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3,4-Methylenedioxymethamphetamine (MDMA) and Metabolites Disposition in Blood and Plasma Following Controlled Oral Administration

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Abstract

3,4-Methylenedioxymethamphetamine (MDMA) is an illicit phenethylamine ingested for entactogenic and euphoric effects. Although blood is more commonly submitted for forensic analysis, previous human MDMA pharmacokinetics research focused on plasma data; no direct blood-plasma comparisons were drawn. Blood and plasma specimens from 50 healthy adult volunteers (33 males, 17 females, 36 African-American) who ingested recreational 1.0 mg/kg and 1.6 mg/kg MDMA doses were quantified for MDMA and metabolites 4-hydroxy-3-methoxymethamphetamine (HMMA), 3,4-methylenedioxyamphetamine (MDA), and 4-hydroxy-3-methoxyamphetamine (HMA) by two-dimensional gas chromatography-mass spectrometry (2D–GCMS). Specimens were collected up to 3 h post-dose and evaluated for maximum concentration (C_{max}), first detection time (t_{first}), time of C_{max} (t_{max}), and 3-h area under the curve ($AUC_{0–3h}$); as well as blood metabolite ratios and blood/plasma ratios. Median blood MDMA and MDA C_{max} were significantly greater ($p<0.0005$) than in plasma, but HMMA was significantly less ($p<0.0005$). HMA was detected in few blood specimens, at low concentrations. Nonlinear pharmacokinetics were not observed for MDMA or MDA in this absorptive phase; but HMMA C_{max} and $AUC_{0–3h}$ were similar for both doses despite the 1.6-fold dose difference. Blood MDA/MDMA and MDA/HMMA significantly increased ($p<0.0001$) over the 3-h time course, and HMMA/MDMA significantly decreased ($p<0.0001$). Blood MDMA C_{max} was significantly greater in females ($p=0.010$) after the low dose only. Low-dose HMMA $AUC_{0–3h}$ was significantly decreased in females' blood and plasma ($p=0.027$), and in African-Americans' plasma ($p=0.035$).

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These data provide valuable insight into MDMA blood-plasma relationships for forensic interpretation, and evidence of sex- and race-based differential metabolism and risk profiles.

Keywords

MDMA; ecstasy; pharmacokinetics; metabolites; blood; plasma

INTRODUCTION

3,4-Methylenedioxymethamphetamine (MDMA, ecstasy) is an illicit amphetamine derivative ingested orally for its entactogenic, stimulant, and hallucinogenic properties [1–5]. In 2010, 0.9 million people took MDMA for the first time in the United States [6]; worldwide, 10–28 million people ages 15–64 have taken the drug. MDMA was found in 0.09% of nighttime drivers in the 2007 US National Roadside Survey [5].

There are two primary Phase I MDMA metabolic pathways. The first is O-demethylenation (mainly mediated by CYP2D6) to 3,4-dihydroxymethamphetamine (HHMA), followed by O-methylation to 4-hydroxy-3-methoxymethamphetamine (HMMA). The second is N-demethylation (CYP1A2) to 3,4-methylenedioxyamphetamine (MDA), followed by O-demethylenation to intermediate 3,4-dihydroxyamphetamine (HHA) and O-methylation to 4-hydroxy-3-methoxyamphetamine (HMA) [7–8].

Administered at recreational doses in human experimental laboratory studies, MDMA increases heart rate (HR) and blood pressure (BP), subjective energy level, closeness to others, and “high,” and causes perceptual changes [9–10]. Subjective effects peak 1–2 h post-administration [9,11]. As a stimulant, some driving performance aspects may improve, but others (e.g., speed adaptation ability) may be impaired [12]. Although blood is a forensically relevant matrix for MDMA and metabolites evaluation, previous clinical and preclinical studies primarily focused on plasma [10,13–14,7,15,11].

We developed a sensitive two-dimensional gas chromatography-mass spectrometry (2D-GCMS) method with cryotrapping for simultaneously quantifying MDMA, HMMA, MDA, HMA, and 3,4-methylenedioxyethylamphetamine (MDEA) in whole blood. We employed this 2D–GCMS method for studying MDMA and metabolite whole blood pharmacokinetics, and correlated MDMA and metabolites blood and plasma concentrations for the first time after controlled MDMA administration. These data will be useful for forensic toxicologists interpreting MDMA blood test results.

MATERIALS AND METHODS

Reagents

MDMA-d_0, MDA-d_0, MDEA-d_0, MDMA-d_5, MDA-d_5, and MDEA-d_6 were purchased from Cerilliant Corp. (Round Rock, TX), racemic HMA-d_0 and HMMA-d_0 from Lipomed, Inc. (Cambridge, MA), and *p*-hydroxymethamphetamine (pholedrine) from Sigma (St. Louis, MO). Isopropanol, sodium acetate and ethyl acetate were acquired from Sigma; GC-grade *n*-heptane, dibasic potassium phosphate, acetic acid, and HPLC-grade methanol were

from Fisher Scientific (Fair Lawn, NJ). Hydrochloric acid, sodium hydroxide, ammonium hydroxide, and monobasic potassium phosphate were obtained from JT Baker (Phillipsburg, NJ), triethylamine (>99.5% purity) from Thermo Scientific (Rockford, IL), and heptafluorobutyric acid anhydride (HFAA) from Regis Technologies, Inc. (Morton Grove, IL). Styre Screen™ 6 mL 50 mg DBX solid-phase extraction (SPE) columns were purchased from United Chemical Technologies (Bristol, PA). Blank human plasma and blood were obtained from the National Institutes of Health blood bank.

Participants

Fifty healthy adult participants provided written informed consent for this National Institute on Drug Abuse (NIDA) Intramural Research Program (IRP) Institutional Review Board-approved research (Table 1). Participants underwent comprehensive medical and psychological examinations, including medical and drug histories, as previously described [16]. Eligibility requirements included intake of ≥5 lifetime MDMA tablets (self-report, ≥1 tablet within the past month), ages 18–40 years, and medically acceptable birth control or abstinence (females) throughout the study. Serum pregnancy tests were administered at screening, and urine pregnancy tests preceded each dose administered to females. Exclusion criteria included nursing or pregnant; current medical condition or history of neurologic illness; axis I psychiatric diagnosis other than nicotine, cannabis, or MDMA abuse or dependence; ingestion of a CYP2D6 or CYP3A4 inhibitor or CYP3A4 inducer in the 30 days prior to MDMA administration; abnormal cardiovascular parameters; and serum transaminase greater than three times normal.

Study Procedures

Participants resided on a secure clinical research unit up to 23 days (continuous stay encompassing all dosing sessions, or separate stays separated by ≥1 week). Admission to the residential unit was ≥12 h prior to MDMA dosing. Participants ingested placebo (0 mg/kg), low (1.0 mg/kg), or high (1.6 mg/kg) dose MDMA (Lipomed, Arlesheim, Switzerland) in a randomized, counterbalanced, and double-blind manner. Active drug was administered as the hydrochloride salt; placebo contained lactose. For safety, a maximum 150-mg dose limit was enforced in six participants whose weight exceeded 93.75 kg.

Blood Collection

Blood was collected in sodium heparin (green-top) vacutainers at −0.25, 0.5, 1.0, 1.5, 2.0, 2.5, and 3 h after dosing, and placed on ice immediately after collection. Plasma was collected after centrifuging blood at 2000g, 4°C for 10 min within 2 h after collection. Blood was collected for only 3 h due to a 450 mL maximum total blood collection limit per participant as required by the ethical committee. Extended plasma pharmacokinetics (up to 143 h) from this study were previously published [7]. Blood and plasma were stored frozen (−20°C) until analysis.

Plasma Analysis

MDMA, MDA, HMMA, HMA, and MDEA concentrations were quantified with a validated, modified 2D–GCMS method [17]. Briefly, 1 mL plasma was fortified with internal standard

(MDMA-d5, MDA-d5, and pholedrine) and hydrolyzed with 1 mL 0.5 mol/L HCl at 100°C for 40 min. After cooling, 1 mL 0.1 mol/L phosphate buffer (pH 6.0) and 50 μL 10 mol/L sodium hydroxide were added. After centrifugation, supernatant was decanted onto preconditioned polymeric Styre Screen DBX SPE columns. Columns were washed, dried, and extracts eluted with ethyl acetate:isopropanol:ammonium hydroxide (90:6:4, v/v/v). Methanolic HCl (15 μL, 0.12 mol/L) was added prior to evaporation. Residues were reconstituted with 100 μL 0.1 mol/L triethylamine in heptane, and incubated with 10 μL HFAA (derivatizing reagent) at 60°C for 30 min. Phosphate buffer (200 μL, pH 7.4) was added to cooled samples and the aqueous phase discarded. Samples were injected onto an Agilent 6890 GC-5973 mass selective detector instrument equipped with microfluidic Deans switch and flame ionization detector. Instrument parameters were previously reported by Kolbrich et al [17]. Linear ranges were 2.5–400 μg/L (MDMA and HMMA), 2.5–100 μg/L (HMA), and 1–100 μg/L (MDA). Extraction efficiencies were ≥85%, inter- and intra-assay imprecision coefficients of variation (N=20) were ≤6.7%, and analytical bias was between 14.4–7.2% of target.

Blood Analysis

MDMA, MDA, HMMA, HMA, and MDEA were quantified in blood by the plasma method described above with minor modifications. Extraction was performed with 1 mol/L pH 4.5 acetate buffer and reconstitution in 0.2 mol/L triethylamine in heptane.

Method validation was performed to reflect guidelines recently established by the Scientific Working Group for Forensic Toxicology (SWGTOX) [18], and included determining dynamic linear ranges for all compounds, limits of detection (LOD) and quantification (LOQ), recoveries, accuracy and imprecision, endogenous and exogenous interference studies, carryover, stability, and dilution integrity. Parameters were determined with low, medium, and high quality control (QC) samples (Table 2). Detailed validation procedures and results are presented in Online Resource 1. Supplemental Table 1 (within Online Resource 1) provides specific calibration concentrations for each analyte.

Data Analysis

Statistical analyses were performed with IBM® SPSS® Statistics 19.0.0 for Windows. Data displayed non-normal distribution by visual inspection and Kolmogorov-Smirnov tests; thus, nonparametric analyses were utilized. Observed time of first detection (t_{first}), maximum concentration (C_{max}), time of maximum concentration (t_{max}), and 3-h area under the curve ($AUC_{0–3h}$, calculated by linear trapezoidal method) were compared with the Wilcoxon signed-ranks test. Between-group sex and race comparisons were performed with the Mann-Whitney *U* test. Because only two participants identified as unknown/Hispanic and/or mixed-race/ethnicity (insufficient for additional categories), these individuals were included in a group with white participants. To avoid ambiguity, cases where no analyte was detected were excluded from t_{first} and t_{max} calculations. Blood/plasma ratios were calculated only when analytes were quantifiable in both matrices. Low and high dose blood/plasma ratios, overall and by time, were compared with the Wilcoxon signed-ranks test. Analyte ratios (MDA/MDMA, HMMA/MDMA, and MDA/HMMA) also were evaluated with Wilcoxon signed-ranks test, and temporal differences were evaluated by independent samples median

test. Linear least-squares plasma vs. blood concentration (MDMA, MDA, HMMA) regressions were evaluated in GraphPad Prism 5.02 (GraphPad Software Inc.).

RESULTS

Participants

Fifty volunteers (33 males, 17 females) participated; 41 completed all three sessions. Demographic and amphetamines use history are presented in Table 1.

Blood Method

The optimized blood MDMA and metabolites assay was achieved by modifying the plasma method to optimize the pH for extraction and triethylamine concentration for derivatization. Extraction at pH (6.0) utilized for plasma analyses revealed poor HMA extraction efficiency with non-detectable HMA at 3.0 μg/L; >25.2% HMA extraction efficiency was achieved with pH 4.5 buffer [19]. Validation data are presented in Table 2 and Online Resource 1.

Blood and Plasma Specimens

Blood (144 after placebo, 321 after low, and 297 after high dose MDMA) and plasma (145 after placebo, 321 after low, and 300 after high dose MDMA) specimens were analyzed for MDMA, HMMA, MDA, and HMA. There were 757 (144 placebo, 316 low, 297 high) concurrently collected blood and plasma specimens. Specimens were analyzed until there were three consecutive negative specimens, resulting in fewer total placebo analyses. Rigorous plasma pharmacokinetic analysis (17 participants, up to 143 h post-dose) was previously published [7]. Blood was collected up to 3 h post-dose. Fig. 1 depicts blood time courses for MDMA and metabolites. Metabolite HMMA concentrations were similar after the two doses, but MDMA and MDA concentrations were dose-dependent. HMA was only detected in 4 blood specimens.

MDMA and MDA

Median blood MDMA and MDA C_{max} were significantly greater than in plasma ($p<0.0005$, Table 3). Overall median MDMA blood and plasma C_{max} achieved after the high dose were 1.7 and 1.6 times those after the low dose, reflecting the 1.6-fold difference in MDMA administered. Median MDA 3 h observed C_{max} in both blood and plasma also were 1.6-fold higher after the high dose. Median t_{max} occurred at 2.5 h for MDMA and 3.0 h for MDA, consistent with the demethylation process requiring additional time to peak. Similarly, t_{first} was non-significantly earlier (0.5 h) for the parent compound than the metabolite (1.0 h); and MDA after the low, but not high, dose revealed a significantly earlier t_{first} ($p=0.002$) in blood than plasma. Median MDMA $AUC_{0–3h}$ were 1.7 and 1.6-fold higher in blood and plasma after the high than low dose, respectively, consistent with C_{max}. Median MDA $AUC_{0–3h}$ high v. low dose ratios were lower (1.5 and 1.4 for blood and plasma, respectively).

Median MDMA blood C_{max} was significantly greater in females than males ($p=0.010$) after the low dose only. Plasma C_{max} and $AUC_{0–3h}$ did not differ significantly by sex for MDMA

or MDA; nor did blood MDA C_{max} or $AUC_{0–3h}$. No racial differences in MDMA or MDA C_{max} or $AUC_{0–3h}$ were observed.

Two participants (16 and 24) had consistent and quantifiable MDMA from baseline throughout the placebo session (Online Resource 2). Self-reported last-consumed MDMA was 2 days (16) and 16–20 h (24) prior to first specimen collection. MDA was detected in both individuals' plasma, and in blood for participant 16 only. Participant 11 chewed and expectorated the high-dose capsule. No MDMA, MDA, or HMA was detected in his blood or plasma. HMMA was still positive from a previous dose; and concentrations remained within ±16% and ±9% of baseline for blood and plasma, respectively. This session was excluded from t_{first}, C_{max}, and t_{max} calculations.

HMMA and HMA

Blood HMMA C_{max} was significantly less than in plasma ($p<0.0005$) at both MDMA doses (Table 3). HMMA concentrations were not linear with dose, achieving median high-dose C_{max} and $AUC_{0–3h}$ 1.0 and 1.1 times those of the low dose (Table 3, Fig. 1a). T_{max} and t_{first} did not differ between doses or matrices. Few specimens were positive for HMA (blood, n=4/618; plasma, n=41/621). HMA only slightly exceeded the 2.5 μg/L LOQ when positive (C_{max} 3.1, 2.6 μg/L [low, high blood]; 3.2, 3.5 μg/L [low, high plasma]). The trend ($p=0.070$) for slightly lower HMA concentrations in blood vs. plasma after the low dose became significant ($p=0.012$) at the high dose. There were too few positive HMA specimens to statistically compare doses or matrices for t_{first} or t_{max}.

The only significant sex difference observed was with HMMA concentrations after the low MDMA dose, which showed lower $AUC_{0–3h}$ in females than males ($p=0.027$, blood and plasma). Also for the low dose only, plasma HMMA $AUC_{0–3h}$ was significantly lower ($p=0.035$) in African-American participants. No blood matrix or high-dose racial differences were observed.

MDEA

The MDMA capsules administered in the study did not contain MDEA, and no MDEA was detected in blood or plasma.

Blood/Plasma Ratios

Median [range] blood/plasma ratios for paired low and high-dose MDMA (N=253 and 236), HMMA (N=267 and 250), and MDA (N=212 and 199) were 1.17 [0.42–1.89] and 1.12 [0.86–2.33]; 0.75 [0.45–1.12] and 0.74 [0.03–1.44]; and 1.49 [0.63–4.83] and 1.63 [0.60–5.43], respectively. At most time points, blood/plasma ratios did not vary significantly by dose (Fig. 2). In this primarily-absorptive phase, only MDA displayed a significantly greater overall blood/plasma ratio after the high than the low dose ($p<0.0005$). Blood MDMA and MDA concentrations were always greater than plasma (ratio >1); HMMA blood concentrations were always less than those of plasma. Despite substantial inter-subject variability, ratios were relatively consistent over time, particularly MDMA. Linear least-square regression equations for blood-plasma concentration relationships were y=0.9287x-5.280 (MDMA), y=0.5214x+0.7891/y=0.6570x-0.1834 (MDA low/high), and

y=1.342x+3.094 (HMMA), with respective R^2=0.9512, 0.5889/0.7409, and 0.8964 (Online Resource 3). All correlations were significant (p<0.0001). There was only one paired HMA specimen (high dose, t=3 h, blood/plasma=0.72).

Analyte Ratios

Blood MDA/MDMA (Fig. 3a) and MDA/HMMA (Fig. 3c) ratios significantly increased (p<0.0001) over the 3-h time course; whereas HMMA/MDMA ratios significantly decreased overall (p<0.0001), but were relatively stable after the first h post-dose (Fig. 3b). Low vs. high-dose time-course slopes were not significantly different for MDA/MDMA, MDA/HMMA, or HMMA/MDMA. Beginning at 1.0 h, HMMA/MDMA was significantly greater (p<0.005) after the low than the high dose, and after 1.5 h MDA/HMMA was significantly greater (p<0.005) after the high dose. MDA in blood was almost twenty-fold lower than blood MDMA. Overall MDA/MDMA, MDA/HMMA, and HMMA/MDMA ratios all displayed significant dose differences (p<0.0005), although mean low vs. high dose MDA/MDMA differed by only 8.5% (0.052 vs. 0.047). Thus, despite statistical significance, this difference was not clinically significant. Mean MDA/MDMA low vs. high dose was 26.7% greater (1.46 vs. 1.15), and mean MDA/HMMA low vs. high was 21.3% lower (0.088 vs. 0.111).

DISCUSSION

We established and validated a sensitive, specific 2D-GCMS method for simultaneously quantifying MDMA, MDA, HMMA, HMA, and MDEA in blood and utilized this method to analyze specimens after controlled MDMA administration. We believe this to be valuable analytically novel information in that it contains, to our knowledge, the first published whole blood method for simultaneous quantification of MDMA and its metabolites HMMA, MDA, and HMA, as well as MDEA [20,14,21–27]. MDMA and MDA are weak bases (pK_a approximately 9.9 [28] and 9.67 [29], respectively); as are probably HMA and HMMA, based on their amine groups. Thus, these analytes were more effectively protonated (ionized) at the lower extraction pH, allowing more consistent cation exchange SPE column functionality. Doubling the triethylamine concentration in the reconstitution solvent also improved HMA quantification. Extraction efficiencies from blood were substantially diminished compared to our previously-published plasma results [17]. We attempted to improve recovery by testing QC specimens extracted at pH 6 (n=3), and after protein-precipitation with ice-cold acetonitrile after hydrolysis (n=3); neither condition improved extraction efficiency. We were able to achieve desired sensitivity with acceptable analytical performance despite low analyte recoveries. The recent publication of the SWGTOX guidelines on method validation were followed. These new comprehensive guidelines demonstrate proper validation procedures in a concise and simplified manner. This information combining both analysis and interpretation of blood MDMA following controlled MDMA administration will benefit the field.

We present, for the first time, *in vivo* human blood vs. plasma relationships for MDMA and metabolites MDA, HMMA, and HMA. We also examined metabolite ratios in blood, and directly compared sex and racial differences in MDMA pharmacokinetics. This study was

limited by the 3 h post-dose blood collection window imposed by a total blood collection limit of 450 mL per participant over the entire study. This prevented extended blood pharmacokinetic analysis. In cases where t_{max} was reported as 3 h, the true t_{max} could have been later; C_{max} were observed 3 h data. Another limitation was long-term storage prior to analysis. Although stored frozen at −20°C in the dark, blood and some plasma specimens were not analyzed until 1–7 years after collection. MDMA and MDA were determined to be stable frozen ≥17 weeks in serum and 5 weeks in blood [30–31], but longer-term MDMA and metabolites stabilities are not fully characterized

To our knowledge, blood-plasma relationships were previously determined in only two *in vitro* studies [31–32]. MDMA and MDA concentrations were significantly ($p<0.0005$) higher in blood than plasma (Table 3, Fig. 3). Garrett et al [31] documented similar red blood cell (RBC)-plasma coefficients for MDMA and MDA (1.48 and 1.45, respectively), corroborating our findings. Values are higher than the blood/plasma ratios we determined, possibly because reported results were partition coefficients for separated RBCs vs. plasma rather than whole blood vs. plasma concentrations. Plasma-protein binding was reported to be 34–40% for both compounds. In contrast, Belhadj-Tahar et al [32] reported the (haematocrit-normalized) RBC vs. whole blood partition coefficient for MDA (MDMA not reported) as 30%, the free fraction 27%, and the plasma protein-bound fraction 71%.

We found significant dose-dependent differences in MDA blood/plasma ratios and concentration correlations (Fig. 2, Online Resource 3). Based on previous findings [31], which showed MDA and MDMA plasma-protein binding was not dose-dependent, the relative increase may relate more to RBC affinity than to plasma-protein binding. Taken together, these results suggest that higher concentrations may gradually exceed RBC binding capacity, allowing more free drug to partition into plasma. Correlation coefficients were relatively high for MDMA (0.9512) and HMMA (R^2=0.8964) and somewhat lower for MDA (>0.58). This suggests greater inter-subject variability in MDA blood-plasma partitioning than for MDMA and HMMA, possibly contributing to these findings.

To our knowledge, no HMMA or HMA partition coefficients were reported to date, but our results (blood-plasma ratio <1 and significantly lower blood C_{max}) indicate HMMA partitions more readily into plasma. The significant HMMA blood/plasma ratio dose difference observed at 1.5 h (Fig. 2) represented a minor fluctuation likely caused by inter-subject variability, and appears to lack clinical significance. HMA is a relatively minor metabolite, with concentrations never exceeding 3.5 μg/L during the 3-h time course, even after the high dose. The single paired-positive HMA result (0.72 blood/plasma ratio) agreed with the slightly lower C_{max} in blood, but is insufficient to draw conclusions.

The two participants positive for MDMA, HMMA, and MDA after the placebo capsule (Online Resource 2) self-reported ingesting an average 8 MDMA pills/month in the prior 3 months (Table 1). Our closed research unit prevented drug intake within 12 h prior to controlled MDMA/placebo administration, and these participants self-reported last MDMA ingestion >16 h prior to the placebo dose. Although blood and plasma concentrations observed here did not result from controlled administration and should be interpreted with caution, they offer blood information from a longer post-dose window. The data collected

from these individuals corroborates the MDMA and HMMA findings from controlled administration; blood concentrations were higher and lower than in plasma, respectively. MDA blood and plasma concentrations were near the LOQ throughout these sessions. Previous studies reported MDMA, HMMA, and MDA plasma $t_{1/2}$ 5.9–11.8 [10,7,11,33], 9.8–10.4 [7,33], and 9.3–17.7 h [10,7,33], respectively, with substantial $t_{1/2}$ inter-subject variability [7]. Farre et al [10] documented longer MDMA and MDA $t_{1/2}$ 24–48 h after MDMA administration than in the first 24 h (8.8 vs. 7.0 and 14.1 vs. 12.8 h, respectively), indicating a longer secondary distribution and excretion phase.

Blood MDA/MDMA ($p<0.0005$) and MDA/HMMA ($p<0.01$) ratios significantly increased after dosing, while HMMA/MDMA significantly decreased ($p<0.01$), reaching a plateau after approximately 1.5 h (Fig. 3).The statistically significant ($p=0.010$) low-high MDA/MDMA dose difference observed at 3 h was minor and not clinically significant. Although initially unexpected that HMMA/MDMA decreased during the 3 h post-dose, this agrees with previous plasma findings [7] showing greater initial ratios that decreased for the first few hours post-dose as MDMA increased. HMMA/MDMA gradually increased during the 10–40 h post-dose window. Previous studies showed MDMA inhibits its own metabolism [7,10–11] through CYP2D6 inhibition of the primary pathway to HMMA. This inhibition occurs within 2 h [11]. By 4 h after 1.5 mg/kg MDMA, CYP1A2 activity increased 20–40% [34]. We hypothesize this process likely explains the patterns observed. As the major HMMA metabolic pathway was inhibited, the MDA pathway increased, thereby increasing MDA ratios. For the same reason, HMMA/MDMA was greater—and MDA/HMMA less—in the low dose relative to the high. Greater MDMA doses more rapidly inhibited the 2D6 enzyme.

Prior research [7] indicated median plasma t_{max} >3 h for MDA (7.1 h [low and high]) and HMA (11.0 h [low] and 12.0 h [high]). Our blood results appear to emulate those findings, since C_{max} did not appear within the 3 h window. Similar observed $t_{max,}$ occurring prior to 3 h, suggests true MDMA and HMMA C_{max} are reported. $AUC_{0–3h}$ represents the MDMA absorption window, so our results matched the 1.6-fold dose difference. The nonlinear plasma pharmacokinetics we previously observed [7] appear to extend from inhibited metabolism and decreased clearance, causing lasting elevated concentrations that impacted the later time course. Our HMMA results for both matrices corroborate those previously described, showing equal C_{max} and slightly-elevated (1.1 high/low ratio) $AUC_{0–3h}$. The 1.3 previously-reported plasma AUC_{∞} ratio reflects longer-term metabolism inhibition. MDA patterns were similar, linearly reflecting the dose difference for this 3-h window despite showing greater differences in extended C_{max} and AUC_{∞}. HMA was detected in few blood specimens, corroborating a longer formation time. Extended plasma pharmacokinetics revealed 79% and 100% of 17 participants' plasma specimens were HMA-positive 23 h after the low and high dose, respectively [7].

Between-subjects sex and race C_{max} and $AUC_{0–3h}$ comparisons revealed significant differences after the low dose only, agreeing with our previous findings [7]. The greater blood MDMA C_{max} in females ($p=0.010$) did not significantly alter $AUC_{0–3h}$. We did not observe the previously-reported significantly increased female plasma MDA C_{max} or AUC; but our decreased female HMMA low-dose $AUC_{0–3h}$ in whole blood and plasma supports

previous plasma results. Females exhibit a stronger MDMA-induced CYP2D6 inhibition profile than males [35], possibly explaining decreased MDMA clearance after the lower dose only. We hypothesize the higher 1.6 mg/kg dose caused substantial enough enzyme inhibition that sex did not display the differential effect observed after the low dose.

Previously, we had too few participants to examine racial differences. In this study our sample was large enough that for the first time, we were able to directly compare and show a significant MDMA pharmacokinetic difference between African-American and white/Hispanic or mixed-race individuals. We observed a significantly lower ($p=0.035$) plasma HMMA $AUC_{0–3h}$ in African-American participants after the low dose, suggesting increased toxicity risk due to decreased metabolism. Although this was the only statistically-significant racial difference observed, it is possible that the diminished post-dose time course did not allow racial metabolic differences to fully manifest. As noted with sex differences, the observation that only the low dose showed a differential racial effect may indicate that the 1.6 mg/kg dose caused substantial enough enzyme inhibition that race did not display the differential effect observed after the low dose.

Genetic polymorphisms, particularly in the CYP2D6 enzyme, influence pharmacokinetic profiles of MDMA and its metabolites. A recent study by Pardo-Lozano et al [36] demonstrated that individuals carrying two functional alleles (FA) for CYP2D6 produce significantly higher HMMA plasma concentrations relative to carriers with one FA. It is likely that the patterns observed in plasma would be reflected in blood; i.e., higher blood HMMA concentrations (and possibly increased MDMA clearance) would be observed in cases of extensive metabolizers. However, we believe that MDMA inhibition of its own metabolism is a more important factor in explaining our findings, as also reported by Yubero-Lahoz et al [35]. We do not anticipate that this would alter blood vs. plasma findings.

In summary, we present for the first time blood MDMA and metabolites pharmacokinetics after controlled oral MDMA administration. The MDMA and metabolite blood/plasma correlation data presented here are highly relevant and, as far as we are aware, the first available in the literature. This is the first direct comparison between paired blood and plasma specimens, and the first evidence of racial differences in MDMA metabolism. The racial and sex differences we observed warrant further investigation in order to determine the extent of greater toxicity risk in females and African-Americans. These controlled MDMA administration data provide valuable information for interpretation of clinical and forensic data. This information fills a critical knowledge gap of MDMA and metabolites analysis and concentrations in blood; previous analytical procedures and results focused almost entirely on plasma. Our blood data and matrix comparisons will be beneficial for interpreting forensic cases, given the prior focus on plasma in the published literature.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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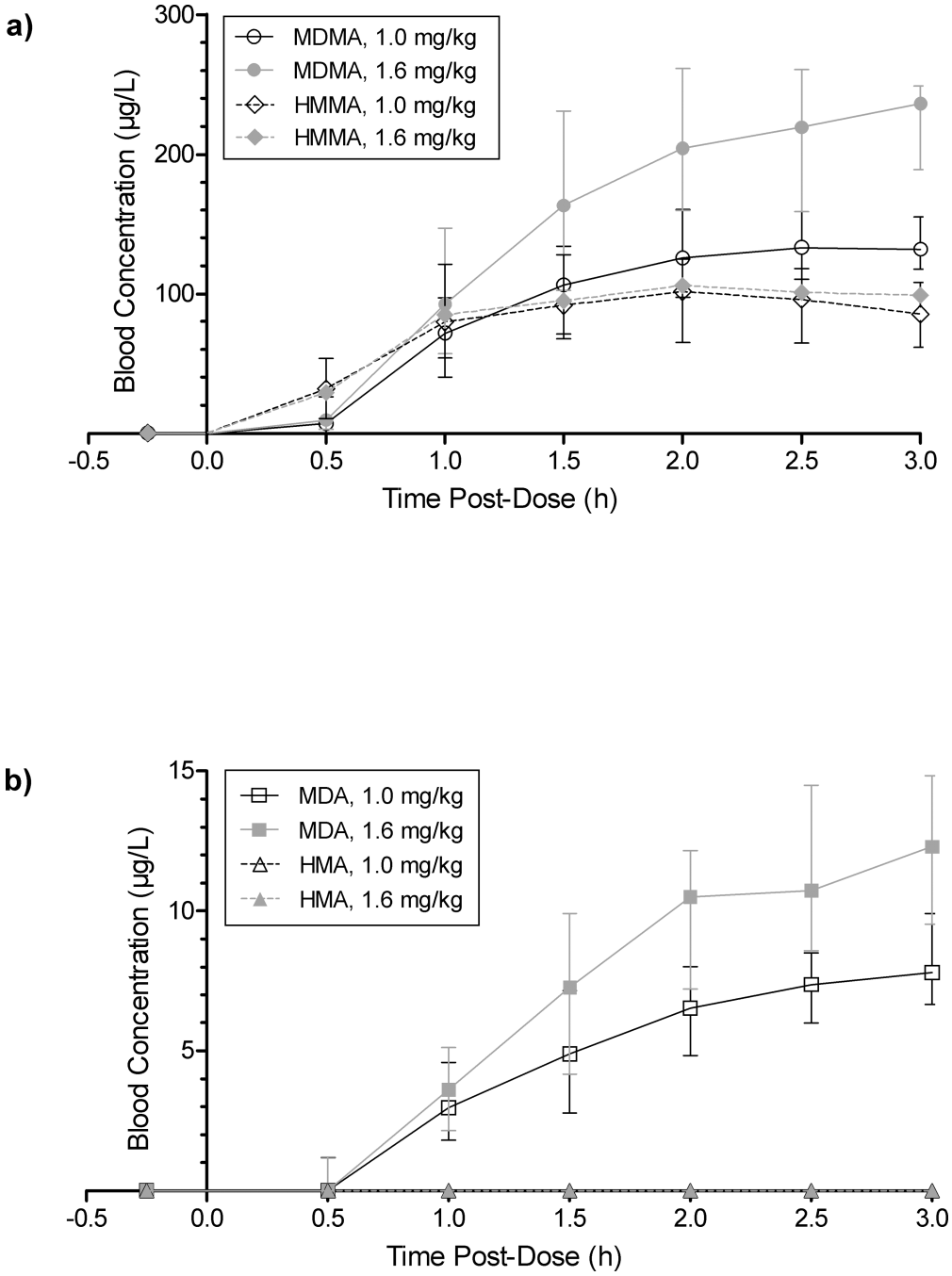

Fig. 1.

Median (interquartile range) a) 3,4-methylenedioxymethamphetamine (MDMA) and 4-hydroxy-3-methoxymethamphetamine (HMMA); and b) 3,4-methylenedioxyamphetamine (MDA) and 4-Hydroxy-3-methoxyamphetamine (HMA), in blood over 3 h after oral 1.0 and 1.6 mg/kg MDMA administration to adult users

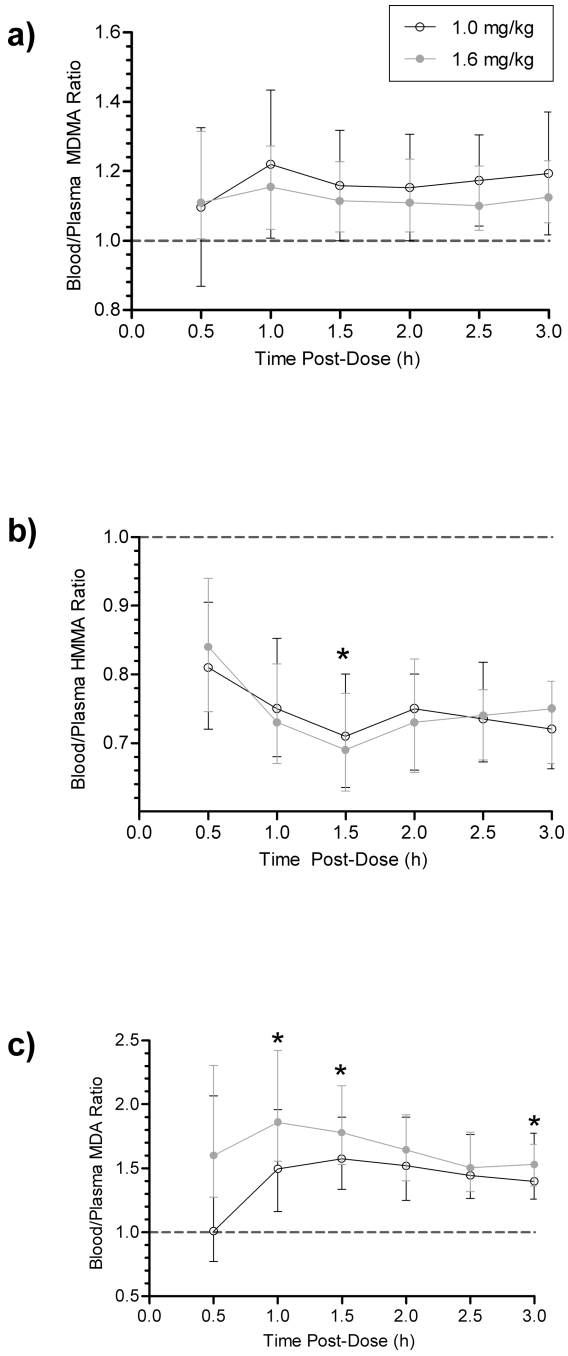

Fig. 2.

Median (interquartile range) blood/plasma 3,4-methylenedioxymethamphetamine (MDMA) (a), 4-hydroxy-3-methoxymethamphetamine (HMMA) (b), and 3,4-methylenedioxyamphetamine (MDA) (c) ratios for 3 h after controlled MDMA administration. Changes over time were significant after the 1.6 mg/kg dose for HMMA and MDA (p=0.013 and p=0.021), but not for MDMA. No changes over time were significant after the 1.0 mg/kg dose. Note: y-axes do not begin at 0. *Denotes p<0.05 (low vs. high)

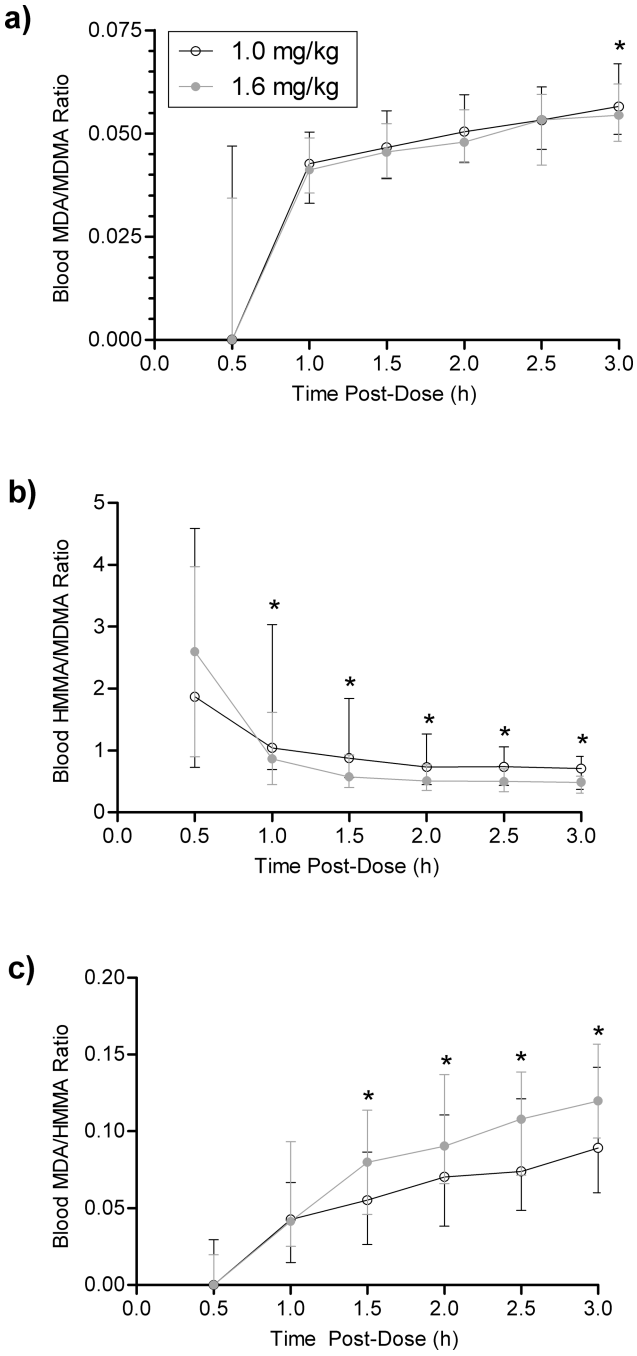

Fig. 3.

Median (interquartile range) blood MDA/MDMA (a), HMMA/MDMA (b), and MDA/MDMA (c) ratios over 3 h after controlled MDMA administration. *Denotes significant difference (low vs. high MDMA dose), $p<0.05$. Changes over time were significant ($p<0.0005$, $p<0.01$, and $p<0.01$ for MDA/MDMA, HMMA/MDMA, and MDA/HMMA, respectively). Abbreviations: MDA, 3,4-methylenedioxyamphetamine; MDMA, 3,4-methylenedioxymethamphetamine; HMMA, 4-hydroxy-3-methoxymethamphetamine

Table 1

Participant demographic data and self-reported current MDMA administration.

Participant	Race & Ethnicity	Sex	Age	BMI	Self-reported MDMA Consumption in past 14 days[a]	Self-reported average MDMA tablets per month in past 3 months[a]	Sessions Completed
1	U & H	M	19.7	22.4	1	2	3
2	B	M	22.3	21.9	3	1	3
3	B	M	19.5	26.5	2	12	3
4	W	F	19.4	18.6	2	36	3
5	B	M	24.6	23.1	1	3	3
6	B	F	26.1	34.2	5	4	3
7	W	M	20.5	28.2	1	4	3
8	W	M	18.6	21.0	0	2	3
9	B	M	20.0	27.3	8	12	3
10	B	M	21.2	24.8	0	4	3
11	B	M	25.0	19.4	2	2	3[b]
12	B	F	20.1	28.2	4	12	3
13	B	M	18.3	24.4	1	2	3
14	B	F	20.0	24.1	1[c]	4	2
15	B	F	22.2	28.2	10	16	3
16	B	F	27.6	23.3	6	8	3
17	B	F	23.2	19.1	0	32	3
18	B	F	21.8	18.0	1	8	3
19	B	M	23.8	29.5	1	28	3
20	B	F	20.0	26.4	2	2	1
21	W	F	20.7	18.8	1	2	1
22	W	M	34.9	24.4	2[c]	4	3
23	B	M	26.0	21.7	10	96	1
24	B	M	28.3	28.1	4	8	3
25	W	M	18.5	19.7	1	6	3

Participant	Race & Ethnicity	Sex	Age	BMI	Self-reported MDMA Consumption in past 14 days[a]	Self-reported average MDMA tablets per month in past 3 months[a]	Sessions Completed
26	W	M	21.9	24.4	1	2	3
27	B	M	24.9	31.6	1	3	3
28	B & H	F	23.9	35.8	6	24	3
29	B	M	28.6	24.2	1	1	3
30	B	M	20.8	27.5	4	12	1
31	B	F	21.5	31.0	4	5	3
32	W	M	18.9	20.7	1	5	3
33	W	F	27.3	25.6	1	0.3	2
34	B	M	22.2	20.7	0	2	3
35	B	M	25.3	19.9	1	4	2
36	B	M	28.5	25.7	1	6	3
37	B	M	25.4	23.0	4	36	3
38	B	M	22.6	22.6	0	16	3
39	B	M	23.7	28.2	1	3	1
40	B	F	25.9	19.8	1	0.3	3
41	B	M	28.3	30.6	2	16	3
42	W	F	24.5	22.5	0	1	3
43	W	F	25.9	20.5	5	8	3
44	B	M	35.7	28.3	4	16	3
45	B	M	27.5	30.7	4	8	3
46	B	M	28.7	25.1	3	8	3
47	B	M	29.5	30.1	0	0.7	1
48	B	M	27.7	26.9	6	20	3
49	W	F	23.6	25.1	1	0.3	3
50	B	M	28.6	25.0	5	36	3
Mean ± SD			24.1 ± 4.0	24.9 ± 4.2	2.5 ± 2.4	10.9 ± 15.8	
Median [Range]			23.8 [18.3–35.7]	24.6 [18.0–35.8]	1 [0–10]	5 [0.3–96]	

[a]Screening data

[b] Participant expectorated high-dose tablet

[c] Participant reported MDMA use as "hallucinogens"

Abbreviations: MDMA, 3,4-methylenedioxymethamphetamine; B, Black or African American; H, Hispanic or Latino; U, Unknown; W, White or Caucasian

Table 2

Calibration curves, extraction efficiency, and intra- and inter-day imprecision from a 2D–GC/electron impact MS method with cryotrapping for simultaneous MDMA, MDA, HMMA, HMA, and MDEA quantitation in blood.

	MDMA-*d5*	**MDMA**	**MDA-*d5***	**MDA**	**Pholedrine**	**HMMA**	**HMA**	**MDEA-*d6***	**MDEA**
				Dynamic Range (μg/L)					
	IS (MDMA)	2.5–400	IS (MDA)	1.0–100	IS (HMA, HMMA)	2.5–400	2.5–100	IS (MDEA)	2.5–400
				QC Concentrations (μg/L)					
Low		7.5		3.0		7.5	3.0		7.5
Medium		40		15		40	15		40
High		200		75		200	75		200
				Calibration Curve Characteristics, Mean (SD) (n = 7)					
Slope		0.041 (0.001)		0.225 (0.012)		0.020 (0.002)	0.047 (0.008)		0.045 (0.001)
Intercept		−0.011 (0.003)		0.115 (0.037)		0.001 (0.005)	0.026 (0.016)		−0.014 (0.002)
r^2		0.996 (0.002)		0.997 (0.003)		0.995 (0.002)	0.990 (0.007)		0.996 (0.002)
				Extraction Efficiency (%) (n = 6)					
Low	50.0	53.1	46.4	52.7	39.5	27.8	31.9	49.7	51.2
Medium	52.0	55.1	48.9	51.5	40.9	27.7	25.2	51.7	53.9
High	54.0	57.8	53.1	56.0	43.9	30.3	27.7	53.4	55.9
				Inaccuracy (%) (N = 21)					
Low		0.2		10.0		2.4	−1.6		−1.4
Medium		−0.5		8.7		4.0	−0.3		2.3
High		5.3		2.6		10.9	0.8		5.4
				Intra-Day Imprecision (%)					

	MDMA-*d5*	MDMA	MDA-*d5*	MDA	Pholedrine	HMMA	HMA	MDEA-*d6*	MDEA
				(n = 3)					
Low		1.9		3.5		2.5	5.9		1.7
Medium		2.0		2.3		3.2	3.5		2.2
High		1.5		2.1		3.6	7.3		1.5
				Inter-Day Imprecision (%) (N = 21)					
Low		2.9		4.8		5.0	6.3		2.6
Medium		2.9		3.6		5.6	5.6		2.9
High		3.6		2.6		5.1	8.6		3.5

Abbreviations: 2D–GC, 2-dimensional gas chromatography; MS, mass spectrometry; MDMA, 3,4-methylenedioxymethamphetamine; MDA, 3,4-methylenedioxyamphetamine; HMMA, 4-hydroxy-3-methoxymethamphetamine; HMA, 4-hydroxy-3-methoxyamphetamine; MDEA, 3,4-methylenedioxyethylamphetamine

Table 3

Median [range] MDMA, MDA, HMMA and HMA observed C_{max}, t_{max}, t_{first}, and $AUC_{0–3h}$ in blood and plasma after 1.0 (low) and 1.6 (high) mg/kg controlled oral MDMA administration.

		Blood	N	Plasma	N	p-value (blood vs. plasma)	Blood	N	Plasma	N	p-value (blood vs. plasma)
		MDMA					MDA				
Observed C_{max} (μg/L)											
Overall	Low	144.9 [90.3–358.2]	46	126.3 [66.9–276.1]	46	<0.0005	8.0 [4.0–18.3]	46	5.5 [2.3–11.3]	46	<0.0005
	High	241.6 [58.5–461.9]	41	205.1 [46.3–465.3]	41	<0.0005	13.0 [3.0–24.1]	41	8.6 [2.0–21.0]	42	<0.0005
p-value (low vs. high)		<0.0005		<0.0005			<0.0005		<0.0005		
Females	Low	159.0[b] [111.7–239.3]	13	129.8 [97.0–243.6]	13		8.0 [6.0–11.7]	13	6.1 [3.7–8.4]	13	
	High	240.2 [153.6–461.9]	14	194.6 [125.6–465.3]	15		12.5 [4.4–24.1]	14	8.5 [2.6–21.0]	15	
Males	Low	131.9[b] [90.3–358.2]	32	120.1 [66.9–276.1]	32		8.0 [4.0–18.3]	32	5.3 [2.3–8.5]	32	
	High	241.6 [58.5–390.9]	27	205.5 [46.3–361.9]	27		13.0 [3.0–20.4]	27	8.7 [2.0–12.9]	27	
African American	Low	144.4 [90.3–358.2]	33	127.3 [66.9–276.1]	33		8.0 [5.3–18.3]	33	5.3 [2.6–8.4]	33	
	High	244.0 [117.0–461.9]	28	213.4 [95.6–465.3]	28		12.7 [4.4–24.1]	28	8.8 [3.7–21.0]	28	
White, Hispanic, or Mixed[a]	Low	137.7 [91.1–176.4]	12	113.3 [74.5–164.4]	12		7.9 [4.0–11.7]	12	5.9 [2.3–8.5]	12	
	High	241.0 [58.5–345.3]	13	199.6 [46.3–296.4]	14		13.0 [3.0–19.2]	13	7.8 [2.0–12.6]	14	
Observed t_{max} (h)											
Low		2.5 [1.5–3.0]	46	2.5 [1.5–3.0]	46	ns	3.0 [1.5–3.0]	45	3.0 [2.0–3.0]	46	ns
High		2.5 [1.0–3.0]	41	2.5 [1.0–3.0]	42	ns	3.0 [1.5–3.0]	41	3.0 [2.0–3.0]	42	ns
p-value (low vs. high)		ns		ns			ns		ns		
Observed t_{first} (h)											
Low		0.5[−0.25–1.5]	47	0.5[−0.25–1.5]	46	ns	1.0[−0.25–2.0]	47	1.0[−0.25–2.5]	46	0.002
High		0.5[−0.25–2.0]	41	0.5[−0.25–2.5]	42	ns	1.0 [0.5–3.0]	41	1.0 [0.5–3.0]	42	ns
p-value (low vs. high)		ns		ns			ns		ns		
$AUC_{0–3h}$ (h•μg/L)											

		Blood	N	Plasma	N	p-value (blood vs. plasma)	Blood	N	Plasma	N	p-value (blood vs. plasma)
Overall	Low	248.9 [120.0–536.4]	45	220.7 [98.7–480.7]	45	<0.0005	12.9 [4.1–26.4]	45	8.8 [2.5–15.2]	45	<0.0005
	High	419.0 [20.0–831.7]	40	352.7 [16.2–846.3]	42	<0.0005	19.4 [0.8–43.6]	40	11.9 [0.5–36.6]	42	<0.0005
p-value (low vs. high)		<0.0005		<0.0005			<0.0005		<0.0005		
Females	Low	279.3 [148.8–469.2]	14	237.3 [119.8–480.7]	14		13.9 [6.7–18.3]	14	9.1 [2.6–15.2]	14	
	High	450.8 [187.7–818.2]	14	384.9 [85.0–846.3]	15		18.7 [6.9–43.6]	14	11.8 [0.7–36.6]	15	
Males	Low	235.6 [120.0–536.4]	32	212.0 [98.7–441.3	32		12.5 [4.1–26.4]	32	8.0 [2.5–13.9]	32	
	High	413.7 [20.0–831.7]	26	341.6 [16.2–734.9]	27		19.5 [0.8–39.0]	26	12.4 [0.5–20.4]	27	
Black or African American	Low	251.3 [120.0–536.4]	34	223.9 [98.7–480.7]	34		13.4 [6.6–26.4]	34	8.9 [2.6–15.2]	34	
	High	419.1 [192.4–831.7]	27	352.7 [180.9–846.3]	28		19.07 [6.9–43.6]	27	12.9 [3.4–36.6]	28	
White, Hispanic, or Mixed[a]	Low	245.9 [126.4–326.5]	12	189.9 [107.7–302.3]	12		12.8 [4.1–19.0]	12	8.7 [2.5–12.4]	12	
	High	418.8 [20.0–668.0]	13	352.9 [16.2–581.1]	14		20.0 [0.8–37.4]	13	11.0 [0.5–20.4]	14	
		HMMA					**HMA**				
Observed C_{max} (µg/L)											
Overall	Low	107.0 [24.9–231.1]	46	143.7 [35.6–304.9]	46	<0.0005	0.0 [0.0–3.1]	46	0.0 [0.0–3.2]	46	0.070
	High	111.0 [19.8–222.3]	41	147.5 [19.8–299.2]	42	<0.0005	0.0 [0.0–2.6]	41	0.0 [0.0–3.5]	42	0.012
p-value (low vs. high)		ns		ns			ns		ns		
Females	Low	98.1 [24.9–231.1]	13	117.7 [35.6–259.5]	13		0.0 [0.0–3.1]	13	0.0 [0.0–2.7]	13	
	High	109.8 [19.8–222.3]	14	135.6 [19.8–265.1]	15		0.0 [0.0–2.6]	14	0.0 [0.0–3.2]	14	
Males	Low	110.1 [49.0–211.5]	32	153.5 [67.3–304.9]	32		0.0 [0.0–0.0]	32	0.0 [0.0–3.2]	32	
	High	111.0 [53.4–197.9]	27	161.2 [78.8–299.2]	27		0.0 [0.0–2.5]	27	0.0 [0.0–3.5]	27	
Black or African American	Low	105.6 [24.9–211.5]	33	125.8 [35.6–304.9]	33		0.0 [0.0–0.0]	33	0.0 [0.0–3.2]	33	
	High	113.6 [19.8–197.9]	28	147.5 [19.8–299.2]	28		0.0 [0.0–2.5]	28	0.0 [0.0–3.5]	28	
White, Hispanic, or Mixed[a]	Low	112.2 [33.1–231.1]	12	174.6 [42.0–259.5]	12		0.0 [0.0–3.1]	12	0.0 [0.0–2.9]	12	
	High	111.0 [73.9–222.3]	13	152.1 [85.3–265.1]	14		0.0 [0.0–2.6]	13	0.0 [0.0–2.8]	14	
Observed t_{max} (h)											
Low		2.0 [1.0–3.0]	46	2.0 [1.0–3.0]	46	ns	3.0	1	3.0 [2.0–3.0]	8	--

		Blood	N	Plasma	N	p-value (blood vs. plasma)	Blood	N	Plasma	N	p-value (blood vs. plasma)
High		2.0 [1.0–3.0]	41	2.0 [1.0–3.0]	42	ns	3.0 [3.0–3.0]	2	3.0 [2.5–3.0]	9	--
p-value (low vs. high)		ns		ns			--		--		
Observed t_{first} (h)											
Low		0.5[−0.25–1.0]	47	0.5[−0.25–1.0]	46	ns	3.0	1	2.0 [1.0–3.0]	8	--
High		0.5[−0.25–2.0]	42	0.5[−0.25–2.0]	42	ns	2.5 [2.0–3.0]	2	2.5 [1.5–3.0]	9	--
p-value (low vs. high)		ns		ns			--		--		
$AUC_{0–3h}$ (h•μg/L)											
Overall	Low	217.4 [40.7–459.9]	45	302.0 [59.4–596.0]	45	<0.0005	0.0 [0.0–0.8]	45	0.0 [0.0–6.5]	45	0.012
	High	249.4 [37.8–500.1]	40	322.0 [37.8–646.8]	42	<0.0005	0.0 [0.0–1.9]	40	0.0 [0.0–5.1]	42	0.014
p-value (low vs. high)		ns		ns			ns		ns		
Females	Low	200.9[c] [40.7–450.5]	14	256.3[c] [59.4–477.4]	14		0.0 [0.0–0.8]	14	0.0 [0.0–2.0]	14	
	High	233.9 [37.8–500.1]	14	270.8 [37.8–588.4]	15		0.0 [0.0–1.9]	14	0.0 [0.0–5.1]	15	
Males	Low	238.6[c] [95.6–459.9]	32	333.2[c] [128.6–596.0]	32		0.0 [0.0–0.0]	32	0.0 [0.0–6.5]	32	
	High	250.1 [87.8–470.8]	26	349.1 [120.9–646.8]	27		0.0 [0.0–0.6]	26	0.0 [0.0–4.0]	27	
Black or African American	Low	203.0 [40.7–459.9]	34	271.9[d] [59.4–596.0]	34		0.0 [0.0–0.0]	34	0.0 [0.0–6.5]	34	
	High	252.3 [37.8–470.8]	27	322.0 [37.8–646.8]	28		0.0 [0.0–0.6]	27	0.0 [0.0–5.1]	28	
White, Hispanic, or Mixed[a]	Low	253.4 [55.4–450.5]	12	375.5[d] [67.1–511.8]	12		0.0 [0.0–0.8]	12	0.0 [0.0–3.5]	12	
	High	246.5 [87.8–500.1]	13	321.5 [82.4–588.4]	14		0.0 [0.0–1.9]	13	0.0 [0.0–2.1]	14	

Abbreviations: MDMA, 3,4-methylenedioxymethamphetamine; MDA, 3,4-methylenedioxyamphetamine; HMMA, 4-hydroxy-3-methoxymethamphetamine; HMA, 4-hydroxy-3-methoxyamphetamine; C_{max}, observed maximum concentration; t_{max}, time of observed C_{max}; t_{first}, time of first detection; $AUC_{0–3h}$, 3 h area under the curve; ns, not significant.

[a] Incorporated into one category due to insufficient number identifying as Hispanic or Mixed

[b] Low blood MDMA C_{max} was significantly higher in females vs. males (p=0.010).

[c] Low blood and plasma HMMA $AUC_{0–3h}$ was significantly lower in females vs. males (p=0.027 blood and p=0.027 plasma).

[d] Low plasma $AUC_{0–3h}$ was significantly lower in African-Americans vs. white/Hispanic or mixed race individuals (p=0.035).