Identification and quantitative determination of active ingredients in drug exhibits by LC-MS^n

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Introduction

Besides routine screening of body fluids, the analysis of tablets and powders is of forensic interest, especially in intoxication or post mortem cases where illicit drug preparations or drug paraphernalia were found. Screening methods usually applied for this purpose like GC-MS often require more laborious sample preparation, like multiple derivatization steps for different compounds classes. The LC-MS^n screening method applied in this project allows the identification of about 1000 compounds within a single 11-minutes run, including emerging New Psychoactive Substances (NPS) like designer benzodiazepines, "bath salts", and designer opioids. Overdosing of these NPS, most notably compounds of the opioid and the synthetic cannabinoid class, pose severe health threats especially after unintentional uptake, e.g. when mixed into other drug preparations, or uptake of highly potent, pure research chemicals.

The aim of this project was to develop a fast and easy-to-use method for identification and quantitation of the active ingredients in illicit drug preparations.

Experimental

Semi-Quantitative Screening

For semi-quantitative screening, the MS^1 peak area of the molecular ion of a detected compound and an assigned internal standard are used. For this purpose, the software script carries out the following steps:

- Extracted ion chromatogram (EIC) of the $[M+H]^+$ of the respective compound
- Peak detection at the retention time of the screening result using routine peak finding algorithms, subsequent smoothing and calculation of the peak area
- Calculation of the concentration according to calibration data stored in a .csv-file.

In order to set up this semi-quantitative data evaluation, the followings steps have to be performed:

Evaluation of the LOD

As the majority of samples analyzed with this approach will be methanolic solutions diluted with eluent, limit of detection (LOD) was evaluated by analyzing decreasing concentrations of analytes in eluent A:B 50:50 (v:v).

Evaluation lower and upper LOQ

The linear range was assed by analyzing calibration curves within the desired concentration range. Linearity of the calibration was checked using linear regression coefficients calculated by Excel.

Quantitation by .csv-file

Quantitation of screening results was carried out using entries of a pre-defined .csv-file. Generating quantitative results could either be performed by stored calibration curves (slope and intercept) or by single-point-calibration. Data for slope and intercept can be derived from the previous analysis carried out for the evaluation of LLOQ und ULOQ. For this project, automated daily single-point-calibration was chosen.

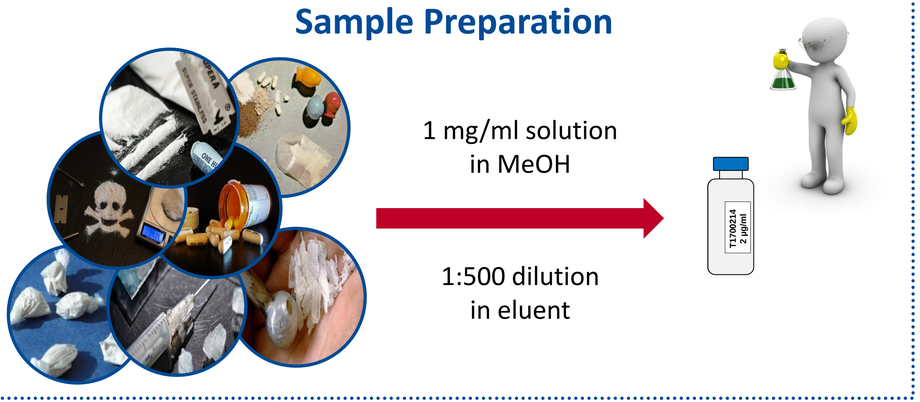
	A	B	C	D	E	F	G	H	I	J	K	L	M
1	Analyte Name	ISTD	Slope	Intercept	LLOQ	ULOQ	Unit	Calibration Concentratio	Quant m/z	Cal_Slope_1			
2	6-O-Acetylcodeine	D4-Haloperidol	4,28E+10		0,1	2,5	µg/mL	1		4,28E+10			
3	6-O-Acetylmorphine	D3-Doxepin	3,74E+10		0,1	2,5	µg/mL	1		3,74E+10			
4	Alpha-PVP	D4-Haloperidol	3,46E+10		0,1	2,5	µg/mL	1		3,46E+10			
5	Amphetamine	D3-Doxepin	1,41E+09		0,1	2,5	µg/mL	1		1,41E+09			
6	Benzoylecgonine	D4-Haloperidol	3,30E+10		0,1	2,5	µg/mL	1		3,30E+10			
7	Caffeine	D3-Doxepin	3,59E+08		0,1	2,5	µg/mL	1		3,59E+08			
8	Cocaine	D4-Haloperidol	6,99E+10		0,1	2,5	µg/mL	1		6,99E+10			
9	Codeine	D3-Doxepin	3,88E+09		0,1	2,5	µg/mL	1		3,88E+09			
10	Diltiazem	D4-Haloperidol	1,31E+11		0,1	2,5	µg/mL	1		1,31E+11			

LC Conditions	
LC system	Thermo Dionex Ultimate 3000 RSLC
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	0.5 ml/min
Injection volume	2 µl
Gradient:	0.0 to 1.0 min: 1% B
	1.0 to 8.0 min: 1% B to 95% B, linear
	8.0 to 9.0 min: 95% B
	9.0 to 9.1 min: 95% B to 1% B, linear
	9.1 to 11 min: 1% B, linear

MS Conditions	
Mass spectrometer	Bruker amaZon speed™ ion trap
Ion source	Electrospray Ionisation (ESI)
Polarity	Positive ESI mode
Scan mode	UltraScan at 32.500 m/z sec^{-1}
Scan range	70 - 800 m/z
MS^n Acquisition	Data dependent MS^2 and MS^3 with Scheduled Precursor List (SPL)

Data Evaluation	
Software	Bruker DataAnalysis
Identification	Toxtyper Workflow using an updated spectral library
Quantitation	One-point-calibration by a DataAnalysis semi-quant script

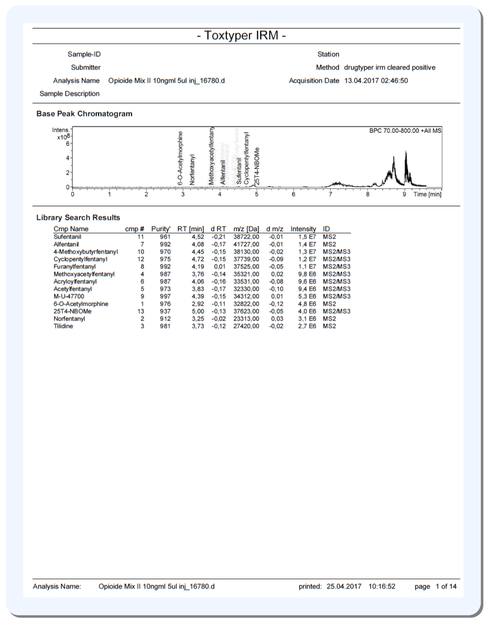
Sample Preparation



Results

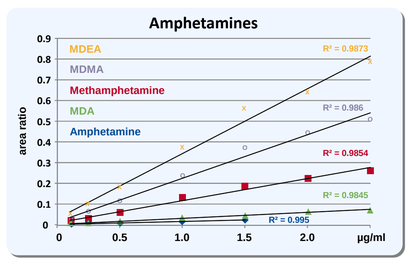
Limits of detection

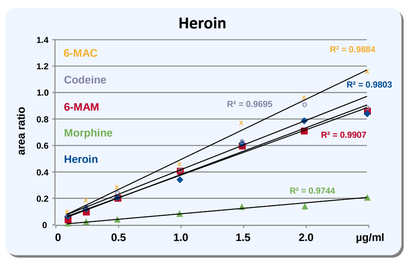
Limits of detection were determined for the most common drugs and selected drugs of different substance classes like stimulants, designer opioids, and synthetic cannabinoids. Also, varying drug preparations or methanolic solvations of different drug paraphernalia like packing material or syringe filters may cause different matrix load, LODs reaching down to 0,5 weight percent which equals 10 ng/ml, respectively, were determined in "matrix free" solution of analytes in LC eluent.

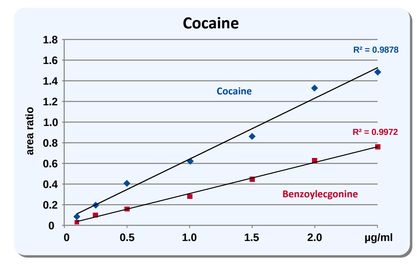


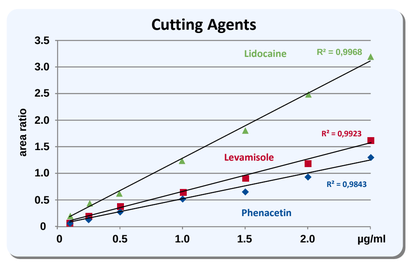
Linear calibration range (LLOQ and ULOQ)

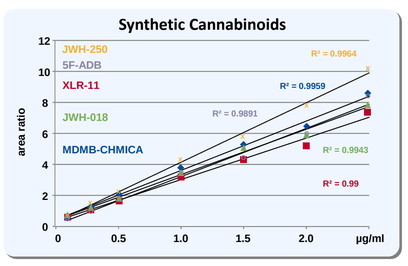
Analytes were assigned to selected isotope labelled internal standards (IS) according to their retention time and peak areas of the $[M+H]^+$ were normalized with the one of its respective IS. The tested calibration range from 5 to 100 weight percent (0.1 - 2.0 µg/ml) was linear for almost all compounds with $R^2 > 0.96$.

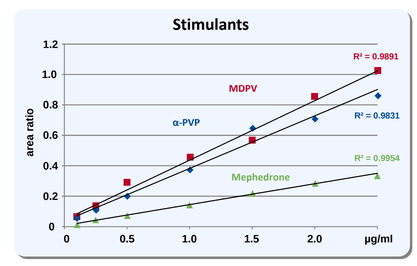












Quantitation results

Quality of the semi-quantitative results were evaluated using QC samples with concentrations levels over the whole linear range of the respective analyte. The following table shows the deviation in % from the target concentration.

	0.1 µg/ml ≙ 5.0 %	0.25 µg/ml ≙ 12.5 %	0.5 µg/ml ≙ 25 %	1.0 µg/ml ≙ 50 %	1.5 µg/ml ≙ 75 %	2.0 µg/ml ≙ 100 %	2.5 µg/ml (≙ 125 %)
Amphetamine	44	22	-13	21	-1	outside linear range	
Methamphetamine	-	-11	1	-7	-11	-15	-10
MDEA	8	-3	-24	-24	-19	-23	-24
MDA	-	64	19	6	4	9	-26
MDMA	16	18	26	9	7	-4	-10
Heroin	67	62	32	50	22	6	-
Cocaine	46	27	0	9	-7	-15	-
LSD	48	6	21	18	4	-2	-
α-PVP	33	14	6	24	9	17	-
MDPV	56	14	9	27	2	6	-2
MBDB	23	15	-1	-3	-3	-9	-5
JWH-250	44	18	24	28	20	-1	-9
Mephedrone	11	-1	-8	15	8	-1	-6
Pentylone	54	-2	2	-5	-15	-21	-17
MDMB-CHMICA	39	3	6	4	-1	-7	-20
5F-ADB	44	22	13	-1	4	-2	-16
JWH-018	16	9	11	-1	7	-14	-21
XLR-11	31	19	17	8	6	-14	-13

± 0 - 25 % | ± 26 - 50 % | > 50 % | „ - " no semi-quant. Value (< LLOQ or > ULOQ)

The results of the QC samples show, that concentration values around the LLOQ deviate most from the spiked concentration. Therefor, for quantitation of drug material with expected concentrations below 10 weight % the dilution step during sample prep has to be adjusted for some compounds.

Conclusion

The presented method allows the automated identification and semi-quantitative determination of the active ingredients and cutting agents of drug preparations. The proposed workflow facilitates quantitative analysis of drug samples with active ingredient contents ranging from 5 to 100%. If lower levels are expected, the dilution step during sample preparation can easily be adjusted to match the linear calibration range of the calibration.

The quality of the (semi-)quantitative data allows assessing the potency and thereby potential health risks of the investigated powders or tablets, e.g. in the frame of drug checking projects. However, for determination of the absolute amount of drug in the preparation with regard to legal limits, fully validated methods with higher accuracy should be applied.