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Drug Monitoring and Toxicology

Duration of Detectable Methamphetamine and Amphetamine Excretion in Urine after Controlled Oral Administration of Methamphetamine to Humans

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Background: **Confirmation of a workplace drug test requires urinary methamphetamine (MAMP) and amphetamine (AMP) concentrations ≥500 and 200 μg/L, respectively, but cutoffs at half those values (250/100 μg/L) have been proposed. We determined the urinary excretion of MAMP after oral ingestion and examined the effect of using lower cutoffs on detection of exposure.**

Methods: **Volunteers (n = 8) ingested four 10-mg doses of MAMP · HCl daily over 7 days, and five of them ingested four 20-mg doses 4 weeks later. After ingestion, the volunteers collected all urine specimens for 2 weeks. After solid-phase extraction, MAMP and AMP were measured by gas chromatography–positive chemical ionization mass spectrometry with dual silyl derivatization.**

Results: **MAMP and AMP were generally detected in the first or second void (0.7–11.3 h) collected after drug administration, with concentrations of 82–1827 and 12–180 μg/L, respectively. Peak MAMP concentrations (1871–6004 μg/L) after single doses occurred within 1.5–60 h. MAMP ≥500 μg/L was first detected in the first or second void (1–11 h) at 524-1871 μg/L. Lowering the MAMP cutoff to 250 μg/L changed the initial detection time little. AMP ≥200 μg/L was first detected in the 2nd–13th (7–20 h) post-administration voids. At a cutoff of 100 μg/L, AMP was first confirmed in the second to eighth void (4–13 h). Reducing the cutoff to 250/100 μg/L extended terminal MAMP detection by up to 24 h, increased total detection time by up to 34 h, and increased the total number of positive specimens by 48%.**

Conclusions: **At the lower cutoff, initial detection times are earlier, detection windows are longer, and confirmation rates are increased. Elimination of the AMP requirement would increase detection rates and allow earlier detection.**

According to the 1999 Drug Abuse Warning Network (DAWN) report, D-methamphetamine (MAMP)[4] is among the five most common illicit drugs mentioned in emergency department and medical examiner settings in the US *(1)*. The popularity of illicit methylenedioxymethamphetamine, other designer amphetamines, and MAMP as "club drugs" at dance club "raves" has grown. Most deaths associated with club drugs involved abuse of MAMP in combination with heroin/morphine, cocaine, and/or ethanol. According to the Substance Abuse and Mental Health Services Administration (SAMHSA) *(2)*, the lifetime rate of MAMP use among 12- to 17-year-olds also doubled between 1996 and 1997. Worldwide, trafficking of amphetamine-type stimulants increased more than any other drug class during the 1990s, and seizures quadrupled *(3)*. Abuse of the stimulant MAMP is attrib-

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[4] Nonstandard abbreviations: MAMP, D-methamphetamine; SAMHSA, Substance Abuse and Mental Health Services Administration; AMP, D-amphetamine; GC-MS, gas chromatography–mass spectrometry; BSTFA, *N*,*O*-bis(trimethyl)trifluoroacetamide; TMCS, trimethylchlorosilane; MTBSTFA, *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide; TBDMCS, *tert*-butyldimethylchlorosilane; SPE, solid-phase extraction; PCI, positive chemical ionization; NIDA, National Institute on Drug Abuse; TMS, trimethylsilyl; TBDMS, *tert*-butyldimethylsilyl; and LOQ, limits of quantification.

utable to its potent dopaminergic and sympathomimetic effects, including euphoria, improved cognitive and sensory performance, generalized improvement in mood, increased physical endurance, and appetite suppression *(4–8)*. The drug's strong reinforcing and addictive potential contributes to abuse, and tolerance to its psychotropic effects leads to use of toxic doses *(6, 9–14)*. Negative sequelae are generally attributable to exaggerated central nervous system and cardiovascular effects *(4, 6, 8, 9, 11, 15–19)* and include psychosis, paranoia, hyperpyrexia, seizures, stroke, tachycardia, arrhythmias, pericarditis, myocardial ischemia, hypertension, aortic and cerebral aneurysm dissection, and myocardial infarction.

MAMP is excreted primarily in urine, with little biliary excretion of the parent drug or metabolites *(20)*. In normal urine (pH 6–8), 37–54% of a dose is excreted as parent drug and 4–7% as D-amphetamine (AMP) *(21–24)*. Each unit increase or decrease in urinary pH produces a respective 7-h increase or decrease in the MAMP plasma half-life *(21)*. Therefore, the percentage of the dose excreted as parent drug can range from as low as 2% in alkaline (pH ≥8.0) to 76% in acidic urine (pH ≤5.0). After administration of AMP, urinary excretion of AMP is similarly affected by urinary pH (1% and 74% excretion in alkaline and acidic urine, respectively) *(25)*. It has also been reported that the renal clearance of MAMP exceeds that of the average renal filtration rate, suggesting the involvement of an active transport mechanism in the excretion of MAMP *(23)*.

Current SAMHSA requirements for a positive workplace urine test include an amphetamines screen ≥1000 μg/L (or ng/mL) confirmed by a MAMP concentration ≥500 μg/L and an AMP concentration ≥200 μg/L (500/200 cutoff) by gas chromatographic–mass spectometric (GC-MS) analysis *(26)*. AMP requirements were instituted because of the potential for false-positive analytical results with specimens containing other sympathomimetic amines *(27)*. SAMHSA has recently proposed new workplace screening cutoff concentrations of 500 μg/L and confirmation cutoffs of 250 and 100 μg/L for MAMP and AMP, respectively (250/100 cutoff) *(28)*.

The object of this study was to characterize the urinary excretion of MAMP and its major active metabolite, AMP, in humans after controlled oral administration. Urine concentration data were evaluated to determine detection windows and confirmation rates based on current and proposed confirmation cutoffs and cutoffs based on MAMP requirements alone.

Materials and Methods

CHEMICALS AND REAGENTS

Chemicals were obtained from the following sources: MAMP, AMP, D,L-[$^2H_{11}$]MAMP, and D,L-[$^2H_{10}$]AMP were from Radian (now Cerilliant); *N*,*O*-bis(trimethyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS), *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) with 1% *tert*-butyldimethylchlorosilane (TBDMCS) were from Pierce Chemical. Solid-phase extraction (SPE) columns (Clean Screen DAU; 200 mg of packing; 10-mL columns) were obtained from United Chemical Technologies. Methanol, methylene chloride, 2-propanol, and acetonitrile were HPLC-grade chemicals. All other chemicals were reagent grade.

INSTRUMENTATION

Qualitative and quantitative analyses were performed on a Hewlett-Packard 6890 gas chromatograph interfaced with a Hewlett-Packard 5973 mass selective detector. A split-splitless capillary inlet system operated in the splitless mode and a HP-1 [12 m × 0.2 mm (i.d.); 0.33-μm film thickness] or Phenomenex ZB-1 [15 m × 0.25 mm (i.d.); 0.10-μm film thickness] dimethylpolysiloxane fused-silica capillary column were used for chromatographic isolation of analytes. The single quadrupole mass spectrometer was operated in positive chemical ionization (PCI) mode with methane as reactant gas as described previously *(29)*.

HUMAN SUBJECTS

This study was conducted at the Intramural Research Program, National Institute on Drug Abuse (NIDA), in Baltimore, MD as part of a broader study of the clinical pharmacokinetics and pharmacodynamics of MAMP after oral administration. The NIDA Institutional Review Board approved the protocol. Participants were healthy volunteers with a history of stimulant and opioid use. Participants were evaluated medically and psychologically before admission to the clinical ward to establish their health. Volunteers provided informed consent and resided on a closed research unit for ~10 weeks. Their vital signs were monitored daily, and routine blood testing (CHEM Profile 2) was performed on alternate weeks for medical evaluation throughout the study. Participants were not physically dependent on illicit drugs. During the study, periodic urine specimens were tested to ensure that there was no self-administration of drugs.

STUDY PROTOCOL

The first 2 weeks of the study served as a clearance phase for previously self-administered drugs. Morning urine specimens were collected during this time. Drug administrations occurred in weeks 3 and 7. In week 3 (low-dose week), participants (n = 8) received four daily oral administrations of MAMP · HCl (Desoxyn® Gradumet®; 10 mg) at 1030 in the morning. As part of the pharmacodynamic study, participants were administered placebo MAMP capsules in week 6. Lactose was substituted for sustained-release MAMP to contrast physiologic and subjective responses after active and placebo MAMP. During week 7 (high-dose week), participants (n = 5) received four 20-mg oral administrations of MAMP · HCl. Participants were scheduled to receive four consecutive daily administrations of the sustained-release formulation, the only form of MAMP available for therapeutic administra-

tion at the time of this study. Abbott Laboratories discontinued production of Desoxyn Gradumet in 1999; the Desoxyn formulation is currently available. If physiologic measures (i.e., heart rate, blood pressure, and temperature) did not meet NIDA Intramural Research Program medical dosing criteria on the scheduled administration day, drug administration was sometimes delayed by 24 h. However, participants always received four doses once daily within 7 days. We therefore have grouped the data into three categories: single, consecutive, and nonconsecutive administration regimens (see section on data analysis). The total base equivalent amounts of MAMP administered in the low- and high-dose regimens were 32.2 and 64.3 mg, respectively. Three participants did not receive the high-dose regimen because of personal choice, medical disqualification, or behavioral misconduct. All drug administrations were conducted under subject-blind conditions. Urine specimens were collected ad libitum after initiation of the dosing regimen. Specimens were collected in polypropylene bottles, and void volumes were recorded. Approximately 15 mL was aliquoted into polypropylene cryotubes and stored at −20 °C until analysis.

CALIBRATORS AND CONTROLS

Calibrators and controls were prepared in urine that was collected from laboratory volunteers and was demonstrated to be negative for MAMP and AMP by GC-MS analysis before use in the preparation of calibrators and controls. Two dynamic linear ranges were established for the assay. Quantitative analysis was performed by use of split curves at concentration ranges of 2.5–100 and 100-1000 μg/L. Specimen samples were analyzed using the curve with the appropriate dynamic range. Specimens with concentrations >1000 μg/L were diluted with 0.5 mol/L sodium acetate buffer (pH 4.0) and reanalyzed to bring them within the dynamic range of the assay. [$^2H_{11}$]MAMP and [$^2H_{10}$]AMP were added as internal standards to each sample (100 ng in 0.1 mL of H_2O). MAMP and AMP curves were prepared in duplicate at drug concentrations ranging from 1.25 to 1000 μg/L. Calibrators and controls were prepared from different commercially available drug lots and by different analysts. Duplicate controls at 10, 100, and 250 μg/L were prepared and analyzed at the beginning and end of each sample batch. Linear regression analysis of control samples containing 10 and 100 ng of drug were performed using the 2.5–100 μg/L curves, and 250 ng controls were analyzed using the 100-1000 μg/L curves. Quantitative results for all controls across sample batches were within 20% of their target concentrations.

MAMP AND AMP ISOLATION FROM URINE

Calibrators, controls, and specimens were processed by SPE according to a previously published method *(30)*. Briefly, 1 mL of urine was aliquoted, 100 ng of each internal standard was added, and samples were adjusted to acidic pH with 0.5 mol/L sodium acetate buffer (pH 4.0). Analytes were isolated on preconditioned SPE columns and eluted with methylene chloride–2-propanol–ammonium hydroxide (80:20:2 by volume). After SPE, AMP analogs were stabilized by addition of 20 μL of MTBSTFA + 1% TBDMCS to each tube. Samples were evaporated under nitrogen in a water bath at 40 °C until dry. A 500-μL aliquot of acetonitrile was added, and tubes were vortex-mixed to recover drug from centrifuge tube walls. After evaporation to dryness, 20 μL of acetonitrile was added, tubes were vortex-mixed and centrifuged, and samples were transferred to autosampler vials. A 20-μL aliquot of MTBSTFA + 1% TBDMCS was added to each vial. Vials were loosely capped and placed in a heat block at 80 °C for 15–20 min. A 20-μL aliquot of BSTFA + 1% TMCS was added, vials were crimp-capped, and samples were heated at 80 °C for 45 min. One microliter was injected for analysis under splitless GC/PCI-MS conditions.

This dual derivatization scheme was originally developed as a method for the analysis of multiple basic drug classes. Addition of MTBSTFA to samples after SPE and before evaporation was found to greatly reduce volatility losses of the amphetamines. *tert*-Butyldimethylsilyl (TBDMS) derivatization of the primary amine of AMP proceeds readily. However, formation of the TBDMS derivative of MAMP, a secondary amine, is sterically hindered, similar to the normetabolites of cocaine and the opiates *(31)*. Consequently, BSTFA was used in a second derivatization step to produce the trimethylsilyl (TMS) derivative of MAMP. Under the derivatization conditions described, there was selective formation of the TMS derivative of MAMP and the TBDMS derivative of AMP. Time course experiments demonstrated that >95% of MAMP was converted to the TMS derivative and >95% of AMP was converted to the TBDMS derivative.

METHOD SPECIFICITY AND SENSITIVITY

Samples containing single analytes in acetonitrile were derivatized and analyzed by GC/PCI-MS in full scan mode for spectral characterization. The ions chosen as quantitative ions for MAMP, [$^2H_{11}$]MAMP, AMP, and [$^2H_{10}$]AMP were *m/z* 130, 136, 158 and 162, respectively. Confirming ions, retention order, and derivatives are listed in Table 1. Urine specimen analyte concentrations were determined in selected ion monitoring mode. Urine controls to which MAMP and AMP had been added were

Table 1. MAMP and AMP derivatives, elution order, and target and confirming ions.

Analyte	Derivative	Elution order	Target ion, *m/z*	Confirming ions, *m/z*
MAMP-d_{11}	TMS	1	136	217, 231, 261
MAMP	TMS	2	130	206, 220, 250
AMP-d_{10}	TBDMS	3	162	244, 260, 202
AMP	TBDMS	4	158	234, 250, 192

serially diluted (0.5–10 μg/L) and analyzed in quadruplicate to determine the limits of quantification (LOQ). The following criteria were used to determine LOQ: (*a*) quantifying ion signal-to-noise ratio (determined by peak height) was >3:1; (*b*) 75% of control replicates (n = 4) calculated within ±25% of the target concentration; and (*c*) confirming ion ratios were within 20% of those observed for a within-run 10 μg/L (167 pg on column) calibrator. LOQ for MAMP and AMP were 2.5 μg/L (42 pg on column). Sensitivity and specificity criteria were met by both the HP-1 and Phenomenex ZB-1 dimethylpolysiloxane GC capillary columns.

DATA ANALYSIS

Data were analyzed using method LOQ, the 500/200 and 250/100 cutoffs, and cutoffs requiring only 500 or 250 μg/L MAMP. Initial and final detection times, peak concentrations, percentage detection rates, and AMP-MAMP percentage ratios were determined. The initial detection time after first administration and final detection time after last administration were defined as the first and last specimens positive at or above respective cutoffs. The percentage detection rate was determined by dividing the number of positive specimens collected over a time period by the total number of specimens collected over the same time period and multiplying by 100. Mean detection rates were determined by averaging rates from all participants over a collection period. Total detection rates were also determined by dividing the sum of all positive specimens from all participants by the total number of specimens collected over a collection period. AMP-MAMP concentration ratios were multiplied by 100 to determine percentage ratios. Individual, mean, and total 12-h detection rates were determined after single doses. Ranges and means for final detection times and void number, total urine specimens collected from first drug administration to last positive specimen, total positive specimens from initial dosing to last positive specimen, and total detection rates across participants and doses were also determined.

Specimen volumes were multiplied by the respective MAMP and AMP concentrations to determine the total amount of MAMP and AMP excreted per void. These products were then summed to determine the total base amounts of MAMP and AMP excreted after the low- or high-dose regimens. The percentage of MAMP eliminated in urine after each administration regimen was determined by dividing the total amount of MAMP excreted by the total amount of base drug administered in the low- and high-dose regimens (32.2 and 64.4 mg, respectively) and multiplying the quotient by 100 (percentage of dose excreted as MAMP). The percentage of AMP eliminated in urine was determined by dividing the moles of AMP excreted by the total moles of MAMP administered and multiplying the quotient by 100 (percent molar dose excreted as AMP).

Because of postponements that occurred in daily drug administration schedules for several participants, the data were categorized by regimen into three subsets: four consecutive administrations (n = 8), four nonconsecutive administrations within 7 days (n = 5), and single administrations (n = 5). All single-administration data were collected after low-dose administrations. Administrations were considered single if specimens from five consecutive urine voids collected immediately before the next dosing were negative for both MAMP and AMP at their respective LOQ. Nonconsecutive administrations had random 2- to 4-day intervals between administrations.

Results

EXCRETION PATTERN, LOQ DETECTION TIMES AND RATES, AND AMP-MAMP RATIOS

With the 2.5 μg/L LOQ, initial detection of MAMP generally occurred in the first urine void. Only two participants had initial detection of MAMP later than the first void, and they were also the only participants from whom specimens were collected <0.5 h after drug administration. After the first drug administration of each regimen, measurable concentrations of both MAMP and AMP initially occurred in the first to fourth void collected, and no dose–detection time or regimen–detection time relationship was observed (Tables 2 and 3).

MAMP was initially measured at concentrations ranging from 82 to 1827 μg/L. Peak concentrations after initial low- and high-dose drug administration ranged from 1871 to 6004 μg/L and occurred in the 2nd to 15th void collected at 1.5–43.7 h after dosing. Peak MAMP concentrations after single-administration regimens ranged from 2310 to 14 660 (mean ± SD, 4720 ± 2302) μg/L, respectively. Peak MAMP concentration ranges after consecutive low- and high-dose regimens were 5317–7003 (5338 ± 1652) μg/L and 5092–13 824 (9466 ± 3639) μg/L, respectively. Peak MAMP concentrations after nonconsecutive low-dose regimens were 3598–10 905 (6936 ± 3005) μg/L, and the peak MAMP concentration after one nonconsecutive high-dose regimen was 18 468 μg/L.

AMP was initially detected in the first to fourth urine specimen collected after single administrations of low and high doses at much lower concentrations (12–180 μg/L). Peak AMP concentrations after single-administration regimens occurred in the 2nd to 29th void collected at 12.0–58.3 h and ranged from 264 to 660 μg/L. Peak AMP concentrations after consecutive low- and high-dose regimens were 422-2872 and 1195–4016 μg/L, respectively. Peak AMP concentrations after nonconsecutive low-dose regimens were 370-1649 μg/L, and the peak AMP concentration after one nonconsecutive high-dose regimen was 5992 μg/L.

After consecutive daily administrations, successive trough MAMP and AMP concentrations increased, indicating accumulation. Fig. 1 illustrates the within-regimen variability in MAMP and AMP excretion over the urinary elimination time course for volunteer S after single, consecutive, and nonconsecutive drug administrations.

Table 2. Initial MAMP detection times and initial detection voids at different cutoff concentrations after the first 10-mg MAMP · HCl administration.

			Initial detection time, h		Initial detection void	
Cutoff[a]	Regimen[b]	Volunteers, n	Range	Mean ± SD	Range	Mean
LOQ	Single	5	0.7–4.2	2.8 ± 1.6	1–2	1.4
	Nonconsecutive	4	1.4–11.3	4.6 ± 4.5	1–2	1.5
	Consecutive	4	4.0–9.1	5.6 ± 2.3	1–4	1.8
	Across regimens[c]	13	0.7–11.3	4.2	1–4	1.6
250	Single	5	0.5–4.2	2.0 ± 1.4	1–2	1.2
	Nonconsecutive	4	1.4–11.3	4.9 ± 4.4	1–2	1.8
	Consecutive	4	3.2–9.1	5.2 ± 2.6	1–3	1.5
	Across regimens[c]	13	0.5–11.3	3.9	1–3	1.5
250/100	Single	5	0.5–4.3	3.0 ± 1.7	1–4	1.8
	Nonconsecutive	4	4.3–17.2	10.3 ± 5.4	1–9	4.5
	Consecutive	4	6.6–10.1	8.5 ± 1.9	2–5	3.3
	Across regimens[c]	13	0.5–17.2	6.9	1–9	3.1
500	Single	5	1.4–4.3	3.0 ± 1.3	1–2	1.8
	Nonconsecutive	4	1.4–11.3	4.9 ± 4.4	1–2	1.8
	Consecutive	4	3.2–9.1	6.2 ± 2.8	1–3	1.8
	Across regimens[c]	13	1.4–11.3	4.6	1–4	1.8
500/200	Single	5	4.2–12.0	8.3 ± 3.3	1–7	4.8
	Nonconsecutive	4	9.3–17.2	15.0 ± 4.1	2–9	6.5
	Consecutive	4	11.4–19.6	12.7 ± 5.2	3–8	4.5
	Across regimens[c]	13	4.2–19.6	11.7	1–9	5.2

[a] LOQ, concentrations of MAMP and AMP at 2.5 μg/L; 250, 250 μg/L MAMP; 250/100, 250 μg/L MAMP and 100 μg/L AMP; 500, 500 μg/L MAMP; 500/200, 500 μg/L MAMP and 200 μg/L AMP.

[b] Consecutive, four consecutive daily administrations; Nonconsecutive, four nonconsecutive daily administrations within 7 days; Single, single administration preceded by five or more specimens negative for MAMP and AMP at their respective LOQ.

[c] Mean values are weighted.

The total amount of MAMP excreted over the LOQ detection window ranged from 8.9 to 29.5 mg and 16.1 to 27.2 mg, equivalent to 25–92% and 25–43% of the low and high doses, respectively. The total amount of AMP excreted ranged from 1.2 to 4.8 and 3.2 to 8.6 mg, representing 5–20% and 7–18% of the molar MAMP dose for the low and high doses, respectively.

Large intersubject variability in AMP-MAMP ratios were observed, whereas intrasubject ratios after consecutive administration consistently increased over time. Ini-

Table 3. Initial MAMP detection times and initial detection voids at different cutoff concentrations after the first 20-mg MAMP · HCl administration.

			Initial detection time, h		Initial detection void	
Cutoff	Regimen	Volunteers, n	Range	Mean ± SD	Range	Mean
LOQ	Nonconsecutive	1	6.1	6.1	1	1
	Consecutive	4	1.2–8.8	4.6 ± 3.2	1–4	1.9
	Across regimens[a]	5	1.2–8.8	4.9	1–4	1.7
250	Nonconsecutive	1	3.3	3.3	1	1
	Consecutive	4	1.2–8.8	4.0 ± 3.3	1–4	1.8
	Across regimens[a]	5	1.2–8.8	3.9	1–4	1.6
250/100	Nonconsecutive	1	3.3	3.3	1	1
	Consecutive	4	8.8–13.1	10.5 ± 1.8	1–12	5
	Across regimens[a]	5	3.3–13.1	9.1	1–12	4.2
500	Nonconsecutive	1	3.3	3.3	1	1
	Consecutive	4	1.2–8.8	4.6 ± 3.2	1–4	2.0
	Across regimens[a]	5	1.2–8.8	4.3	1–4	1.8
500/200	Nonconsecutive	1	7.9	7.9	2	2
	Consecutive	4	12.0–19.6	15.7 ± 3.8	2–13	7.5
	Across regimens[a]	5	7.9–19.6	14.1	2–13	6.4

[a] Mean values are weighted.

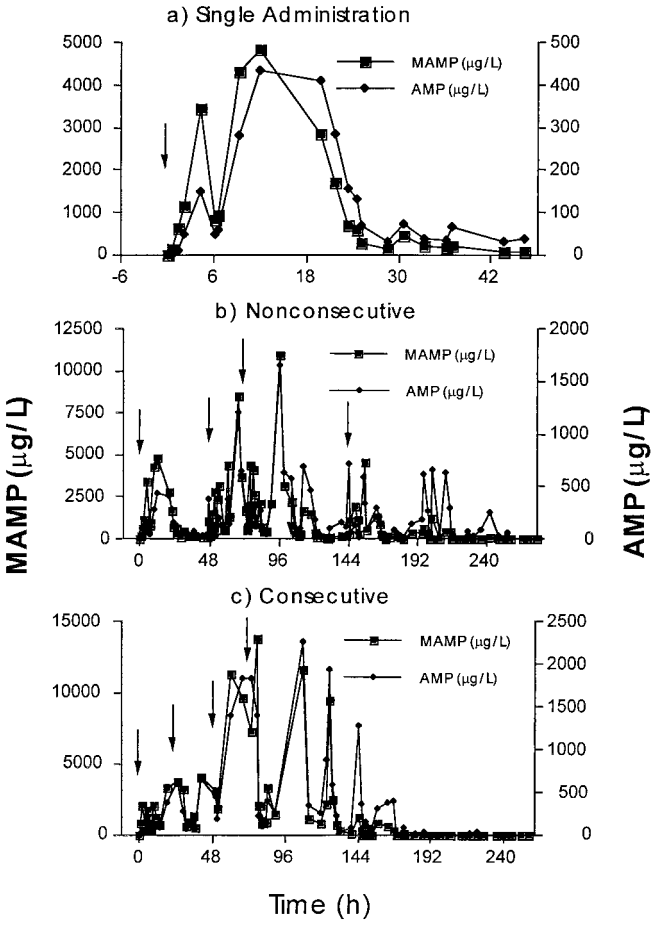

Fig. 1. Urinary excretion profiles for volunteer S after single (*a*) and four nonconsecutive daily oral administrations of 10 mg (*b*) or four consecutive daily oral administrations of 20 mg (*c*) of sustained-release MAMP · HCl.

Arrows indicate MAMP · HCl administration times.

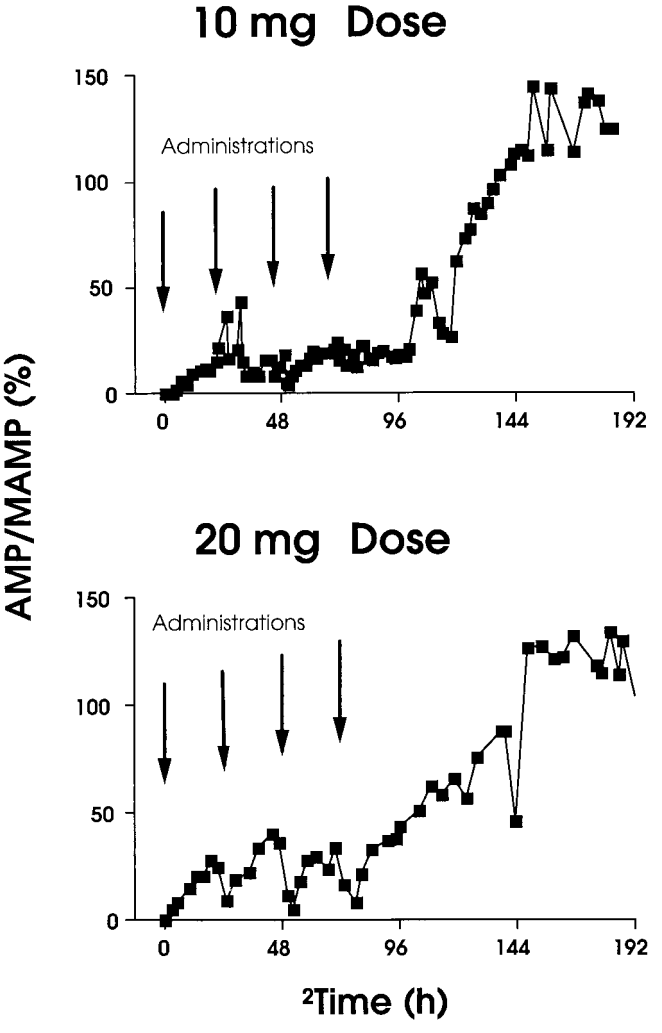

Fig. 2. AMP-MAMP ratios for volunteer BB after four consecutive administrations of 10 (*top*) or 20 mg (*bottom*) of sustained-release MAMP · HCl.

tial AMP-MAMP ratios ranged from 1% to 18%. AMP-MAMP ratios increased throughout the elimination time course after a single dose. With consecutive dosing, a sharp decrease in AMP-MAMP ratios occurred immediately after each successive MAMP dose, followed by a return to the pattern of gradual increase observed after single dosing. In urine specimens containing peak MAMP concentrations, AMP-MAMP ratios had increased to 7–42%, and in the last urine specimen positive for both analytes, AMP-MAMP ratios were 25–260%. Fig. 2 illustrates the increase in AMP-MAMP ratios after a single dose and the immediate decrease observed after each successive dose in volunteer BB. This volunteer was the only participant to receive consecutive administrations of the low and high doses, but these patterns were consistent across participants.

Individual urinary 12-h detection rates after the first administration were 67–100% (mean ± SD, 91.2% ± 10.0%) after low-dose regimens and 63–100% (87.6% ± 17.5%) after high-dose regimens (Tables 4 and 5). A correlation between regimen and detection rate was not observed. Of the 100 specimens collected within 12 h of initial MAMP administration, 87 were positive, for a total 12-h detection rate of 87%. Lack of AMP detection was responsible for most negative results early in the excretion of MAMP. With the MAMP and AMP LOQ, no significant differences were observed in final detection times (range, 46–169 h) between doses or after single, consecutive, or nonconsecutive regimens (Tables 6 and 7). With the 2.5 μg/L cutoffs (LOQs) for MAMP and AMP, the mean final detection time from the last drug administration was 110.7 h, and the total detection rate across doses and regimens was 94% (Table 8).

Detection times based on 500/200 μg/L cutoff concentrations

Initial detection voids and times after the first low and high MAMP administration are listed in Tables 2 and 3. Across doses and regimens with a 500/200 cutoff, MAMP

Table 4. Individual, mean, and total 12-h detection rates after the first administration of 10 mg of MAMP · HCl.

Volunteer	Regimen	Specimens collected after initial drug administration, n	12-h detection rate, %				
			LOQ	250 cutoff	250/100 cutoff	500 cutoff	500/200 cutoff
S	Single	8	88	88	25	88	25
S	Single	7	86	86	43	72	28
Y	Single	2	100	100	100	100	50
Z	Single	7	100	100	14	86	14
AA	Single	7	86	86	14	86	0
Mean ± SD	Single	6.2	92.0 ± 7.3	92.0 ± 7.3	39.2 ± 36.0	86.4 ± 9.9	23.4 ± 18.5
S	Nonconsecutive	8	88	88	25	88	25
Y	Nonconsecutive	1	100	100	100	100	0
Z	Nonconsecutive	6	83	83	17	83	0
AA	Nonconsecutive	8	88	75	0	63	0
Mean ± SD	Nonconsecutive	5.8	89.8 ± 7.2	86.5 ± 10.5	35.5 ± 44.2	83.5 ± 15.4	6.3 ± 12.5
V	Consecutive	4	100	100	50	75	25
W	Consecutive	2	100	100	100	100	50
X	Consecutive	4	100	100	50	100	50
BB	Consecutive	3	67	67	0	67	0
Mean ± SD	Consecutive	3.3	92.8 ± 16.5	92.8 ± 16.5	50.0 ± 40.8	85.5 ± 17.1	31.25 ± 23.9
Mean[a]	Across regimens	5.1	91.2 ± 10.0	90.2 ± 11.0	41.4 ± 37.1	85.2 ± 12.9	20.5 ± 20.1
Total	Across regimens	67	89.6	86.6	26.9	82.1	19.4

[a] Mean values are weighted.

and AMP were initially detected in specimens 1–13 (mean, 5.6) collected 4.2–19.6 h (mean, 12.4 ± 4.2 h) post drug administration. MAMP and AMP concentrations were 1574–6004 (3344 ± 1304) and 219-1556 (380 ± 304) μg/L, respectively. Initial AMP-MAMP ratios ranged from 7% to 26%. There were no significant differences observed in final detection times from last drug administration across regimens or doses (Tables 6 and 7). The time to last positive from the last drug administration was 22–66 (42.7 ± 22.8) h, 27–92 (53.3 ± 20.6) h, and 46–70 (58.5 ± 9.8) h after single, consecutive, and nonconsecutive administrations, respectively. Although individual total detection rates varied substantially, as evidenced by the data shown for single nonconsecutive administration of the high dose (89%; Table 7), across all participants and doses (Tables 6 and 7), the 500/200 total detection rate after consecutive administrations (59%) was higher than that after nonconsecutive (39%) or single regimens (39%). Individual 12-h detection rates (Tables 4 and 5) ranged from 0% to 67% (20.2% ± 23.8%). Sixteen of 100 total specimens collected from all participants within 12 h of initial drug administration were positive, for a total 12-h detection rate of 16%. Of the 591 specimens collected from initial drug administration to last positive across participants, doses, and regimens, 292 were confirmed positive by the 500/200 cutoff, for a total detection rate of 49% (Table 8). Detection rate also appeared to be correlated with micturition interval. Generally, detection rate increased with longer micturition intervals.

The initial detection void and time means and ranges after low and high doses are listed by regimen in Tables 2 and 3, with elimination of the 200 μg/L AMP requirement. Individual initial detection voids and times also were determined across regimens and doses when the 200

Table 5. Individual, mean, and total 12-h detection rates after the first administration of 20 mg of MAMP · HCl.

Volunteer	Regimen	Specimens collected after initial drug administration, n	12-h detection rate, %				
			LOQ	250 cutoff	250/100 cutoff	500 cutoff	500/200 cutoff
S	Consecutive	9	100	100	22	78	0
Y	Consecutive	1	100	100	100	100	0
AA	Consecutive	12	75	67	8	58	0
BB	Consecutive	8	63	100	25	75	12
Mean ± SD	Consecutive	7.5	84.5 ± 18.6	91.8 ± 16.5	38.8 ± 41.5	77.8 ± 17.3	3.0 ± 6.0
W	Nonconsecutive	3	100	100	100	100	67
Mean[a]	Across regimens	5.1	87.6 ± 17.5	93.4 ± 14.8	51.0 ± 45.2	82.2 ± 17.9	15.8 ± 29.1
Total	Across regimens	33	81.8	87.9	27.3	72.7	9.1

[a] Mean values are weighted.

Table 6. Final detection times and total detection rates after administration of 10 mg of MAMP · HCl.

Cutoff	Regimen	Volunteers, n	Final detection time from last administration, h		Specimens from initial administration to last positive		Total detection rate, %
			Range	Mean ± SD	Total	Positive	
LOQ	Single	5	46–144	87.2 ± 51.0	138[a]	125[a]	91[a]
	Nonconsecutive	4	71–169	123.0 ± 49.3	247	232	94
	Consecutive	4	102–142	125.7 ± 19.2	277	253	91
	Across regimens	13	46–169	110.2[b]	524	485	93
250	Single	5	31–87	55.6 ± 27.9	88[a]	68[a]	77[a]
	Nonconsecutive	4	59–71	65.8 ± 5.9	254	182	72
	Consecutive	4	44–73	58.2 ± 14.2	171	160	93
	Across regimens	13	31–87	59.5[b]	425	342	80
250/100	Single	5	25–77	51.6 ± 26.3	83[a]	51[a]	61[a]
	Nonconsecutive	4	34–71	55.3 ± 15.6	245	123	50
	Consecutive	4	44–73	58.2 ± 14.2	169	137	81
	Across regimens	13	25–77	54.8[b]	414	260	63
500	Single	5	25–77	48.4 ± 23.8	80[a]	54[a]	68[a]
	Nonconsecutive	4	34–59	52.1 ± 12.3	242	142	59
	Consecutive	4	31–55	43.7 ± 10.4	158	138	87
	Across regimens	13	25–77	48.1[b]	400	280	70
500/200	Single	5	22–66	42.7 ± 22.8	72[a]	28[a]	39[a]
	Nonconsecutive	4	46–65	54.2 ± 9.5	236	77	33
	Consecutive	4	27–55	40.4 ± 13.5	152	87	57
	Across regimens	13	22–66	42.7[b]	391	164	42

[a] Contributes to total after nonconsecutive dosing regimen.
[b] Mean values are weighted.

μg/L AMP requirement was eliminated (data not shown) and led to earlier detection of MAMP exposure [0–17.2 (7.7 ± 4.8) h or 0–10 (3.8) voids]. Tables 6 (10-mg dose) and 7 (20-mg dose) list the final detection times from the last dose by regimen. Across doses, final detection times were 25–77 (48.4 ± 23.8) h, 31–92 (55.0 ± 18.7) h, and 34–70 (61.2 ± 6.0) h after single, consecutive, and nonconsecutive administrations, respectively. Individual 12-h detection rates across doses (Tables 4 and 5) were 58–100% (84.0% ± 14.0%). With elimination of the 200 μg/L AMP requirement, the total 12-h detection rate across doses increased from 16% to 79%. In addition, the total number of positive specimens collected from initial drug administration to last positive across doses and regimens

Table 7. Final detection times and total detection rates after administration of 20 mg of MAMP · HCl.

Cutoff	Regimen	Volunteers, n	Final detection time from last dose, h		Specimens from initial dosing to last positive, n		Total detection rate, %
			Range	Mean ± SD	Total	Positive	
LOQ	Nonconsecutive	1	169	169	42	40	95
	Consecutive	4	94–137	99.5 ± 27.3	209	200	96
	Across regimens		94–169	113.4[a]	251	240	96
250	Nonconsecutive	1	94.5	94.5	31	29	94
	Consecutive	4	56–96	77.3 ± 18.8	192	165	86
	Across regimens		56–96	80.7[a]	223	194	87
250/100	Nonconsecutive	1	94.5	94.5	31	29	94
	Consecutive	4	56–96	77.3 ± 18.8	186	142	76
	Across regimens	5	56–96	80.7[a]	217	171	79
500	Nonconsecutive	1	70	70	28	25	89
	Consecutive	4	46–92	66.2 ± 19.2	172	143	83
	Across regimens	5	46–92	67.0[a]	200	168	84
500/200	Nonconsecutive	1	70	70	28	25	89
	Consecutive	4	46–92	66.2 ± 19.2	172	103	60
	Across regimens	5	46–92	67.0[a]	200	128	64

[a] Mean values are weighted.

Table 8. Final detection times and total detection rates across MAMP doses and administration regimens.

	Final detection time from last dose, h		Specimens from initial dosing to last positive, n		Total detection rate, %
Cutoff	Range	Mean[a]	Total	Positive	
LOQ	46–169	110.7	775	725	94
250	31–96	65.2	648	536	83
250/100	25–96	64.1	631	431	68
500	25–92	54.8	600	448	75
500/200	22–92	51.7	591	292	49

[a] Mean values are weighted.

increased from 292 to 448, and the total detection rate also increased, from 49% to 75% (Table 8), without the AMP requirement.

DETECTION TIMES BASED ON 250/100 CUTOFF CONCENTRATIONS

Initial detection based on the 250/100 cutoff (Tables 2 and 3) occurred in the 1st to 12th (mean=3.7) post-drug administration specimens, collected at 1–17 (7.5 ± 3.0) h. Reducing the cutoff from 500/200 to 250/100 shifted initial confirmation to the left by up to five voids or 11 h. Lowering the AMP cutoff requirement was responsible for earlier MAMP detection in all cases. Individual 12-h detection rates (Tables 4 and 5) ranged from 0% to 100% (44.1% ± 38.4%); the total 12-h detection rate was 28%. Final detection time from last drug administration was 25–77 (mean, 51.6) h, 44–96 (67.8) h, and 34–95 (70.5) h after single, consecutive, and nonconsecutive administrations, respectively (Tables 6 and 7). Low MAMP and AMP concentrations were equally likely to limit final detection. In 9 of 18 sessions, the specimen collected immediately after the last positive specimen had a MAMP concentration <250 μg/L with an AMP concentration >100 μg/L. When we compared the 500/200 and the 250/100 cutoffs, the number of positive specimens from initial drug administration to last positive specimen for eight participants across doses and regimens increased from 292 to 431 and the total detection rate increased from 49% to 68% (Table 8).

Elimination of the 100 μg/L AMP cutoff requirement (250 μg/L MAMP cutoff) shifted initial MAMP detection by up to seven voids (Tables 2 and 3), occurring in voids 1–4 (mean, 1.5), collected 0.5–11.3 (mean ± SD, 3.9 ± 2.7) h after drug administration. In three instances, final detection time also increased by 6–10 h. The mean individual 12-h detection rate was 91% (range, 67–100%); the total 12-h detection rate was 88%. When we compared the 500/200 and 250 cutoffs across doses and participants, the total number of positive specimens collected from initial drug administration to last positive increased from 292 to 536 and the total detection rate increased from 49% to 83% (Table 8).

Discussion

MAMP is abused for its euphoric and performance effects. Reports indicate that truck drivers, construction workers, young professionals, and students commonly self-administer single doses of MAMP to improve performance and to prevent or reverse somnolence *(32)*. However, even low single doses (0.3 mg/kg) of MAMP can lead to physiologic and behavioral changes *(33)*. Rapid development of tolerance to the "feel good" and "high" drug effects after subchronic administration of low to moderate doses has been demonstrated in humans *(11, 22, 34)* and nonhuman primates *(12, 13)*. Identification of both habitual and occasional abusers is an important component of medical, workplace, criminal justice, military, and forensic drug testing programs. Therefore, it is important to describe the urinary excretion of MAMP and AMP after controlled administration of single and multiple doses of MAMP and to evaluate the effects of cutoff changes on detection times and rates after these regimens. Results from these studies should help determine interindividual differences in metabolism and excretion of MAMP. Compilation of MAMP excretion data after controlled administration will also provide a scientific database for improved interpretation of urine drug testing and will aid in the development of drug testing policy.

MAMP is abused via the smoked, intravenous, and oral routes of administration. The hydrochloride salt is highly water soluble, and unlike cocaine, which must be converted from its salt to base form before smoking, MAMP · HCl can be smoked, injected, or administered orally. AMP is a primary metabolite of MAMP in humans *(35)* as well as a commonly abused stimulant. It is also a pyrolytic product formed when MAMP is smoked *(36–38)*. MAMP · HCl is more prone to pyrolytic N-demethylation than is MAMP base because of its protonated nitrogen and nucleophilic chlorine *(36)*.

This study was part of a larger study on the pharmacokinetics and disposition of MAMP in various biological matrices. Because MAMP and AMP concentrations in many of these matrices were expected to be low, we used PCI-MS to achieve the required sensitivities. However, the impact of confirmation cutoffs on the interpretation of data would be the same with any mass spectrometric method for which adequate sensitivity and specificity have been demonstrated.

In a report by Wu et al. *(39)*, 83 urine specimens that had screened positive for MAMP by immunoassay were analyzed by GC/ion-trap MS to determine the effect of the 200 μg/L AMP cutoff requirement on MAMP confirmation testing. The authors did not define the ionization technique used. The limits of detection and quantification of their assay were 50 and 100 μg/L, respectively. They found that 12 specimens contained AMP alone and 52 met the 500/200 cutoff requirements. However, two of the specimens, with MAMP concentrations of 1621 and 2169 μg/L, had AMP concentrations <200 μg/L, and 17 of the

specimens, with MAMP concentrations of 714-5538 μg/L, contained no detectable AMP.

The initial 12-h urinary excretion profile of MAMP in naive humans has been reported after two single oral administrations of 30 mg/70 kg MAMP · HCl separated by 7 days to 10 volunteers *(40)*. Using a LOQ of 27.5 μg/L, the authors reported that the time to the MAMP c_{max} was 4–6 h. Approximately 20% of a dose was recovered in the urine as MAMP and 2% as AMP. AMP concentrations plateaued by 4–6 h, and contrary to reports after AMP administration *(25)*, changes in urinary pH had no effect on urinary excretion of the metabolite after this dose. In the initial 12 h after dosing, all study participants excreted ~2% of the dose as AMP, leading the authors to conclude that N-demethylation of MAMP to AMP and/or the renal excretion of AMP were saturable processes. When specimens collected up to 12 h post drug administration were analyzed based on current SAMHSA cutoff concentrations, 90% of specimens with MAMP concentrations ≥500 μg/L failed to confirm because of AMP concentrations below the SAMHSA required cutoff (200 μg/L). The authors postulated that saturation of AMP excretion mechanisms led to low early urine AMP concentrations that were, in turn, responsible for postponing initial detection times and reducing 12-h detection rates. Low concentrations of MAMP were detected in some specimens collected just before the second drug administration, indicating that MAMP excretion could extend to 7 days after this dose.

The urinary excretion pharmacokinetics of MAMP have also been determined and compared after administration of single smoked and intravenous doses to six male volunteers who were experienced MAMP users *(22)*. For two volunteers, there was an initial lag time of 0.8 and 1.8 h for MAMP disposition in urine. Mean peak urine concentrations occurred at 5–20 h (determined at the midpoint of each specimen collection interval) after both smoking and intravenous administration. Over a 72-h collection period, 37–45% of a dose was excreted as MAMP and ~7% was excreted as AMP. Initial AMP-MAMP ratios were <5%, and there was a multiphasic increase in the ratio of AMP to MAMP after both routes of administration, with AMP-MAMP ratios increasing to ~10% by 10 h. Both analytes were still detectable at 60 h. The authors postulated that after a single drug administration to a single participant, a low ratio might be indicative of recent MAMP exposure. However, they recognized that this relationship might not stand for multiple-administration regimens given to a population. These data were also compared with previous urine data collected after controlled subchronic oral MAMP administration. Single doses of oral MAMP-d_3 were administered to eight human volunteers before and after 13 consecutive daily oral administrations of 0.125 or 0.25 mg/kg sustained-release MAMP *(23)*. Specimens were collected after both MAMP-d_3 doses, and data were compared to determine the effect of subchronic MAMP administration on MAMP pharmacokinetics. Urinary excretion kinetics did not appear to change after subchronic dosing. Excretion rates for MAMP (in mg/h) were highest 3–6 h after administration, consistent with the time to peak plasma concentrations. Urinary MAMP concentrations peaked in the first 3 h and remained increased through 12–24 h. AMP-d_3 excretion rates were greatest 6–24 h after administration, and urine concentrations peaked during the same time period. All specimens collected <72 h after drug administration were positive for MAMP-d_3 and AMP-d_3 at concentrations above the assay LOQ (2.3 μg/L). The percentage of the dose excreted as parent drug was 56–66% greater for the low dose compared with the high dose. Analysis of covariance showed that renal clearance of MAMP-d_3 was dependent on urine pH, renal flow, and dose. In both studies, the fraction of bioavailable MAMP excreted in urine decreased with increasing dose.

In the present study, comparison of peak concentrations, total MAMP and AMP excreted, and detection times demonstrated that there was no significant intra- or intersubject dose–urine concentration relationship. Additionally, although there was a trend toward higher MAMP urine concentrations after multiple drug administrations, no administration regimen–concentration relationship could be demonstrated for MAMP or AMP. MAMP reached peak or near-peak concentrations in the first or second void, but peak concentrations of AMP generally occurred later. Consequently, low AMP concentrations were the main cause of low 12-h detection rates. AMP-MAMP ratios were also initially low, but slowly increased over time. In approximately one-half of the dosing sessions, AMP concentrations exceeded MAMP concentrations in specimens collected late in the excretion phase. For every participant, AMP-MAMP ratios did increase over time, supporting the proposal of Cook et al. *(22)* that low AMP-MAMP ratios may be indicative of recent MAMP exposure after controlled single doses. However, in the present study, high intra- and intersubject variability in initial and final AMP-MAMP ratios precluded use of a single AMP-MAMP ratio as a measure of chronologic exposure across a population or even within an individual, as also suggested by Cook et al. These data also demonstrate that parent drug-metabolite kinetics become more complicated when a drug is readministered before the total elimination of parent and metabolites, as was the case after both consecutive and nonconsecutive drug administrations. Given the variability in dose and administration regimen of MAMP abusers and the different routes of drug administration, establishing a correlation between analyte ratios and time of MAMP exposure would, therefore, be difficult to make from a single urine AMP-MAMP ratio.

Application of the current 500/200 SAMHSA cutoffs to these urine excretion data produced a low total 12-h detection rate (16%), similar to that reported by Valentine et al. *(40)* after single oral administrations (10%). In both

studies, low 12-h detection rates were attributable almost entirely to failure of specimens to meet the AMP requirement. A dramatic example from the current study are the data from volunteer S after initial administration of the high dose, in which four of the first seven specimens collected (2–22 h post drug administration), with MAMP concentrations ranging from 566 to 2422 μg/L, had AMP concentrations <100 μg/L. Additionally, across all participants, only 49% of the specimens collected from initial dosing to last positive specimen could be confirmed by use of current cutoffs.

In the present study, there were relatively large decreases in initial detection times and increases in 12-h detection rates when the AMP requirement was eliminated. When we used the 500 μg/L MAMP requirement only, MAMP was initially detected an average of four voids or ~9 h earlier; 12-h detection rates also increased from 16% to 79%. Final detection times were only minimally extended when AMP requirements were eliminated, but total detection rates across the collection period increased from 49% to 75%. Across participants, the total detection window increased, and the number of positive specimens collected through terminal excretion also increased by 53% (from 292 to 448 specimens).

Reduction of cutoffs from the current 500/200 cutoff to the proposed 250/100 cutoff also reduced initial detection times and improved both 12-h and total detection rates. Mean initial detection time shifted by 1.9 voids or 4.9 h; the mean 12-h detection rate increased by 132%, from 19% to 44%; and 12-h total detection rate increased by 75%, from 16% to 28%. Mean final detection time increased from 52 to 64 h. The total number of true-positive specimens from initial drug administration to last positive specimen increased by 48%. However, improvements in times and rates, especially early in elimination, were not as marked compared with use of a 500 μg/L MAMP requirement alone.

The earliest and most sensitive detection was noted with a 250 μg/L MAMP requirement alone. Initial detection times occurred in the first to fourth voids. Detection rates across participants were comparable to rates produced by the 2.5 μg/L LOQ cutoff. The mean and total 12-h detection rates improved by 379% (from 19% to 91%) and 450% (from 16% to 88%), respectively, compared with 500/200 detection rates. The mean final detection time increased by ~16 h, and the total number of specimens positive from initial dosing to last positive specimen increased by 84% compared with the 500/200 cutoff.

In conclusion, lowering cutoff requirements from 500/200 to 250/100 will substantially improve detection rates and increase both initial and final detection times for determining MAMP exposure. Generally, reduction in the MAMP requirement extended both initial and final detection times and increased detection rates. Reduction in the AMP requirement had a more pronounced effect on detection times and rates for specimens collected later than 12 h after initial drug administration. However, MAMP confirmation within the first 12 h of administration will be limited whenever confirmation of AMP is required. In this study, elimination of AMP requirements produced dramatic improvements in initial detection times and early detection rates. These data indicate that use of a lower 250 μg/L MAMP cutoff with no AMP requirement would substantially increase detection rates and lengthen detection windows provided that artifactual MAMP production and misidentification in the presence of over-the-counter amphetamine analogs is eliminated with adequately validated confirmation methods.

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