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

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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℃ 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 ℃ and delivered at a flow rate of 850 L/h. Cone gas (N_2) 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 transfered 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℃ with N_2 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.

Results

UPLC-MS/MS method

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

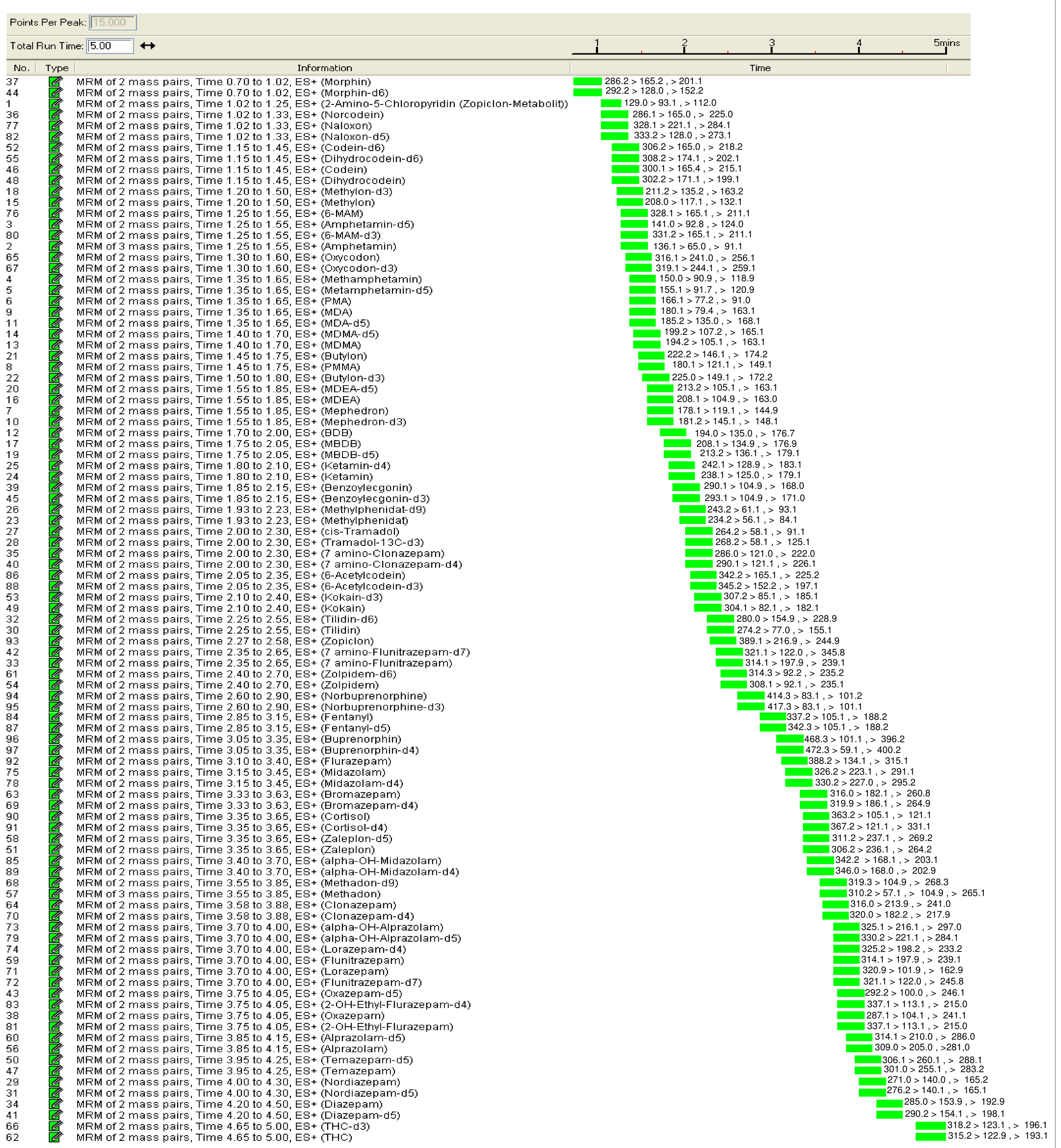


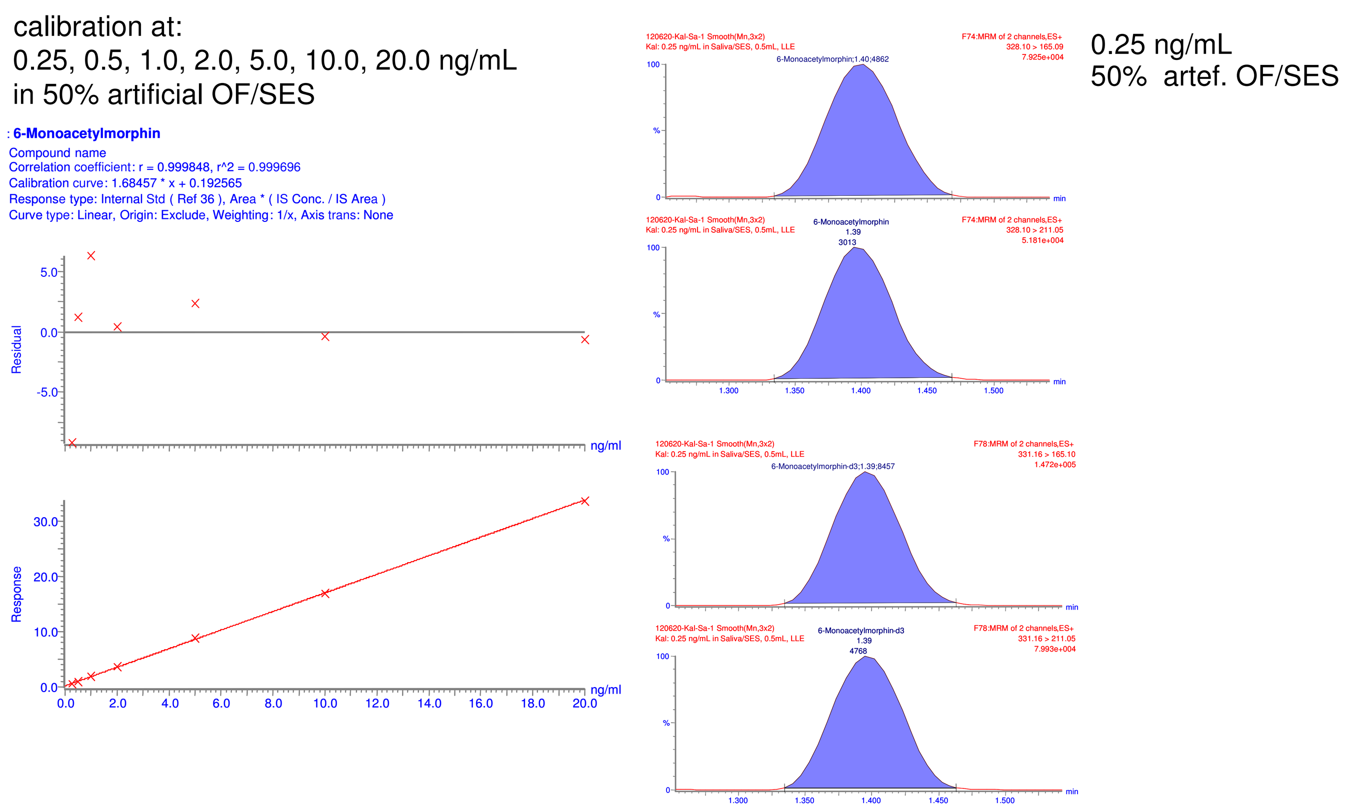
Fig.2 Target analytes in OF sorted by substance class

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-Amino-clonazepam, 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

n = 49 + 45 deuterated IS

Fig.3 6-Acetylmorphine: example for matrix calibration



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)

-- 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.

Tab.2 Positive rates OF compared to urine samples

	OPC	OPC	OPC	ALL	ALL	ALL
	saliva % pos. spls.	urine % pos. spls.	urine no. of spls.	saliva % pos. spls.	urine % pos. spls.	urine no. of spls.
Amphetamines	9.3	3.3	1082	10.3	4.1	7396
Benzodiazepines	11.0	14.4	958	25.7	22.4	6891
Cocaine	5.2	3.9	1075	9.8	7.2	8295
Opiates	13.5	13.5	968	17.6	21.7	6977
Methadone saliva EDDP urine	**86.6**	85.2	953	**85.4**	88.0	8938
THC	26.9	-	-	30.5	31.3	598
Opioids	1.2	-	-	2.1	-	-
Others	0.8	-	-	1.4	-	-
Buprenorphine	**12.3**	-	-	**16.9**	73.1	640
	n = 902			**n = 1072**		

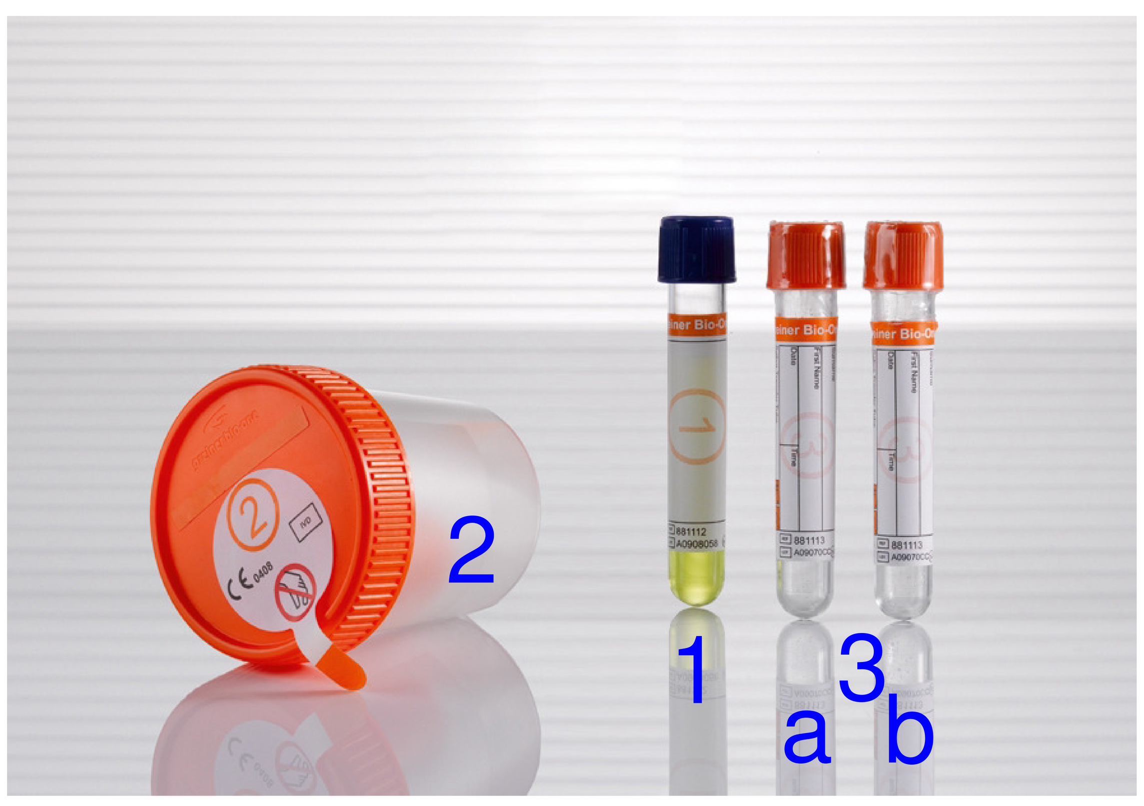
Methadone/EDDP was positive in both matrices where expected. However, **Buprenorphine** was negative in 8 OF samples from 2 OPC pats. in low dose therapy (0.4 and 1.0 mg/d).
Cutoff <0.1 ng/mL?

← Tab.3 → Detailed results for OF samples

	OPC	OPC	ALL	ALL
	saliva % pos. spls.	saliva % from pos.	saliva % pos. spls.	saliva % from pos.
Amphetamines	9.3	100	10.3	100
Amphetamine	8.9	95.2	9.4	91.8
Methamphetamine	1.4	15.5	3.0	29.1
MDMA	1.0	10.7	0.9	9.1
MDA	0.8	8.3	0.4	3.6
PMA	0.1	1.2	-	-
PMMA	0.1	1.2	-	-
Benzodiazepines	11.0	100	25.7	100
Nordiazepam	10.3	93.9	21.8	85.1
Diazepam	7.2	65.7	17.8	69.5
Oxazepam	3.8	34.3	7.9	30.9
Temazepam	3.3	30.3	4.7	18.2
7-Aminoflunitrazepam	0.4	4.0	3.5	13.5
Clonazepam	0.2	2.0	-	-
7-Aminoclonazepam	0.2	2.0	-	-
Flunitrazepam	0.2	2.0	1.8	6.9
Bromazepam	0.1	1.0	0.9	3.6
Alprazolam	-	-	0.3	1.1
Lorazepam	0.1	1.0	1.0	4.0
Cocaine	5.2	100	9.8	100
Benzoylecgonine	5.0	95.7	8.8	89.5
Cocaine	3.9	74.5	8.6	87.6

	OPC	OPC	ALL	ALL
	saliva % pos. spls.	saliva % from pos.	saliva % pos. spls.	saliva % from pos.
Opiates	13.5	100	17.6	100
Morphine	13.3	98.4	16.9	95.8
6-Acetylmorphine	10.3	**76.2**	13.4	**76.2**
Codeine	8.2	60.7	12.8	72.5
6-Acetylcodeine	3.7	**27.4**	4.8	**27.3**
Norcodeine	2.8	20.5	4.1	23.3
Dihydrocodeine	0.1	0.8	0.1	0.5
Methadone saliva **EDDP** urine	86.6	100	85.4	100
THC	26.9	100	30.5	100
Opioids	1.2	100	2.1	100
Fentanyl	-	-	0.5	21.7
Tramadol	0.8	63.6	0.8	34.8
Tilidine	0.2	18.2	0.4	17.4
Naloxone	0.2	18.2	0.4	17.4
Oxycodone	-	-	0.2	8.7
Others	0.8	100	1.4	100
Ketamine	0.7	85.7	0.6	40.0
Zopiclone	0.1	14.3	0.4	26.7
Zolpidem	-	-	0.3	20.0
Methylphenidate	-	-	0.2	13.3
Buprenorphine	12.3	100	16.9	100

Fig. 4 Saliva Collection System (SCS) pH 4.2



(1) rinsing oral cavity with Saliva Extraction Solution (SES) for 2 minutes
(2) spitting OF/SES into beaker
(3) transfer OF/SES into evacuated tubes (a+b sample) containing bacteriocids and send to lab.
(4) after centrifugation Amylase and OF concentration (Fig.5) are determined on an Olympus AU680.

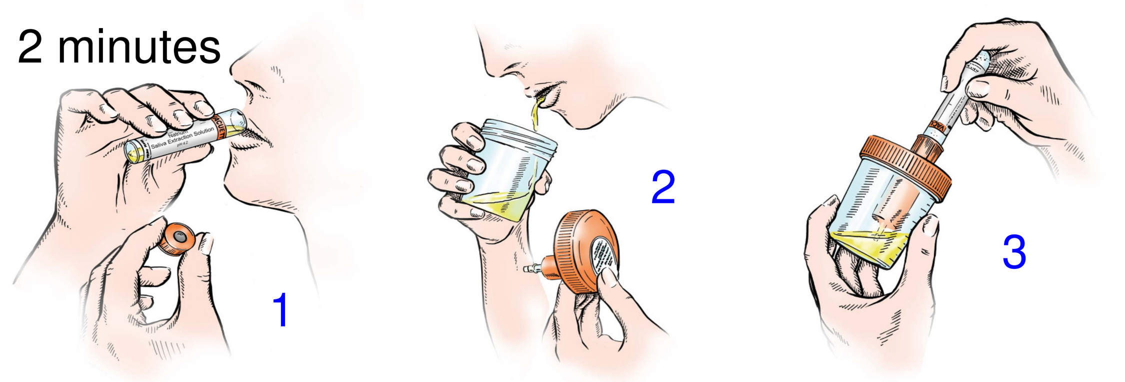


Fig.5 Calibration curve OF concentration