Interpreting oral fluid drug results from prisoners: monitoring current drug intake and detection times for drugs self-administered prior to detention

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Abstract

Purpose Urine is the most common matrix for prisoner drug testing, although oral fluid offers a possible alternative. Identifying new drug intake by a prisoner results in negative sanctions. Detection times in oral fluid after chronic drug intake may be extended. Within the prison admission population are chronic drug users. Our aim was to investigate drugs of abuse detection windows in oral fluid from prisoners.

Methods Nineteen frequent drug abusing prisoners provided oral fluid and urine at admission, and each morning for 9 consecutive days.

Results The most positive findings were for amphetamine/ methamphetamine, cannabis and benzodiazepines. Maximum detection times in oral fluid were ≥ 9 days for diazepam, methadone and methamphetamine, with corresponding urinary detection times of ≥ 9, 7 and 6 days. Maximum oral fluid detection times were nine days for clonazepam, eight for oxazepam, three for amphetamine and nitrazepam and two for tetrahydrocannabinol, with positive urinary detection times of 8, ≥ 9, 5, 7 and ≥ 9 days, respectively. Cocaine, morphine and 6-acetylmorphine were positive only one day in oral fluid, and one and two days, respectively, in urine, while 6-acetylmorphine was not detected in urine.

Conclusion We confirmed oral fluid as a viable matrix for monitoring drugs of abuse in prisoners. Windows of detection for benzodiazepines and amphetamines were up to one week, an important consideration for evaluating oral fluid drug testing results. Some likely new drug exposures were observed based on urine and oral fluid drug results, but there are few data guiding these interpretations.

Introduction

Prisoners are frequently drug tested, with urine as the preferred matrix. Observed urine collections are time consuming and many donors consider it as an intrusion of privacy. Due to advances in analytical technology for oral fluid testing, this biological matrix is now a viable alternative to urine testing in several disciplines [1-5]. The easy, fast and gender-neutral oral fluid sample collection can take place in almost any location, with less embarrassment for the donor, giving oral fluid significant advantages over urine.

In Norway, urine is collected on admission to prison, and creatinine-corrected urine sample concentrations taken at regular intervals thereafter, are interpreted to determine if results are likely to represent new intake within the prison or residual excretion from intake before imprisonment. Replacement of urine with oral fluid as the testing matrix requires a scientific basis, and although data exist on drug elimination in oral fluid from controlled administration studies, these results might not be representative for samples collected from prisoners with chronic and/or high drug intake.

Drug windows of detection in oral fluid are considered short, and more similar to blood than urine [6; 7]. The detection periods are thus highly dependent upon both the chosen cut-off concentrations, and the dose ingested [8; 9]. Multiple studies documented that oral fluid is a viable matrix for drugs of abuse detection [10-15]. Single and low doses are typically administered in controlled drug studies [16-26], although others investigating drug elimination purported high doses from patients admitted for drug detoxification [27-30] or after chronic frequent use [31; 32] reported increased drug detection times. Since many

prisoners use high and/or chronic doses of drugs of abuse before incarceration, elimination and detection times of drugs of abuse in oral fluid from this population provide relevant data for future interpretation of oral fluid tests. The aim of this study was to investigate drugs of abuse windows of detection in oral fluid after possible ingestion of high doses or chronic frequent drug use, at the time of prisoner incarceration and the following 9 days. Drug use is prohibited in prison and inmates are under sustained and monitored abstinence.

Materials and methods

Study group

In total, 19 inmates from three prisons were enrolled in the study. Drug consumption prior to incarceration was self-reported. Information regarding prescribed drugs during the study was provided by the prison physician. The only relevant medications reported were buprenorphine and methadone for opioid-dependence treatment and oxazepam.

Positive drug test results produced no negative consequences for participants, as the prisons did not receive results. Participants received no payment for providing samples. Each participant had a unique code linked to their self-report data and samples, and only one person in each prison had access to these data. Everyone else was blind to prisoner identity, and only participants´ unique codes were reported.

Sample collection

Sampling occurred the day of and for 9 days after prison admission (reported as day 0 to day 9), for a total of 10 oral fluid samples per participant. Since drug intake might have occurred

on day 0, positive oral fluid samples collected on day 1 were considered as having a detection time of 1 day. Oral fluid samples were collected each morning, and if possible, each first voided urine also. Oral fluid samples were collected with the commercially available Intercept® Oral Specimen Collection Device (OraSure Technologies, Bethlehem, PA, USA). The cotton pad on a stick was placed between the cheek and gum for 2 min to sample oral fluid according to manufacturer's recommendations. All samples were weighed to obtain the amount of oral fluid collected. The collection pad contains preservatives and citric acid, stimulating oral fluid production, and collecting a mixture of saliva, gingival crevicular fluid and mucosal transudate. After collection, the pad was placed into a vial containing 0.8 mL stabilizing buffer solution and stored at -20˚C until analysis. The urine sample was collected in a 120 mL BD-Vacutainer urine collection cup with integrated transfer device (Becton, Dickingson and Company, Franklin Lakes, NJ, USA) and transferred to Vacuette® vials without additives (Med-Kjemi A/S, Asker, Norway) before transport to the laboratory.

Analytical methods

Urine samples were screened for amphetamines (EMIT DAU reagents, Siemens, Healthcare AS, Oslo, Norway), cannabis, cocaine, methadone, opiates (EMIT II Plus reagents, Siemens Healthcare AS) and benzodiazepines (CEDIA reagents, Thermo Fisher Microgenics, Fremont, CA, USA) by immunological methods on the Hitachi 917 analyzer (Hitachi, Tokyo, Japan). In addition, pH (DRI® pH-Detect Test; Thermo Fisher Microgenics) and creatinine (DRI ® Creatinine-Detect® Test; Thermo Fisher Microgenics) were measured. γ-hydroxybutyrate, GHB, was screened by ultrahigh performance- liquid chromatography–tandem mass spectrometry (UHPLC-MS/MS) [33]. Confirmation analyses were performed by liquid-chromatography–tandem mass spectrometry (LC–MS/MS) for benzodiazepines [34] and

UHPLC–MS/MS for opiates and cocaine [35]. Amphetamines, methadone and 11-nor-9-carboxy-Δ9- tetrahydrocannabinol, THCCOOH, were analysed by internally-validated UHPLC-MS/MS and gas chromatography–mass spectrometry (GC–MS) methods, respectively. Oral fluid samples were analysed for drugs of abuse by a quantitative LC-MS-MS method [36]. Cut-off concentrations in oral fluid and urine are shown in Table 1. Urine validation data for amphetamine, methamphetamine and THC-COOH are presented in Table 4, together with urine validation data for the other compounds presented in figures 2 to 4.

Statistics

The data were analysed using IBM SPSS Statistics 23 (IBM). Pearson's correlation was used to investigate the relationship between concentrations in oral fluid and urine.

Results

Demographic data and self-reported prior drug intake for the 17 male and 2 female participants are shown in Table 2. Fifteen subjects provided biological samples for all ten days of the study and the remaining four for five, seven, eight and nine days, respectively. The longest detection times for each drug and/or metabolite are reported in Table 3. It is important to emphasize that drugs might have been consumed prior to admission (day 0), and for those drugs still detected on day nine, detection times might be longer, because later samples were not collected or analysed.

Amphetamine/methamphetamine

Amphetamine and methamphetamine were detected together in 11 participants' oral fluid and/or urine, while one participant's biological samples contained only amphetamine, subject

15, and one only methamphetamine, subject 5. Amphetamine was identified in oral fluid from day 0 to 3 days, and for methamphetamine from day zero to nine days. Amphetamine was detected in urine from day zero to day five, and for methamphetamine from day zero to six days. The longest amphetamine detection time was in urine, while for methamphetamine it was in oral fluid. As seen in Fig. 1, the biological sample with the longest detection time varied between subjects. The prisoners self-reported previous amphetamine, but not methamphetamine use. If self-reported ingestion times were considered, detection times were longer, with a maximum of ten days for amphetamine and 15 days for methamphetamine.

<Figure 1 here>

Opioids

Morphine and/or 6-acetylmorphine (6-AM) were detected in two participants' samples. Heroin is metabolised rapidly to 6-AM and morphine, and later morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G). Subject 8 self-reported heroin consumption the day before admission (day –1) and morphine, M3G and M6G were identified in his/her urine through day two, Table 2. Opioids were not detected in any of his/her oral fluid samples. Subject 11 self-reported heroin ingestion three days before imprisonment (day –3); 6-AM was identified in oral fluid on day 1, but not in any urine sample. Morphine was detected in the oral fluid sample on day 0 and in urine until day 1. Considering the self-reported time of the last exposure, morphine's window of detection was four days in urine, and the detection time for morphine/6-AM was three days in oral fluid. These estimates are, however, uncertain.

Methadone was detected in samples from two participants. Subject 2 allegedly ingested massive amounts of drugs prior to admission (methadone, heroin, cannabis and diazepam; doses and times of ingestion were not given) and had a nonfatal overdose. Urine samples showed decreasing methadone concentrations detectable until day 7, and methadone was detected in oral fluid through day 9. The other participant, subject 4, received opioid-dependence treatment during the study, and no detection time window can be given.

In all cases where buprenorphine was detected, it was given as opioid-dependence treatment. This makes it impossible to estimate the window of detection for buprenorphine.

Δ9-Tetrahydrocannabinol (THC)

In 15 participants 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THCCOOH) was detected in urine on day 1. Of these, only two participants had detectable THC in oral fluid, and only on day 0. Seven participants had detectable THCCOOH in urine throughout the study. However, most participants claimed last cannabis intake several days before admission. One participant tested positive for THC in oral fluid on days 4 and 5, with prior negative samples. Oral fluid concentrations corrected for dilution were 7.7 and 14.2 µg/L, respectively. Urine creatinine-corrected THCCOOH increased from 15 ng/mg on day 1 and <cut-off on day 2, to 78,171 and 203 ng/mg creatinine on days three, four and five, respectively. Day 3 urine concentrations were assessed as new cannabis intake using the reference values of U2/U1 ratios reported by Smith et al. [37]. Clearly positive THC results in oral fluid on days 4 and 5 also indicate new cannabis intake in prison, Fig. 2.

<Figure 2 here>

Benzodiazepines

Clonazepam

Clonazepam was identified in seven prisoners' urine or oral fluid samples, and none received medical treatment with clonazepam in prison. Maximum clonazepam detection time was eight days in urine (range one to eight days), and in oral fluid, at least nine days (range one to nine days) if the positive clonazepam samples for subject 15 on days 0 and 9 only are included. Clonazepam and 7-aminoclonazepam oral fluid concentrations in one participant on day 8 were 97 and 6.4 µg/L, respectively, while all prior urine and oral fluid samples were negative. This suggested a new clonazepam intake after admission to prison.

Nitrazepam

Nitrazepam/7-aminonitrazepam were detected in oral fluid from three participants. In urine, detection times ranged from two to seven days, and in oral fluid from one to three days. Considering the subjects' self-reported last intake, the detection time did not change. Fig. 3 shows the elimination curves for nitrazepam and 7-aminonitrazepam in oral fluid from participants 14 and 18, the prisoners with the longest detection times, and the corresponding creatinine-normalized urine elimination curves for 7-aminonitrazepam.

<Figure 3 here>

Oxazepam

Eleven participants had oxazepam in either urine or oral fluid. Two subjects used oxazepam when admitted to prison. No information about doses was available for subject 7, who, according to our information, stopped taking oxazepam during the study; however, the date

was not given. For subject 10, 25 mg Sobril® was prescribed morning and evening during the study, making it impossible to determine detection times in either matrix. For the other inmates, oxazepam was found with other diazepam metabolites. Windows of detection for oxazepam ranged from two to ≥nine days in oral fluid and urine, with generally longer detection times in urine than oral fluid samples. Additionally, it was difficult to distinguish the source of oxazepam, as it also is a metabolite of other benzodiazepines including diazepam. One person (subject 14) disclosed oxazepam ingestion the day before incarceration to prison, with detection times of six days in oral fluid and seven days in urine; however, presence of other diazepam metabolites demonstrates that there was intake of other benzodiazepine(s) also.

Diazepam/*N*-desmethyldiazepam

Diazepam or metabolites were identified in eight inmates' samples. Maximum diazepam detection times in oral fluid ranged from four to ≥nine days, and in urine from one to ≥nine days. Participant 4 only had positive *N*-desmethyldiazepam in oral fluid, and 3-hydroxydiazepam and oxazepam in urine, and did not declare diazepam intake. For four participants, diazepam and its metabolites were detected in oral fluid for the entire study period, but there was no self-report of time of last intake. However, one person with positive samples during the entire study claimed that the last diazepam ingestion was at least 13 days before admission.

Cocaine

Cocaine and its metabolite benzoylecgonine were detected in subject 5's oral fluid and benzoylecgonine in a urine sample only on day 3, after negative tests the days before. Oral

fluid concentrations corrected for dilution were 4.4 µg/L for cocaine and 9.9 µg/L for benzoylecgonine. In urine samples, cocaine was negative, while the benzoylecgonine concentration was 2262 µg/L and the creatinine-normalized result was 1122 ng/mg. This finding was interpreted as ingestion of cocaine after admission.

Correlation between oral fluid and urine results

Oral fluid and urine samples collected on the same days were compared. For most drugs, both matrices were initially positive, but last detection varied according to matrices. Cannabis was an exception, as there were many positive urine samples without matching positive oral fluid samples. For amphetamine, a trend towards longer detection time in urine could be seen, while methamphetamine tended to have longer detection times in oral fluid, Fig. 1. Oxazepam had longer detection times in urine, while for *N*-desmethyldiazepam evaluation was difficult as most samples were positive in both oral fluid and urine at the end of the study. For the other compounds, the number of cases was too small to infer any trends. Direct comparison of quantitative results for oral fluid and creatinine-corrected urine concentrations for the four most prevalent drugs is shown in Fig. 4. Pearson's correlation was used to investigate the relationship between concentrations in oral fluid and urine, and we found correlation coefficients of 0.612 (methamphetamine), 0.314 (amphetamine), 0.535 (7-aminoclonazepam) and 0.553 (*N*-desmethyldiazepam). The correlations were significant ($p<0.01$) for methamphetamine, 7-aminoclonazepam and -desmethyldiazepam, but not ($p=0.086$) for amphetamine.

<Figure 4 here>

Discussion

We investigated the detection times of drugs of abuse in oral fluid and urine samples using 19 prisoners with a history of drug abuse, while under constant supervision. Individual elimination curves (Figs. 2 and 3) of creatinine-normalized urine results were used for comparison to see if variation in oral fluid results was likely to be the result of new intake during the study. There was a larger variability in elimination curves in oral fluid as compared to creatinine corrected urine curves, in line with the previous findings [27-30]. In addition, after ingestion of high and repeated drug doses, detection times could be several days. Despite significant correlation between oral fluid and urine concentrations for more of the drugs, it is not possible to infer the concentration in urine from oral fluid and vice versa, Fig. 4. At the end of the elimination curve of a drug, a positive sample following after a negative can be found in any matrix, as the concentration fluctuates around the limit of quantification/detection. Oral fluid concentrations tend to be more variable than e.g. blood concentrations, and this effect, is therefore more pronounced in oral fluid. Negative samples interspersed with positive findings were encountered for some in our study, which is consistent with other elimination studies. [16; 27-31]. Detection times were longer than in controlled single dose administration studies [16; 38; 39]. As many prison inmates have a chronic drug problem, these data are important because they represent long term intake of high drug doses based on self-report.

Benzodiazepines

Benzodiazepines are popular drugs of abuse, and frequently included in drug testing programs. Few studies investigated windows of benzodiazepine detection in oral fluid [6; 7;

29; 40; 41]. A summary by Kidwell et al. [42] reported detection times for diazepam and nitrazepam of 48 and 70 h, respectively, after ingestion of single doses. This is comparable to our previous study of patients undergoing drug detoxification, where diazepam was found as *N*-desmethyldiazepam in oral fluid for the entire nine days, applying a cutoff of 1.3 µg/L [29]. Our current research documented a window of detection for diazepam of at least 9 days in oral fluid and urine, and *N*-desmethyldiazepam had the longest detection time in oral fluid compared to urine.

Detection times for clonazepam were at least 6 days in oral fluid and 8 days in urine, Table 3. One participant was positive for clonazepam in oral fluid on admission day and day 9 only; 7-aminoclonazepam fluctuated around the cutoff, extending the detection time to at least 9 days in oral fluid. This is comparable to our previous study of patients undergoing drug detoxification, where 7-aminoclonazepam was detected for 6 days [29], with a cutoff of 1.3 µg/L.

Few data are available for nitrazepam elimination in oral fluid. Nitrazepam oral fluid C_{max} was 1.9 µg/L after 5 mg nitrazepam and the drug was quantifiable up to 70 h (approx. 3 days) with a limit of quantification (LOQ) of 0.5 µg/L [43]. As Fig. 3 shows, we also found low 1-2.5 µg/L initial nitrazepam concentrations that decreased over three days. No data were provided about the time of intake of nitrazepam in our study. Nitrazepam/7-aminonitrazepam was detected for three days in oral fluid and 7 days in urine, and 7-aminonitrazepam had higher concentrations than nitrazepam in all samples.

Opioids

Only one study to our knowledge investigated heroin's oral fluid window of detection [30], but others reported that 6-AM is more frequently detected in oral fluid as compared to urine [44]. Opioids were only found in samples from two participants. In one case, 6-AM was detected in oral fluid, but not in urine. In the other case, the opposite situation occurred. This documents individual variability that must be taken into account when interpreting results. Our window of detection for methadone in oral fluid of at least 9 days, Table 2, subject 2, is similar or longer than the five and eight days previously reported from patients undergoing drug detoxification [30].

Amphetamines

Few studies investigated windows of detection for amphetamines in oral fluid. Huestis and Cone [24] showed that after sequential daily dosing of 20 mg methamphetamine for four days, a clear accumulation of methamphetamine in oral fluid was observed. Positive specimens were reported for approximately 24 h at a 2.5 µg/L cut-off. Schepers et al. [16] also reported detection times in oral fluid for amphetamine and methamphetamine up to 24 h at the same cut-off after a 20 mg dose of methamphetamine. Methamphetamine was measurable for 36 – 72 h after the last of four doses. As could be expected assuming higher intake, we found a much longer 9 day methamphetamine window of detection than reported in clinical studies, with a 8 µg/L cutoff, Table 3. This is slightly longer than in our previous study from patients undergoing drug detoxification, where the detection window was up to eight days [27]. For amphetamine, a shorter detection window of up to three days was found, Table 3, as compared to the previously reported detection window of up to 8 days for patients undergoing drug detoxification [27]. It might be difficult to differentiate the effects of amphetamine and methamphetamine [45]; thus there was consistency between the

participants' self-reports regarding methamphetamine/amphetamine ingestion and the actual findings in oral fluid/urine.

THC

THC is metabolized to the inactive metabolite THCCOOH, which can be detected in urine for weeks after stopping chronic frequent cannabis intake [46]. Lee et al. [31] showed that the detection time for THC in oral fluid among chronic frequent cannabis smokers ranged from 48 h to 28 days, with negative samples (<0.5 µg/L) interspersed with a few positive samples, raising into question the possibility of reuse despite 24 h surveillance on a closed research unit. In patients undergoing detoxification, Andås et al. [28] reported an oral fluid THC window of detection of 8 days (0.3 µg/L LOQ). In the present study, a 0.9 µg/L cutoff was applied, and THC was detected only in oral fluid samples from two subjects, with the longest detection time of 1 day. The difference could in part attributed to a higher cutoff, but it could also indicate that participants in Lee' and Andås' studies [28; 31] had greater and more frequent cannabis intake.

New cannabis intake was suggested for subject 5, with similar findings of THC in oral fluid and urine on days 4 and 5 after admission, Fig. 2. These data support oral fluid as a matrix to reveal drug use in prison. However, the aforementioned possibility of negative samples interspersed with positive findings must also be considered [31].

Cocaine/benzoylecgonine

Cocaine or benzoylecgonine were only identified in one participant's oral fluid samples on day 3, Table 2, subject 5; these results were interpreted as new cocaine intake in prison. The

transfer of cocaine from blood to oral fluid depends on oral fluid pH. Cocaine has a short detection time in oral fluid, as also occurs for this analyte in blood and urine [22; 47; 48].

Limitations

The limitations of the study include the number of participants and single oral fluid and urine samples each day. However, valuable oral fluid detection time data from individuals with histories of potentially high and repeated drug intake are included, as well as comparison of paired oral fluid and urine data. Limited studies investigated this population. Detection times for benzodiazepines and amphetamines in oral fluid were consistent with or somewhat longer than previously reported data, while detection times for opiates and THC were shorter. It is important to emphasize that the study period was 10 days, leading to maximum detection times of at least 9 days (Table 3), while intake was varied prior to imprisonment.

Conclusions

Oral fluid was a viable alternative to urine for monitoring drugs of abuse in prison. Oral fluid is easier to collect and much less subject to adulteration than urine. Our study confirms that long detection times, especially for amphetamines and benzodiazepines, can be encountered in this population, although oral fluid cannabinoid results had a much lower prevalence than urine tests. From daily oral fluid concentrations, it might be possible to identify new drug intake, but elimination curves were not as consistent as seen in blood [49] or creatinine-corrected urine. Negative oral fluid samples might be interspersed with positive findings as noted with urine samples, especially when concentrations are close to applied cutoffs.

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Ethical approval

The Norwegian Regional Committee for Medical and Health Research Ethics approved the study. Participation was voluntary, and participants could withdraw at any time without penalty. Written informed consent from 19 inmates from three prisons was obtained prior to inclusion after fully informing participants about the study.

Conflict of interest

The authors declare no conflicts of interest.

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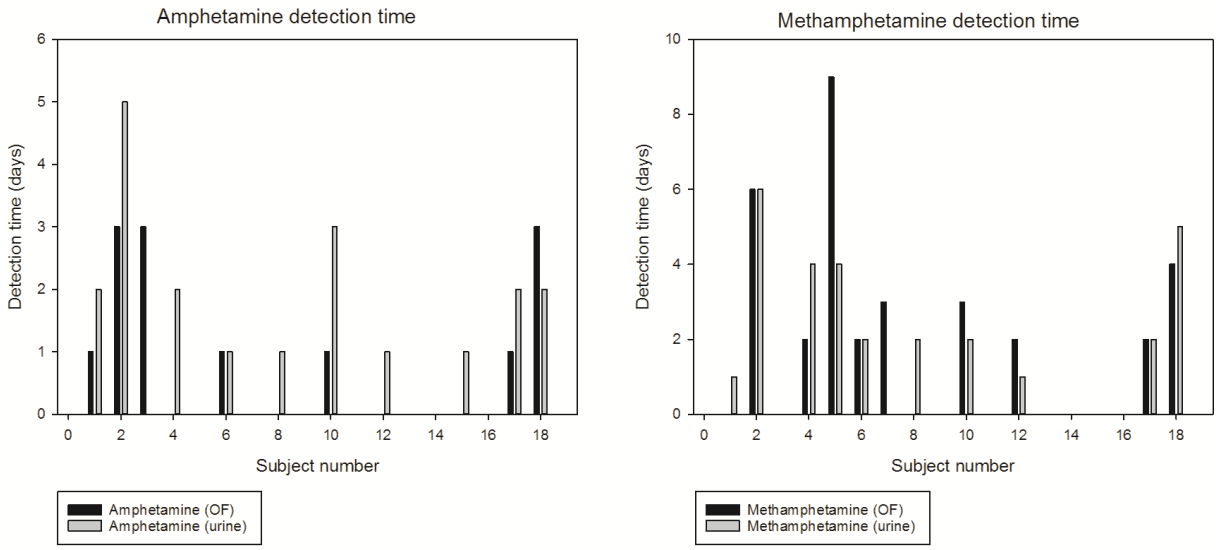
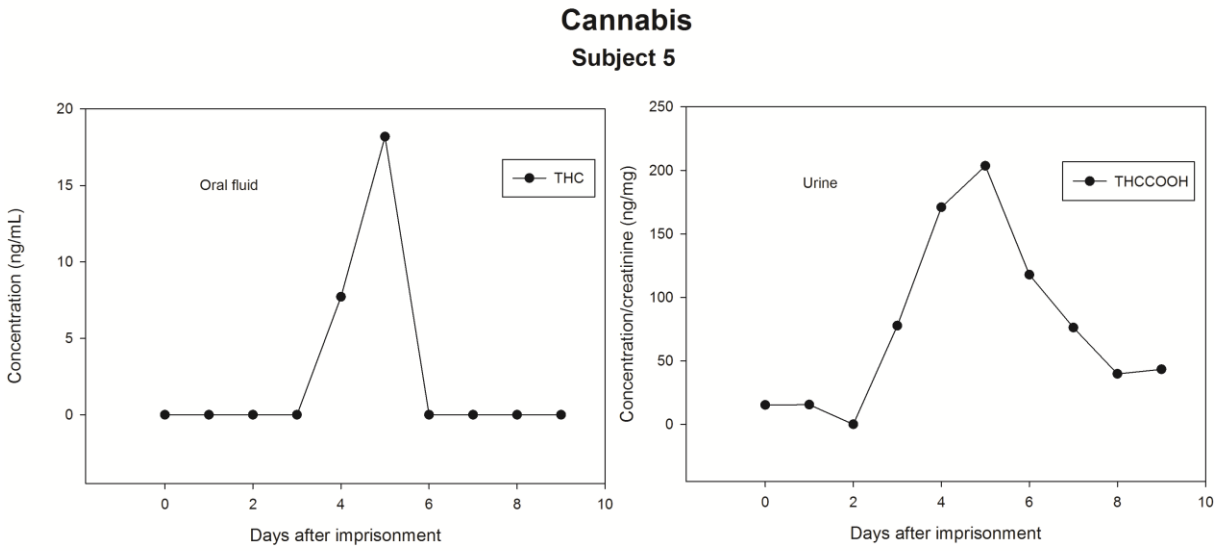
548 **Figures**



549

550 **Fig. 1** Detection time in oral fluid (OF) and urine for amphetamine (left panel) and

551 methamphetamine (right panel)



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553 **Fig. 2** OF and creatinine-normalized urine concentrations from subject 5, with probable new

554 intake of cannabis. THC Δ^9-tetrahydrocannabinol, THCCOOH 11-nor-9-carboxy- Δ^9-

555 tetrahydrocannabinol

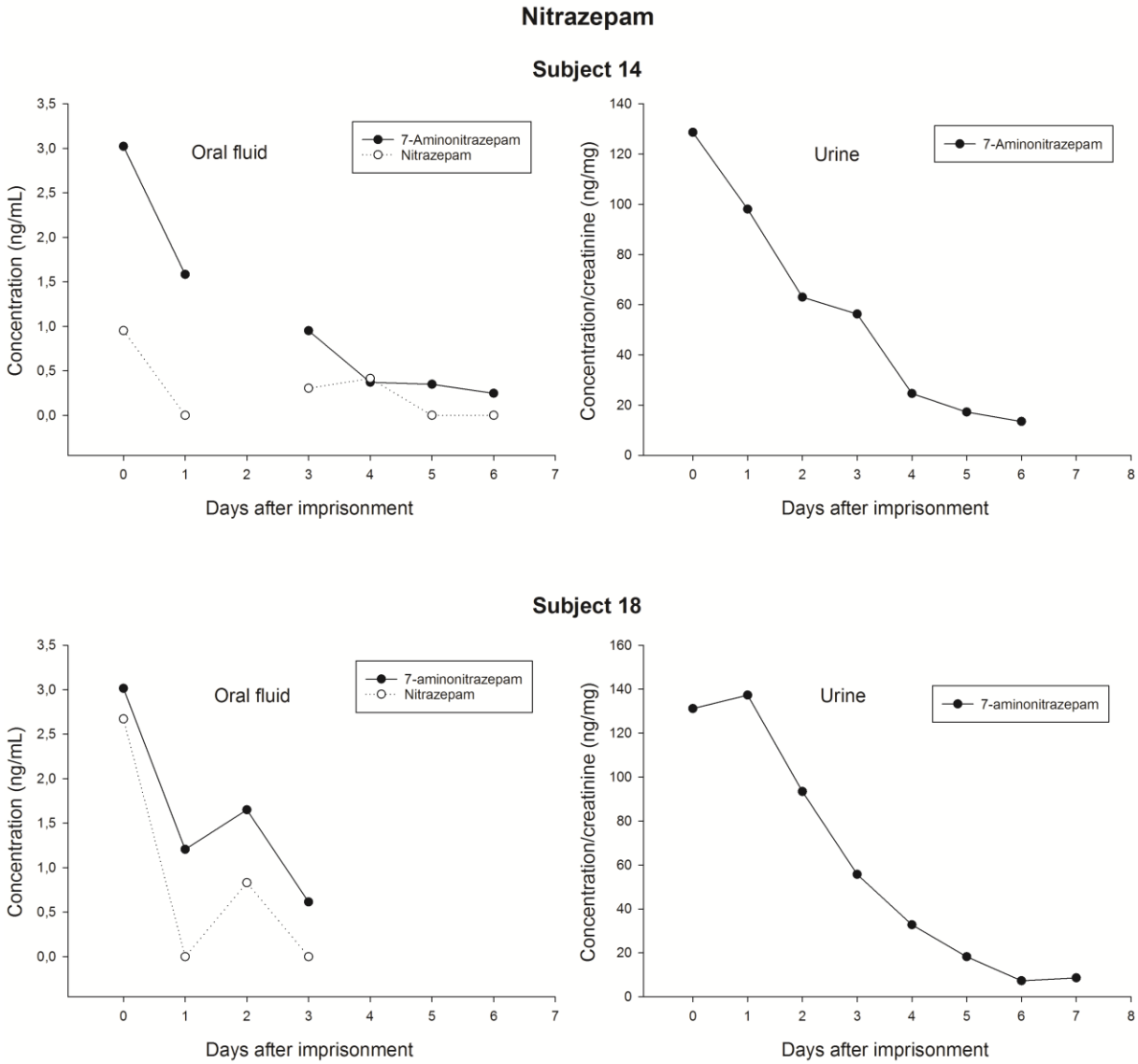
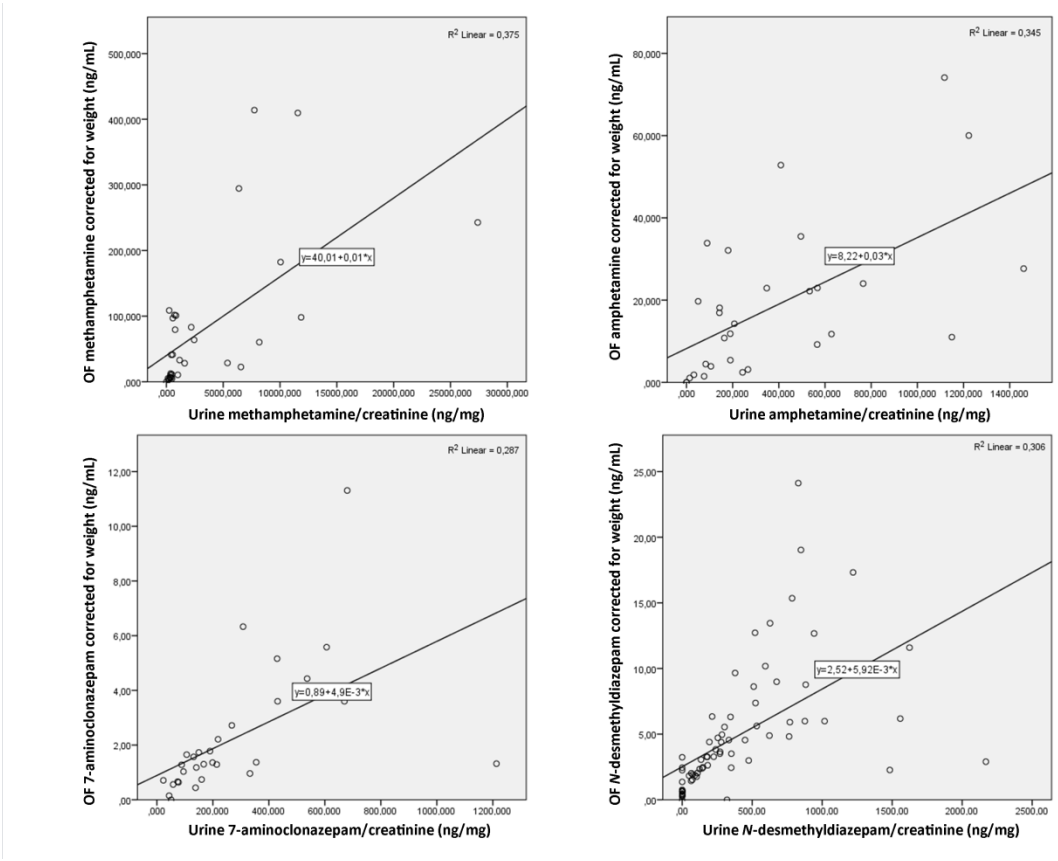


Fig. 3 Elimination curves for nitrazepam and 7-aminonitrazepam in OF and urine samples from participants 14 and 18, with the longest detection times. No OF results were available on day 2 due to an analytical error



562

563 **Fig. 4.** Scatter plots and trend lines of the creatinine-normalized urine and OF concentrations

564 of methamphetamine, amphetamine, 7-aminoclonazepam and *N*-desmethyldiazepam

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