UPLC-MS/MS multi-target screening of 55 commonly abused drugs at different cutoffs in oral fluid from patients in addiction treatment

Michael Böttcher[1], Nicky Günther[1], Stefan Lierheimer[1], Olof Beck[2]

[1] MVZ Labor Dessau GmbH, Dessau-Roßlau, Germany

[2] Karolinska Institute and Karolinska University Laboratory, Stockholm, Sweden

Introduction

Screening for drugs of abuse with immunoassays in urine spls. from pats. in heroin substitution treatment can be regarded as standard practice. Oral fluid (OF) is increasingly accepted as a suitable matrix to test drugs of abuse in clinical, drug treatment and other settings. This is mostly because of ease of collection and less risk for adulteration or substitution of the spl. However, little is known about the required sensitivity in OF especially for the "modern" drugs. Regarding compliance testing in opiates maintenance therapy false negative results for the substitution drugs must be avoided. To disclose possible intentional oral contamination ("clinical false positive") with the substitution drugs, EDDP and Norbuprenorphine has to be included in drug testing methods. We decided for a liquid based OF collection device buffered at acidic pH to assure that sufficient sample volume is collected in a reasonable time when those very often xerostomic pats. are tested. In this study we compared the positive prevalence rate for 55 licit and illicit drugs at our routine OF cutoff (CO) at 1 ng/mL with a 10 ng/mL CO. For EDDP and Norbuprenorphine also a 0.1 ng/mL CO was considered.

Methods

Patient samples: All routine OF spls. during a 3 month period from 2050 pats. were evaluated retrospectively, 91.6 % of the pats. were in substitution treatment with either d-/l-methadone or l-methadone (70.1%) or buprenorphine (29.9%).; see Tab. 2. **Sample collection:** OF spls. were collected using the Greiner-Bio-One (GBO) SCS pH 4.2 device according to the manufacturer. **OF concentration [%]** of the OF/SES mixture was quantified on an Olympus AU680 using the GBO saliva quantification kit. **Multi-target-drug screening** (see Tab. 1) including Cortisol quantification was performed on a Waters Acquity/Xevo™ TQ-S UPLC-MS/MS. Separation was within 6 min linear gradient elution (MoP A = 20 mM ammonium formate + 0.1% formic acid ad pH 3, MoP B = MeOH + 0.1% formic acid) on a BEH Phenyl 1.7 µm, 2.1 x 100 mm column (Waters) kept at 55°C with a flow rate of 0.55 mL/min. The system was operated in ESI+ and SRM mode with 3 transitions monitored per analyte and 2 transitions monitored for internal standards (IS). Capillary voltage was set to 0.2 kV, ion source temperature was 150 °C, and desolvation gas was heated to 650 °C and delivered at a flow rate of 1000 L/h. Cone gas (N_2) was set to 150 L/h and the collision gas (Ar) was maintained at 0.17 mL/min. Matrix calibration (50% artificial OF/SES, GBO) was performed for every analyte at 0.025, 0.050, 0.075, 0.100, 0.125, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 2.0, 2.5, 5.0, 10.0 and 20 ng/mL. **Sample preparation:** 100µL OF/SES was fortified with 10 µL IS (54 deuterated standards, 5 ng/mL each in MeOH). Flurazepam and BDB were refered to Desalkylflurazepam-d4 and MBDB-d5 resp.. The spls. were precipitated with 50µL sulfosalicylic acid (20%) and subsequently 600 µL ACN. Phase separation was achieved by the addition of 100 µL of 10 M ammonium acetate. After centrifugation 550 uL of the organic supernatant was transfered into a 1000 µL glas vial, fortified with 10 µL ethylene glycol and evaporated to a residual volume of 10 µL at 45 °C with N_2 . The concentrate was dissolved with 90 µL 10% methanol and 5 µL was injected into the UPLC system. **QC:** a lyophilized QC spl. was prepared from drug-free pooled OF/SES (OF 60%) by spiking the 55 target analytes ad 0.6 ng/mL OF/SES each (corresponding to 1 ng/mL in neat OF = cutoff conc.) For Amylase and Cortisol the endogenous values were taken.

Conclusion

-- The positive prevalence rates for "Opioids" and "Miscellaneous" (esp. Pregabalin) demonstrated the requirement of including these substances into our routine OF screening method.

-- The CO for Ketamine and Fentanyl should be lowered to 0.1 ng/mL. This could be necessary for some Benzodiazepines also.

-- Increasing the CO from 1 to 10 ng/mL resulted in reduction of the positive rates between 0.3% (Methadone) to 100% (eg.: 7-Aminoflunitrazepam).

-- No false negative results were observed for EDDP and Norbuprenorphine at the 0.1ng/mL CO.

Results

Tab. 1 **Target analytes in OF sorted by substance class**

preliminary Cutoff 1 ng/mL neat OF, IS = 0.5 ng/mL OF/SES

- **Authenticity marker:** Cortisol
- **Substitution drugs (n = 4):** D-/L-Methadone, EDDP Buprenorphine, Norbuprenorphine
- **Amphetamines (n = 11):** Amphetamine, Methamphetamine, MDMA, MDA, MBDB, BDB, MDEA, Butylone, Mephedrone, Methylone, MDPV
- **Benzodiazepines (n = 13):** Diazepam, Nordiazepam, Oxazepam, Midazolam, Desalkylflurazepam, Flurazepam, Temazepam, 7-Aminoclonazepam Alprazolam, Flunitrazepam, 7-Aminoflunitrazepam, Bromazepam, Lorazepam
- **Cocaine (n = 3):** Cocaine, Benzoylecgonine, Lidocaine
- **Opiates (n = 6):** Morphine, Codeine, 6-Acetylmorphine, 6-Acetylcodeine, Norcodeine, Dihydrocodeine
- **Opioids (n = 9):** Naloxone, Tilidine, Nortilidine, Hydromorphone O-D-Tramadol, Oxycodone, Noroxycodone, Fentanyl, Tramadol
- **Cannabinoids (n = 1):** THC
- **Miscellaneous (n = 8):** Zolpidem, Zopiclone, Zaleplon, Ketamine, Methylphenidate, Ritalinic acid, Pregabalin, Gabapentin
- **56 analytes (3 transitions) + 54 deuterated IS (2 transitions)**
- **"Peri-analytics":** sample volume, % OF in SES
  2nd authenticity marker: **Amylase** (enzymatic ; Olympus AU680)

Tab. 2 **Patients and samples**

Samples: 5355

from pats. in maintenance therapy:	4954 spls. = 92.5% of all spls.
from Methadone/Polamidone™ pats.:	3671 spls. = 68.5% of all spls.
from Buprenorphine pats.:	1283 spls. = 24.0% of all spls.

Patients: 2050

male: 1455 (71.0%), female: 595 (29.0%)

in maintenance therapy:	1877 pats. = 91.6% of all pats.
male:	1347 pats. = 65.7% of all pats.
female:	530 pats. = 25.9% of all pats.
Methadone/Polamidone™ pats.:	1315 pats. = 64.1% of all pats.

male: 924 (63.5%), female: 391 (36.5%)

Buprenorphine pats.:	562 pats. = 27.5% of all pats.

male: 423 (75.3%), female: 139 (24.7%)

Tab. 3 **Opiates :**

CO 1 ng/mL: 610 pos. samples = 11.4% CO 10 ng/mL: 397 pos. samples = 7.4%

a sample was defined positive when at least one analyte was >= CO

Positive samples rate reduced by 34.9%

No. of spls.	Analytes >= CO 1 ng/mL	No. of spls.	Analytes >= CO 10 ng/mL	reduced by
597	Morphine	376	Morphine	37.0%
494	6-Acetylmorphine	237	6-Acetylmorphine	52.0%
396	Codeine	217	Codeine	45.2%
173	6-Acetylcodeine	100	6-Acetylcodeine	42.2%
129	Norcodeine	10	Norcodeine	92.2%
11	Dihydrocodeine	6	Dihydrocodeine	45.2%
81.0%	of all Opiate positive samples contained 6-Acetylmorphine thus proving Heroin abuse.	60.0%	of all Opiate positive samples contained 6-Acetylmorphine thus proving Heroin abuse.	
34.7%	of all 6-Acetylmorphine positive samples contained 6-Acetylcodeine thus proving "Street Heroin" abuse.	42.2%	of all 6-Acetylmorphine positive samples contained 6-Acetylcodeine thus proving "Street Heroin" abuse.	

Tab. 4 **Benzodiazepines :**

CO 1 ng/mL: 731 pos. samples = 13.7% CO 10 ng/mL: 415 pos. samples = 7.7%

a sample was defined positive when at least one analyte was >= CO

Positive samples rate reduced by 43.2%

No. of spls.	Analytes >= CO 1 ng/mL	No. of spls.	Analytes >= CO 10 ng/mL	reduced by
663	Nordiazepam	336	Nordiazepam	49.3%
536	Diazepam	239	Diazepam	55.4%
343	Oxazepam	51	Oxazepam	85.1%
182	Temazepam	17	Temazepam	90.7%
38	Lorazepam	18	Lorazepam	52.6%
32	7-Aminoclonazepam	17	7-Aminoclonazepam	46.9%
30	Bromazepam	24	Bromazepam	20.0%
12	Alprazolam	5	Alprazolam	58.3%
5	7-Aminoflunitrazepam	0	7-Aminoflunitrazepam	100.0%
1	Midazolam	0	Midazolam	100.0%

Most of the positive samples are related to Diazepam ingestion. Because of its elimination half-life (~100 h) and its better OF/plasma-ratio when compared with the other Diazepam metabolites, Nordiazepam determines the positive sample rate. Nordiazepam is the target analyte in OF to detect Diazepam consumption. The Lorazepam cutoff should perhaps be lowered. For the other Benzodiazepines more data are needed.

Tab. 5 **Amphetamines:**

CO 1 ng/mL: 487 pos. samples = 9.1% CO 10 ng/mL: 349 pos. samples = 6.5%

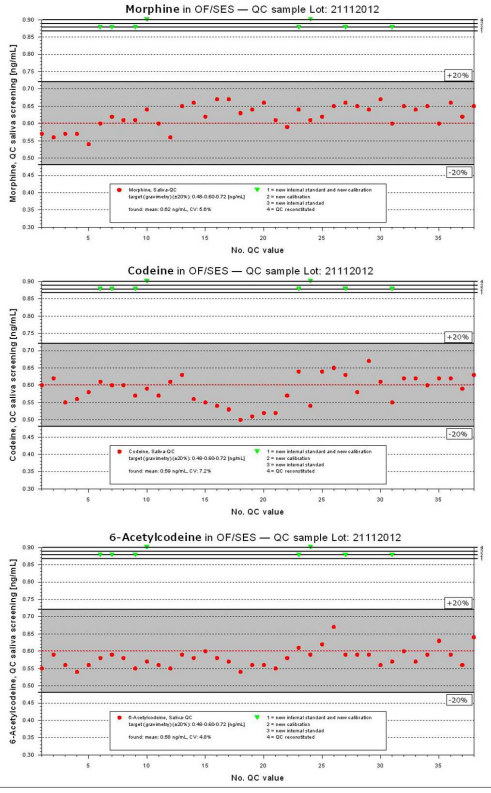
a sample was defined positive when at least one analyte was >= CO

Positive samples rate reduced by 28.3%

No. of spls.	Analytes >= CO 1 ng/mL	No. of spls.	Analytes >= CO 10 ng/mL	reduced by
415	Amphetamine	278	Amphetamine	33.0%
276	Methamphetamine	202	Methamphetamine	26.8%
34	MDMA	16	MDMA	52.9%
21	MDPV	13	MDPV	61.9%
15	MDA	8	MDA	46.7%
7	Mephedrone	4	Mephedrone	42.9%
1	Methylone	1	Methylone	0.0%
1	Butylone	0	Butylone	100.0%

The cutoffs for MDMA and MDPV should perhaps be lowered.
For Mephedrone, Methylone and Butylone more data are needed.
MBDB, BDB and MDEA seems to be without relevance in the investigated patient population.

Fig. 1 **Cutoff QC: QC-chart examples**



Tab. 6 **Miscellaneous:**

CO 1 ng/mL: 294 pos. samples = 5.5% CO 10 ng/mL: 204 pos. samples = 3.8%

No. of spls.	Analytes > CO 1 ng/mL	No. of spls.	Analytes > CO 10 ng/mL	reduced by
136	Pregabalin	116	Pregabalin	14.7%
90	Methylphenidate	46	Methylphenidate	48.9%
88	Ritalinic acid	30	Ritalinic acid	65.9%
33	Zopiclone	25	Zopiclone	24.2%
32	Ketamine	12	Ketamine	62.5%
21	Gabapentin	13	Gabapentin	38.1%
6	Zolpidem	2	Zolpidem	66.6%

Pregabalin cutoff at 1 ng/mL seems to be sufficient.
Methylphenidate itself is the target analyte in OF.
The Ketamine cutoff should perhaps be lowered. For the other substances more data are needed.
Due to the acidic collection buffer Zopiclone is stable and therefore the target analyte.

Tab. 7 **Cocaine/Benzoylecgonine :**

CO 1 ng/mL: 339 pos. samples = 6.3% CO 10 ng/mL: 197 pos. samples = 3.7%

a sample was defined positive when at least one analyte was >= CO

Positive samples rate reduced by 41.9%

No. of spls.	Analytes >= CO 1 ng/mL	No. of spls.	Analytes >= CO 10 ng/mL	reduced by
331	Cocaine	123	Cocaine	62.8%
287	Benzoylecgonine	177	Benzoylecgonine	38.3%
76 (28)	Lidocaine without Cocaine/Benzoylecgonine		Detector linearity ends at 3 ng/mL, thus no evaluation was performed for Lidocaine.	

Tab. 8 **THC :**

CO 1 ng/mL: 1399 pos. samples = 26.1% CO 10 ng/mL: 871pos. samples = 16.3%

No. of spls.	Analytes >= CO 1 ng/mL	No. of spls.	Analytes >= CO 10 ng/mL	reduced by
1399	THC	871	THC	37.7%

Tab. 9 **Opioids :**

CO 1 ng/mL: 231 pos. samples = 4.3% CO 10 ng/mL: 133 pos. samples = 2.5%

No. of spls.	Analytes >= CO 1 ng/mL	No. of spls.	Analytes >= CO 10 ng/mL	reduced by
131	Naloxone	58	Naloxone	55.7%
51	Tramadol	44	Tramadol	13.7%
45	O-D-Tramadol	32	O-D-Tramadol	28.9%
39	Fentanyl	25	Fentanyl	38.5%
18	Oxycodone	13	Oxycodone	27.8%
18	Noroxycodone	11	Noroxycodone	38.9%

High positive rate for Naloxone is mostly due to the prescription of Suboxone™.

CO 1 ng/mL: 19 pos. samples = 0.4% CO 5 ng/mL: 11 pos. samples = 0.2%

No. of spls.	Analytes >= CO 1 ng/mL	No. of spls.	Analytes >= CO 5 ng/mL	reduced by
18	Nortilidine	11	Nortilidine	38.9%
16	Tilidine	7	Tilidine	56.3%

Detector linearity for Tilidine and Nortilidine ends at 5 ng/mL, thus separate evaluation was performed for these analytes.

Tab. 10 **Substitution drugs:**

	Cutoff 0.1 ng/mL	Cutoff 1 ng/mL	Cutoff 10 ng/mL
EDDP	3671 (68.5%)	3031 (56.6%)	698 (13.0%)
pos. rate reduced		17.4%	81.0%
Methadone		3671 (68.5%)	3660 (68.3%)
pos. rate reduced			0.3%
Norbuprenorphine	1283 (24.0%)	822 (15.4%)	44 (0.8%)
pos. rate reduced		35.9%	96.6%
Buprenorphine		1283 (24.0%)	615 (11.5%)
pos. rate reduced			52.0%

In compliance testing unintentional oral contamination (nurse: sampling post dosing) must be differentiated from intentional oral contamination by the patient ("self" dosing prior sampling). Therefore the concentration of substitutes metabolites EDDP and Norbuprenorphine resp. should be "somehow" in agreement to the parent drug concentration. This esp. is of importance at high parent drug concentrations.On the other hand a false negative result for the metabolites could lead to falsely assumed non-compliance of the patient and must be avoided. This is of importance when regarding pats. in low-dose therapy. At the 0.1 ng/mL CO EDDP and Norbuprenorphine will be detected when the patient is in steady-state.