Automated semi-quantitative screening of drugs consumed in drug consumption rooms in Frankfurt, Germany using LC-ion trap-MS

Ronja Peter[1,2], Markus Meyer[3], Louis Maljers[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

Hochschule Offenburg offenburg.university

BRUKER

UNIVERSITÄTS KLINIKUM FREIBURG

Institute of Forensic Medicine Forensic Toxicology

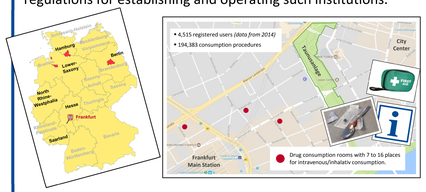
OBJECTIVES

- Analysis of type and quality of street drugs in Frankfurt
- Development of an easy-to-use quantitative screening using LC-ion trap-MS with automated reporting
- Constant updating of the method with a focus on NPS

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 main station 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. These rooms are also 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.

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 rooms are established - Berlin, Hamburg, Hesse, Saarland, Lower Saxony and North Rhine-Westphalia - passed additional regulations for establishing and operating such institutions.



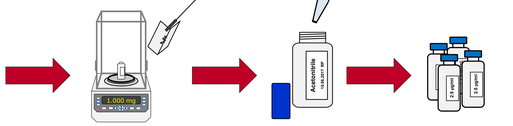
While the German Narcotics Law explicitly prohibits the analysis of drugs from/for users ("Drug Checking"), authorities agreed on anonymous analysis of drugs consumed in three consumption rooms around Frankfurt main station and a scientific evaluation of the findings in cooperation with the drug department of the City of Frankfurt.

The main objective of the project is to gather 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.

Drug packing material and used filters were collected by the staff of the three consumption rooms and sent to our institute.

METHODS

Sample Preparation

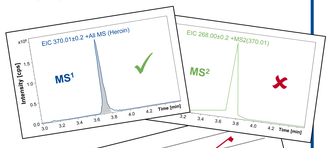


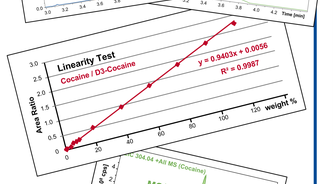
Weighable amounts of powder were dissolved in acetonitrile (c = 1 mg/ml) and subsequent dilutions in LC eluent (c = 2.5 µg/ml) were used for quantitative analysis. Packings with trace amounts of powder were rinsed with acetonitrile, diluted in eluent after reweighing of the dried residue and analyzed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

Setting up the Quantitative Screening Approach

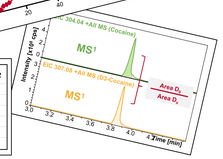
The original Toxtyper™ 2.0 approach was modified by adding about 200 compounds - mostly designer stimulants and synthetic opioids - and switching the ion source to ESI positive mode only to obtain more data points per peak. Due to data dependent acquisition in autoMS^n mode including active exclusion of precursors, in contrast to other MS/MS approaches only MS^1 full scan data is available for quantitation.

To set up the quantitative part of the screening the linear range of each analyte has to be evaluated first. Therefore, the peak area ratio of the molecular ion of the compound and the corresponding deuterated internal standard (ISTD) was used (Cal_Slope_1). The upper (ULOQ) and lower limits (LLOQ) as well as the concentration of the calibration sample were added to a .csv-file linked to the DataAnalysis script of the method.





Analyte Name	ISTD	Slope	Intercept	LLOQ	ULOQ	Unit	Calibration Concentration	Quant m/z	Cal_Slope_1	Cal_Slope_2
Heroin	D9-Heroin	3.20E-02		1	120	Gew-%	50		3.20E-02	
Morphine	D3-6-Acetymorphine	1.02E-02		1	120	Gew-%	50		1.02E-02	
Codeine	D3-6-Acetymorphine	2.04E-02		1	120	Gew-%	50		2.04E-02	
6-Acetylcodeine	D9-Heroin	4.64E-02		1	120	Gew-%	50		4.64E-02	
6-Acetymorphine	D3-6-Acetymorphine	2.33E-02		1	120	Gew-%	50		2.33E-02	
.....										

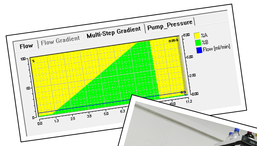


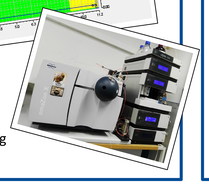
This .csv-file is crucial for automated quantitation of screening results using one-point-calibration. If marked as a calibrator, all qualitative findings in a sample are checked for an entry in the .csv-file and the area ratio of the compound and its assigned ISTD is recorded, subsequently. If marked as an unknown, all qualitative findings are checked for an calibration entry (Slope) and the calculated concentration is reported. Positive findings below or above the linear range are reported as '< cal_{Low}' or '> cal_{High}', respectively.

LC - MS^n 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
Column: Acclaim® RSLC 120 C18 2,2 µm 120A 2.1x100 mm
Total flow: 500 µL/min
Gradient: 1 % to 95 % B in 8 min; 11 min runtime

MS-System: Bruker amaZon speed™ ion trap
Ion source: ESI source, positive mode
Scan mode: UltraScan (70 - 600 Da at 32.500 Da/s), Auto MSn mode (n = 3)
Scheduled Precursor List to trigger data dependent acquisition of MS^2- and MS^3-spectra





Data Evaluation and Reporting

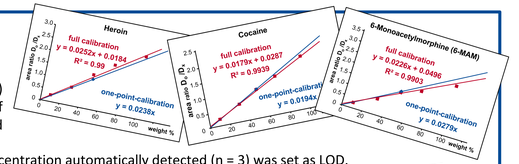
DataAnalysis 4.1 software package for automated data processing and result reporting according to the Toxtyper workflow including an automated quantitation script.

RESULTS

Method Development

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.

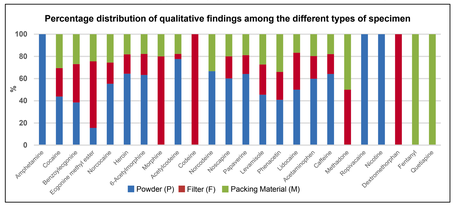
LODs - especially for synthetic opioids - were evaluated by analyzing standard solutions in decreasing concentrations. The lowest concentration automatically detected (n = 3) was set as LOD.



Analysis of Drug Samples - Qualitative Findings

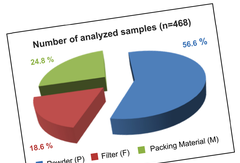
Up to now, 409 different drug samples were sent in for analysis. Samples consisted of powder residues (P), syringe filters (F) or packing material (M) only, or varying combinations of the latter. Taking into account samples with multiple specimens, we analyzed a total of 468 different samples. As expected, 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 a mixture of both or vice versa.

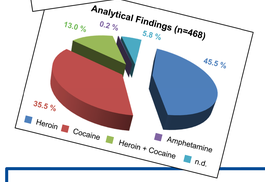
Among a total of 2415 hits, 24 different substances could be identified: Three major active agents (amphetamine, heroin and cocaine), 10 typical by-products and 11 other compounds like commonly used extenders.

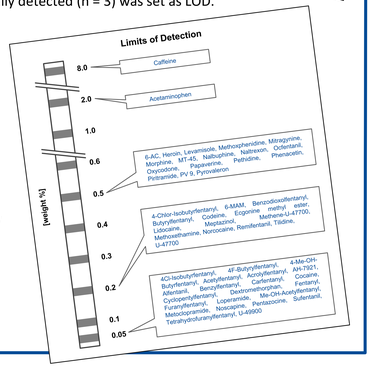


Heroin could be detected in 213 samples (P: n=158, F: n=24, M: n=31), cocaine in 166 samples (P: n=83, F: n=34, M: n=49), and cocaine plus heroin in 61 samples (P: n=17, F: n=25, M: n=19). There was one single amphetamine finding and 27 samples where no drugs could be found at all.

Up to now, no NPS, like designer fentanyls or stimulants could be detected.

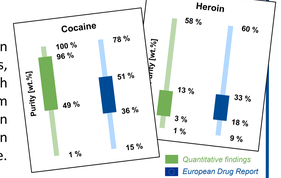






Analysis of Drug Samples - Quantitative Results

We got 265 specimen with weighable amounts of powder, 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 conc. ranged from 1 to 58 wt.%, with 50 % of the findings between 3 and 13 wt.% (shown in green). For comparison, the purity of seized cocaine and heroin according to data from the European Drug Report 2017 is shown in blue.



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 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.

CONCLUSION

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 presented LC-MS^n approach allows automated identification and quantitative determination of the active ingredients and cutting agents of drug preparations with active ingredients contents down to 1 % by weight. If lower levels are expected and quantification is of interest, the dilution step during sample preparation can easily be adjusted to match the linear calibration range of the calibration. LODs are typically in the range of 0.5 to 0.05 wt.% which is of particular interest for detecting highly potent opioids like fentanyl derivatives potentially added to heroin preparations.

In addition to this study, this method has also been used in forensic casework dealing with seized materials e.g. pills or powders containing designer stimulants and synthetic cannabinoids.

The approach is not considered to be used in 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 may serve as valuable tool to asses the potential harm of street drugs.

ACKNOLEDGEMENTS

We would like to thank the staff members and users of the three drug consumption rooms for their collection of sample material and the City of Frankfurt for financial support of this ongoing study.