLC

Billerica, MA · USA
Phone +1 (978) 663-3660

ms.sales.bdal@bruker.com – www.bruker.com