

Monitoring drug abuse of patients in substitution therapy: comparison of UPLC-MS/MS screening in oral fluid and urine testing with immunoassay

Michael Böttcher, Stefan Lierheimer

MVZ für Mikrobiologie, Labordiagnostik und Hygiene Dessau GmbH, Dessau, Germany

Introduction

Screening for drugs of abuse with immunoassays in urine samples from patients in heroin substitution treatment can be regarded as standard practice. Oral fluid (OF) gains increasing interest in drugs of abuse testing of these patients and within other settings. The ease of non-invasive sampling under close supervision decreases the chances for adulteration or substitution of the sample by the patient. However, little is known about the required sensitivity of the possible screening methods to reach comparable positive rates or so to speak similar detection times. We therefore developed a sensitive multi-component UPLC-MS/MS method for OF screening and compared the positive rates to standard urine testing of substituted outpatients from the Berlin area for a three month period. This OF multi-target-method furthermore screened for additional analytes which could be of abuse relevance and are normally not part of our standard urine screening. 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 patients are tested. The absence of detergents which could interfere in our chromatographic method or in the sample preparation procedures is another advantage of this collection system.

Methods

Patient samples: routine urine and OF sampels from 2 patient groups were compared ; see Tab.1. Sample collection: OF samples were collected using the Greiner-Bio-One (GBO) SCS pH 4.2 device according to the manufacturer (see Fig.4). % OF concentration of the OF/SES mixture was quantified on an Olympus AU680 using the GBO saliva quantification kit. Urine drug screening was conducted on an Olympus AU680 with immunoassays from ThermoFisher according to the manufacturer (cutoffs see Tab.1). Multi-target-drug screening (n = 49) including Cortisol quantification was performed from an alkaline OF/SES extract on a Waters Acquity/Xevo UPLC-MS/MS. Separation was within 5min gradient elution (MoP A = 5 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 50 °C with a flow rate of 0.4mL/min. The system was operated in ESI+ and SRM mode with at least 2 transitions monitored per analyte (Fig.1). Capillary voltage was set to 0.6 kV, ion source temperature was 150 ℃, and desolvation gas was heated to 600 °C and delivered at a flow rate of 850 L/h. Cone gas (N₂) was set to 20 L/h and the collision gas (Ar) was maintained at 0.25 mL/min. Matrix calibration was performed for every analyte at 0.25, 0.5, 1.0, 2.0, 5.0, 10.0 and 20 ng/mL (compare Fig.3) from spiked 50% SES/arteficial saliva (GBO). Extraction ("stationary LLE"): 375µL OF/SES was fortified with 7.5µL IS solution (45 analytes at 25ng/mL, equals 0.5ng/mL spl.) and 37.5µL ammonia (32%) and then transferred to a MTPL with 0.45µm glass fiber filters (Phenomenex) and ~235mg CHEM TUBE-HYDROMATRIX (Agilent) per cavity. Incubation time was 10min. Sequential elution into glass vials containing 10µL ethylene glycol was done with 4x0.2mL ethyl acetate/heptane and 3x0.2mL methylene chloride. Eluates were evaporated to dryness at 45 °C with N₂ and then re-dissolved with 75µL MeOH/water/ammonia (50/48/2). Injection volume was 10µL

Conclusion

- -- The positive rates for OF and urine were comparable at the selected cutoffs suggesting that OF is of equal value. The positive rate for **Amphetamines** is higher in OF.
- -- The cutoff for Buprenorphine in OF at 0.1 ng/mL needs further evaluation
- -- 6-AM positive rate in OF was high: 76% of all Opiates positives. Interestingly, 6-Acetylcodeine ("street heroin" marker) could be detected in 27% of Opiates positive OF samples.
- -- Opiates, Benzodiazepines and surprisingly Amphetamines revealed the highest positive rates.

UPLC-MS/MS method

Fig.1 RT windows (functions) for analytes and their transitions

rotarnu	ın Tim	e: 5.00	↔								Í		4
No. T	[ype					Info	ormation		I			Time	
1		MRM of 2 MRM of 2	mass pairs mass nairs	, Time () Time ()).70 to 1) 70 to 1	.02, ES-	+ (Morphin) + (Morphin-d6)			286.2 > 165	5.2 , > 201.1 8.0 . > 152.2		
	Ż	MRM of 2	mass pairs	, Time 1	.02 to 1	.25, ES-	+ (2-Amino-5-Chl	oropyridin (Zopi	clon-Metabolit))	129.0 :	> 93.1 , > 112.0)	
i I	Z	MRM of 2 I	mass pairs	, Time 1	.02 to 1	.33, ES-	+ (Norcodein)			286.	1 > 165.0 , > 2	25.0	
		MRM of 21 MRM of 21	mass pairs mass nairs	, Time 1 Time 1	1.02 to 1 1.02 to 1	.33, ES-	+ (Naloxon) + (Naloxon-d5)			328.	1 > 221.1 , > 20 2 > 128.0 . > 2	84.1 73.1	
		MRM of 21	mass pairs	, Time 1	1.02 to 1	.45, ES	+ (Codein-d6)			30	6.2 > 165.0 , >	218.2	
	Z	MRM of 2 i	mass pairs	, Time 1	.15 to 1	.45, ES-	+ (Dihydrocodein	-d6)		30	8.2 > 174.1 , >	202.1	
	K	MRM of 21 MPM of 21	mass pairs	, Time 1	1.15 to 1	.45, ES-	+ (Codein) • (Dibydrocodoin)	、 、		30	10.1 > 165.4 , > 12 2 > 171 1 - >	215.1	
		MRM of 21	mass pairs	, Time 1 . Time 1	1.15 to 1	.40, E8-	+ (Methvlon-d3)	,		2	11.2 > 135.2 , >	> 163.2	
İ		MRM of 2	mass pairs	Time 1	.20 to 1	.50, ES-	+ (Methylon)			20	08.0 > 117.1 , >	> 132.1	
	<u>Ľ</u>	MRM of 21 MRM of 21	mass pairs	, Time 1	1.25 to 1	.55, ES-	+ (6-MAM) • (Americatomic d	(E)			328.1 > 165.1 ,	> 211.1	
		MRM of 21	mass pairs mass pairs	, Time 1 . Time 1	1.25 to 1	.55, ES-	+ (Amphetamin-u + (6-MAM-d3)	io)			331.2 > 165.1	, > 211.1	
	2	MRM of 3	mass pairs	, Time 1	.25 to 1	.55, ES-	+ (Amphetamin)				136.1 > 65.0 ,	> 91.1	
	4	MRM of 21	mass pairs	, Time 1	1.30 to 1	.60, ES	+ (Oxycodon)				316.1 > 241.0	, > 256.1	
		MRM of 21	mass pairs mass pairs	Time 1	1.30 to 1 1.35 to 1	.60, ES	F (Oxycodon-d3) ⊦ (Methamphetar	nin)			150.0 > 90.9	, > 259.1 . > 118.9	
i	Ż	MRM of 2	mass pairs	Time 1	.35 to 1	.65, ES-	• (Metamphetam	in-d5)			155.1 > 91.7	, > 120.9	
	Z	MRM of 2 i	mass pairs	, Time 1	.35 to 1	.65, ES-	+ (PMA)				166.1 > 77.2	, > 91.0	
		MRM of 21 MRM of 21	mass pairs mass nairs	, Time 1 Time 1	1.35 to 1 1.35 to 1	.65, ES-	F (MDA) F (MDA-d5)				180.1 > 79.4	, > 163.1 0. > 168.1	
		MRM of 21	mass pairs	, Time 1	.40 to 1	.70, ES-	+ (MDMA-d5)				199.2 > 107	.2 , > 165.1	
	Z	MRM of 2	mass pairs	, Time 1	.40 to 1	.70, ES-	+ (MDMA)				194.2 > 105	5.1, > 163.1	
	y	MRM of 21 MPM of 21	mass pairs	, Time 1	1.45 to 1	.75, ES-	+ (Butylon)				222.2 > 140	0.1,> 1/4.2 21.1.> 149.1	
		MRM of 21	mass pairs mass pairs	, nme 1 . Time 1	r.⇔p to 1 I.50 to 1	.75, ES .80, ES	+ (ElwiwA) ⊦ (Butvion-d3)				225.0 > 14	49.1 , > 172.2	
		MRM of 2	mass pairs	, Time 1	1.55 to 1	.85, ES	+ (MDEA-d5)				213.2 > 1	105.1 , > 163.1	
	Ø	MRM of 2 i	mass pairs	, Time 1	.55 to 1	.85, ES	+ (MDEA)				208.1 > 1	104.9 , > 163.0	
		MRM of 21 MRM of 21	mass pairs mass nairs	, Time 1 Time 1	1.55 to 1 1.55 to 1	.85, ES-	+ (Mephearon) + (Menhedron-d3)			1/8.1 > 1	119.1, > 144.9	
	Ż	MRM of 2	mass pairs	Time 1	.70 to 2	2.00, ES-	+ (BDB)	/			194.0	0 > 135.0 , > 176.	7
	Z	MRM of 2 i	mass pairs	, Time 1	1.75 to 2	2.05, ES·	+ (MBDB)				208.	1 > 134.9 , > 176.	.9
		MRM of 21 MRM of 21	mass pairs mass nairs	, Time 1 Time 1	1.75 to 2 1.80 to 2	2.05, ES [.] 210 ES:	+ (MBDB-05) + (Ketamin-d4)				213	2.1 > 128.9 . > 18	3.1
	7	MRM of 21	mass pairs	, Time 1	1.80 to 2	2.10, ES·	• (Ketamin)				238	8.1 > 125.0 , > 179	Э.1
	Z	MRM of 2 i	mass pairs	, Time 1	.85 to 2	2.15, ES·	+ (Benzoylecgoni	n)			29	0.1 > 104.9 , > 16	8.0
	les -	MRM of 21 MPM of 21	mass pairs	, Time 1 Time 1	l.85 to 2 ⊢02 to 2	2.15, ES·	+ (Benzoylecgoni • (Mothylphopida)	n-d3) + d0)			29)3.1 > 104.9 , > 17	1.0
		MRM of 21	mass pairs	, Time 1 , Time 1	1.93 to 2	2.23, ES·	• (Methylphenida)	t)			2	34.2 > 56.1 , > 84	.1
		MRM of 2 i	mass pairs	, Time 2	2.00 to 2	2.30, ES·	+ (cis-Tramadol)					264.2 > 58.1 , > 9	91.1
	<u>Ľ</u>	MRM of 21 MRM of 21	mass pairs	, Time 2	2.00 to 2	2.30, ES·	+ (Tramadol-13C	-d3)				268.2 > 58.1 , > 1	25.1
		MRM of 21	mass pairs mass pairs	, Time 2 . Time 2	2.00 to 2	2.30, ES· 2.30, ES·	+ (7 amino-Ciona + (7 amino-Ciona	azeparn) azeparn-d4)				290.1 > 121.1 , >	226.1
	2	MRM of 2	mass pairs	, Time 2	2.05 to 2	2.35, ES-	+ (6-Acetylcodein))				342.2 > 165.1 , >	225.2
	4	MRM of 21 MDM of 21	mass pairs	, Time 2	2.05 to 2	2.35, ES·	+ (6-Acetylcodein - (Kokoin d2)	-d3)				345.2 > 152.2 , >	197.1 185.1
		MRM of 21	mass pairs mass pairs	, Time 2 . Time 2	2.10 to 2 2.10 to 2	2.40, ES· 2.40, ES·	+ (Kokain-03) + (Kokain)				_	304.1 > 82.1 . >	182.1
	2	MRM of 2	mass pairs	, Time 2	2.25 to 2	2.55, ES-	+ (Tilidin-d6)					280.0 > 154.9	9, > 228.9
	4	MRM of 21 MDM of 21	mass pairs	, Time 2	2.25 to 2	2.55, ES·	+ (Tilidin)					274.2 > 77.0	, > 155.1
		MRM of 21 MRM of 21	mass pairs mass pairs	, Time 2 . Time 2	2.27 to 2 2.35 to 2	2.58, ES [.] 2.65, ES [.]	+ (Zopicion) + (7 amino-Flunit	razepam-d7)				389.1 > 216	.9, > 244.9 2.0, > 345.8
i	Z	MRM of 2	mass pairs	, Time 2	2.35 to 2	2.65, ES·	+ (7 amino-Flunit	razepam)				314.1 > 19	7.9 , > 239.1
	4	MRM of 21	mass pairs	, Time 2	2.40 to 2	2.70, ES·	+ (Zolpidem-d6)					314.3 > 92	2.2, > 235.2
		MRM of 21	mass pairs mass pairs	, Time 2 . Time 2	2.40 to 2 2.60 to 2	2.70, ES· 2.90, ES·	+ (Zoipiderri) + (Norbuprenorpi	hine)				414.3	2.1, > 233.1 3 > 83.1 . > 101.2
		MRM of 2	mass pairs	, Time 2	2.60 to 2	2.90, ES·	+ (Norbuprenorph	hine-d3)				417.3	> 83.1 , > 101.1
	y	MRM of 21 MPM of 21	mass pairs	, Time 2 Time 2	2.85 to 3	3.15, ES·	+ (Fentanyl) • (Fentanyl dE)					3	37.2 > 105.1 , > 188.2
		MRM of 21	mass pairs	, Time 2 . Time 3	2.05 to 3 3.05 to 3	3.35. ES·	+ (Pentanyi-us) + (Buprenorphin)						468.3 > 101.1 . > 396.2
	Z	MRM of 2	mass pairs	, Time 3	3.05 to 3	3.35, ES-	+ (Buprenorphin-	d4)					472.3 > 59.1 , > 400.2
	y	MRM of 21	mass pairs	, Time 3	3.10 to 3	3.40, ES-	+ (Flurazepam)						388.2 > 134.1 , > 315.
		MRM of 21 MRM of 21	mass pairs mass naire	, rime 3 . Time 3	5.15 to 3 3.15 to 3).40, ES [.] 3.45 ES [.]	r (imidazolam) ⊦ (Midazolam-d4)	1					330.2 > 223.1 , > 291
	Ż	MRM of 21	mass pairs	, Time 3	3.33 to 3	3.63, ES·	• (Bromazepam)	, ,					316.0 > 182.1 , >
	Z	MRM of 2 i	mass pairs	, Time 3	3.33 to 3	3.63, ES-	+ (Bromazepam-	d4)					319.9 > 186.1 , >
		MRM of 21 MRM of 21	mass pairs mass pairs	, Time 3 Time 3	3.35 to 3 2.35 to 3	8.65, ES· 2 66 ES·	+ (Cortisol) • (Cortisol-d4)						363.2 > 105.1 , >
		MRM of 21	mass pairs	, Time 3 . Time 3	3.35 to 3	3.65, ES-	+ (Zalepion-d5)						311.2 > 237.1 , >
		MRM of 2 i	mass pairs	, Time 3	3.35 to 3	3.65, ES-	+ (Zaleplon)						306.2 > 236.1 , >
	g	MRM of 2 I	mass pairs	, Time 3	3.40 to 3	3.70, ES-	+ (alpha-OH-Mida	azolam)					342.2 > 168.1 , :
		MRM of 21 MRM of 21	mass pairs mass nairs	, Time 3 Time 3	3.40 to 3 3.55 to 3	3.70,ES [.] 885 ES:	⊦ (alpha-OH-Mida ⊦ (Methadon-d9)	azolam-d4)					346.0 > 168.0 , >
	Ż	MRM of 3	mass pairs	, Time 3	3.55 to 3	3.85, ES·	• (Methadon)						310.2 > 57.1
		MRM of 2	mass pairs	, Time 3	3.58 to 3	3.88, ES-	+ (Clonazepám)						316.0 > 213.9
	y	MRM of 21 MRM of 21	mass pairs	, Time 3 Time 2	3.58 to 3	3.88, ES·	+ (Clonazepam-d	(4) (14)					320.0 > 182.2
		MRM of 21	mass pairs mass pairs	, Time 3 . Time 3	3.70 to 4	1.00, ES-	• (alpha-OH-Alpr: • (alpha-OH-Alpr:	azolarn) azolam-d5)					330.2 > 22
i	2	MRM of 2	mass pairs	, Time 3	3.70 to 4	1.00, ES·	+ (Lorazepam-d4)					325.2 > 19
	4	MRM of 21	mass pairs	, Time 3	8.70 to 4	1.00, ES-	• (Flunitrazepam))					314.1 > 19
		MRM of 21	mass pairs mass naire	, Time 3 . Time 3	5.70 tO 4 3.70 to 4	1.00, ES [.] 1.00, ES [.]	• (Eurazepam) • (Flunitrazenam-	-d7)					320.9 > 10
		MRM of 2	mass pairs	, Time 3	3.75 to 4	1.05, ES-	+ (Oxazepam-d5)	,					292.2 > 1
į		MRM of 2	mass pairs	, Time 3	3.75 to 4	1.05, ES-	+ (2-OH-Ethyl-Flu	razepam-d4)					337.1 > 1
	K	MRM of 21 MRM of 21	mass pairs	, Time 3 Time 2	3.75 to 4 3.75 to 4	1.05, ES-	+ (Oxazepam) + (2-OHLEther Eth	razanam)					287.1 > 1
		MRM of 21	mass pairs mass pairs	, Time 3 , Time 3	3.85 to 4	.00, E8 .15, E8-	• (Alprazolam-d5	талерани)					314.1 >
İ		MRM of 2	mass pairs	, Time 3	3.85 to 4	.15, ES-	+ (Alprazolam)	<i>.</i>					309.0 >
ļ	Ø	MRM of 2 i	mass pairs	, Time 3	3.95 to 4	.25, ES	• (Temazepam-d	5)					306.1
	les al al al al al al al al al al al al al	MRM of 21 MRM of 21	mass pairs	, Time 3 Time 4	3.95 to 4 1.00 to 4	1.25, ES∙ L30 ⊑⊝	+ (Temazepam) + (Nordistonam)						271
	7	MRM of 21	mass pairs	, Time 4	1.00 to 4	1.30, ES-	+ (Nordiazepam-	d5)					276.
į		MRM of 2	mass pairs	, Time 4	4.20 to 4	1.50, ES-	+ (Diazepam)	r.					
	y	MRM of 21 MPM of 21	mass pairs	, Time 4	1.20 to 4	1.50, ES-	+ (Diazepam-d5) המשמה אמצ						
	1000	WIRKIW OT Z I	mass pairs	, inne 4	+.00105	1.00, ES.	r (THC-03)						

Resuits

Tab.1 Study conditions / patients population

-- three month observation period

-- urine cutoffs: Amphs 500 ng/mL, Benzos (enzym. hydrolysis) 100 ng/mL, Coca 50 ng/mL, Opi 100 ng/mL, EDDP 100 ng/mL, Bupre 2 ng/mL, THC-COOH 25 ng/mL.

-- saliva cutoffs: 1 ng/mL (neat OF)

Tab.2 Positive rates OF compared to urine samples

-- Patients from:

- **1.** an outpatient clinic (**OPC**) where the drug testing was stepwise moved from urine to OF:
- 194 patients (26 Bupre, 67 Metha, 101 Pola), 902 OF spls.
- 182 patients (25 Bupre, 66 Metha, 91 Pola), 1119 urine spls.
- **2.** other outpatient clinics (**ALL**) with more random selection between the two matrices:
 - 612 patients from 23 clinics (116 Bupre, 265 Metha, 231 Pola), 1072 OF spls.
 - 1463 patients from 40 clinics (285 Bupre, 673 Metha, 505 Pola), 9008 urine spls.

xpected.

therapy

										-					
				OPC (OPC OPC			ALL			ALL		
	saliva urine		าย	urine	saliva		urine		urine						
	% pos. spls.		ls.	% pos. spls.		no. of spls.	% pos. spls.		% pos. spls.		no. of spls.				
Ampheta	9.3			3.3		1082	10.3		4.1		7396				
Benzodia	11.0			14.4		958	25.7		22.4		6891				
Coca	5.2			3.9		1075	9.8		7.2		8295				
					12.5		069	176		01.7		6077			
Opia	Oplates				13.3		900 17		0.1	21.7		09//			
Methadon EDDP	86.6			85.2		953	85.4		88.0		8938				
TH	26.9			-		-	30	30.5		31.3		598			
Opio	1.2			_		-		2.1		-		_			
Othe	0.8			-		-		1.4		_		_		Methadone/EDDP was positive	
Bupreno	12.3			_		-	16.9		73.1		640		However, Buprenorphine was negative in 8 OF samples from		
	n	= 902					n = 1072							2 OPC pats. in low dose therapy (0.4 and 1.0 mg/d). Cutoff <0.1 ng/mL?	
	OPC	OPC	ALL	ALI	L					OF	PC	OPC	ALL	ALL	
	saliva	saliva	saliva	saliv	/a					sal	iva	saliva	saliva	saliva	
	% pos. spis. %	from pos.	% pos. spis.	% from	pos.				Onistas	% pos	. spis.	% from pos	. % pos. spis.	% from pos.	
Amphetamines	9.3	100	10.3	100					Morphine			98.4	17.0	95.8	
Amphetamine	8.9	95.2	9.4	91.8	8					$\frac{10}{10}$		90.4 76.2	12.4	95.0 76.0	
Methamphetamine	1.4	15.5	3.0	29.	1				Codoino		0.0 0	60.7	10.4	70.2	
MDMA	1.0	10.7	0.9	9.1							-2	00.7	12.0	72.0	
MDA	0.8	8.3	0.4	3.6	6				6-Acetyicode	eine 3.	.7	27.4	4.8	27.3	
PMA	0.1	1.2	-	-		←	Tah ? —		INorcodeir	ie 2.	8	20.5	4.1	23.3	
PMMA	0.1	1.2	-	-					Methadone s	saliva	.1	0.8	0.1	0.5	
Benzodiazepines	11.0	100	25.7	100	\mathbf{D}_{1}	etai	iled resu	ults	EDDP urin	ne 86	5.6	100	85.4	100	
Diazonom	7.0	95.9 65.7	17.0	60	F F		_	-	THC	26	6.9	100	30.5	100	
Diazepain	7.2	00.7	17.0	09.)r ()	F samn	les	Opioids	; <u>1</u> .	.2	100	2.1	100	
	3.8	34.3	7.9	30.	9				Fentany	-		-	0.5	21.7	
Iemazepam	3.3	30.3	4.7	18.	2				Tramado		.8	63.6	0.8	34.8	
7-Aminoflunitrazepam	0.4	4.0	3.5	13.	5				Tilidine	0	2	18.2	0.4	17.4	
Clonazepam	0.2	2.0	-	-					Nalovon		2	18.2	0.4	17 /	
7-Aminoclonazepam	0.2	2.0	-	-									0.4	۲ <i>.</i> .+	
Flunitrazepam	0.2	2.0	1.8	6.9)						Q	-	1.4	100	
Bromazepam	0.1	1.0	0.9	3.6	3				Utners		.0		1.4	100	
Alprazolam	-	-	0.3	1.1							./	85./	0.6	40.0	
Lorazepam	0.1	1.0	1.0	4.0)					e 0.	.1	14.3	0.4	26.7	
Cacaina	5.2	100	0.0	10/					Zolpidem	1 -		-	0.3	20.0	
	5.2		9.8	100	5				Methylpheni	date -	-	-	0.2	13.3	
I Benzoylecgonine	5.0	95./	8.8	89.	5				Duproporal		0	100	16.0	100	

Fig.2 Target analytes in OF sorted by substance class

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

- Peri-analytics: sample volume, % OF in SES, Amylase, Cortisol
- Substitution drugs (n = 2): D-/L-Methadone, Buprenorphine (0.1 ng/mL)
- Amphetamines (n = 12): Amphetamine, Methamphetamine, MDMA, MDA, MBDB, BDB, MDEA, PMA, PMMA, Butylone, Mephedrone, Methylone
- Benzodiazepines (n = 16): Diazepam, Nordiazepam, Oxazepam, Midazolam, 1-OH-Midazolam, 2-OH-Ethylflurazepam, Flurazepam, Temazepam, Clonazepam, 7-Aminoclonazepam, Alprazolam, 1-OH-Alprazolam, Flunitrazepam, 7-Aminoflunitrazepam, Bromazepam, Lorazepam
- Cocaine (n = 2): Cocaine, Benzoylecgonine
- Opiates (n = 6): Morphine, Codeine, 6-Acetylmorphine, 6-Acetylcodeine, Norcodeine, Dihydrocodeine
- Opioids (n = 5): Naloxone, Tilidine, Tramadol, Oxycodone, Fentanyl
- Cannabinoids (n = 1): THC
- Others (n = 5): Zolpidem, Zopiclone (via ACP), Zaleplon, Ketamine, Methylphenidate

Fig. 4 Saliva Collection System (SCS) pH 4.2

8.6

87.6

74.5

3.9

Cocaine



(1) rinsing oral cavity with Saliva Extraction Solution (SES) for 2 minutes spitting OF/SES into beaker (2)transfer OF/SES into evacuated tubes

16.9

100

n = 49 + 45 deuterated IS

Fig.3 6-Acetylmorphine: example for matrix calibration

calibration at: 0.25, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0 ng/mL in 50% artificial OF/SES

curve: 1.68457 * x + 0.19256 nal Std (Ref 36), Area * (IS Conc. / IS Area





0.25 ng/mL 2 channels,ES+ 328.10 > 165.09 50% artef. OF/SES

316.0 > 182.1 . > 260 319.9 > 186.1 , > 264.9 363.2 > 105.1 , > 121 367.2 > 121.1 . > 331

342.2 > 168.1 . > 203346.0 > 168.0 , > 202.9 319.3 > 104.9 , > 268.3 **310.2** > 57.1 , > 104.9 , > 265.1

316.0 > 213.9 , > 241.0 320.0 > 182.2 , > 217.9

325.1 > 216.1 , > 297.0 330.2 > 221.1 . > 284.

325.2 > 198.2 . > 233.2

314.1 > 197.9 , > 239.1 320.9 > 101.9 , > 162.9

321.1 > 122.0 , > 245.8 292.2 > 100.0 , > 246.

337.1 > 113.1 . > 215.0 287.1 > 104.1 . > 241. 337.1 > 113.1 . > 215.0 314.1 > 210.0 , > 286.0 309.0 > 205.0 , >281,0 306.1 > 260.1 , > 288.1 301.0 > 255.1 , > 283.2 271.0 > 140.0 , > 165.2 276.2 > 140.1 , > 165.1 285.0 > 153.9 , > 192.9 290.2 > 154.1 , > 198.1

318.2 > 123.1 , > 196. 315.2 > 122.9 , > 193

> (a+b sample) containing bacteriocids and send to lab.

after centrifugation Amylase and OF (4) concentration (Fig.5) are determined on an Olympus AU680.

12.3

Buprenorphine

100

Fig.5 Calibration curve OF concentration

