Urinary Excretion of Amphetamine after Termination of Drug Abuse*

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Abstract

Important issues in urinary drug testing are the variability between consecutive urine specimens, the duration of positive specimens after last intake, and the usefulness of creatinine concentration to correct for variability in urine concentration. These issues were addressed in the present study with amphetamine as the drug of abuse. Drug users who were starting their sentences in prison participated in the study. Urine specimens were collected 1 to 5 times per day. Screening was performed by EMIT d.a.u. (cutoff, 0.30 µg/mL) and EMIT II (cutoff, 1.00 µg/mL), and confirmation was performed with gas chromatography–mass spectrometry. Creatinine and pH were recorded. Amphetamine was demonstrated in seven subjects. The highest concentration was 135 µg/mL. The last positive-screened specimen was observed by EMIT d.a.u. after almost 9 days of imprisonment and by EMIT II after 3 days. Large concentration differences could be found between consecutive specimens, accompanied by considerable differences in creatinine and pH. The individual curves were generally smoother after creatinine correction of concentrations. As expected, urinary pH was observed to influence the excretion.

Introduction

Amphetamine is the second most frequently abused illegal drug in Norway, after cannabis, as judged from the frequency of positives out of a total of 30,000 urine samples analyzed annually at the National Institute of Forensic Toxicology. The mainstream knowledge of amphetamine pharmacokinetics and excretion has been presented (1–8), clearly demonstrating the importance of urinary pH in drug excretion. However, these data are insufficient for the purpose of interpretation of urinary drugs-of-abuse testing results. Important issues are normal variability between consecutive urine samples, the duration of positive specimens after intake, and creatinine concentration as a correction factor for variability in urine concentration.

To address these issues, self-reported drug users who started to serve their prison sentences were subjected to daily urine testing until consistently negative specimens were obtained.

Methods

Participants

Drug users (n = 22) were recruited when they commenced serving their sentences in the prison of Trondheim, Norway (population approximately 140,000) over a three-year period, November 1991–1994. There was never more than one participant in the study at one time. The participants answered a questionnaire concerning the duration of drug abuse and drug habits prior to imprisonment. Frequently, only the date of the last intake was given. In those cases, the time of intake was set at noon. The time of imprisonment was also set to noon at the day the sentence started unless clearly stated otherwise. The participants were given an entrance number into the project. At the end of the project, each entrance number was assigned an arbitrary alphabetical letter to conceal the identities of the participants.

Urine sampling

Subjects yielded a urine specimen 1–5 times daily. The time of collection was decided by the prison inspector from a practical point of view. Specimens were not collected between 8 pm and 7 am. Positive results were not to be (and were not) reported back to the prison authorities, and thus did not have any negative consequences for the participant. Urine collection was performed under strict surveillance to avoid manipulation. The need for the testing of urinary specimens to control drug intake within the prison was organized independently of the present study. The results of those samples were reported back to the prison.

Ethics

Written informed consent was obtained, and the subject could withdraw from the study at any time during the testing period, as required by the Declaration of Helsinki. The urine test results

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were by agreement kept confidential between the participants and the investigators. The participants received compensation for each specimen (approximately $5.00). The study protocol was approved by the Ethics Committee of Health Region 2, the Norwegian Data Inspectorate, and the Board of Professional Secrecy and Science.

Urine analysis

All samples were analyzed at the National Institute of Forensic Toxicology by immunological screening. Confirmation by gas chromatography–mass spectrometry (GC–MS) was carried out on all positive-screened samples and on negative-screened samples when indicated. The sampling of each individual was terminated when several consecutive specimens screened negative. Amphetamine screening was performed with the polyclonal EMIT d.a.u. kit from Syva (Behring, San José, CA) using a 0.30-µg/mL cutoff. The system was run in a semiquantitative mode with calibrators at 0.00, 0.30, 1.15, and 2.00 µg/mL. A Monarch autoanalyzer (Instrumentation Laboratories, Lexington, MA) operating at 30°C was used. In this system, the observed coefficient of variance (CV) of the cutoff calibrator was 7%, and the CV of a sample spiked to 0.41 µg/mL was 12% (*d,l*-amphetamine). The assay had equal sensitivity towards the *d*- and *l*-amphetamine. Screening was also performed with the monoclonal EMIT II amphetamine/methamphetamine assay, which had cutoff 1.00 µg/mL; the calibrator substance was *d*-methamphetamine. This system was also run in a semiquantitative mode with calibrators at 0.00, 1.00, 2.00, and 3.00 µg/mL. The assay was directed towards *d*-methamphetamine and *d*-amphetamine and had a lower sensitivity towards *l*-amphetamines. The Monarch reported concentrations above the high calibrator as such (greater than highest calibrator). The actual screening with EMIT II amphetamine/methamphetamine assay was done at a later stage than the original screening (EMIT II was introduced into the Norwegian market in 1993). However, several samples were rerun with EMIT d.a.u. at the same time, and these results were consistent with the original screening results.

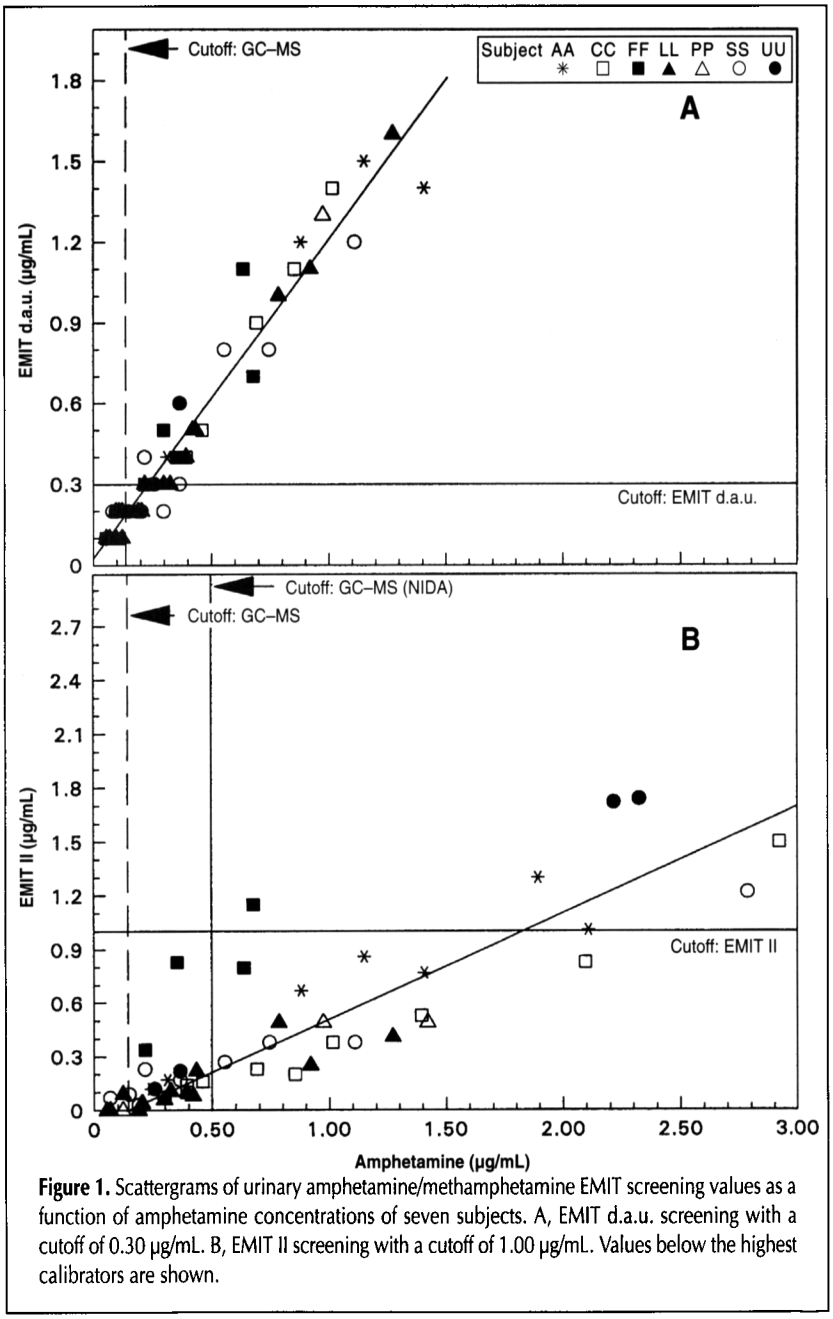

Figure 1. Scattergrams of urinary amphetamine/methamphetamine EMIT screening values as a function of amphetamine concentrations of seven subjects. A, EMIT d.a.u. screening with a cutoff of 0.30 µg/mL. B, EMIT II screening with a cutoff of 1.00 µg/mL. Values below the highest calibrators are shown.

Quantitative confirmation analysis (9), which typically uses 0.1 mL of urine, was done by GC–MS in the selected ion monitoring mode with a Hewlett-Packard (Palo Alto, CA) 5890 GC equipped with a 5940A MS Chem system. Deuterated amphetamine was used as internal standard. This system did not discriminate between *d*- and *l*-amphetamine. The limit of amphetamine detection was 0.04 µg/mL, the cutoff used was 0.14 µg/mL, and the CV was 8% at the concentration of 0.28 µg/mL. For comparison, it should be mentioned that the NIDA cutoff used for confirmation analysis was 0.50 µg/mL. The calibration curve was linear over the range 0.14–2.7 µg/mL. Samples containing higher amphetamine concentrations were usually diluted. Positive and negative controls were run with samples for both immunological and GC–MS methods.

All specimens were analyzed for creatinine by the alkaline picrate method (IL Test™ Instrumentation Laboratories, Milan. Italy), with a CV of 6%. Acidity was measured with a pH meter (pH 10, Beckman, Fullerton, CA) or with indicator paper (Neutralit® pH 5–10, Merck, Dermstadt, Germany).

Results

Twenty-two drug abusers agreed to participate in the study: 21 men (aged 16–47) and

1 woman (aged 32). Most of the 22 participants reported themselves to be multidrug users. Amphetamine was found in urine samples from seven male subjects. All seven also had cannabinoid-positive urines; three subjects had benzodiazepine-positive urines; and one subject (FF) received chlorpromazine, promethazine, and carbamazepine by prescription from the start of imprisonment. We have previously reported that promethazine and chlorpromazine metabolites may crossreact with the EMIT II amphetamine/methamphetamine assay (10). The amphetamine-positive subjects had a median age of 32 (range, 16–38). Five of these subjects reported use of amphetamine before imprisonment, and the last doses taken were reported to be in the range of 200 to 400 mg "street quality" (usually 40–50% purity). Two subjects out of the five (AA, CC) reported daily intake of amphetamine. The median time between last reported intake of amphetamine and first specimen taken was 1.7 days (range, 0.1–4.4 days; $n = 5$), and the median time between imprisonment and first specimen was 0.2 days (range, 0.1–0.9; $n = 7$).

Figure 1 shows the correlation between urinary amphetamine concentration (GC–MS) and the EMIT d.a.u. and EMIT II values. Only points occurring within the interval between amphetamine detection limit (0.04 µg/mL) and the highest calibrator of the EMIT assays are included. EMIT d.a.u screening and confirmation results corresponded well in the interval shown in Figure 1A; the regression line is described by $y = 1.19x + 0.02$, $r^2 = 0.96$ ($n = 71$). The equation shows that EMIT d.a.u. crossreacting material was about 20% higher than the concentration of amphetamine itself, which could indicate the presence of immunoreactive amphetamine metabolites. All positive-screened samples were also positive after confirmation. Eight of the confirmed positive specimens were screening negative (EMIT d.a.u. = 0.20 µg/mL). EMIT II screening and confirmation results are shown in Figure 1B. The correspondence between screening and confirmation results is described by the line $y = 0.59x - 0.08$, $r^2 = 0.92$ ($n = 53$). Subject FF was not included in the calculation because of his interfering medication. The equation shows that slightly more than half the amphetamine present was EMIT II crossreacting material, consistent with the fact that the assay mainly reacts with *d*-amphetamine.

The median duration of EMIT d.a.u. screening positive urines (cutoff, 0.30 µg/mL) for those who reported last intake ($n = 5$) and after imprisonment ($n = 7$) were 4.8 days (range, 4.4–6.3) and 2.9 days (range, 1.0–8.8), respectively. Using the by EMIT II assay, one of the subjects never screened positive for amphetamine after imprisonment (4.4 days after intake). Three subjects (AA, CC, UU) screened positive by EMIT II for 2.8–4.8 days after last intake. After imprisonment, the median duration of EMIT II positive specimens was 1.6 days (range, 0.0–2.8; $n = 6$)

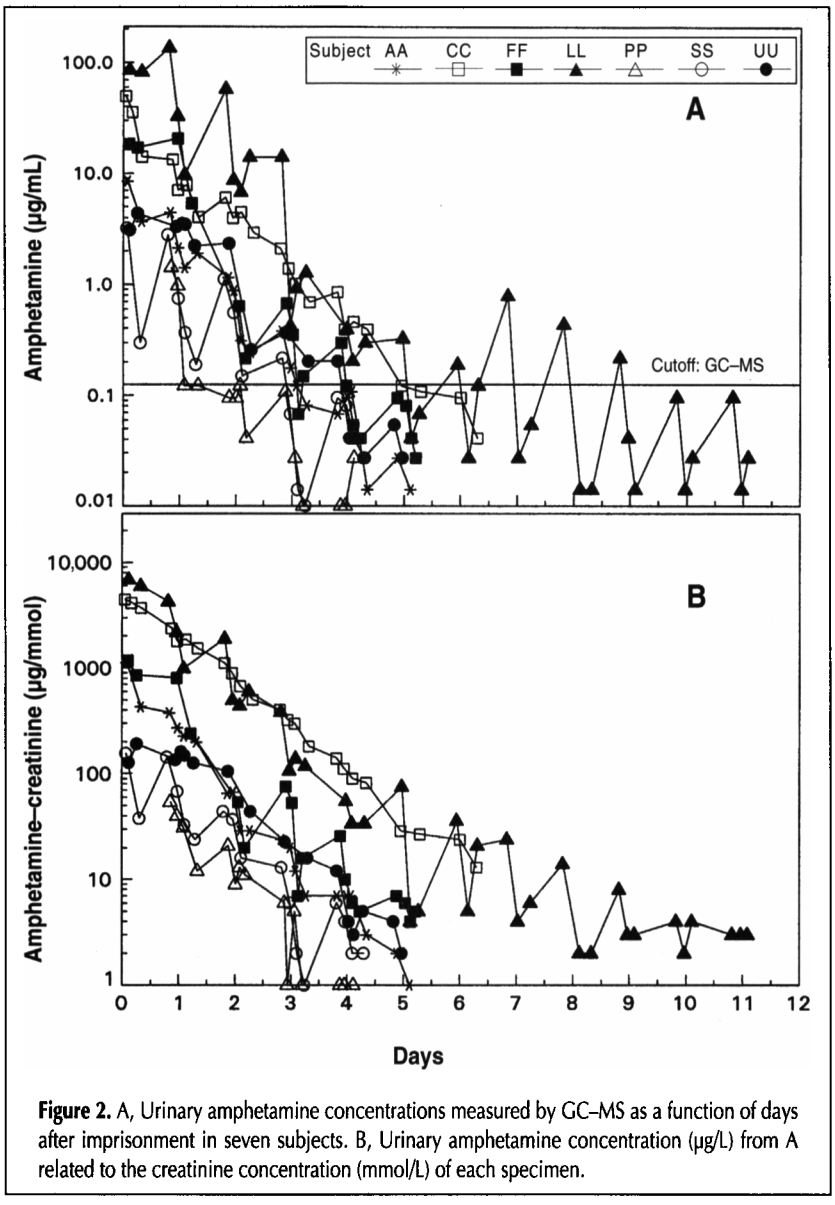

Figure 2. A, Urinary amphetamine concentrations measured by GC–MS as a function of days after imprisonment in seven subjects. B, Urinary amphetamine concentration (µg/L) from A related to the creatinine concentration (mmol/L) of each specimen.

The individual urinary amphetamine concentration curves (GC–MS) are shown in Figure 2A. Three subjects had initial amphetamine concentrations above 10 µg/mL. One of these (CC) reported daily intake of amphetamine and cannabinoids, and last amphetamine intake was a few hours before imprisonment. A second subject (FF) reported daily intake of cannabinoids and heroin and occasional intake of amphetamine; the last reported dose was taken 1.5 days before imprisonment. The third subject (LL) reported daily use of cannabinoids and benzodiazepines (by prescription), but there was no report of amphetamine intake.

Fluctuations in individual amphetamine concentrations over time (Figure 2A) occurred quite frequently. In the total material of 94 specimens, which included the last positive for each subject, there were 30 occasions of amphetamine concentration increasing from one specimen to the subsequent one. Both EMIT d.a.u. and EMIT II negative-screened samples were

followed by positive-screened samples in various subjects. In one of the subjects, an amphetamine concentration below the EMIT d.a.u. cutoff was followed by a positive test on four occasions (subject LL).

Extreme variations in concentrations between consecutive samples were sometimes observed as illustrated by two cases shown in Table I. One subject fluctuated from a highly positive specimen to a negative screen and then again to a highly positive sample. The other shows the difference in amphetamine concentrations between two specimens taken less than 4 h apart.

Subject LL, who had the highest amphetamine concentration observed, was also positive for the longest time. His amphetamine concentrations showed a pattern of fluctuations with high concentrations found in the morning when the acidity (low pH) and creatinine concentration were high. This seemed to be the trend for most of the participants. Furthermore, between 5.1 and 6.8 days of imprisonment (in subject LL), there were five consecutive EMIT d.a.u. negative-screen urines, after which a positive specimen (screening, 1.00 µg/mL and confirmation, 0.78 µg/mL) occurred (data not shown).

Figure 2B shows the time curves for the ratios between urinary amphetamine and the corresponding creatinine concentrations. The curves were generally sloping downward with time in a smoother fashion than in Figure 2A. The increases of the curves still observed were associated with an increase in urinary acidity. The curves of the amphetamine to creatinine ratios did not in any case indicate any new intakes of amphetamine when the changes in urinary acidity were taken into account. In one subject (CC), the urinary acidity varied little throughout the sampling period, but in another subject (LL), there was a shift from an average urinary pH of 6.2 (3 days) to pH 7.2 (next 3 days) to pH 6.5 for the rest of the sampling period. In some of the subjects, the creatinine concentrations tended to be higher during the first few days than the last days, which might suggest dehydration at the start of the testing period. High urinary acidity (low pH) was regularly associated with high amphetamine values, which was consistent with the well-known pH dependency of urinary amphetamine excretion. Conversely, low acidity (high pH) was associated with lower amphetamine concentrations. Low acidity was also often associated with low creatinine concentrations (data not shown).

Rough estimates of amphetamine urinary half-life were carried out on the basis of curves of the ratio of amphetamine to creatinine in subjects AA, CC, LL, and UU in Figure 2B. The shortest urinary half-life was approximately 10 h (AA), and the longest was approximately 25 h (LL).

Discussion

The present study showed amphetamine positive urines for more than 48 h. Using the low cutoff of the immunological EMIT d.a.u. and confirmation (GC–MS) methods, one subject was found to have amphetamine-positive urine for almost 9 days. Literature claims that urinary amphetamine remains detectable by immunological screening assays for 24–48 h after last intake (11,12). Typically, in controlled studies, 10–20 mg amphetamine sulfate has been given (1–3,5), although Änggård et al. (4) and Gunne and Änggård (6) gave 160–200 mg amphetamine sulfate to some of their volunteers. The polyclonal EMIT d.a.u. screening cutoff level (0.30 µg/mL) prolonged the median duration of positive urines by more than one day compared with the monoclonal EMIT II assay (1.00 µg/mL), which had higher specificity towards the *d*-isomer. This isomer is shown to be excreted into urine to a considerably lower extent than the *l*-isomer over a collection period of 60 h (1,13).

Because of the amphetamine pK_a, alkaline urine decreases ionized amphetamine and enhances renal tubular reabsorption with a subsequent decrease in the excretion rate. Pharmacokinetic studies (4) have shown an increase in amphetamine plasma $t_{1/2}$ by approximately 7 h for each urinary pH unit in the interval from 5.0 to 7.2 with a maximum plasma $t_{1/2}$ of 33.6 h observed (4). The half-lives estimated from urine data in the present study fit with values reported in that study.

Physiological fluctuations in urinary pH are common, with a range of 4.9–8.3 observed by Beckett and Rowland (1). Typically, low pH values occurred early in the morning. Higher values were seen later in the morning and in the afternoon (1). Our data are in accordance with these observations.

The urinary amphetamine concentration results depended on several factors working in different directions. Dehydration decreased urinary output and flow-dependent excretion of amphetamine. The urines had high solute content, low pH, and hence, high acid-dependent excretion. Finally, because of the high concentration of the urine, the amphetamine concentration was high. Conversely, with good hydration, flow-dependent amphetamine excretion was high; pH was closer to neutrality, which decreased the acid-facilitated excretion of amphetamine; and amphetamine will be diluted in the ensuing larger volume of urine. Fluctuations in pH and flow variables could occur within hours, which explained large differences in amphetamine concentrations from specimens taken with short time intervals.

Repeated use of amphetamine was reported to have little effect on the

Table I. Extremes in Amphetamine Concentrations between Consecutive Specimens

Subject	Time of imprisonment (h)	EMIT d.a.u. screening* (µg/mL)	Amphetamine (µg/mL)	pH	Creatinine (mmol/L)
SS	1.8	2.00	3.11	5.7	20.4
	6.4	0.20	0.30	7.9	7.2
	11.6	2.00	2.78	5.5	19.5
LL	67.6	2.00	14.0	5.5	36.6
	71.3	0.50	0.42	7.0	3.9

*Highest calibrator was 2.00 µg/mL.

fraction of the dose recovered in urine (1). Hepatic metabolism of amphetamine appeared to be independent of the dose, and there were no major differences between regular and nonregular users (5,6). Whether the presence of cannabinoids and other drugs might affect the metabolism of amphetamine would only be speculative (14–16).

In the present study, there was good correlation between amphetamine concentrations determined by GC–MS and EMIT d.a.u. up to 2.00 µg/mL. EMIT d.a.u. seemed to react to amphetamine metabolites in addition to amphetamine itself, which was in accordance with previous findings (17). EMIT II, which mainly reacted with *d*-amphetamine, resulted in the confirmation of only rather high concentrations of urinary amphetamine. In Norway, *l*-amphetamine is not medically available; the prescription drug selegiline, however, is metabolized to *l*-methamphetamine and *l*-amphetamine.

New intakes of amphetamine during the specimen collecting period of our subjects could not be entirely ruled out, but seemed unlikely because changes in creatinine and pH could explain most of the variations observed. For this reason, we believe that correction for creatinine and pH measurements are useful tools in determining if there has been a new intake or if a high or increased amphetamine concentration could be explained by physiological factors only.

Conclusion

Several factors determined the duration of an amphetamine-positive urine sample at a given intake: the specificity of the immunological screening test the cutoffs used for screening and confirmation analyses, whether the urine was diluted or concentrated, and urinary pH. The ratio between amphetamine and creatine concentrations and pH measurements was useful in assessing the likelihood of a new intake shortly after imprisonment, start of treatment, or when one specimen had a higher amphetamine concentration than the previous one.

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