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Comparative LCMS/MS Drug Analysis of paired Urine, Oral Fluid and Capillary Blood Samples in Addiction Therapy

Bernd Huppertz^{1*},
Michael Bottcher², Stefan
Lierheimer², Prisca
Gottwald³, Sergej Bleicher⁴
and Martin Schafer³

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Abstract

Background: For many years, drug of abuse analysis was typically performed on urine using enzyme immunoassays with Gas Chromatography–Mass Spectrometry (GCMS) confirmation. However, analytical instruments improved significantly over the last decades, allowing analysis of smaller sample volumes and other matrices, such as capillary blood and oral fluid, with lower drug concentrations. To our knowledge, the present study is the first to compare the matrices urine, capillary blood and oral fluid using similar analytical techniques and paired samples.

Method: Samples were collected from patients admitted for detoxification to Huysens-Stiftung, Essen, Germany over a period of maximum 14 days. Each patient provided samples of all three matrices at almost the same time during each collection cycle. All analyses were performed with Liquid Chromatography with tandem Mass Spectrometry (LCMS/MS).

Results: Detection periods vary between the matrices depending on the analytes and several positive results in one matrix could not be confirmed in one or two of the others. In particular capillary blood showed some implausible results.

Conclusion: It depends on the formulation of the question which of the matrices is better suitable for any given purpose. The highest positive rates, for example, for 6-Monoacetylmorphine (6-MAM) can be found in oral fluid, and the longest detection periods for cannabis and benzodiazepine detection can be found in urine. Although, again depending on the formulation of the question, the very long detection periods may make it advisable to use a cut off for urine in the range of 10 to 50 ng/ml, depending on the analyte.

Ethical approval: The study was authorized by the ethics commission of the Medical Faculty, University of Duisburg-Essen/Germany on July 15th, 2016

Keywords: Urine, Oral fluid, Capillary blood, LCMS/MS

- ¹ Forensic toxicology and special chemistry, MVZ Labor Dr. Quade and Kollegen, Cologne, Germany
- ² Analysis of legal and illicit drugs, MVZ Medizinische Labore Dessau Kassel GmbH, Dessau, Germany
- ³ Psychiatry, psychotherapy, psychosomatics and addiction medicine, Evang. Kliniken Essen-Mitte, Essen, Germany
- ⁴ Drug analysis/forensic toxicology, Synlab Medizinisches Versorgungszentrum Weiden GmbH, Weiden in der Oberpfalz, Germany

*Corresponding author:

Bernd Huppertz

✉ huppertz@lab-quade.de

Department of Clinical and Toxicological Analysis, University of Cologne, MVZ Laboratory Dr. Quade & Colleagues, Aachener Str. 338, 50933 Cologne, Germany

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Introduction

Drug screenings play an important part in adherence monitoring in addiction therapy. Biological samples are analyzed to detect concomitant use and observe therapy progress. But what guides a clinician's choice of matrix?

For many years, drug of abuse analysis was typically performed on urine using enzyme immunoassays with GCMS confirmation. In 1984, for example, Richard L. Hawks described the general use of immunoassays and chromatography in urine drug detection

and recommended using a confirmation analysis for all samples that screened positive [1]. A certain preference for urine may be attributed to ease of collection as well as high concentrations of drugs and metabolites which allow for comparatively long detection times [2]. However, several substances require urine samples to be hydrolyzed. Furthermore, urine is prone to various forms of manipulation [3]. Also, to avoid sample substitution in an

attempt to submit 'clean' urine, collection needs to be executed either under direct supervision or using a chemical marker to verify the identity of the sample donor [4].

As the sensitivity of new analytical devices such as LCMS/MS and high-resolution mass spectroscopy improved over the last years, it became possible to investigate materials with lower concentrations of addictive drugs and low sample volumes. Verstraete [5] determined the detection periods for various drugs in oral fluid, urine and blood using different cut offs from different studies of up to 48 h in blood and plasma, up to 50 h for oral fluid and 96 h for urine.

Numerous publications regarding the detection of drugs in urine, blood and oral fluid using various analytical procedures have been available for a long time already. Reviews were provided e.g. by Moeller et al. [6] in 1998 or Schramm et al. [7], Dolan et al. [8]. Over the past years, one focus has been directed at the investigation of capillary blood for various drugs of abuse, including the analysis of Dried Blood Spots (DBS) [9-11].

Comparative measurements between capillary blood and DBS showed a significantly broader fluctuation rate and lower concentrations for cocaine and benzoylecgonine in DBS than in venous blood [12]. Furthermore, the hematocrit has an influence on formation of the DBS and the determined concentration of drugs [13,14]. In our investigation capillary blood was collected in EDTA coated tubes so that hematocrit has no significance.

The concentration of the drugs in oral fluid depends on the amount of free, unbound drugs and lipophilic metabolites in the blood. The oral fluid concentrations are a function of the drugs' pKa values and of the pH values of the blood as well as the oral fluid and the protein-bound parts of the drugs. The saliva/plasma ratio (S/P ratio) can be calculated using the Henderson-Hasselbalch equation [15]. The pH value in the saliva depends on the salivation flow. Stimulated saliva has a higher pH value than unstimulated saliva [16]. Saliva secretion is controlled by the parasympathetic nervous system and the sympathetic nervous system and can be influenced by various stimuli. For example, patients taking medications affecting the nervous system may show a different oral fluid composition [17]. Please note that even though the terms saliva and oral fluid may have been used in an interchangeable manner in literature quoted in this article, for the purpose of this investigation the term oral fluid is used.

The aim of the study was to evaluate the advantages and disadvantages of the matrices urine, capillary blood and oral fluid with paired samples and comparable, sensitive analytical methods as High-Performance Liquid Chromatography (HPLC) coupled with mass-spectroscopy. The method of sample collection for each matrix is described in 2.2.

To our best knowledge, comparative measurements from urine, capillary blood and oral fluid with paired samples and comparable analytical methods as performed in this study have not been investigated before.

Methods

General overview: Paired samples of urine, oral fluid and capillary blood produced by 46 patients investigated for drugs.

Patients

The paired samples originated from 46 patients who were admitted to the addiction ward of Huysdens-Stiftung, Essen, Germany, for addictive drug detoxification. 41 patients were male and 5 female. 16 were between 30-39 years of age, 17 patients between 40-49, and 9 between 50-59. Four patients were younger than 30 or older than 60 years of age, respectively. 24 of the patients had used two, 15 three, 6 one and 1 four substances in the past. 33 patients were addicted to heroin, 19 to benzodiazepines, 18 to cocaine, 13 to Tetrahydrocannabinol (THC) and 4 to amphetamines.

Samples

Urine samples were collected without direct observation using the Ruma® Marker-System (Ruma GmbH, Cologne, Germany) [18], oral fluid samples were collected using the Greiner (Greiner Bio One GmbH, Kremsmünster, Austria) collection system, and 20 µl capillary blood samples were collected with an ethylenediaminetetraacetic acid (EDTA) coated Minivette® (Sarstedt, Nümbrecht, Germany). Oral fluid samples and capillary blood samples were taken at the same time. The urine samples were passed within one hour before or after collecting the other matrices. The samples were labeled with barcodes and transferred to the respective laboratory in refrigerated containers. The urine samples were analyzed by MVZ Labor Dr. Quade & Kollegen, Cologne, Germany, the oral fluid samples by MVZ Synlab, Weiden, Germany, and the capillary blood samples by MVZ Labor Dessau, Dessau, Germany.

Samples were collected on the day the patients were admitted to the ward (day 1) as well as on days 3, 5, 7, 9, 11 and 13 or 14, respectively. For the first 10 study participants, samples were only taken for a period of 7 days after the patient was admitted to the ward. Since it became apparent that the period of detectability is longer than 7 days in several matrices, the period of investigation was extended to 2 weeks for the following patients. No further samples were collected after a patient tested negative in all three matrices for two consecutive days. Patients who stopped therapy before the end of the 2 week period were excluded.

Analytical Method

The analytical method used for all three matrices was triple quad mass spectroscopy after HPLC or LCMS/MS. Depending on the executing laboratory, equipment by different manufacturers was used. Agilent (Santa Clara, CA, USA) for oral fluid, Shimadzu (Kyōto, Japan) for urine and Waters (Milford, MA, USA) for capillary blood. Additionally, the urine samples were also tested using the Siemens enzyme multiplied immunoassay technique (EMIT). The results of the immunoassay are shown in **Table 1**.

In urine and oral fluid the drug concentrations were quantified. For capillary blood a semiquantitative method was used, respectively. The concentration in capillary blood was estimated without considering the hematocrit value because DBS were not used.

Our investigation covered a wide range of substances or substance groups. Five individual substances from the substance group of opiates were investigated. These were morphine, codeine,

Table 1: The number of positive samples of the different analytes in the three matrices at the first and last day. It is also shown how many of the positive samples were negative at day 3 and the day the last positive result occurred. The cut off values are in ng/ml. If not shown the cut off for capillary blood was 1 and for oral fluid 0.5 ng/ml.

Substance		Urine EIA	Urine	Urine >	Oral Fluid	Capillary Blood
THC/THC-COOH	Cut Off	20	0.6	10	0.5	1
	positive d1	13	16	12	5	11
	from that negative d3	1	2	5	3	3
	positive d13/14	7	11	4	0	1
	last positive	>=14	>=14	>=14	3	>=14
Cocain/BZE	Cut Off	35	1.7	35	0.5	1
	positive d1	11	18	12	14	15
	from that negative d3	4	0	2	3	1
	positive d13/14	0	6	1	1	3
	last positive	11	>=14	>=14	>=14	>=14
Morphine/Opi	Cut Off	100	0.4	25	0.5	1
	positive d1	23	23	23	23	23
	from that negative d3	8	0 / d5=1	2	9	1
	positive d13/14	0	11	0	0	6
	last positive	5	>=14	7	11	>=14
Codeine	Cut Off		0.8	25	0.5	1
	positive d1		19	18	17	18
	from that negative d3		5	17	16	7
	positive d13/14		1	0	0	3
	last positive		>=14	3	3	>=14
6-MAM	Cut Off		1.8		0.5	1
	positive d1		19		25	9
	from that negative d3		18		14	5
	positive d13/14		0		0	2
	last positive		3		7	9/>=14
Acetylcodeine	Cut Off		0.3		0.5	1
	positive d1		12		16	1
	from that negative d3		12		16	1
	positive d13/14		0		0	0
	last positive		1		1	1
Amphetamine/s	Cut Off	500	0.7	50	5	1
	positive d1	5	8	6	5	9
	from that negative d3	3	3	2	2	3
	positive d13/14	0	3	0	0	2
	last positive	5	>=14	7	7	>=14
Oxazepam/BZO	Cut Off	200	0.3	50	0.5	1
	positive d1	14	16	11	1	9
	from that negative d3	0	0	0	0	0
	positive d13/14	7	11	6	1	4
	last positive	>=14	>=14	>=14	7/>=14	>=14
Diazepam	Cut Off		0.5		0.5	1
	positive d1		12		10	13
	from that negative d3		2		0	1
	positive d13/14		3		7	1
	last positive		>=14		>=14	>=14
Nordiazepam	Cut Off		1.3	50	0.5	1
	positive d1		13	7	6	11
	from that negative d3		2	3	0	0

	positive d13/14		8	2	5	8
	last positive		≥ 14	≥ 14	≥ 14	≥ 14
Temazepam	Cut Off		1	50	0.5	1
	positive d1		15	12	1	8
	from that negative d3		1	3	0	0
	positive d13/14		9	5	0	1
	last positive		≥ 14	≥ 14	5	≥ 14
Clonazepam(NH)	Cut Off		2.6		0.5	1
	positive d1		6		2	5
	from that negative d3		2		0	2
	positive d13/14		0		0	0
	last positive		11		7	9

dihydrocodeine, 6-monoacetylmorphine and acetylcodeine. In capillary blood, morphine glucuronide and codeine glucuronide were additionally analyzed as plausibility checks. For cocaine detection, cocaine and the main metabolite benzoylecgonine were measured in capillary blood and oral fluid, in urine only benzoylecgonine. For the detection of cannabis consumption, oral fluid and capillary blood were tested for THC and the main metabolite 11-Nor-9-carboxy- Δ^9 -tetrahydrocannabinol (THC-COOH). Urine was tested only for the metabolite THC-COOH. With all three matrices, amphetamine, methamphetamine, Methylenedioxyamphetamine (MDA), Methylenedioxy-Methylamphetamine (MDMA) and Methyl Diethanolamine (MDEA) were analyzed for the class of amphetamines. For the detection of benzodiazepines, investigation was performed for diazepam and metabolites (nordiazepam, temazepam and oxazepam), bromazepam, flunitrazepam, nitrazepam, clonazepam, midazolam, flurazepam, alprazolam, lorazepam, clobazam, triazolam or their metabolites, respectively. Urine was primarily analyzed for the metabolites, whereas capillary blood and oral fluid were primarily analyzed for the parent substances. The results of the Siemens EMIT urine screening procedure were not explicitly included in the evaluation as they are semiquantitative group tests. However, they were used as a comparison to LCMS/MS testing.

The results were individually analyzed for each substance. Only those substances were considered in the evaluation that showed values above the cut off or the detection limit, respectively, on the first day in at least one matrix (**Table 2**). The evaluation provides an analysis of samples collected over two weeks of the individual matrices for the various analytes. As the last day of sample collection varied between day 13 and day 14, the results of those two days were pooled. Data on specific substances for patients who did not reach the endpoints – negative for the substance on two consecutive days of measurement in at least two matrices or sample collection over 13/14 days – was not included.

All materials and solvents were of LCMS grade. Analytical standards were purchased from Merck (Darmstadt, Germany) and LGC Standards GmbH (Wesel, Germany). Solvents of analytical grade purchased at Carl Roth (Karlsruhe, Germany) or Merck (Darmstadt, Germany) as well as urine and serum controls from ACQ Science GmbH (Rottenburg, Germany) and Medichem (Rendsburg, Germany) were used.

Detection of drugs in urine: Urine analysis was carried out on a Shimadzu LCMS 8050 with a Multiple Reaction Monitoring (MRM) method with two or three transitions for each analyte. A liquid/liquid extraction procedure was utilized for sample preparation. All drugs were measured in one analysis run. Most of the results are quantitative. Some analytes (acetylcodeine, OH-bromazepam, NH-clonazepam) were measured semiquantitatively. 100 μ l urine was mixed with 10 μ l internal standard (5-50 ng/ml of each analyte), 10 μ l buffer, 10 μ l BG-turbo β -glucuronidase and 40 μ l methanol. The mixture was incubated for 14 h at 60 °C. After incubation, 25 μ l 3 M NH₃, 1.6 ml saturated NaCl-solution and 2 ml ethyl acetate/dichloromethane (1:1) were added to the sample in a glass vial. The vial was mixed in an overhead mixer for 10 min and then centrifuged for 5 min at 1500 rpm. The supernatant was transferred in a separate vial. In a second step 2 ml ethyl acetate/diethylether (1:1) were added to the residue in the original vial and mixed overhead for 10 min. Then, the sample was again centrifuged for 5 min at 1500 rpm and the supernatant of the second step was added to the supernatant of the first step. 50 μ l 10% Hydrochloric Acid (HCl) was added and the sample was dried under nitrogen at 37 °C. The residue was solved in 50 μ l methanol/water. 5 μ l of the sample was applied on a Restek biphenyl column 150*3 mm 2.7 μ l and separated with a water-methanol gradient containing 0.1% acetic acid and 2 mmol ammonium formate. Eluent A was containing 100% water and eluent B 100% methanol. The column was first flushed with 10% eluent B for 0.5 min. Thereafter, as a linear gradient, the percentage of eluent B was increased in a first step to 40% at 2.5 min, in a second step to 90% B after 5.5 min and held until 8.5 min. After 9 minutes the eluent contained 10% B again until the method ended at 10 minutes. The flow decreased from 0.35 ml/min to 0.2 ml/min after 9 minutes. The method target analytes were accredited according to DIN EN ISO 17025 regulations.

Detection of drugs in oral fluid: The samples were collected with the oral fluid collection system (Greiner Bio One GmbH, Kremsmunster, Austria). The particular advantage of this kit is the active stimulation of salivary flow by a sampling fluid which itself is an aqueous solution containing citric acid and the ternary dye tartrazine, buffered to pH 4.2. During our investigation the probands rinsed their mouth with the sampling fluid for a period of one to two minutes. After spitting it out into a collection cup, the sample was transferred to evacuated tubes where sodium azide was added as a preservative. Since the sampling fluid was

Table 2: Level of detection (ng/ml) of the tested analytes in urine and cut off (ng/ml) for the tested drugs in oral fluid and capillary blood. Analytes with no value in one or more matrices were not measured in this matrix.

Analyte	Urine: limit of detection	Capillary blood: cut off	Oral fluid: cut off
Morphine	0.4	1.0	0.5
Codeine	0.76	1.0	0.5
6-MAM	1.75	1.0	0.25
Acetylcodeine	0.29	1.0	0.25
THC-COOH	0.6	1.0	-
THC		1.0	1
Benzoylcegonine	1.65	1.0	0.5
Cocaine		1.0	0.5
Diazepam	0.5	1.0	0.5
Nordiazepam	1.26	1.0	0.5
Oxazepam	0.3	1.0	0.5
Temazepam	0.93	1.0	0.5
NH-Clonazepam	2.61	1.0	0.5
Clonazepam		1.0	
Bromazepam		1.0	0.5
OH-Bromazepam	3.09	-	-
NH-Flunitrazepam	0.16	0.1	0.5
Flunitrazepam		1.0	0.5
Amphetamine	0.68	1.0	5
Methamphetamine	0.11	1.0	5
MDA	0.19	1.0	5
MDMA	0.13	1.0	5

part of the sample, the proportion of saliva was determined in the laboratory by photometry. Because of the frequent occurrence of very viscous and sometimes slimy samples, extraction *via* a solid phase appeared more suitable here than a liquid/liquid extraction. The selected solid phase, Isolute HXC, 300 mg (Biotage, Uppsala, Sweden), contained two components for the retention of neutral and slightly basic substances. The solid phase was first conditioned with 1 mL of methanol and equilibrated to pH 4 with 1 mL ammonium formate buffer (380 mg/L with 0.01% formic acid). 1 mL sample was added with deuterated standards (LGC and Lipomed) and diluted with 1 mL ammonium formate buffer in 2 mL Eppendorf tubes, mixed and then centrifuged (5 min, 14000 rpm). The solid phase was loaded with the sample, which is slowly forced through the column bed by overpressure (UTC, Positive Pressure Manifold, up to 800 kPa, 0.5 mL/min). This was followed by two washes with 1 mL of ammonium formate buffer and 3 mL of a 1:1 mixture of methanol and ammonium formate buffer. The column bed was dried with high nitrogen flow for 5 min. The analytes were extracted from the solid phase by 1 mL of methanol with 5% ammonia (Merck, 25 %, for analysis). The extract was evaporated to dryness at 40 °C and gentle nitrogen flow and then taken up with 100 µL of a mixture of ammonium formate buffer and MeOH (60:40) and centrifuged in 1.5 mL reaction tubes (5 min, 14000 rpm). Part of the extract was used for analysis and the remainder is stored in the refrigerator in order to be able to carry out a reinjection in case of any possible carry-over. 5 µL were injected into the LC/MSMS instrument.

All analytes were measured by liquid chromatography (Agilent, 1260 Infinity II) coupled tandem mass spectrometry (Agilent, 6470 A). The analytes were separated by a gradient starting with 95% of an aqueous ammonium formate solution (380 mg/L, 0.01%

formic acid) to 100% of acetonitrile (Carl Roth, HPLC degree) with 0.01% formic acid over 10 min on an analytical separation column (Agilent, Zorbax Eclipse Plus C18, 100 mm × 2.1 mm × 1.8 µm). The molecules were ionized by electrospray ionization (ESI) in positive mode. For this purpose, the parameters of the ESI source were optimized for the ionization of THC. At least three MS/MS transitions were recorded.

The method target analytes were accredited according to DIN EN ISO 17025 regulations.

Detection of drugs in capillary blood: The capillary blood samples were collected from the finger pad of ring or middle finger. Prior to the finger prick the finger was wiped with a disinfectant solution containing ethanol. After that, 20 µL of sample were taken twice each time using an EDTA coated Minivette® (Sarstedt, Nümbrecht, Germany). The sample volume was subsequently transferred into a 1.5 mL reaction vial and sent to the laboratory *via* postal service. The sample arrived dried in most cases. The residue was dissolved by 1 min vortexing with 220 µL acetonitrile and 10 µL deuterated internal standard in methanol. Internal standard concentrations were chosen to be 50% below cut off concentration in whole blood. After centrifugation the supernatant was transferred into a 96 well micro-titer plate where 10 µL ethylene glycol has been previously added to each cavity. Subsequently, evaporation was performed within 60 min at 30°C with the plate positioned into a centrifugal evaporator (Eppendorf AG, Hamburg). The remaining ethylene glycol was solved with 80 µL water and 10 µL methanol. After vortexing the plate 10 µL of the sample was injected into an ultraperformance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) system consisting of an UPLC I-Class connected to an Xevo

TQ-XS (both Waters GmbH, Eschborn, Germany) operating in selected reaction monitoring (SRM) and positive ionization mode. Separation was conducted on an ACQUITY UPLC® BEH Phenyl 1.7 µm, 2.1 mm x 100 mm column (Waters GmbH, Eschborn, Germany) within 11-minute gradient elution, followed by a 0.5-minute re-equilibration step. Mobile phase A consisted of 20 mM ammonia formate with 0.1% formic acid and mobile phase B was methanol with 0.1% formic acid. Three transitions were recorded for all analytes and two transitions for the internal standards. The method targets 65 analytes and were accredited according to DIN EN ISO 15189 regulations.

Results

The results of the different matrices were separately compared for each analyte.

Table 1 shows a comparison of positive results for the three matrices over the 14 days of investigation. The number of positive results the first day and the used cut off was specified. Moreover, the numbers of patients who were positive the first day and negative at day three and the number of positive patients the last day were listed. If no patient was positive the last day, the day was specified when at a minimum one patient was positive for the analyte. Because of the high positive rates and long detection times for urine using the level of detection, the positive rates for a higher cut off were also investigated. The lower level of quantification of the German chemical toxicological investigation (Chemisch-Toxikologische Untersuchung, CTU) criteria of the medical psychological investigation program for recovering the driver's license after driving under drug influence (CTU criteria) [19] was used as an alternative urine cut off. In comparison to the LCMS/MS testing the popular enzyme immunoassay was evaluated. The EMIT drug tests by Siemens have been used with the manufactures recommended cut offs.

The results for THC or THC-COOH, respectively, regarding urine with LCMS/MS and a 10 ng/ml cut off, enzyme immunoassay (EIA) and capillary blood were similar the first 3 days. After 14 days the positive rates in urine were higher, even with EIA, than in the other matrices. The detection rates and time in oral fluid were lower than for the other matrices. The last positive result in oral fluid was detected after three days. The S/P-ratio for THC is 0.03, so this result was expected [20].

Cocaine or its metabolite benzoylecgonine show comparable detection times and rates for all the matrices. The highest positive rates were found for urine if the Limit of Detection (LOD) was applied. 6 of the 18 positive patients who were positive on the first day were even positive for benzoylecgonine after 14 days. The S/P-ratio for cocaine is 1, so the findings were plausible [20].

23 patients were morphine positive the first day in all matrices. Positive morphine results the last day in urine and capillary blood could be found. The number of positive patients after 3 and more day was lower for oral fluid, which can be explained by the S/P-ratio of 7 for morphine. The S/P-ratio for codeine is also 7 which matches similar positive rates seen for codeine [20].

For 6-MAM and acetylcodeine the highest number of positive results was observed in oral fluid, for 6-MAM also the longest time of detection.

The number of patients positive for amphetamine in oral fluid was comparable to the number of patients positive in urine with a 50 ng/ml cut off. This also applied to the detection time. In capillary blood and urine without a cut off used, there were patients positive after 14 days. As the S/P-ratio of amphetamine is nearly 7 [20] a longer detection time in oral fluid was expected.

Diazepam metabolites had the highest positive rates and longest detection times in urine, for oxazepam and temezepam even if a 50 ng/ml cut off was used. Nordazepam appeared similar in all matrices. Just on the first day the positive rate was lower for oral fluid. Diazepam was comparable on the first day for all matrices. However, it had the highest number of positive results in oral fluid. The S/P ratio is 0.02 so this result was surprising [20].

Clonazepam or its metabolite had a good correlation between all matrices. For urine, instead of clonazepam itself only the metabolite 7-amino-clonazepam was measured because it can be detected for a longer period of time. For temezepam urine seemed to be the most appropriate matrix for detection. In oral fluid temezepam was difficult to detect because only one patient was positive.

The figures not only show the number of positive samples for one matrix at the different days. They also show the correlation between the different matrices. Specifically, how many samples were positive for all matrices or only in two or one matrix, respectively? In the example of **Figure 1** at day 14, 3 samples were positive for urine and 2 for capillary blood, but only one of the samples was positive for both matrices. Over all, four samples were positive.

Amphetamines

Figures 1 and 2 the best correlation between the matrices was observed during the first days. After 9 days, urine samples investigated with a cut off 50 ng/ml no longer registered as positive and only capillary blood samples were positive. It was astonishing to us that the number of positive capillary blood samples increased from day 9 to day 11. However, implausible results were measured in capillary blood for one patient on day 9 and two other patients on day 11. These three patients were only positive on that respective day and only in capillary blood, not in urine or oral fluid.

Cannabis

Figure 3 the number of positive cannabis (THC or THC-COOH) results was less for oral fluid than for the other matrices. None of the patients was positive only in oral fluid the first five days. At day seven one patient showed a high THC concentration in oral fluid even after he was negative at day five. This patient showed decreasing THC-COOH concentrations on day seven compared to day five in urine. Some patients were positive in capillary blood and urine but after seven days most of the cannabis positive patients had only a positive result in urine. After 14 days 10 of the 14 positive patients from the first day were positive in urine, only one in capillary blood. For half of the urine positive patients concentrations >10 ng/ml were measured.

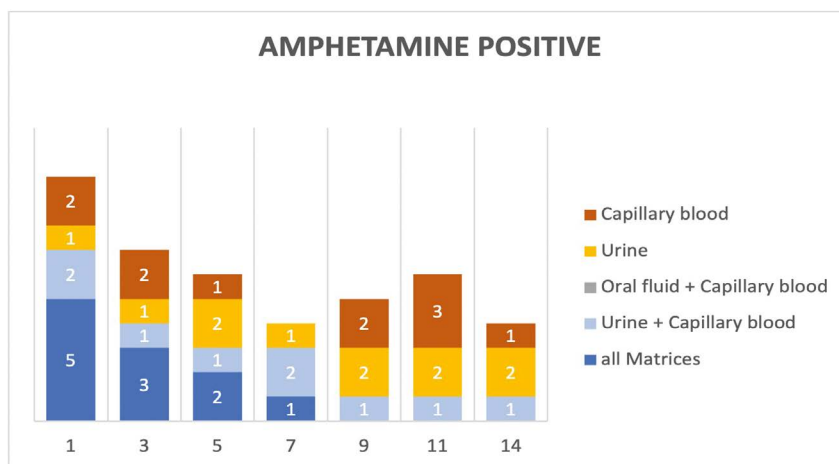


Figure 1 Number of patients amphetamine positive only one (capillary blood; urine), two (oral fluid+capillary blood; urine+capillary blood) or all three matrices. For urine a LOD of 0.65 ng/ml, and a 1 ng/ml cut off for capillary blood and 5 ng/ml for oral fluid was used.

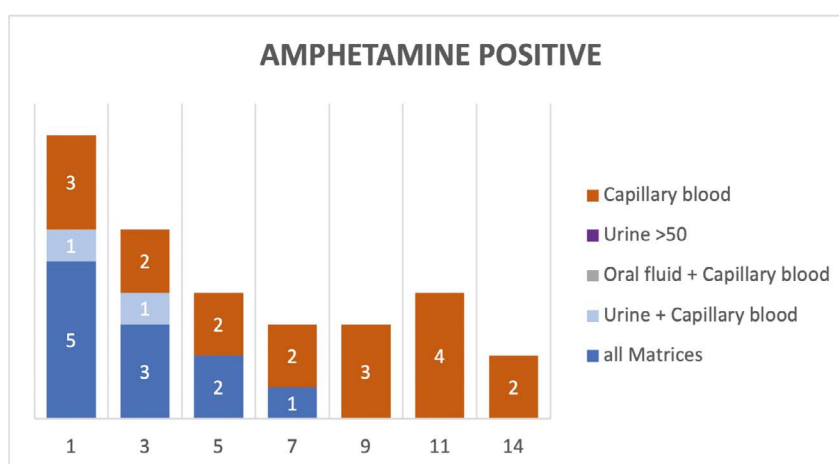


Figure 2 Number of patients amphetamine positive in only one (capillary blood), two (oral fluid and capillary blood; urine and capillary blood) or all three matrices. A 50 ng/ml cut off for urine, 1 ng/ml for capillary blood and 5 ng/ml for oral fluid was used.

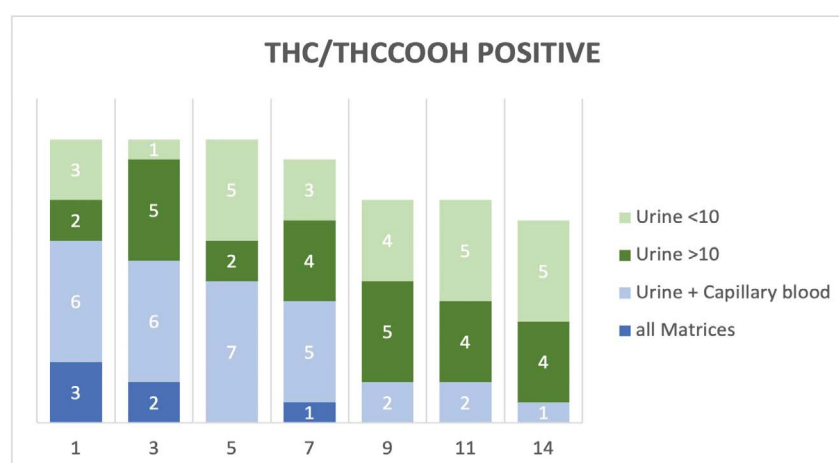


Figure 3 Number of patients THC/THC-COOH positive in only one (urine), two (urine+capillary blood) or all three matrices. A urine LOD of 0.6 ng/ml, a 1 ng/ml cut off for capillary blood and 0.5 ng/ml for oral fluid was used.

Morphine

Figures 4 and 5 all 23 morphine positive patients meeting the criteria for the study were positive in all three matrices on the first day (**Figure 4**). The third day 22 patients were urine positive, 13 of them in all matrices, 9 in capillary blood and urine and one in oral fluid and urine. After 7 days two patients had positive results in all matrices, 13 in capillary blood and urine, and 7 only in urine. In total, 22 of the 23 positive patients from day 1 were positive in at least one matrix. At the 9th day one patient was only positive in oral fluid and capillary blood, respectively, 17 in urine only. Even after 14 days 14 of the patients showed a positive result in one [11] or two matrices [3]. The number of patients positive in urine and oral fluid decreased over the investigated time. In capillary blood the number of positive patients increased from the 9th to the 14th day from three to six. That meant patients were positive again after they were negative the days before. For most of the capillary blood positive patient's only morphine and no morphine glucuronide was found. A positive result after a negative one the days before normally was a hint for a relapse. By contrast, the results in urine showed that this was not the case. Three of the patients were only positive in capillary blood.

Figure 5 shows the results when urine cut off 25 ng/ml was used. The first three days were quite similar to the results without cut off. The number of patients with positive urine results decreased much faster. The last patient positive in oral fluid was detected on day 9. After 11 and 14 days some patients were only positive in capillary blood. As mentioned before, it was amazing that the positive capillary blood samples increased from day 9 to 14.

6-MAM

Figure 6 at the first day 25 patients were measured 6-MAM positive in at least one matrix. All 25 patients were positive in oral fluid, 19 in urine and 9 in capillary blood. At the 5th day patients were only positive in oral fluid and capillary blood but only one patient in both matrices. Amazingly, one patient was capillary blood positive only that day and only in capillary blood. On day 7, three patients showed positive results in oral fluid and one in capillary blood. Two of the oral fluid positive patients from day 1 were negative at day 5.

After 9 days one patient was positive only in capillary blood. But this patient was negative at day 5 and 7. No patient was positive for 6-MAM in any matrix on the 11th day but 2 patients were positive at day 14 in capillary blood. One of them was never 6-MAM positive in capillary blood before, only in oral fluid until day 7.

Acetylcodeine

The street-heroin marker acetylcodeine was only positive the first day. One patient was positive in all matrices, 9 in oral fluid and urine, and 5 in oral fluid only and one only in urine.

Codeine

Codeine was only positive for patients with higher morphine concentrations and an impurity of the heroin used by the patients. The codeine concentrations found were quite low. Oral fluid and urine at a cut off at 25 ng/ml have nearly the same positive rates

and the last positive patient result after three days. The positive urine samples after 7 and more days showed concentrations less than 1.1 ng/ml. Only in capillary blood patients showed codeine concentrations between 2 and 12 ng/ml after 14 days, though the concentrations were lower or negative at day 9 and 11.

Diazepam

Figure 7 in figure 7, the number of positive results of diazepam or one of its metabolites is shown. Patients rated as positive if diazepam or one of its metabolites nordiazepam, oxazepam or temazepam was positive. The first 7 days most of the positive patients were positive in all three matrices. The time of detection was longer in urine and capillary blood than in oral fluid. Even in oral fluid, diazepam or nordiazepam was measurable with 5 of the 10 positive patients. 8 of these patients were positive in capillary blood and all of them in urine. The target analytes for oral fluid were diazepam and nordiazepam, whereas oxazepam and temazepam were positive for one patient each. The number of positive patients was also lower for capillary blood for oxazepam and temazepam in comparison to diazepam and nordiazepam. Only in urine the number of positive patients was similar for all metabolites but lower for diazepam.

Clonazepam

Figure 8 the confirmation of clonazepam intake was carried out measuring clonazepam in oral fluid and capillary blood and its metabolite 7-aminoclonazepam in urine. Clonazepam itself can be found in urine but was not measured in this study. Two patients were clonazepam or metabolite positive in all three matrices for seven days. The last positive patient was found after 11 days in urine. This patient was positive in urine and capillary blood at the 9th day and positive in all matrices at day seven. The time of detection for most patients was longer in urine than for capillary blood, which had longer detection times than oral fluid.

Cocaine

Figures 9-11 in urine, only the cocaine metabolite benzoylecgonine was detected because cocaine was only detectable in urine a short time after consumption. Both analytes measured in oral fluid and capillary blood. Further cocaine metabolites were not measured.

In **Figures 9 and 10** the number of positive patients is illustrated for urine at the limit of detection and with a cut off at 35 ng/ml (CTU criteria). During the first week of investigation most of the cocaine positive patients were positive in three or two matrices. The patients who were positive in urine only, had benzoylecgonine concentrations lower than 10 ng/ml aside from one patient at days 11 and 14.

Two patients at the 7th day were only positive in oral fluid with a concentration of approx. 0.5 ng/ml of cocaine. The patients with positive results for capillary blood showed concentrations between 1.2 and 8 ng/ml of cocaine or benzoylecgonine, respectively. Some of these patients were cocaine negative on one day and positive again some days later. For example one patient was capillary blood positive for benzoylecgonin at a concentration of approx. 5 ng/ml at day 5 and showed benzoylecgonine and cocaine positive results at 20 ng/ml benzoylecgonine and 7.5

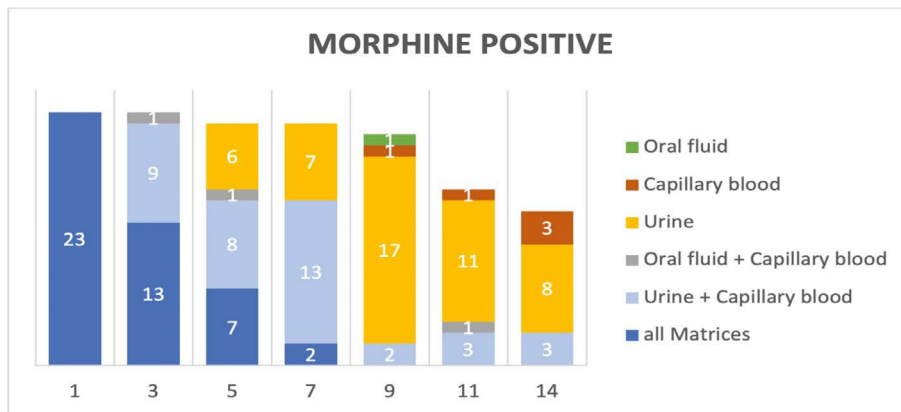


Figure 4 Number of patients positive for morphine in only one (capillary blood; oral fluid; urine), two (urine+oral fluid; urine+capillary blood) or all three matrices. Urine LOD of 0.4 ng/ml, a cut off at 1 ng/ml for capillary blood and 0.5 ng/ml for oral fluid was used.

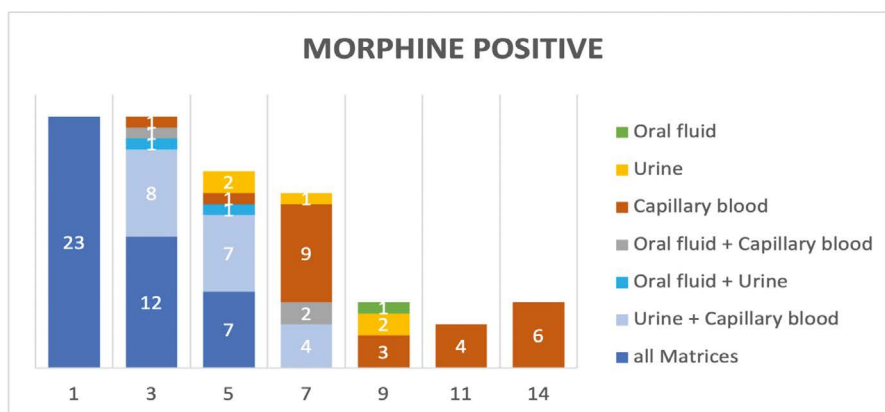


Figure 5 Number of patients positive for morphine in only one (capillary blood; oral fluid; urine), two (urine + oral fluid; oral fluid + capillary blood; urine + capillary blood) or all three matrices. 25 ng/ml for urine, 1 ng/ml for capillary blood and 0.5 ng/ml for oral fluid were used as cut offs.

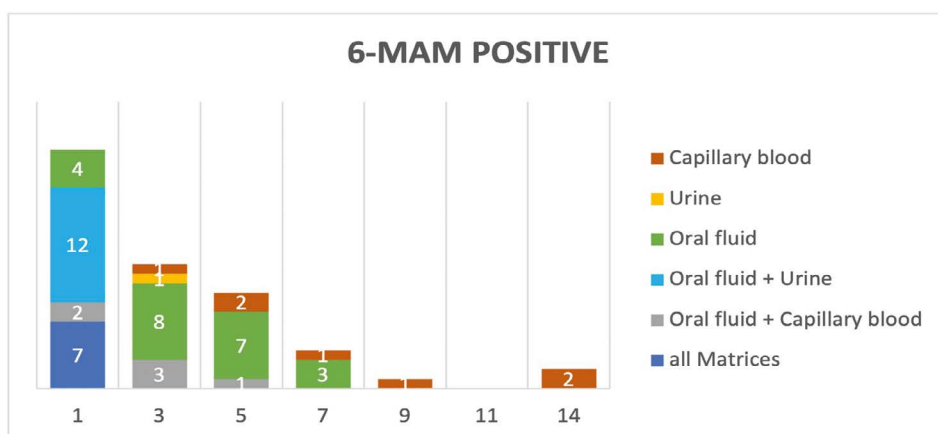


Figure 6 Number of patients positive for 6-MAM in only one (capillary blood; oral fluid; urine), two (urine+ oral fluid; oral fluid+capillary blood) or all three matrices. Urine LOD of 1.8 ng/ml, a 1 ng/ml cut off for capillary blood and 0.5 ng/ml for oral fluid was used.

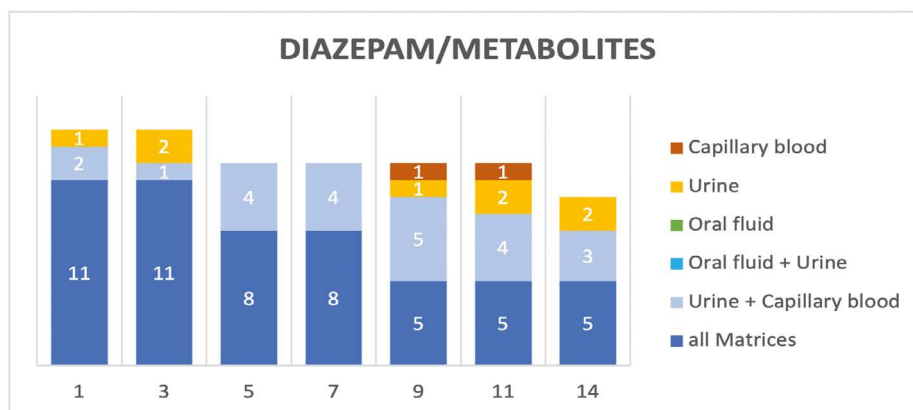


Figure 7 Number of patients positive for diazepam or metabolite in only one (capillary blood; oral fluid; urine), two (urine + oral fluid; urine + capillary blood) or all three matrices. A urine LOD of 0.5 ng/ml, a 1 ng/ml cut off for capillary blood and 0.5 ng/ml for oral fluid was used.

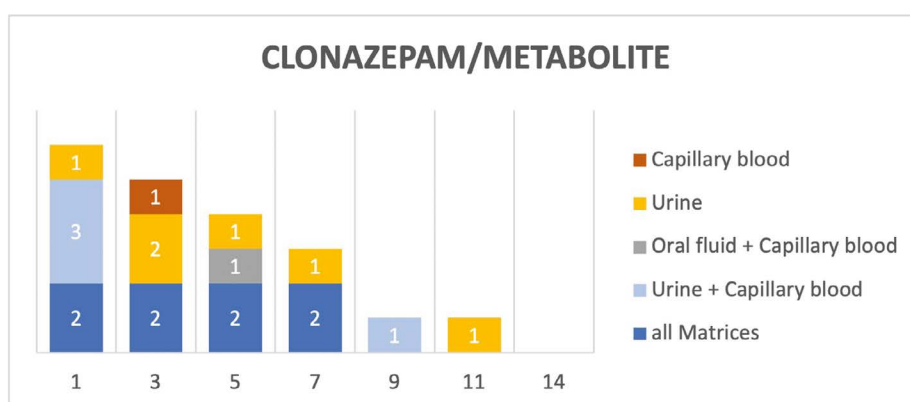


Figure 8 Number of patient's clonazepam or 7-amino-clonazepam positive in only one (capillary blood; oral fluid; urine), two (oral fluid+capillary blood; urine+capillary blood) or all three matrices. All positive urine concentrations of 7-amino-clonazepam were > 10 ng/ml. A 1 ng/ml cut off for capillary blood and 0.5 ng/ml for oral fluid was used.

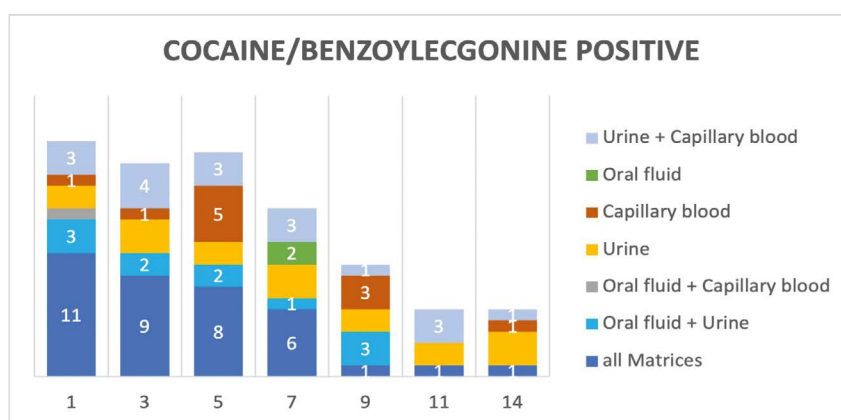


Figure 9 Number of patients positive for cocaine (oral fluid) or benzoylecgonine (urine, capillary blood) in only one (capillary blood; oral fluid; urine), two (urine + oral fluid; oral fluid+capillary blood; urine capillary blood) or all three matrices. Urine LOD of 1.65 ng/ml, and 1 ng/ml cut off for capillary blood and 0.5 ng/ml for oral fluid was used.

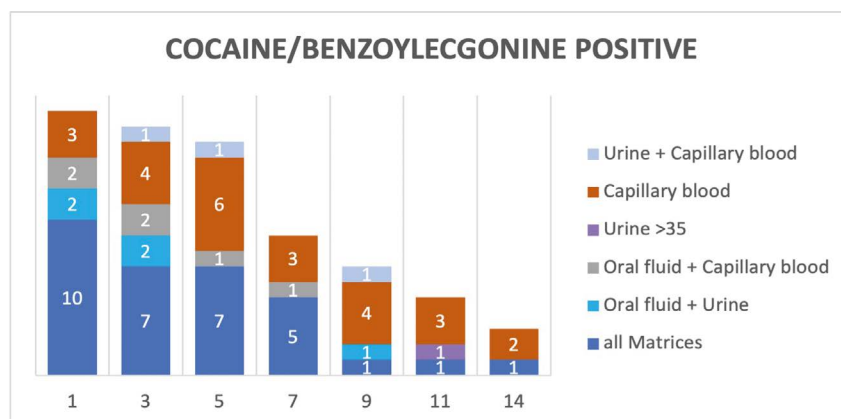


Figure 10 Number of patients positive for cocaine (oral fluid) or benzoylecgonine (urine, capillary blood) in only one (capillary blood; oral fluid; urine), two (urine+oral fluid; oral fluid+capillary blood; urine+capillary blood) or all three matrices. 35 ng/ml used as cut off for urine, 1 ng/ml for capillary blood and 0.5 ng/ml for oral fluid.

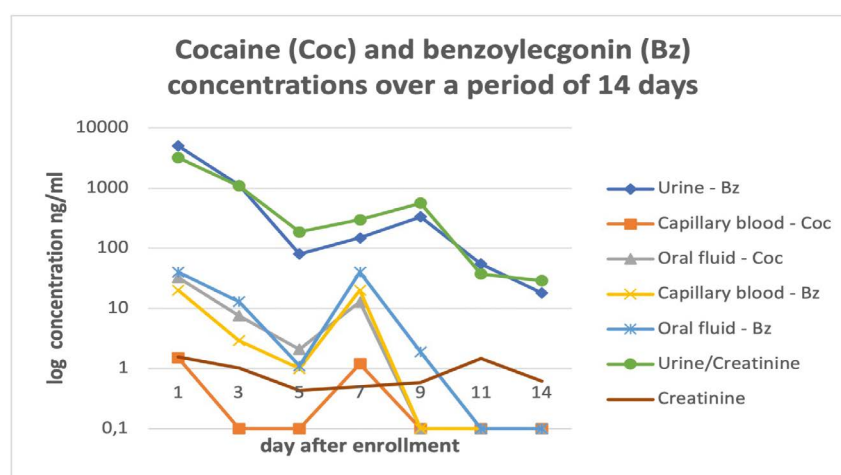


Figure 11 Cocaine and Benzoylecgonine concentrations over 14 days for a patient with cocaine intake during therapy. Urine - Bz – benzoylecgonine in urine; Capillary blood-Coc–cocaine in capillary blood; Capillary blood Bz–benzoylecgonine in capillary blood; Oral fluid-Bz–benzoylecgonine in oral fluid; Creatinine–creatinine; Urine/Creatinine–urine concentration of benzoylecgonine in relation to the creatinine concentration. The LOD for urine was 1.7 ng/ml, the cut off for capillary blood was 1 ng/ml and for oral fluid 0.5 ng/ml.

ng/cocaine, while cocaine was negative before. The other matrices showed no increase of the concentration of cocaine or benzoylecgonine. At day five there were more positive patients than on day 3 because a patient was negative in all matrices at day 3 and positive for benzoylecgonine only in capillary blood on day 5 (Figure 9).

Figure 11 shows an example for a relapse of patient consuming cocaine. The concentration of cocaine or its metabolite increased for all matrices at the 7th day. In urine the concentration also rose on day 9 whereas the concentrations in the other matrices decreased at day 9.

Discussion

The performed comparative measurements show that no procedure has only advantages. More often, the optimal choice

of matrix rather depends on the question to investigate. Some possible questions which influence the selection of matrix are the detection time, the substance and the way of sample collection. If heroin is the target analyte, oral fluid shows the best results and longest detection times. However, for benzodiazepines and THC urine should be preferred.

Urine collection is not invasive. However, urine is also prone to manipulation attempts in various forms. For example, the samples may be swapped for substance-free specimen, diluted or contaminated with chemical agents to present as 'clean'. And even though these attempts mostly prove ineffective in the end, they force investigators to include additional analytical steps. Sample substitution may be detected using either direct observation or, more reliably, using urine markers [3]. The latter even prove effective considering volume of fluid intake, alcohol

consumption or urine flow [21]. Chemical manipulation also proves detectable [22]. Still, countering manipulation attempts is costly and time-consuming. The necessary manipulation checks impede the process though. Either direct observation or a marker such as the Ruma[®] marker with subsequent manipulation testing has to be used. Both procedures do not provide 100% security against manipulation. However, the marker additionally helps to detect sample substitution. During the course of our investigation we detected two results for one patient that indicated swapped samples, day 1 and 3, using the Ruma[®] Marker-System. Another additional implausible urine result that did not contain any marker was also detected. For oral fluid and capillary blood there are significantly less options to manipulate. The influence of the pH value in oral fluid on drug secretion can be avoided by using a collection system that uses a buffered solution to rinse the mouth.

Supervision of oral fluid collection is less invasive than for urine. However, the matrix oral fluid itself has proven to be a challenge for investigators as a vast number of factors influences the production of oral fluid which, in turn, may also have varying effects on the investigated substances. For example, a number of collection systems use acidic media for stimulation and thus alter the pH value of the sample [23,24]. At this time it has not been conclusively determined which effect any chosen collection system may have on the quality of the respective samples. Furthermore, preserving agents or buffer solutions may also have different effects on different substances. And of course, factors regarding the individual test subjects such as metabolism, grade of hydration or form of drug use also have to be considered. And lastly, the amount of oral fluid in the collected sample is not constant, so it has to be measured if quantification is needed. Also, the amylase has to be detected for verification that the sample contains oral fluid. This entails additional work and costs for the laboratory. Drawing capillary blood requires close contact between staff and patient which may be unpleasant at times for both parties involved. Capillary blood is composed of blood and tissue fluid to varying degrees, so for exact quantification the detection of the hematocrit value is discussed. Furthermore, mechanical stimulation of the extraction site should be avoided as it increases the amount of tissue fluid in the sample. Educational materials on clinical chemistry cite a margin of error in volume of up to 15% [25]. Instead, a hyperemic ointment may be used to improve blood flow. However, contamination cannot be excluded [26].

On the analytical side urine has the advantage of higher drug concentrations compared to the other matrices, so the requirements on analytical devices are lower. A time-consuming factor is that samples have to be hydrolyzed before analysis because of several glucuronides. Some benzodiazepines, THC-carbonic acid and opiates and opioids form glucuronides. Matrix effects are a problem when using Electrospray Ionization (ESI) for MS. Oral fluid is the matrix with the lowest matrix effects because it contains less interfering substances like phospholipids, proteins or salts. Consequently, matrix effects on LCMS/MS are less frequent for oral fluid than for capillary blood and urine.. That makes sample preparation easier and the chromatograms are more clearly structured compared to the other matrices.

But some samples are of mucous consistence which may be difficult to handle. In addition to low concentrations and several interfering substances like proteins, phospholipids and others, capillary blood has the smallest sample volume. If only one capillary of blood is drawn, as is usually the case, there is not enough material to perform a second analysis if the first fail.

However, many analytes present the longest detection periods for urine, in particular when the detection limits of a LCMS/MS method is used as basis for the decision between negative and positive. It depends on the question of drug testing. For an addiction therapy the detection time in urine is quite long. The level of quantification the CTU criteria require for abstinence testing is mostly suitable but for analytes like morphine or oxazepam a higher cut off is proposed. An overview of the results is shown in Table 2. On the first day the highest number of positive samples was found for urine with the exception of 6-MAM and acetylcodeine which were more often positive in oral fluid.

The decrease of positive results from day 1 to day 3 is mostly higher in oral fluid.

The longest detection times (last positive) or the highest number of positive samples after 13/14 days, respectively, was found in urine, except 6-MAM and codeine, which were detected longer in oral fluid (6-MAM) and capillary blood (codeine). For example, 5 and 4 instead of 11 patients were still positive for THC-COOH in urine after 14 days at cut off at 10 or 20 ng/ml. In capillary blood and oral fluid the detection period for cannabis was at maximum 9 days. For some diazepam metabolites approx. 50% of the patients were also still positive after 14 days. Our investigation shows that the detection windows in oral fluid are distinctly shorter for many analytes. They are, to some extent, comparable to urine if a cut off between 50 and 100 ng/ml is used. Well liposoluble analytes such as cannabis and some benzodiazepines such as oxazepam and temazepam present distinctly shorter detection windows in oral fluid even with these parameters. To verify heroine consumption through the metabolite 6-MAM, oral fluid appears to be the most suitable matrix with the longest detection period and the highest positive rates on the first day which is quite consistent with the results of Vindenes et al. [27].

Capillary blood often showed long detection windows comparable to urine. However, implausible results were found more often. Patients were positive again at the end of the study after testing negative for these analytes over several days before and negative results in the other matrices. Also, during the investigation period occasional positive results, e.g. for amphetamine, were found only in capillary blood although nothing suggests intermediate consumption. For one patient, cocaine use during therapy was verified in oral fluid and urine by showing strongly increasing concentrations. For capillary blood the patient was above the measuring range during the entire investigation period.

The positive results in capillary blood that cannot be explained by intermediate consumption were found for cocaine, opiates, 6-MAM, THC and amphetamine. Two implausible amphetamine positive patients did not specify using amphetamines before. Such results could not be observed for benzodiazepines which are taken orally. It appears that the contamination of the skin

cannot be removed entirely even by cleaning the finger pad with alcohol prior to sample collection. An analytical contamination is improbable as we verified that the respective samples that had been injected into the LCMS immediately before the implausible samples were processed were negative or so low that a carryover could be excluded. The concentrations in urine are even higher than in capillary blood, so carryover as a reason for implausible results should be higher for urine. But the opposite was the case. A contamination of the skin due to contamination of the clothes might be possible. But the implausible result for 6-MAM can't be explained by the hypothesis above because 6-MAM is produced in the body out of heroin. A residual amount of the drug in one of the upper dermal layers could be an explanation. Implausible drug testing results in capillary blood were found by different authors. During the investigation of finger prints for drugs Costa et al. [28] found a false positive rate of 2.5% with cocaine. Ismail et al. [29] observed positive results with non-drug users of 13% for cocaine, 5% for benzoylecgonine and 1% for 6-MAM. Being wrongly suspected of current drug use could have far reaching consequences for the test subject including but not limited to exclusion from further therapy in the ward, loss of probation privileges and or trust in addiction treatment. Further investigation should be performed to determine whether capillary blood sample collection from the lobe of the ear might provide fewer false results.

Conclusion

The test methods are not only suitable for drug of abuse control during therapy. They also could be used for instance in workplace testing, traffic control, the penal system, probationary services and abstinence monitoring. The sample collection is non-invasive or microinvasive because only low sample volumes are necessary. Urine is the best choice if a longer detection time for a wide range of analytes is wanted. Oral fluid and capillary blood can easily be taken in roadside testing because no toilet or medical personal is required. Oral liquid has short detection times for THC and some benzodiazepines but for some indications this might be no issue. Capillary blood showed longer detection times than oral fluid for most analytes. But the sample volume is so low that a verification analysis of a sample in a laboratory is not possible. Another issue is that isolated implausible results can occur. None of the matrices allows a suitable inference to serum concentrations.

Consent and Ethical Approval

As per university standard guideline participant consent and ethical approval has been collected and preserved by the authors.

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Conflict of Interest

None to declare

References

1. Hawks RL, Chiang CN (1986) Urine Testing for Drugs of Abuse. NIDA Research Monograph 73.
2. Moeller KE, Lee KC, Kissack JC (2008) Urine drug screening: practical guide for clinicians. *Mayo Clin Proc* 83: 66-76.
3. Scholer A (2004) The effect of urine manipulation on substance abuse testing. *ToxChem und KrimTech* 71: 127-137.
4. Baum K, Schüler-Springorum M, Huppertz B (2018) Process Reliability and Psychological Stress in Urine Sample Collection for Drug Testing: A Pilot Study. *J Appl Life Sci Int* 19: 1-12.
5. Verstraete AG (2004) Detection times of drugs of abuse in blood, urine, and oral fluid. *Ther Drug Monit* 26: 200-205.
6. Moeller MR, Steinmeyer S, Kraemer T (1998) Determination of drugs of abuse in blood. *J Chromatogr B Biomed Sci Appl* 713: 91-109.
7. Schramm W, Smith RH, Craig PA, Kidwell DA (1992) Drugs of abuse in saliva: a review. *J Anal Toxicol* 16: 1-9.
8. Dolan K, Rouen D, Kimber J (2004) An overview of the use of urine, hair, sweat and saliva to detect drug use. *Drug Alcohol Rev.* 23: 213-17.
9. Sadones N, Capiou S, De Kesel PM, Lambert WE, Stove CP, et al. (2014) Spot them in the spot: analysis of abused substances using dried blood spots. *Bioanalysis*. 6: 2211-27.
10. Quraishi R, Jain R, Ambekar A (2013) The use of dried Blood Spot Samples in Screening Drugs of Abuse. *Pharmacology and Pharm* 4(2): 152-59.
11. Velghe S, De Troyer R, Stove C (2018) Dried blood spots in therapeutic drug monitoring and toxicology. *Expert Opin Drug Metab Toxicol* 14: 1-3.
12. Ellefsen KN, da Costa JL, Concheiro M, Anizan S, Barnes AJ, et al. (2015) Cocaine and metabolite concentrations in DBS and venous blood after controlled intravenous cocaine administration. *Bioanalysis* 7: 2041-56.
13. De Kesel PM, Sadones N, Capiou S, Lambert WE, Stove CP, et al. (2013) Hemato-critical issues in quantitative analysis of dried blood spots: challenges and solutions. *Bioanalysis* 5: 2023-2041.
14. Daousani C, Karalis V, Malenović A, Dotsikas Y (2019) Hematocrit effect on dried blood spots in adults: a computational study and theoretical considerations. *Scand J Clin Lab Invest* 79(5): 325-333.
15. Spiehler V, Baldwin D, Hand C (2000) Cut-Off Concentrations for Drugs of Abuse in Saliva for DUI, DWI or Other Driving-Related Crimes. *Probl of Forensic Sci* 42: 160-168.
16. Dawes C, Jenkins GN (1964) The effects of different Stimuli on the Composition of Saliva in Men. *J Physiol* 170: 86-100.
17. Aps JK, Martens LC (2005) Review: The physiology of saliva and transfer of drugs into saliva. *Forensic Sci Int* 150(2-3): 119-131.
18. Huppertz B, Gauchel G, Feiertag H, Schweizer H, Krieger H, et al. (2004) Urine labeling with orally applied marker substances in drug substitution therapy. *Clin Chem Lab Med* 42(6): 621-626.
19. Musshoff F (2014) Urteilsbildung in der Fahreignungsbegutachtung - Beurteilungskriterien. *Z für Verkehrssicherheit* 60: 33-37.
20. Langel K, Gjerde H, Favretto D, Lillsunde P, Oiestad EL, et al. (2016) Comparison of drug concentrations between whole blood and oral fluid. *Drug Test Anal.* 6: 461-471.

21. Baum K, Einwachter S, Bibl M, Huppertz B (2018) Urine-kinetics of low molecular polyethylene glycols following an oral capsule ingestion. *J Appl Life Sci Int* 15: 1-6.
22. Huppertz B, Bartling C, Baum K (2018) Adulteration of urine samples, discovery and mitigation. *J Appl Life Sci Int* 16: 1-8.
23. Crouch DJ (2005) Oral fluid collection: the neglected variable in oral fluid testing. *Forensic Sci Int* 150: 165-173.
24. Kato K, Hills Grove M, Weinhold L, Gorelick DA, Darwin WD, et al. (1993) Cocaine and metabolite excretion in saliva under stimulated and nonstimulated conditions. *J Anal Toxicol* 17: 338-341.
25. Müller K, Müller S (2022) Original -Prüfungsfragen mit Kommentar GK 2. *Klinische Chemie*, 15th ed. Stuttgart: Georg Thieme Verl.
26. Zavorsky GS, Cao J, Mayo NE, Gabbay R, Murias JM, et al. (2007) Arterial versus capillary blood gases: a meta-analysis. *Respir Physiol Neurobiol* 155(3): 268-279.
27. Vindenes V, Enger A, Nordal K, Johansen U, Christophersen A, et al. (2015) Very long Detection Times after High and repeated intake of Heroin and Methadone, measured in Oral Fluid. *Scand J Forensic Sci* 20: 34-41.
28. Costa C, Webb R, Palitsin V, Ismail M, de Puit M, et al. (2017) Rapid, Secure Drug Testing Using Fingerprint Development and Paper Spray Mass Spectrometry. *Clin Chem* 63(11): 1745-52.
29. Ismail M, Stevenson D, Costa C, Webb R, de Puit M, et al. (2018) Noninvasive Detection of Cocaine and Heroin Use with Single Fingerprints: Determination of an Environmental Cutoff. *Clin Chem* 64(6): 909-17.