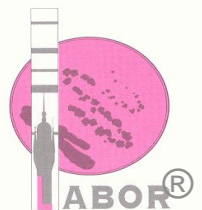# Oral Fluid as an alternative matrix in workplace drug testing: which drugs at which cutoff concentration?

Michael Böttcher

MVZ Labor Dessau GmbH, Dessau, Germany

addiction / abuse relevant substances

drugs of abuse

defined by narcotic law

- Amphetamines / designer-
  drugs
- Heroin
- Cannabinoids / THC
- Cocaine
- LSD
- GHB
- ß-Keto-Amphetamines

-..........

Internet drugs:
Piperazines
„Bath Salts"

„"Legal Highs" on
transit to narcotic law?"

therapeutic drugs

- Methadone
- Buprenorphine
- Dihydrocodeine
- Barbiturates
- Benzodiazepines
- Opioids, Analgesics
- Antidepressive drugs
- Neuroleptics
- Anaesthetics (Propofol, Ketamin)
- Diuretics
- Anabolic steroids

Pregabalin
Zopiclone, Zolpidem,
Zaleplone
Methylphenidate
Lidocain

"Nature drugs"

- Psilocybine
- Meskaline
- "Spice"

- Atropine
- Muskarine
- Myristicine
- Scopolamine
- Kratom/Krypton
- Khat (Cathinon)

Psychedelics

5'-substituted tryptamines
Related to: bufotenin

5-MeO-DMT   5-MeO-DALT
5-MeO-MIPT   5-MeO-MET
5-MeO-DIPT   5-MeO-DPT
5-MeO-AMT
5-MeO-AET

AMT
AET
MIPT
DIPT
DALT
NMT
DET
DPT

4'-substituted tryptamines
Related to: psilocin

4-AcO-DMT   4-HO-DPT
4-AcO-DET   4-HO-DALT
4-HO-MIPT   4-HO-DIPT
4-MES-DMT

NBOMe series
Related to: 2C-x series

25C-NBOMe
25I-NBOMe
25D-NBOMe

PEA

2C-x series
Related to: mescaline

2C-B   2C-D
2C-I   2C-E
2C-T-7 2C-P
2C-B-FLY

Ergolines
Related to: LSD, LSA

ALD-52
LA-SS-Az (LSZ)
PRO-LAD
ETH-LAD

PEA

Psychedelic amphetamines
Related to: 2C-x, amphetamine

DOB   DOM
DOC   DOET
DOI   TMA-2
Bromo-dragonFLY

Stimulants

PEA

Cathinones
Related to: methcathinone, cathinone, amphetamine, MDMA

Mephedrone   Pentedrone
Methylone   Flephedrone
Butylone   bk-PMMA
Benzedrone
4-MEC

Piperazines
Related to: piperazine

BZP   mCPP
MBZP   pFPP
DBZP   MeOPP
MDBZP   TFMPP

5-APB
6-APB
6-ADPB

4-methylaminorex
4-ethylaminorex

Desoxypipradrol

MDAI
MDAT
2-AI
5-IAI

Phenylalkyl-pyrrolidines
Related to: Pyrovalerone, Prolintane

MDPV   α-PPP
α-PVP   MDPVP
α-PBP   MOPVP

Dimethocaine

PEA

Substituted amphetamines
Related to: amphetamine, methamphetamine

4-FA   3-FMA
4-FMA   PMA
3-FA   PMMA

Methiopropamine   Ethylphenidate

Camfetamine

Cannabinoids

Functionally related to naturally occurring cannabinoids including THC

WIN-55,212-2   AB-001

Naphthoylindoles

JWH-018   JWH-019
JWH-073   JWH-081
JWH-122   JWH-200
AM-1221
AM-2201

CP-47,497
CP-47,497, C8 homologue
CP-55,940

JWH-133
JWH-161

Phenylacetylindoles

JWH-250
JWH-251
JWH-203
RCS-8

CB25
CB52

Benzoylindoles

AM-694
AM-1241
AM-2233
RCS-4

JWH-175

Cyclopropanoyl-indoles

UR-144
5F-UR-144
A-834,735
A-796,260

HU-210
HU-211
HU-331

Naphthoylpyrroles

JWH-307
JWH-147
JWH-030

O-1812

Dissociatives

Related to: ketamine, PCP

Methoxetamine
3-MeO-PCP
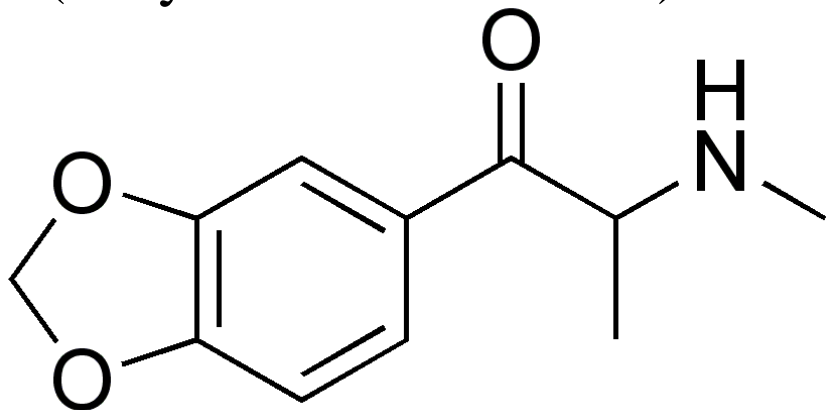4-MeO-PCP
3-MeO-PCE
2-MeO-ketamine

Sedatives
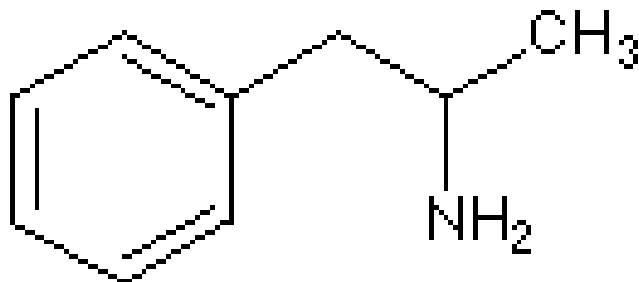
Opioids
Related to: morphine, fentanyl, heroin

α-methylfentanyl
3-methylfentanyl
MPPP
O-desmethyltramadol
7-acetoxymitragynine
Metonitazene
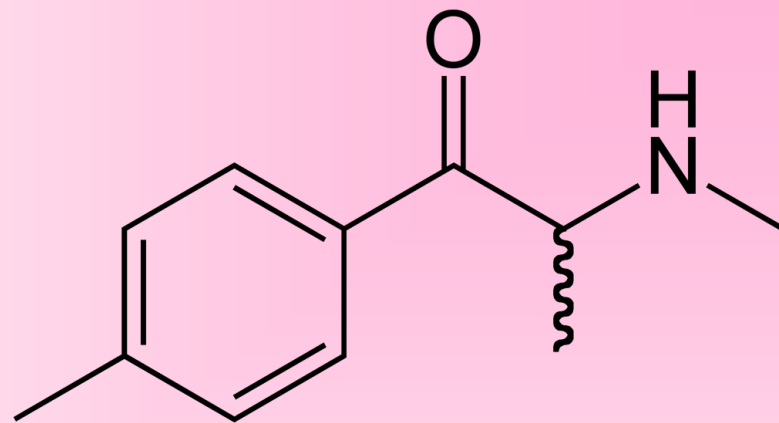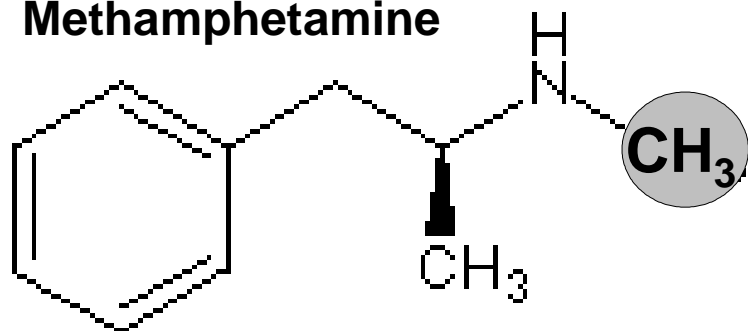AH-7921

Phenazepam Etizolam, Flu-Bromazepam

Methylone = ßk-MDMA
(Butylone = ßk-MBDB)

Amphetamine

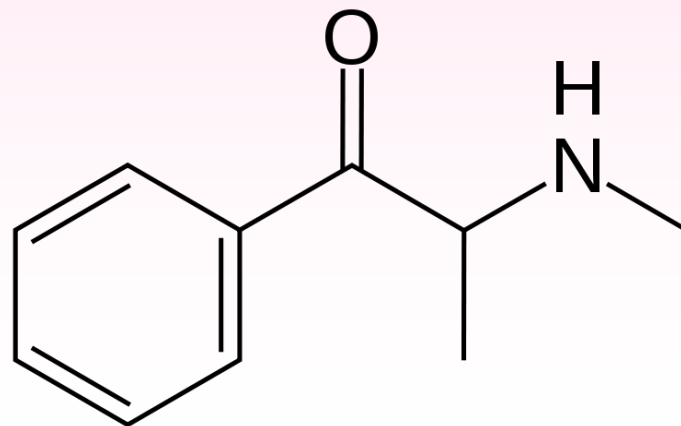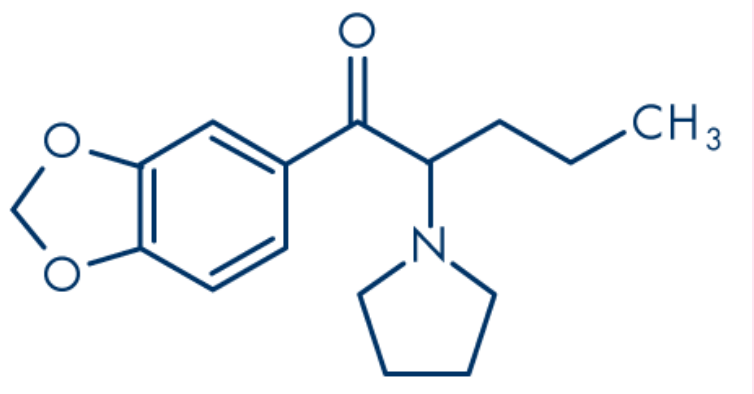**Methamphetamine**

CH₃

Mephedrone
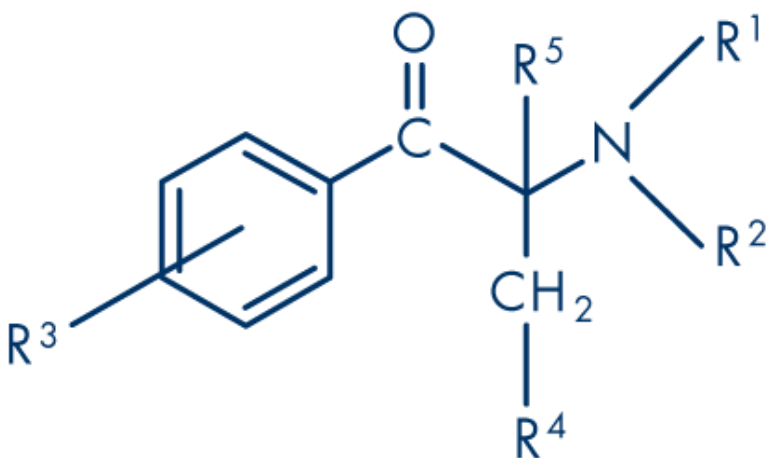
4-Methylmethcathinone, ßk-Methylmethamphetamine)

Ephedrone = Methcathinone

MDPV (3,4-methylenedioxypyrovalerone)

General structure of a cathinone derivative showing substitution patterns

R^1	R^2	R^3	R^4	R^5	Name
H	H	H	H	H	Cathinone
Methyl	H	H	H	H	Methcathinone (ephedrone)
Methyl	Methyl	H	H	H	N,N-Dimethylcathinone (metamfepramone)
Ethyl	H	H	H	H	N-Ethylcathinone (EC)
Methyl	H	H	Methyl	H	Buphedrone
Ethyl	H	4-Methyl	H	H	4-Methyl-N-ethylcathinone
Methyl	H	4-Methyl	H	H	Mephedrone (4-MMC; M-CAT)
Ethyl	Ethyl	H	H	H	Amfepramone
t-Butyl	H	3-Cl	H	H	Bupropion
Methyl	H	3.4-Methylenedioxy	H	H	Methylone (ßk-MDMA)
Ethyl	H	3.4-Methylenedioxy	H	H	Ethylone (ßk-MDEA)
Methyl	H	4-Methyl	Methyl	H	Butylone (ßk-MBDB)
Methyl	H	4-Methoxy	H	H	Methedrone (ßk-PMMA)
Methyl	H	4-F	H	H	Flephedrone (4-FMC)
Methyl	H	3-F	H	H	3-Fluoromethcathinone (3-FMC)
{pyrrolidino}	{pyrrolidino}	H	H	H	a-Pyrrolidinopropiophenone (PPP)
{pyrrolidino}	{pyrrolidino}	4-Methyl	H	H	4-Methyl-a-pyrrolidinopropiophenone (MPPP)
{pyrrolidino}	{pyrrolidino}	4-MeO	H	H	4-methoxy-a-pyrrolidinopropiophenone (MOPPP)
{pyrrolidino}	{pyrrolidino}	4-Methyl	Propyl	H	4-Methyl-a-pyrrolidino-hexanophenone (MPHP)
{pyrrolidino}	{pyrrolidino}	4-Methyl	Ethyl	H	Pyrovalerone
{pyrrolidino}	{pyrrolidino}	4-Methyl	Methyl	H	4-Methyl-a-pyrrolidino-butyrophenone (MPBP)
{pyrrolidino}	{pyrrolidino}	4-Methyl	H	Methyl	4-Methyl-a-pyrrolidino-a-methylpropiophenone
{pyrrolidino}	{pyrrolidino}	3.4-Methylenedioxy	H	H	3,4-Methylenedioxy-a-pyrrolidinopropiophenone (MDPPP)
{pyrrolidino}	{pyrrolidino}	3.4-Methylenedioxy	Ethyl	H	3,4-Methylenedioxypyrovalerone (MDPV)

Which immunoassays (urine) are available?

-- Amphetamin and derivatives (!?)
-- Barbiturates
-- Benzodiazepines
-- Cocaine (Benzoylecgonine)
-- Methadone or better EDDP
-- Opiates
-- 6-Monoacetylmorphine
-- Cannabinoids (THC-COOH)
-- Tramadol
-- Oxycodone
-- Buprenorphine
-- Fentanyl
-- "Spice"

-- LSD

-- Ethanol

-- Phencyclidine
-- Propoxyphene
-- Methaqualone

-- Tricyclic Antidepr.
-- Paracetamol
-- Salicylates

-- Ethylglucuronide

Immunoassay drug testing and urine spls., problems:

-- internal dilution! Creatinine dependent cutoff?!

-- adulteration! sampling under supervision

-- cutoffs: group tests not standardized: accreditation!

-- Xreact.: false positives / **false negatives**

-- increasing no. of different drugs,
   new drug classes

European Laboratory Guidelines for Legally Defensible Workplace Drug Testing - Version 1.0, EWDTS 2002

Appendix E
Recommended maximum cut-off concentrations for Screening Tests appropriate for [country]

Screen Test	Cut-Off Concentration (ng/ml)
Amphetamine group	500
Benzodiazepines group	200
Cannabis metabolites	50
Cocaine metabolites	300
Opiates (total)	300
Methadone or metabolites	300
Barbiturates	200
Phencyclidine	25
Buprenorphine or metabolites	5
LSD or metabolites	1
Propoxyphene or metabolites	300
Methaqualone	300

These recommended cut-off values may be subject to changes as advances in technology or other considerations warrant identification of these substances at other concentrations.

Cut-off levels for substances not indicated in Appendix E will need to be agreed with the customer taking into account the performance of the assays to be used.

European Laboratory Guidelines for Legally Defensible Workplace Drug Testing - Version 1.0, EWDTS 2002

Appendix F

Recommended cut-off concentrations for confirmation tests appropriate for [country]

Confirmation Test	Cut-Off Concentration (ng/ml) (Total)
Amphetamines	
Amphetamine	200
Methylamphetamine	200
MDA	200
MDMA	200
MDEA	200
Other members of the amphetamine group	200
Benzodiazepines	
Temazepam	100
Oxazepam	100
Desmethyldiazepam	100
Others members of the benzodiazepine group by agreement with the customer.	
Opiates (total)	
Morphine	300
Codeine	300
Dihydrocodeine	300
6-Monoacetylmorphine 10	
Cannabis metabolite (11-nor-Ä$_9$-tetrahydrocannabinol-9-carboxylic acid.)	15
Cocaine metabolite (benzoylecgonine)	150
Methadone or metabolites	250
Barbiturates group	150
Phencyclidine	25
Buprenorphine or metabolites	5
LSD or metabolites	1
Propoxyphene or metabolites	300
Methaqualone	300

Problems in drug of abuse testing:

-- new substances, immunoassays do not cover
   no data on abuse pattern in different regions, different
   patients groups, different settings (WDT, prisons etc.)
-- urine: diuresis!, supervision, metabolites
-- matrix saliva (oral fluid)
   - no dilution problems but sampling problem (which device)?
   - easy supervision of sampling
   - only parent drugs needed!(???), easier method devlopment?!
   - „cleaner" matrix: easier method development

   but: which analytes at which concentration?

Develop a sensitive LC/MSMS method for OF
which can be easily adopted to changing requests.

Compare to routine urine drug testing in different settings
Here: patients in opiate maintenance therapy

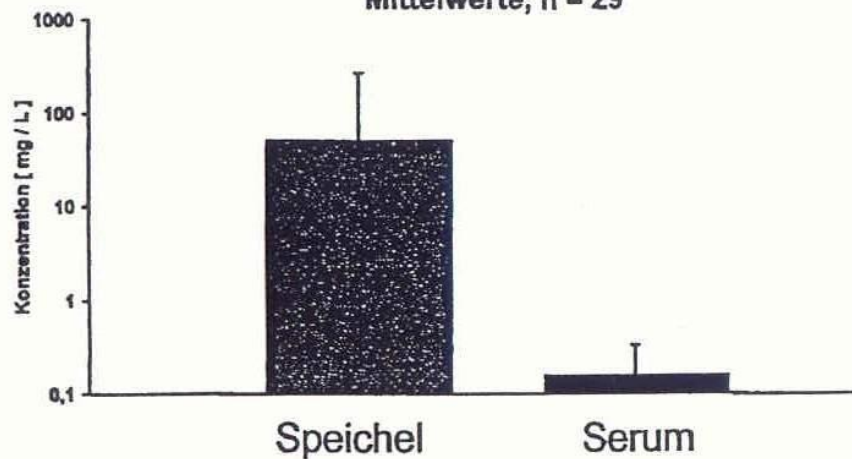**How do drugs get into (mixed) saliva (oral fluid)?**

-- oral contamination

-- from blood by **passive diffusion** across cell membranes

-- active secretion

-- filtration

factors influencing S/P-ratio:
-- **pKa** of substance (acidic-alkaline?)
-- lipid solubility
-- **protein binding**
-- molecular weight

Kauert, Blutalkohol 37, 2000
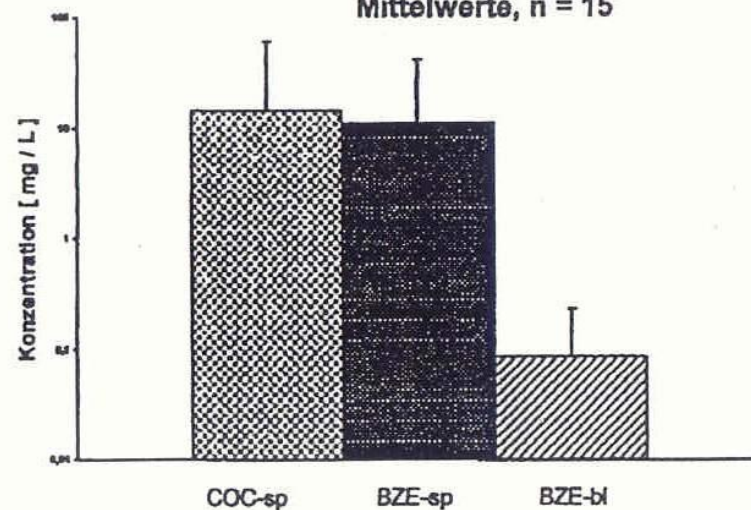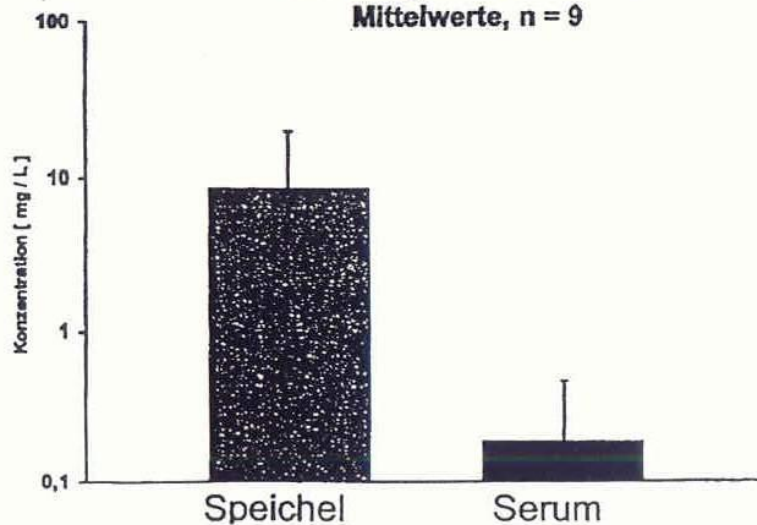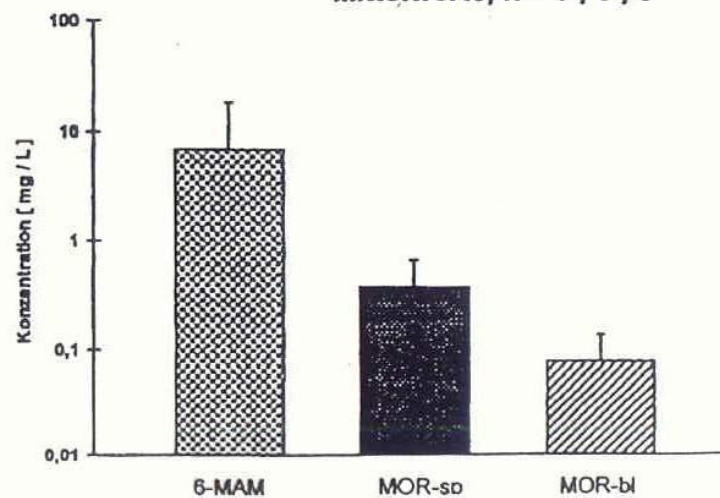
screening for drugs: comparing OF-blood-urine

mixed Oral Fluid = <u>saliva</u> + gingival crevicular fluid + nasal secretions
+ mucosal transudates+ regurgitated gastric secretions

Oral Fluid	Blood (Serum, Plasma)	Urine
non invasive	invasive	supervision needed: privacy!
drug conc. low-high	drug conc. low	drug conc. low-very high
spl. vol. low	spl. vol. low	spl. vol. low-very high
adulteration difficult	no adulteration	adulteration possible
pH-change during collection process may influence Saliva/Plasma-ratio	-----	excretion influenced by urinary pH,drug concentration influenced by (intentional?!) drinking.
mostly parent drugs	parent drugs	mostly metabolites

screening for drugs: comparing OF-blood-urine

Oral Fluid	Blood (Serum, Plasma)	Urine
oral contamination from smoking,intranasal or peroral consumption	-----	-----
correlation with impairment could be possible	correlation with impairment possible	correlation with impairment impossible
screening methods, collection methods, collection devices not fully established and validated Adsorption!?	-----	screening methods, collection methods, collection devices established really standardized??
A+B sample?	-----	-----
collection device closed no contamination	closed device	urine beakers can be contaminated
Xerostomia	-----	"not able to..."

Saliva Collection System (SCS) pH 4.2 Greiner Bio-One

4 ml Saliva Extraction Solution (SES)

contains non-toxic yellow food color and buffer salts

Saliva vacuum collection tubes
contains stabilizing Agents ; A+B sample!

2

1

3

a

b

Saliva Collection Beaker
with integrated saliva transfer device

Saliva sampling with the Greiner Saliva Collection System:

Step 1
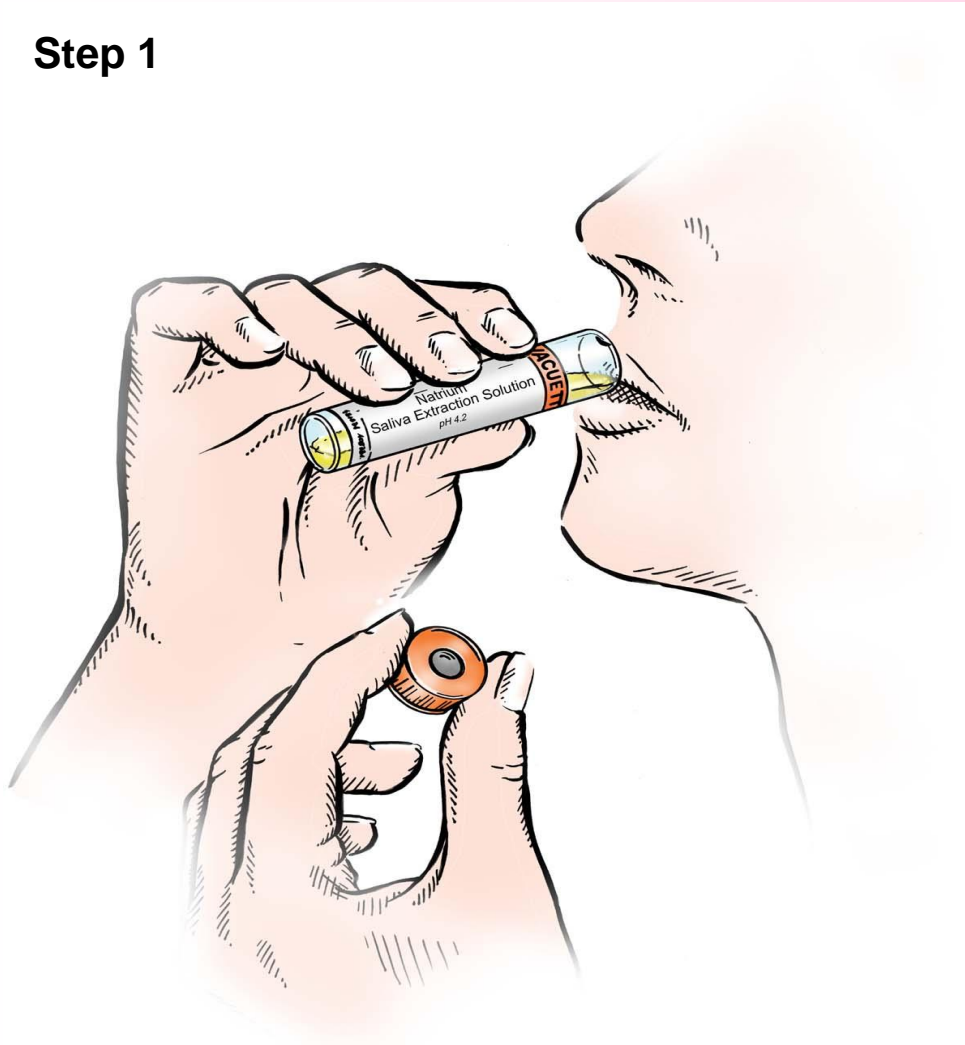
Rinsing of the oral cavity with Saliva Extraction Solution for 2 minutes

Step 2:
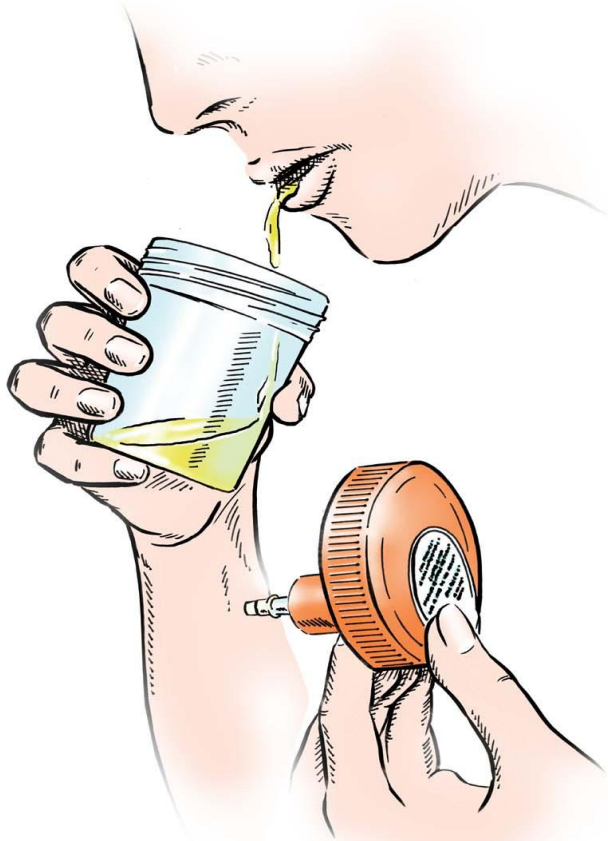
Spitting of the extracted oral fluid into the Saliva Collection Beaker

Step 3
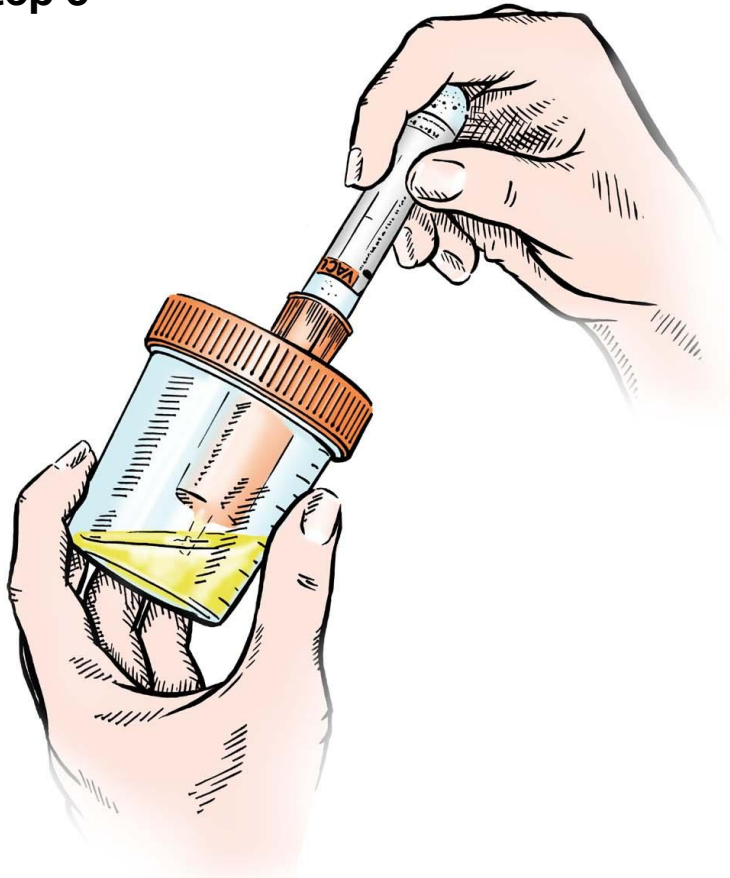
Transfering of the extracted saliva into the evacuated Saliva Collection Tubes

always A + B sample!

Advantages:
-- quick (Xerostomia!), standardized time
-- acidic pH during collection keeps
   pH difference to plasma
-- acidic pH: 6-AM, Cocaine, Zopiclone
   etc. are stable
-- aqueous matrix: less ion suppression,
   rapid SALLE possible

EWDTS draft guidelines for oral fluid
03/2011

screening cutoffs

THC:	10 ng/mL
Cocaine + metabolites:	30 ng/mL
Opiates (Morphine):	40 ng/mL
6-Acetylmorphine:	4 ng/mL
Methadone:	50 ng/mL
Buprenorphine:	5 ng/mL
Amphetamines:	40 ng/mL
Propoxyphene:	40 ng/mL
Barbiturates:	60 ng/mL
Benzodiazepines:	10 ng/mL

high cutoffs:
correlation with impaiment ?!

confirmatory cutoffs

THC:	2 ng/mL
Cocaine-metabolite:	8 ng/mL
Opiates (each):	40 ng/mL
6-Acetylmorphine:	4 ng/mL
Methadone:	20 ng/mL
Buprenorphine:	5 ng/mL
Amphetamines (each):	30 ng/mL
Propoxyphene:	40 ng/mL
Barbiturates:	not mentioned
Benzodiazepines (each):	10 ng/mL

Replacement of immunoassay by LC tandem mass spectrometry for the routine measurement of drugs of abuse in oral fluid

KR Allen[1], R Azad[1], HP Field[1] and DK Blake[2]

Addresses
[1]Department of Clinical Biochemistry, Leeds Teaching Hospitals, Britannia House, Morley, Leeds LS27 0DQ, UK
[2]Applied Biosystems, Warrington, UK

Correspondence
Mr KR Allen
E-mail: keith.allen@leedsth.nhs.uk

Abstract

Background   There is increasing interest in the use of oral fluid as the matrix for the detection of drugs of abuse which requires the use of sensitive immunoassays to achieve the low detection limits required. The use of liquid chromatography linked to tandem mass spectrometry (LC/MS/MS) is explored as a possible replacement for immunoassay in screening for drugs of abuse in oral fluid samples.

Methods   Oral fluid samples collected from 72 subjects attending an addiction clinic were screened for opiates, cocaine, methadone and benzodiazepines using both enzyme-linked immunosorbent assays (ELISA) and LC/MS/MS. The latter analysis used a short gradient elution with individual drugs detected by multiple reaction monitoring using tandem mass spectrometry. Results between the two methods were compared qualitatively using the cut-off concentrations defined by the ELISA assays.

Results   With regard to the ELISA assays which show group specificity, LC/MS/MS detected the presence of 6-monoacetylmorphine, morphine or dihydrocodeine in all but two of 49 samples positive for opiates. Of 55 samples positive for benzodiazepines by ELISA, all but two were confirmed by LC/MS/MS. Overall, LC/MS/MS compared favourably with ELISA for detection of specific drugs or their metabolites in the case of morphine, methadone and the cocaine metabolite benzoylecgonine. Many of the discrepant results between the two assays were a result of samples with drug concentrations near to the cut-off concentrations and the imprecision of these assays at very low concentrations.

Conclusion   LC/MS/MS offers a more flexible, specific and sensitive alternative to the screening of oral fluid samples for drugs of abuse than ELISA. A wide range of drugs and metabolites can be detected from a single sample injection.

Table 1 *Cut-off concentrations for drugs detected by ELISA*

Drug/drug group	ELISA* (μg/L)	SAMHSA[†] (μg/L)
Opiates	10	40
Morphine specific	20	40
Cocaine metabolite	5	20
Methadone	5	—
Benzodiazepines	1	—

*Concentrations allow for a 1 in 4 dilution of oral fluid in collecting device buffer. [†]SAMHSA initial screening test cut-off concentration.

Table 2 *Cut-off concentrations for drugs and drug metabolites detected by LC/MS/MS*

Drug/metabolite	LC/MS/MS* (μg/L)	SAMHSA[†] (μg/L)
6-MAM	1	4
Morphine	20	40
Codeine	10	40
DHC	10	40
Methadone	5	—
EDDP	0.5	—
Cocaine	5	8
Benzoylecgonine	5	8
Diazepam	1	—
Nitrazepam	1	—
Nordiazepam	1	—
Temazepam	1	—
7-aminonitrazepam	1	—

6-MAM, 6-monoacetylmorphine; DHC, dihydrocodeine; EDDP, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine. *Concentrations allow for a 1 in 4 dilution of oral fluid in collecting device buffer. [†]SAMHSA confirmatory test cut-off concentrations.

Simultaneous Screening and Quantification of 29 Drugs of Abuse in Oral Fluid by Solid-Phase Extraction and Ultraperformance LC-MS/MS

Nora Badawi,[1] Kirsten Wiese Simonsen,[1] Anni Steentoft,[1] Inger Marie Bernhoft,[2] and Kristian Linnet[1*]

BACKGROUND: The European DRUID (Driving under the Influence of Drugs, Alcohol And Medicines) project calls for analysis of oral fluid (OF) samples, collected randomly and anonymously at the roadside from drivers in Denmark throughout 2008–2009. To analyze these samples we developed an ultra performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS) method for detection of 29 drugs and illicit compounds in OF. The drugs detected were opioids, amphetamines, cocaine, benzodiazepines, and Δ-9-tetrahydrocannabinol.

METHOD: Solid-phase extraction was performed with a Gilson ASPEC XL4 system equipped with Bond Elut Certify sample cartridges. OF samples (200 mg) diluted with 5 mL of ammonium acetate/methanol (vol/vol 90:10) buffer were applied to the columns and eluted with 3 mL of acetonitrile with aqueous ammonium hydroxide. Target drugs were quantified by use of a Waters ACQUITY UPLC system coupled to a Waters Quattro Premier XE triple quadrupole (positive electrospray ionization mode, multiple reaction monitoring mode).

RESULTS: Extraction recoveries were 36%–114% for all analytes, including Δ-9-tetrahydrocannabinol and benzoylecgonine. The lower limit of quantification was 0.5 μg/kg for all analytes. Total imprecision (CV) was 5.9%–19.4%. With the use of deuterated internal standards for most compounds, the performance of the method was not influenced by matrix effects. A preliminary account of OF samples collected at the roadside showed the presence of amphetamine, cocaine, codeine, Δ-9-tetrahydrocannabinol, tramadol, and zopiclone.

CONCLUSIONS: The UPLC-MS/MS method makes it possible to detect all 29 analytes in 1 chromatographic run (15 min), including Δ-9-tetrahydrocannabinol and benzoylecgonine, which previously have been difficult to incorporate into multicomponent methods.
© 2009 American Association for Clinical Chemistry

Recently, oral fluid (OF[3]; saliva) has been investigated as a sample for drug-of-abuse testing, especially for testing in the workplace and testing individuals suspected of driving under the influence of drugs (1). Substances can be detected in OF for short periods of time, typically 12–24 h after consumption. OF is therefore suitable for detecting recent drug use, e.g., for roadside testing (2). A major advantage of using OF instead of blood samples is the noninvasive nature of the collection procedure and the ability of nonmedical personnel to collect OF samples. Furthermore, OF can be collected under direct observation, which makes it difficult to substitute or adulterate samples.

OF is produced by a number of specialized glands and consists of about 98% water and trace amounts of proteins (normally present in plasma) in addition to electrolytes (1). The pH of OF is typically 6.7 with a range of 5.6–7.9. OF pH affects the concentration of drugs. Several studies have investigated the detection of drugs in OF, as recently reviewed by Drummer (3). Most of these studies focused on detection of amphetamines, cannabis, cocaine, and opiates.

Because only a limited amount of OF is available for drug analysis, it is crucial to have a multicomponent method with a low detection limit for sample analysis. Gunnar et al. reported a multicomponent method that uses GC-MS with fractionated solid-phase extraction

Anal Bioanal Chem
DOI 10.1007/s00216-011-5108-y

UHPLC-ESI-MS/MS method for direct analysis of drugs of abuse in oral fluid for DUID assessment

Sabina Strano-Rossi · Luca Anzillotti ·
Erika Castrignanò · Marialinda Felli ·
Giovanni Serpelloni · Roberto Mollica ·
Marcello Chiarotti

Abstract An ultra-high-performance liquid chromatography–electrospray ionization–tandem mass spectrometry method for the direct analysis in oral fluid (OF) of several abused drugs and metabolites in a single chromatographic run was set up and validated. Amphetamine, methamphetamine, morphine, O-6-monoacetylmorphine, cocaine, codeine, methylenedioxymethamphetamine (MDMA), methylenedioxyethylamphetamine, methylenedioxyamphetamine, methadone, benzoylecgonine (BEG), Δ9-tetrahydrocannabinol (THC), ketamine, and cocaethylene were determined in a single chromatographic run with no sample pretreatment, after addition of the respective deuterated internal standards. The method was designed to perform a confirmation analysis on the residual OF samples after the preliminary on-site screening test, and it was applied on preservative buffers from different devices (Mavand Rapidstat, Concateno DDS, and Greiner Bio-One) or on neat OF samples. The method was suitable to be applied to the small amounts of sample available for the confirmatory analysis after the preliminary on-site screening or on undiluted OF samples. Limits of detection varied from 5 (morphine) to 0.2 ng/mL (methamphetamine, MDMA, BEG, and cocaethylene). The method was linear for all the substances involved, giving quadratic correlation coefficients of >0.99 in all the different preservative buffers checked. In addition, repeatability and accuracy were satisfactory for the majority of the substances, except for a few cases. The developed method was subsequently applied to 466 residual samples from on-site screening performed by police officers. Of these samples, 74 showed the presence of cocaine and metabolites; THC was detected in 49 samples. Two samples showed codeine and morphine while MDMA was detected in 11 samples and ketamine in four samples.

Keywords Forensic toxicology · Oral fluid · UHPLC-MS/MS · DUID

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GBO, SCS pH 4.2

14 substances

direct injection of 20 µL sample into LC-MS/MS!

Table 3 Limits of detection (LODs) and LOQs obtained with different preservative buffer of the collection devices

Analyte	LOD (ng/mL)		LOQ (ng/mL)	
	Rapidstat (DDS)	Pure OF (GBO)	Rapidstat (DDS)	Pure OF (GBO)
Amphetamine	1	0.5	5	5
BEG	0.5	0.2	5	5
Cocaethylene	0.5	0.2	5	5
Cocaine	1	0.5	5	4
Codeine	1	0.5	10	5
Ketamine	0.5	0.4	2	2
MDA	1	1	5	2
MDEA	<0.2	<0.2	5	4
MDMA	<0.2	<0.2	4	2
Methadone	0.5	0.5	5	5
Methamphetamine	0.2	0.2	5	4
Morphine	5	4	10	5
O-6-MAM	2	1	4	2
THC	2	2	10	5

Oral Fluid is a Viable Alternative for Monitoring Drug Abuse: Detection of Drugs in Oral Fluid by Liquid Chromatography–Tandem Mass Spectrometry and Comparison to the Results from Urine Samples from Patients Treated with Methadone or Buprenorphine

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Abstract

Oral fluid is an alternative biological matrix that might have advantages over urine for drug analysis in treatment programs. A liquid chromatography–tandem mass spectrometry (LC–MS–MS) method has been used for screening 32 of the most commonly abused drugs and their metabolites in 0.5 mL preserved oral fluid, and the results were compared to results obtained from urine samples taken at the same time. In all, 164 pairs of oral fluid and urine were obtained from 45 patients stabilized on either methadone or buprenorphine. The total number of detections of drugs other than buprenorphine or methadone was 535 in oral fluid and 629 in urine. Morphine was found more often in urine (n = 66) than in oral fluid (n = 48), whereas the opposite was the case for 6-monoacetylmorphine (n = 20 in urine and n = 48 in oral fluid). Methadone showed the same detection frequency in urine and oral fluid (n = 75), whereas amphetamine (n = 45 in urine and n = 51 in oral fluid), methamphetamine (n = 39 in urine and n = 45 in oral fluid), and N-desmethyldiazepam (n = 37 in urine and n = 51 in oral fluid) were detected slightly more often in oral fluid. The other benzodiazepines, cannabis and cocaine were found more frequently in urine samples. If using a sensitive LC–MS–MS technique, oral fluid might be a good alternative to urine for detection of relatively recent use of drugs.

imens has accelerated over the last decade (1). An advantage with urine samples might be that drug ingestion can be detected for several days, and even weeks later, mainly because of detection of drug metabolites (2–4). However, urine may be difficult to collect; supervision intrudes on donors' privacy; the detection of drugs might be affected by, for example, the dilution of the urine due to fluid intake prior to urine sampling; and adulteration of the urine might render the analytical results worthless. Thus, there has been a growing interest in the use of oral fluid as an alternative to urine, and major technological advances have been made, particularly over the last 10 years (1). Collection of oral fluid is inoffensive, rapid, noninvasive, and easy, and the risk of adulteration is considered to be lower (5). Because of improved analytical techniques with increased sensitivity, a large number of drugs can be analyzed simultaneously in small sample volumes (6).

Oral fluid is a mixture of saliva, gingival crevicular fluid, cellular debris, and other components (5). Healthy adult subjects normally produce 500–1500 mL of oral fluid per day, at a rate of approximately 0.5 mL/min, but several physiological and pathological conditions can modify oral fluid production quantitatively and qualitatively (e.g., smell, taste stimulation, chewing, psychological and hormonal status, drugs, age, hered-

Table I. Cutoff Concentrations for Screening and Confirmation Analysis in Oral Fluid and Urine			
Drug	Oral Fluid Analysis (ng/mL)	Urine Confirmation (ng/mL)	Urine Screening (ng/mL)
3-OH-Diazepam	3	150	
6-MAM	2	33	20
7-Aminoflunitrazepam	0.3	28	
7-Aminoclonazepam	1	29	
7-Aminonitrazepam	1	25	
Alprazolam	1	31	
α-OH-Alprazolam	NA*	32	
Amphetamine	1	135	300
Barbiturates			30
Benzodiazepines			200
Benzoylecgonine	14	58	
Bromazepam	16	32	
Buprenorphine	2		5
Buprenorphine-glucuronide	NA	5	
Norbuprenorphine-glucuronide	NA	12	
Cannabis			20
Carisoprodol†	52	1302	
Clonazepam	1	NA	
Codeine	3	60	
Cocaine	8	61	300
Diazepam	1	NA	
Fenazepam	2	3	
Flunitrazepam	1	NA	
Lorazepam	3	32	
LSD	0.3	0.03	0.50
MDA	36	1434	
MDEA	41	207	
MDMA	39	77	
Meprobamate†	44	1092	
Methadone	15	62	300
EDDP	NA	111	
Methamphetamine	3	149	
Morphine	6	29	
N-Desmethyldiazepam	1	135	
Nitrazepam	1	NA	
Opiates			300
Oxazepam	1	143	
THC-acid	0.3	10	
Zolpidem†	0.3	6	
Zopiclone†	2	4	

* Not analyzed.
† Only analyzed in urine if detected in oral fluid.

-- Intercept
-- LLE

Table II. Comparison of the Results from Oral Fluid and Urine Showing that the Results from the Sample Pairs Primarily Correspond

Drug	Positive OF* and Urine	Negative OF and Urine	Corresponding Results OF and Urine	Positive OF Only	Positive Urine Only
3-OH-Diazepam	6	117	123 (75%)	0	41
6-MAM	19	115	134 (82%)	29 !	1
7-Aminonitrazepam	9	149	158 (96%)	0	6
7-Aminoflunitrazepam	59	83	142 (87%)	3	19
7-Aminoclonazepam	26	122	148 (90%)	2	14
Alprazolam	9	153	162 (99%)	0	2
Amphetamine	45	113	158 (96%)	6	0
Benzoylecgonine	1	158	159 (97%)	0	5
Buprenorphine	67	_†		–	22 ?
Codeine	34	122	156 (95%)	4	4
Cocaine	0	161	161 (98%)	2	1
Methadone	75	89	164 (99%)	–	0
Methamphetamine	39	119	158 (96%)	6	0
Morphine	45	95	140 (85%)	3	CO 6 ng/mL 21
N-Desmethyldiazepam	35	111	146 (89%)	16	2
Oxazepam	41	71	112 (68%)	9	43
THC/THCCOOH‡	81	64	145 (88%)	1	18
Zopiclone	4	106	110 (99%)	1	0 ?

* Oral fluid.
† There were analytical problems with the oral fluid analysis.
‡ THC was analyzed in oral fluid, and THCCOOH was analyzed in urine.

Drug screening in Oral Fluid with LC-MS/MS: Analytes

Analytes in „**Module A",** cutoff 1 ng/mL neat OF, IS = 0.5 ng/mL SA/SES:

- **Peri-analytics:** volume, % saliva in SES, Amylase, Cortisol

- **Substitution drugs:** D-/L-Methadone, EDDP, Buprenorphine, Norbuprenorphine

- **Amphetamines:** Amphetamine, Methamphetamine, MDMA, MDA, MBDB, BDB, MDEA, Butylone, Mephedrone, Methylone, MDPV

- **Benzodiazepines**: Diazepam, Nordiazepam, Oxazepam, Midazolam, Flurazepam, Desalkyl-flurazepam, Temazepam, 7-Aminoclonazepam, Alprazolam, Flunitrazepam, 7-Aminoflunitrazepam, Bromazepam, Lorazepam

- **Cocaine:** Cocaine, Benzoylecgonine, Lidocaine

- **Opiates:** Morphine, Codeine, 6-Acetylmorphine, 6-Acetylcodeine, Norcodeine, Dihydrocodeine

- **Opioids:** Naloxone, Tilidine, Tramadol, O-Desmethyltramadol, Oxycodone, Noroxycodone, Fentanyl, Nortilidine, Hydromorphone

- **Cannabinoids:** THC

- **Others:** Zolpidem, Zopiclone**,** Zaleplone, Ketamine, Methylphenidate, Ritalinic acid, Pregabalin, Gabapentin

actual: **N = 56** (3 transitions) + **54 deuterated IS** (2 transitions)

1st Study: is OF of equal value?

Drug abuse testing of patients in substitution therapy: UPLC-MS/MS screening in OF vs. urine testing with EIA

-- **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 SA.
   - **194 patients** (26 Bupre, 67 Metha, 101 Pola), **902 SA** samples.
   - **182 patients** (25 Bupre, 66 Metha, 91 Pola), **1119 urine** samples.

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 SA** samples.
   - **1463 patients** from 40 clinics (285 Bupre, 673 Metha, 505 Pola), **9008 urine** samples.

Drug abuse testing of patients in substitution therapy: UPLC-MS/MS screening in saliva vs. urine testing with EIA

	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 patients in low dose therapy (0.4 and 1.0 mg/d).
Cutoff 0.1 ng/mL?

2nd study: Cutoff considerations
All routine OF sampels, 3 month

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%)

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.

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%

For Mephedrone, Methylone and Butylone more data are needed.
MBDB, BDB and MDEA seems to be without relevance in the investigated
patient population.

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	Lidocaine			
(28) without Cocaine/Benzoylecgonine				

Detector linearity ends at 3 ng/mL, thus no evaluation was performed for Lidocaine.

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%

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.

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.

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 by			**0.3%**
Norbuprenorphine	1283 (24.0%)	822 (15.4%)	44 (0.8%)
pos. rate reduced by		**35.9%**	**96.6%**
Buprenorphine		1283 (24.0%)	615 (11.5%)
pos. rate reduced by			**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.

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.

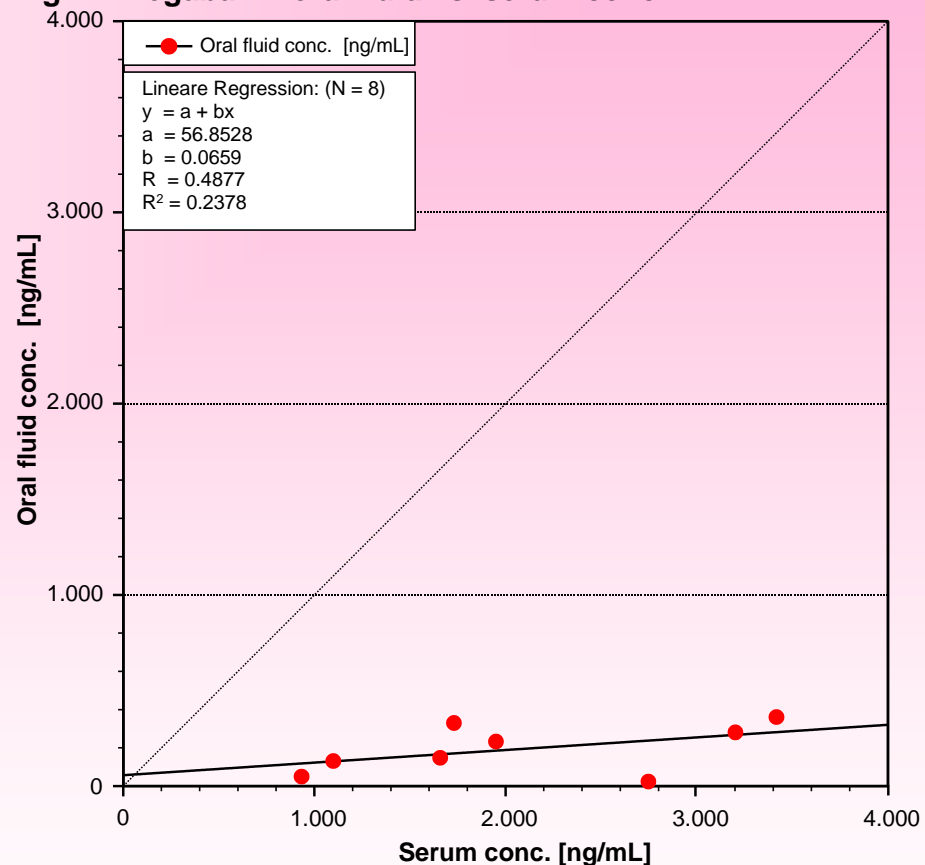# OF/SE ratio of 11 psychoactive therap. drugs: patient data + dose

drug	n male	n female	n patients	age	daily dose [mg] range	no. of pats. without any co-medication
Aripiprazole	3	4	7	26-65	5 - 30	-
Citalopram	3	6	9	31-64	20 - 60	2
Duloxetine	1	9	10	43-81	30 - 120	-
Escitalopram	9	15	24	21-77	10 - 40	4
Mirtazapine	7	7	13	44-76	7.5 - 45	1
Pipamperone	3	6	9	22-77	20 - 100	-
Pregabalin	5	3	8	27-58	50 - 400	-
Promethazine	3	3	6	47-74	unknown	-
Quetiapine	4	10	13	22-81	50 - 700	-
Sertraline	1	3	4	22-73	100 - 150	-
Venlafaxine	17	22	37	22-76	75 - 375	5

Paired SE and OF samples (n=102) were taken from 98 pats. 55 individuals were treated with one (12 without any co-medication), 31 with two and 12 with three of the studied drugs. Samples with values resulting from oral contamination (n = 5) or sampels. from patients obviously not in steady-state (n = 5) were excluded.
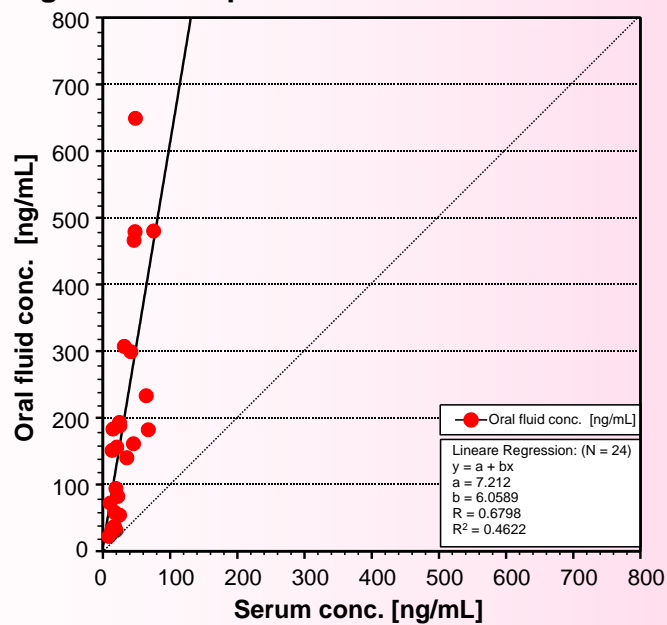
Oral fluid (GBO)/serum conc. ratios of 11 psychoactive therapeutic drugs

drug	n	ratio [OF/SE] mean
Aripiprazole	7	0.10
Citalopram	9	5.17
N-Desmethylcitalopram	9	1.13
Duloxetine	10	0.61
Escitalopram	24	6.10
L-Desmethylcitalopram	22	1.42
Mirtazapine	14	4.52
Pipamperone	9	7.12
Pregabalin	8	0.10
Promethazine	6	3.26
Quetiapine	14	0.94
Sertraline	4	1.07
N-Desmethylsertraline	4	1.07
Venlafaxine	39	8.47
N-Desmethylvenlafaxine	39	2.61

Fig. 1 Pregabalin: oral fluid vs. serum conc.

Lineare Regression: (N = 8)
$y = a + bx$
$a = 56.8528$
$b = 0.0659$
$R = 0.4877$
$R^2 = 0.2378$

Oral fluid conc. [ng/mL]

Fig. 2 Escitalopram: oral fluid vs. serum conc.

Oral fluid conc. [ng/mL]

Oral fluid conc. [ng/mL]

Lineare Regression: (N = 24)
y = a + bx
a = 7.212
b = 6.0589
R = 0.6798
R^2 = 0.4622

Serum conc. [ng/mL]

Fig. 3 Venlafaxine: oral fluid vs. serum conc.

Oral fluid conc. [ng/mL]

Lineare Regression: (N = 39)
y = a + bx
a = -194,3541
b = 10,414
R = 0.8881
R^2 = 0.7887
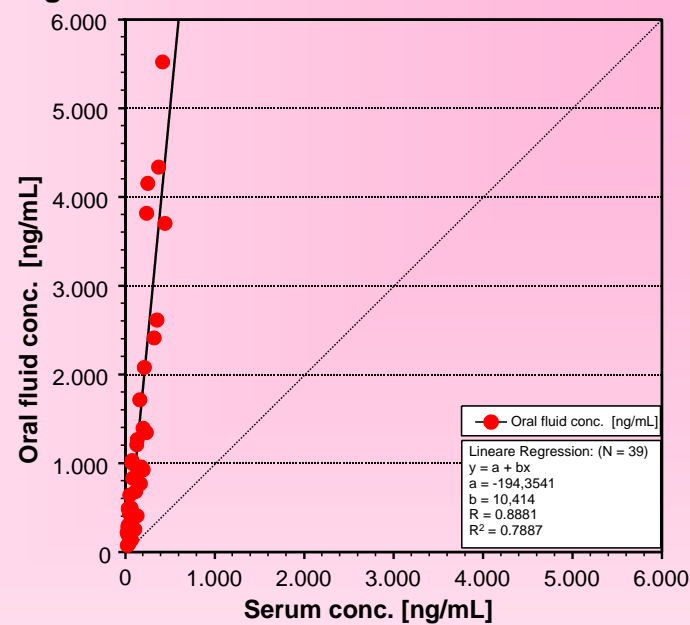
Serum conc. [ng/mL]

Fig. 4 Mirtazapine: oral fluid vs. serum conc.

Oral fluid conc. [ng/mL]

Oral fluid conc. [ng/mL]

Lineare Regression: (N = 14)
y = a + bx
a = 10.1711
b = 4.1158
R = 0.8305
R^2 = 0.6897
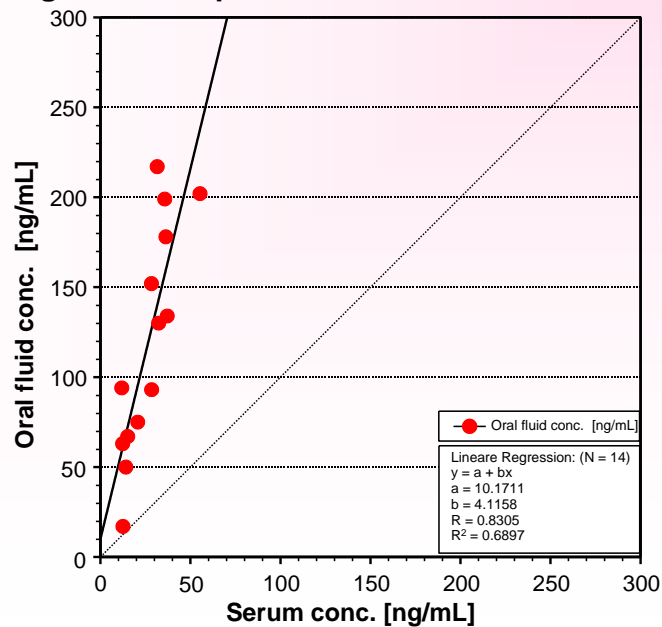
Serum conc. [ng/mL]

Fig. 5 Quetiapine: oral fluid vs. serum conc.

Oral fluid conc. [ng/mL]

Lineare Regression: (N = 14)
y = a + bx
a = -9.407
b = 0.9865
R = 0.9311
R^2 = 0.8559

Serum conc. [ng/mL]
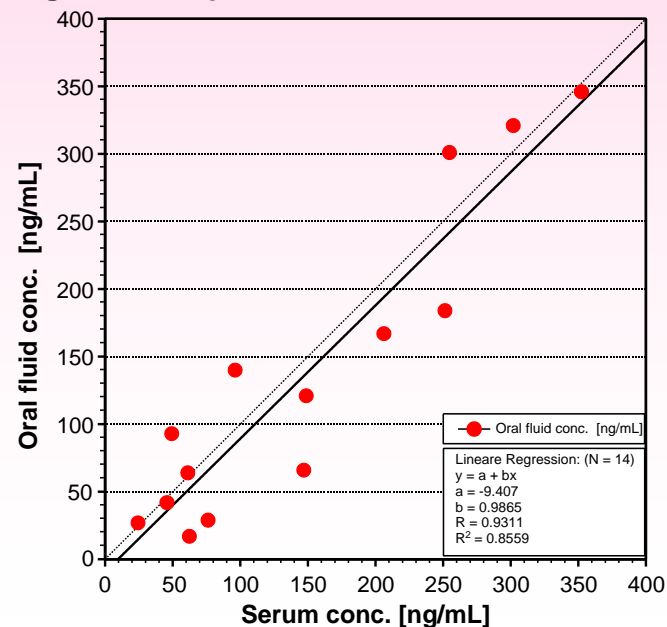
Cocaine-Immunoassay response distribution -- 300- >2500 ng/mL
37081 samples, 6 month

XKOKAI n=37081

cutoff >= 300 ng/mL:
3017 spls. =   8.1%

2310 spls.
> 2500 ng/mL
= 6.2%

Immunoassay calibration:

6-point, 100-2500 ng/mL

no. of samples

class [ng/mL], width = 2
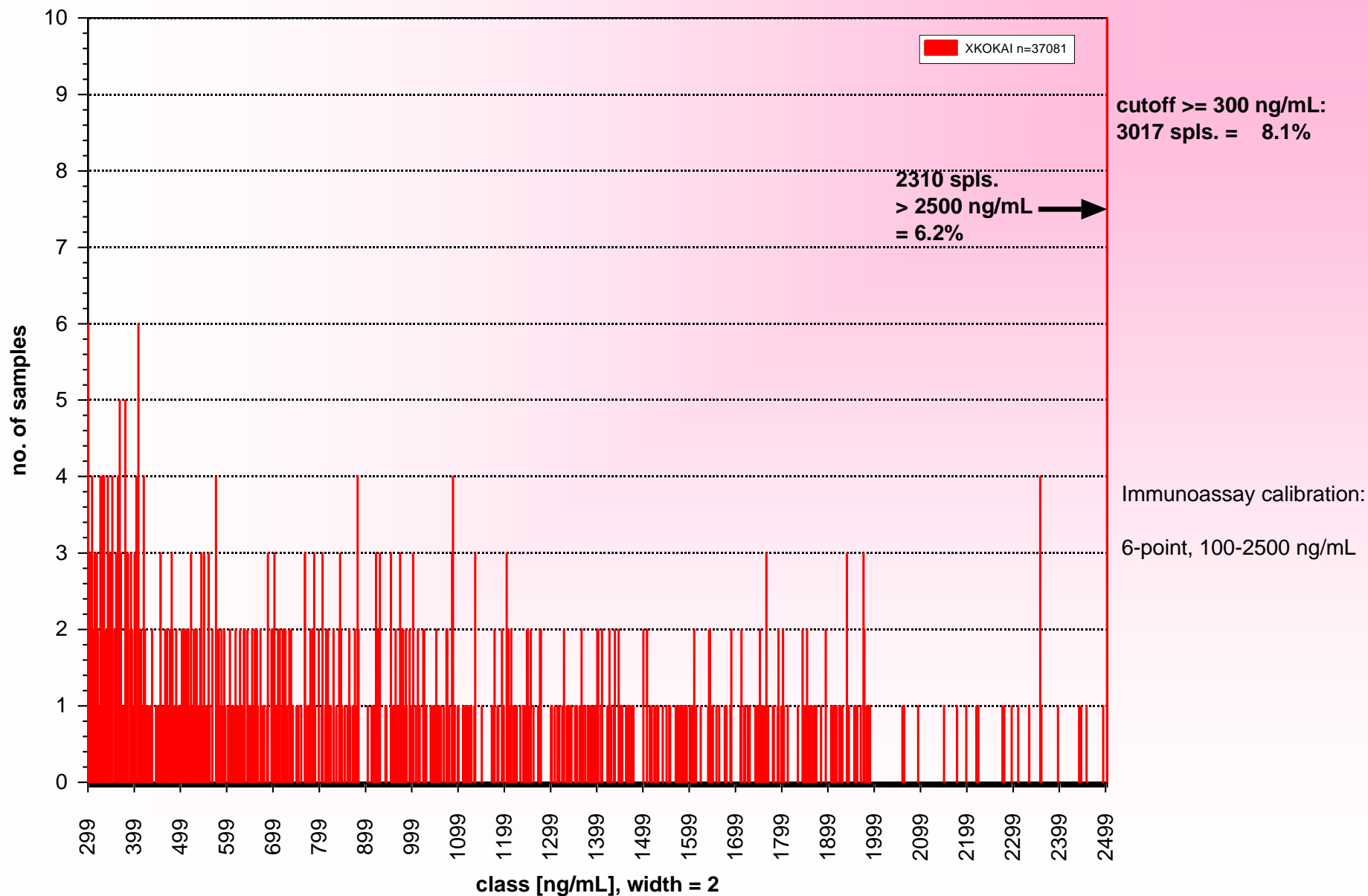
Cocaine-Immunoassay response distribution -- 0-300 ng/mL
37081 samples, 6 month
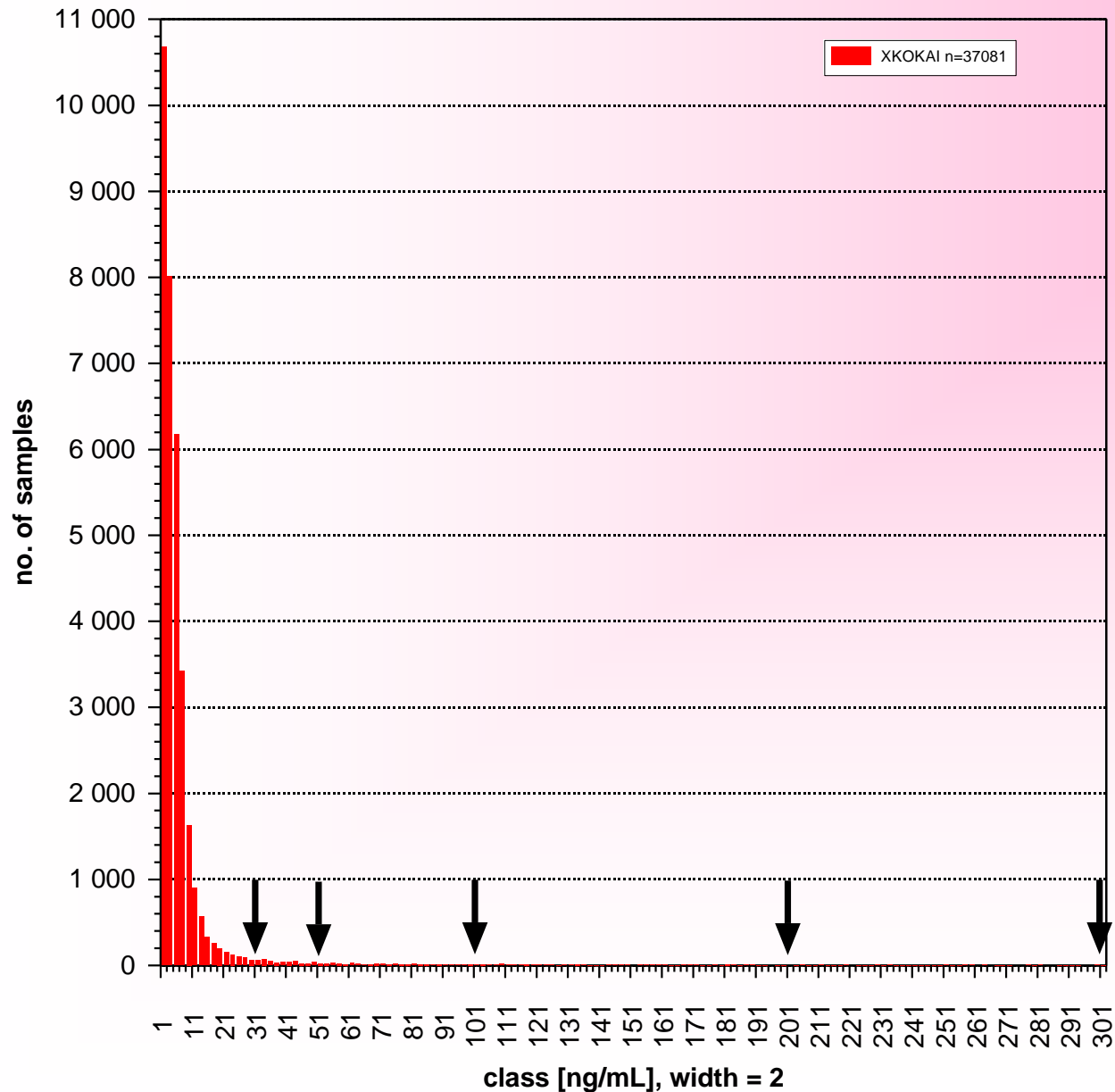
Positive rates:

cutoff >= 300 ng/mL: 3017 spls. =   8.1%
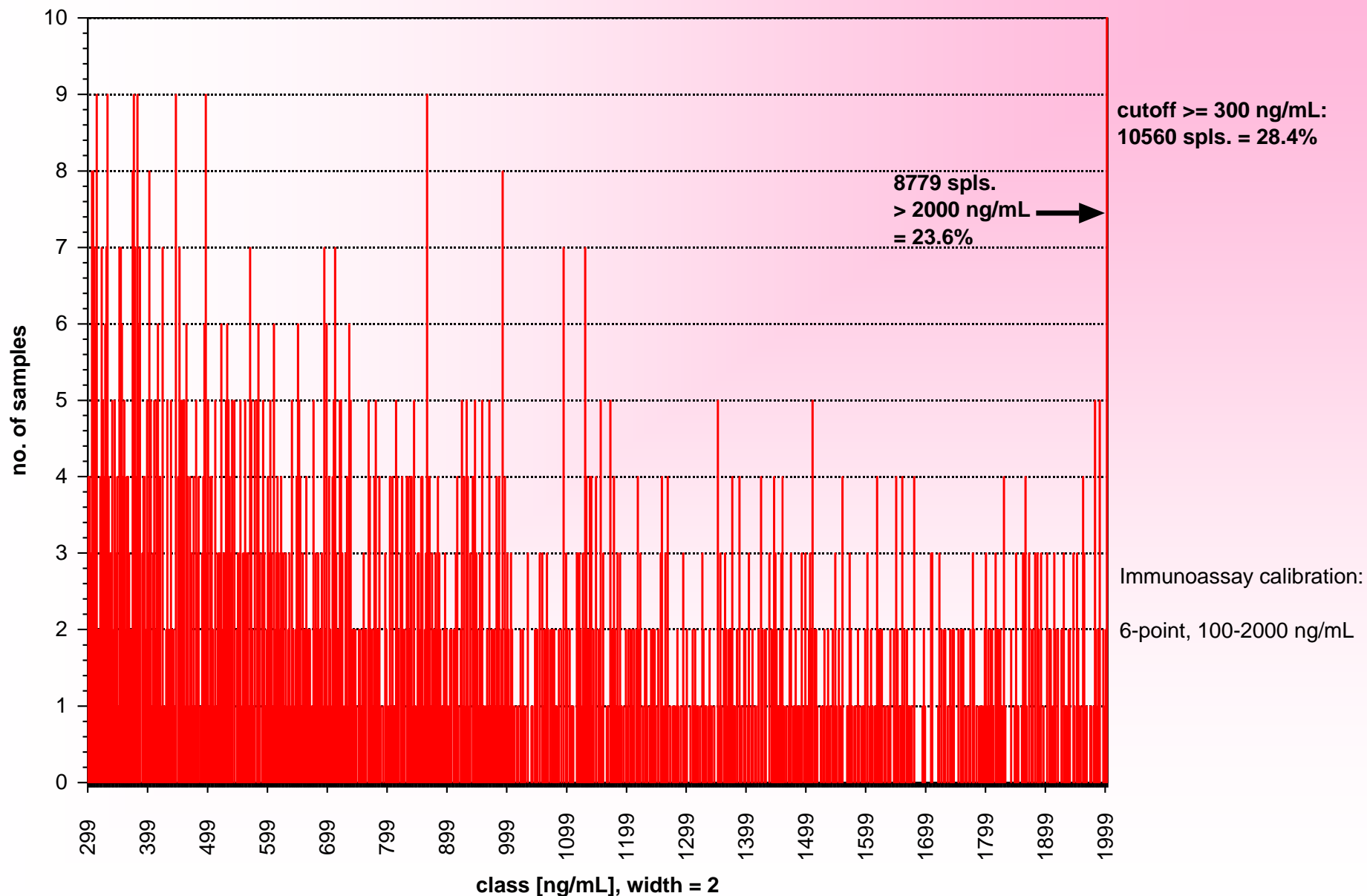
cutoff >= 200 ng/mL: 3169 spls. =   8.5%

cutoff >= 100 ng/mL: 3502 spls. =   9.4%

cutoff >= 50 ng/mL: 3922 spls. = 10.6%

cutoff >= 30 ng/mL: 4361 spls. = 11.8%

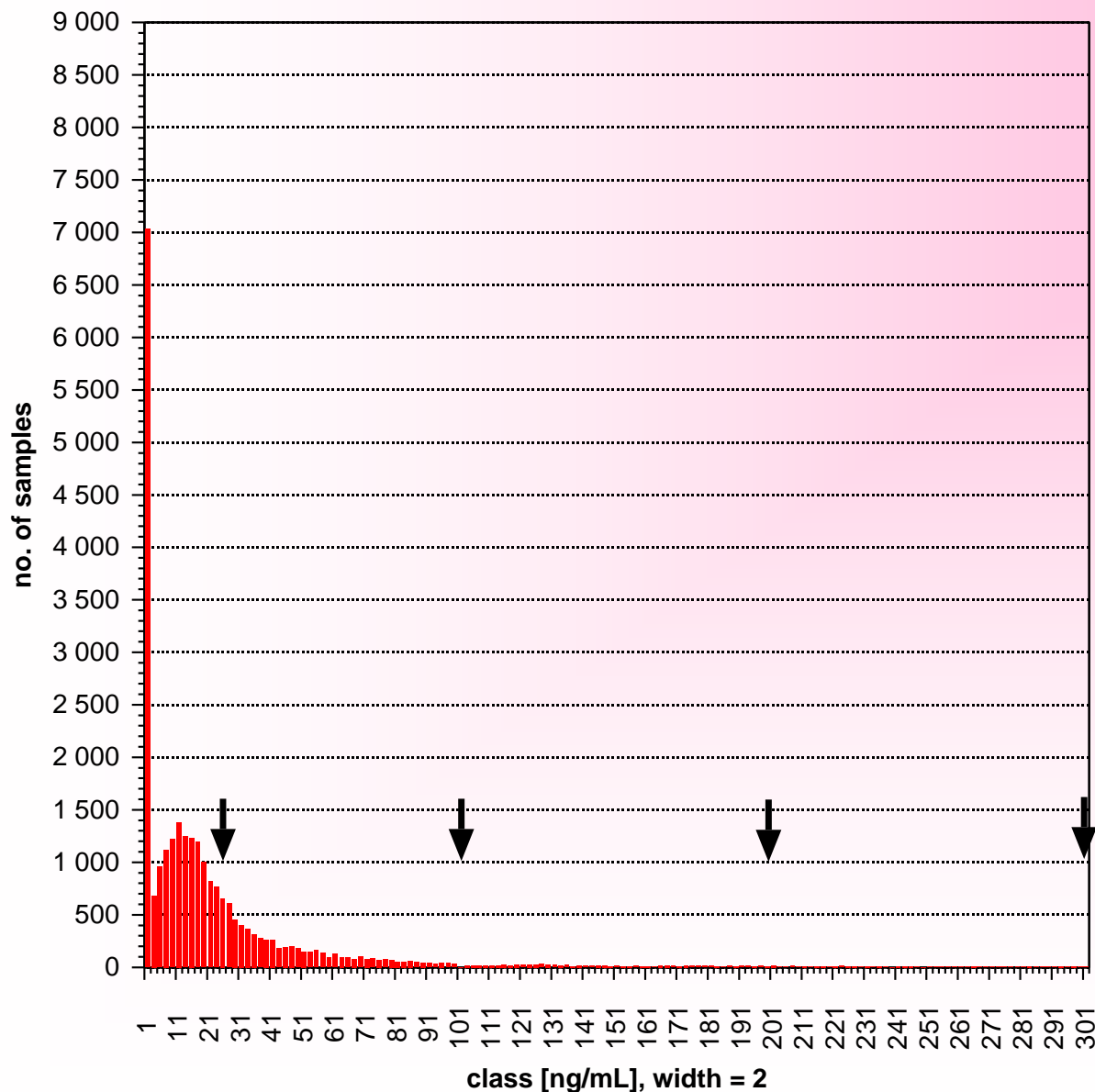XKOKAI n=37081

no. of samples

class [ng/mL], width = 2

**Opiates-Immunoassay response distribution -- 300- >2000 ng/mL
37140 samples, 6 month**

no. of samples

cutoff >= 300 ng/mL:
10560 spls. = 28.4%

8779 spls.
> 2000 ng/mL ➔
= 23.6%

Immunoassay calibration:

6-point, 100-2000 ng/mL

class [ng/mL], width = 2

Opiates-Immunoassay response distribution -- 0-300 ng/mL
37140 samples, 6 month

Positive rates:

cutoff >= 300 ng/mL: 10560 spls. = 28.4%

cutoff >= 200 ng/mL: 10955 spls. = 29.5%

cutoff >= 100 ng/mL: 11750 spls. = 31.6%

cutoff >=   25 ng/mL: 18138 spls. = 48.8%

no. of samples

class [ng/mL], width = 2

Conclusions:

-- positive rates OF (low CO!) vs. urine were comparable

-- 1 ng/mL cutoff recommended for clinical drug testing,
  higher cutoff for workplace testing?
-- lower CO needed for some substances (eg. Fentanyl)

-- OF CO possibly can be adjusted to certain (impairment?)
  serum levels. Studies with paired samples needed

-- multi-target-screening can be quickly adopted to changing
  needs (new drugs, different settings, different CO)

-- scientific societies:
  develop guidelines on method development, accreditation,
  sampling etc.

Thank you for your attention!