

# Application News

LCMS-8050

## Rapid, sensitive and robust UHPLC-LC/MS/MS- screening for 60 drugs of abuse in oral fluids

**No.** SCA-210-029

### Background

Screening for drugs of abuse with immunoassay in urine samples can be regarded as standard practice to test for drugs of abuse in the workplace, drug treatment and other settings. Oral fluid (OF) is increasingly accepted as a suitable alternative matrix. This is mostly because of ease of collection and less risk for adulteration. In addition OF allows a more easy adaptation of the standard screening panel to “modern” drugs within chromatographic methods because the target analytes are mostly parent drugs and commercially available.

This application presents a robust and cost-effective LC/MS/MS assay for the determination of illicit drugs. Analytical challenges like internal dilution and cut-off values were overcome by simple sample preparation.

From 60 compounds, substances linked to heroine consumption were selected as example.

“Street” heroine often presents a mixture of opiates with metabolites that can be traced from minutes to weeks. Nowadays also heroine mixed with cocaine is highly abundant on the market and sold as “snow balls”, consumed orally or nasally. In figure 1, the metabolic pathway is depicted.

In addition, often alcohol or benzodiazepines are consumed at the same time, to alter the drug effect. This whole drug cycle can be monitored using the LC/MS/MS method presented, with a focus on oral fluid testing and also taking extenders and their metabolites into account.

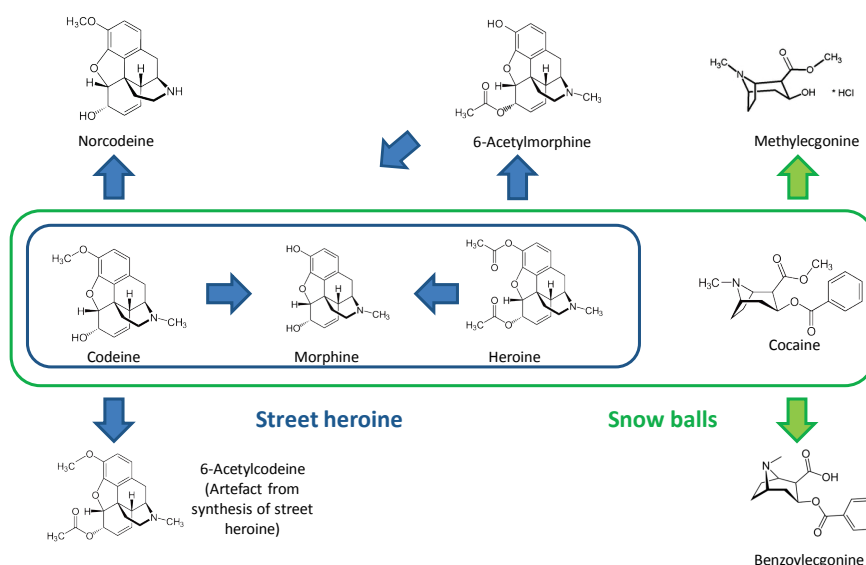


Figure 1: pathway of heroine [2] including exemplary metabolites

## Materials & Methods

Samples were collected using the Saliva Collection System (SCS) pH 4.2 Greiner Bio-One. Authenticity was confirmed by the presence of the saliva specific enzyme alpha-amylase using an immune-assays on an AU680. Saliva samples were prepared for LC/MS/MS analysis as described in figure 2. Sixty substances were analyzed in positive using the LCMS-8050 combined with a Nexera X2 UHPLC System, with a run-time of 6 minutes. Per each analyte 5 transitions were selected, including 2 transitions for the deuterated internal standards. Method details are listed in table 1.

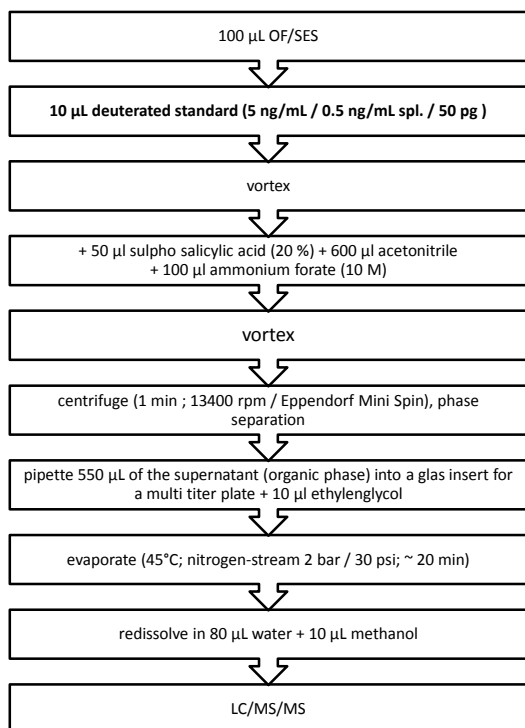


Figure 2: Sample preparation of saliva for LC/MS/MS analysis [1]

## Analytical conditions

Calibration curves fit in the range between 0.05 ng/mL and 20 ng/mL (Figure 4 and Table 2). A positive street heroine sample is shown in figure 5.

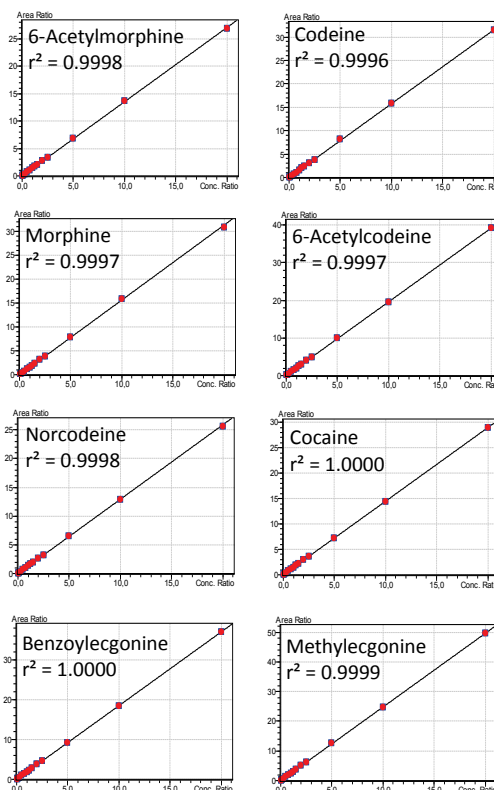


Figure 4: Calibration curves for analytes detected in the saliva sample in figure 5. Calculation in saliva with deuterated internal standard and calculated area ratio. Calibration range 0.05 ng/ml – 20 ng/ml using 16 levels.

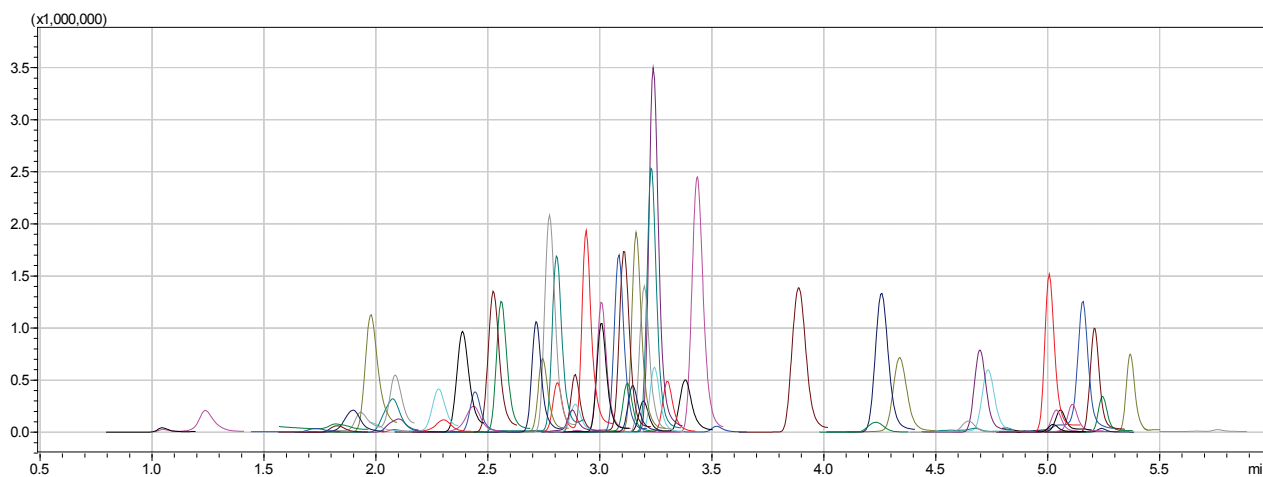


Figure 3: Chromatogram of the Target MRMs of 60 compounds analyzed in saliva samples

Methylecgonine and benzoylecgonine are hydrolysis products of cocaine. Morphine is a metabolite of codeine, but not the other way round, so ingestion of morphine will not account for the presence of codeine. 6-acetylcodeine is an artefact generated during the chemical synthesis of heroine from codeine. If street heroine was consumed, 6-acetylcodeine, 6-acetylmorphine, morphine, codeine and norcodeine can be detected in the corresponding sample. This fingerprint can be found in the positive sample shown in figure 5

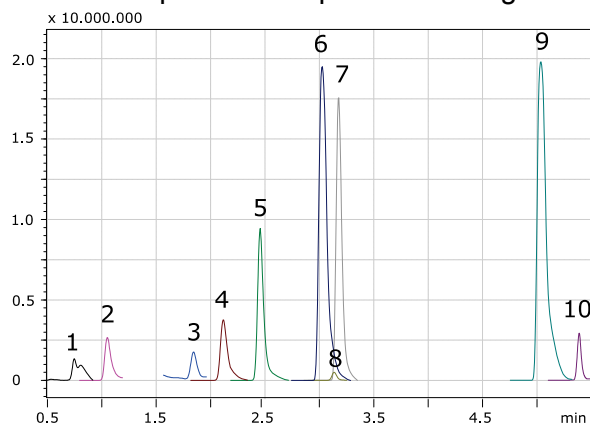


Figure 5: Chromatogram of a street heroine positive saliva sample: 1. methylecgonine (50 x zoom), 2. morphine, 3. norcodeine (50 x zoom), 4. codeine (50 x zoom), 5. 6-acetylmorphine, 6. benzoylecgonine, 7. cocaine , 8. 6-acetylcodeine, 9. methadone, 10. Diazepam

All data were generated in cooperation with the MVZ Labor Dessau GmbH from Stefan Lierheimer and Michael Böttcher.

## Conclusion

A method for the rapid and sensitive screening of 60 drugs of abuse in saliva was shown. It allows to detect multi-drug consumption, monitoring side products as well as extenders and their metabolites.

## Literature

[1] M. Böttcher (2015), Oral fluid as an alternative matrix for monitoring licit and illicit drug abuse, Workshop Nal von Minden/Chiron "New Psychoactive Substances", 12./13.11.2015, Munich

[2] Reisfield, G.M. (2007) Ann Clin Lab Sci Autumn, vol. 37, no. 4, p. 301-314

[3] Monitoring drug abuse of patients in substitution therapy: comparison of UPLC-MS/MS screening in oral fluid and urine testing with immunoassay, Böttcher M, Lierheimer S, SOFT 2012, Boston, 01.-06.07.12

[4] UPLC-MS/MS multi-target screening of 55 commonly abused drugs at different cutoffs in oral fluid from patients in addiction treatment, Boettcher M, Guenther N, Lierheimer S, Beck O, TIAFT Kongress, Madeira, 02.09.-06.09.13

LCMS-8050		Nexera X2 SIL-30ACMP	
Interface Voltage:	+4 kV	Flow:	0.65 ml/min
Q1 Resolution:	Unit (0.7 Da FWHM)	Eluent A:	5 mM ammonium formate + 0,1 % formic acid (pH 3)
Q3 Resolution:	Unit (0.7 Da FWHM)	Eluent B:	methanol, 0.1% formic acid
Desolvation Line Temp.:	150 °C	Column Oven Temp.:	60 °C
Heating Gas:	10 l/min	Inj. Vol.:	20 µl
Interface Temp.:	400 °C	Time Program:	0-0.5 min waste, 0-2 min curve 15-50 %, 2-3.5 min 50 %, 3.5-5 min 50-100 %, 5-5.5 min 100 %
Nebulizing Gas:	3 l/min	Column:	Phenyl-phase, 1.7 µm, 2.1 x 150 mm
Drying Gas:	10 l/min	Other Parameters:	Default (analog tuning file)
Heat Block Temp.:	300 °C		

Table 1: Method parameters

	Calibration range [ng/ml]	Correlation coefficient r <sup>2</sup>		Calibration range [ng/ml]	Correlation coefficient r <sup>2</sup>
6-Acetylcodeine	0.05-20	0.9997	MDEA	0.05-20	0.9999
6-Acetylmorphine	0.05-20	0.9999	MDMA	0.05-20	0.9998
7-Aminoclonazepam	0.05-20	0.9999	MDPV	0.05-20	0.9999
7-Aminoflunitrazepam	0.05-20	0.9987	Mephedron	0.05-20	0.9998
Alprazolam	0.05-20	1	Methadone	0.1-20	0.9994
Amphetamine	0.1-20	0.9994	Methamphetamine	0.05-20	0.9999
Anhydroecgonin-methylester	0.5-20	0.9953	Methylecgonine	0.1-20	0.9999
BDB	0.1-20	0.9998	Methylon	0.05-20	0.9996
Benzoylecgonine	0.05-20	0.9999	Methylphenidat	0.05-20	0.9999
Bromazepam	0.05-20	1	Midazolam	0.05-20	1
Buprenorphin	0.05-20	0.9996	Morphine	0.1-20	0.9997
Bupropion	0.05-20	1	Naloxone	0.05-20	0.9998
Butylon	0.05-20	0.9998	Norbuprenorphine	0.5-20	0.9998
Cocaine	0.05-20	1	Norcodeine	0.05-20	0.9998
Codeine	0.05-20	0.9997	Nordiazepam	0.05-20	0.9999
Cortisol					
(separate calibration)	0.05-20	0.9981	Noroxycodon	0.05-20	1
Desalkylflurazepam	0.05-20	1	Nortilidine	0.05-20	0.9999
Diazepam	0.05-20	1	O-Desmethyltramadol	0.05-20	0.9999
Dihydrocodeine	0.05-20	0.9997	Oxazepam	0.05-20	0.9999
EDDP	0.05-20	0.9984	Oxycodon	0.05-20	1
Fentanyl	0.05-20	1	Phenazepam	0.05-20	0.9999
Flunitrazepam	0.05-20	0.9999	Pregabalin	0.5-20	0.9999
Flurazepam	0.025-20	0.9992	Ritalinsäure	0.05-20	0.9999
Gabapentin	0.75-20	0.9996	Temazepam	0.05-20	0.9999
Hydromorphon	0.05-20	1	THC	0.25-20	0.9994
Ketamine	0.05-20	1	Tilidine	0.05-20	0.9999
Lidocaine	0.05-20	0.9999	Tramadol	0.05-20	0.9997
Lorazepam	0.1-20	0.9999	Zaleplon	0.05-20	0.9999
MBDB	0.05-20	0.9998	Zolpidem	0.025-20	1
MDA	0.1-20	0.9997	Zopiclon	0.05-20	0.9999

Table 2: calibration ranges for all compounds