Sensitive Gas Chromatography-Mass Spectrometry Method for Simultaneous Measurement of MDEA, MDMA, and Metabolites HMA, MDA, and HMMA in Human Urine

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Background: A sensitive gas chromatography-mass spectrometry method was developed and validated for the simultaneous measurement of MDEA, MDMA, and its metabolites, 3,4-methylenedioxy-N-ethylamphetamine (MDEA), 3,4-methylenedioxyamphetamine (MDMA or Ecstasy), and its metabolites, 4-hydroxy-3-methoxyamphetamine (HMA), 3,4-methylenedioxyamphetamine (MDA), and 4-hydroxy-3-methoxyamphetamine (HMMA) in human urine.

Methods: We hydrolyzed 1 mL urine, fortified with MDMA-d5, MDA-d5, and MDEA-d6, with 100 μL of concentrated hydrochloric acid at 120 °C for 40 min, then added 100 μL 10 N sodium hydroxide and 3 mL phosphate buffer 0.1 N (pH 6.0) were added to hydrolyzed urine specimens before solid-phase extraction. After elution and evaporation, we derivatized extracts with heptafluorobutyric acid anhydride and analyzed with gas chromatography-mass spectrometry operated in EI-selected ion-monitoring mode.

Results: Limits of quantification were 25 μg/L for MDEA, MDMA, and its metabolites. Calibration curves were linear to 5000 μg/L for MDEA, MDA, HMA, MDA, and HMMA, with a minimum r² > 0.99. At 3 concentrations spanning the linear dynamic range of the assay, mean overall extraction efficiencies from urine were >85.5% for all compounds of interest. Intra- and interassay imprecisions, produced as CV, were <15% for all drugs at 30, 300, and 3000 μg/L.

Conclusions: This gas chromatography-mass spectrometry assay provides adequate sensitivity and performance characteristics for the simultaneous quantification of MDEA, MDMA, and its metabolites HMMA, MDA, and HMA in human urine. The method meets and exceeds the requirements of the proposed Substance Abuse and Mental Health Services Administration’s guidelines for federal workplace drug testing of MDEA and MDMA in urine.

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Methylenedioxy derivatives of amphetamines, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxyamphetamine (MDEA), and 3,4-methylenedioxyamphetamine (MDMA), constitute a major class of central nervous system stimulants producing well described cardiovascular and behavioral effects (1, 2). The US Drug Enforcement Administration restricted use of these compounds more than 2 decades ago, yet consumption of these drugs has increased dramatically (3, 4). MDMA drug abuse is especially problematic among young people (5). Although acute intoxications have been widely documented (6, 7), the potential for long-term central nervous system toxicity is the subject of much controversy (8).

MDMA is excreted primarily as unchanged drug in urine, with additional N-demethylation to MDA, O-dealkylation to 3,4-dihydroxyamphetamine (HMA), and 3,4-dihydroxyamphetamine (HHA), and O-methylation to 4-hydroxy-3-methoxyamphetamine (HMMA) and 4-hydroxy-3-methoxyamphetamine (HMA) (see Fig.
1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol52/issue9). In determining disposition of xenobiotic compounds in urine, characterization of parent drug and metabolites is useful because both may contribute to toxicity, and metabolites frequently have longer terminal elimination half-lives, increasing the window of drug detection. In support of our controlled human drug administration studies, we developed and validated a method to simultaneously measure MDEA, MDMA, and its most important metabolites.

Gas chromatography/mass spectrometry (GC-MS) is the most common instrumental technique for analysis of amphetamines and derivatives (9–12). Derivatization is required to improve chromatography, sensitivity, and reproducibility of primary and secondary amines (13, 14). Liquid-liquid (10, 15, 16) and solid-phase extraction (SPE) procedures have been published (17–19). Capillary electrophoresis-MS (20, 21) and liquid chromatography-MS (22–25) assays also are available. In general, methods have included a limited number of compounds and a limited linear range. Our clinical pharmacokinetic research requires low limits of quantification (LOQ) to determine terminal elimination half-lives and large dynamic ranges to monitor initial urinary excretion of amphetamines and metabolites. The method is suitable for analysis of MDEA, and MDMA under proposed Substance Abuse and Mental Health Services Administration (SAMHSA) guidelines (26).

We report the development of a robust, rapid, sensitive, and relatively inexpensive GC-MS assay for simultaneous quantification of 5 amphetamine analogs. The method uses a simple and inexpensive acid hydrolysis for recovery of MDMA-conjugated metabolites and is suitable for pharmacokinetic studies and for analysis of urine specimens with a large range of drug concentrations in clinical and forensic toxicology laboratories.

**Materials and Methods**

**REAGENTS**

Solvents were HPLC grade from Mallinkrodt Baker. Ammonium hydroxide, glacial acetic acid, hydrochloric acid, dibasic potassium phosphate, monobasic potassium phosphate, sodium acetate, and sodium hydroxide were American Chemical Society reagent grade, also from Mallinkrodt Baker. Heptafluorobutyric acid anhydride was purchased from Pierce Biotechnology, Inc.

SPE columns (Clean Screen ZSDAU020) and vacuum manifolds were obtained from United Chemical Technologies. Drug-free urine was collected from volunteers and evaluated by GC-MS to ensure the absence of MDMA and metabolites.

**CALIBRATORS AND QUALITY CONTROL SAMPLES**

Reference standards for MDA, MDA-d5, HMMA, MDMA, MDMA-d5, MDEA, and MDEA-d5 were obtained from Cerilliant and for HMA from Lipomed. All stock solutions were 1.0 g/L in methanol, except MDEA-d5, stock solution, which was 100 mg/L in methanol. Intermediate solutions of 100, 10, 1, and 0.1 mg/L were prepared in methanol. Working calibrators at 10, 25, 50, 500, 1000, 2500, 5000, and 10 000 µg/L were prepared by fortifying 1 mL of blank urine with HMA, MDA, HMMA, MDMA, and MDEA. A mixture of deuterated internal standards (50 µL of 1000 µg/L), MDMA-d5, MDA-d5, and MDEA-d5 was added to each urine specimen before hydrolysis.

**ACID HYDROLYSIS PROCEDURE**

We hydrolyzed 1 mL of urine with 100 µL of concentrated hydrochloric acid in a gas chromatograph (GC) oven at 120 °C for 40 min. After specimens cooled to room temperature, 100 µL of 10 N sodium hydroxide and 3 mL of 0.1 N phosphate buffer (pH 6.0) were added. Before application to extraction columns, the pH of samples was checked and adjusted if needed (pH 5–6.5). Tubes were vortex-mixed for 0.5 min and centrifuged at 1850g for 10 min.

**EXTRACTION AND DERIVATIZATION**

SPE columns were conditioned with methanol, deionized water, and 100 mmol/L phosphate buffer (pH 6.0). Supernatants from acid hydrolysis were applied to conditioned SPE columns under gravity flow. Columns were washed with deionized water, 1.0 mol/L acetic acid, and methanol, and dried under vacuum. Analytes were eluted with methylene chloride:iso-propanol:concentrated ammonium hydroxide (78:20:2 v/v). We added 50 µL of 1% hydrochloric acid in methanol were added to eluates before evaporation under nitrogen at 37 °C in a Zymark Turbovap™ LV Evaporator and added 50 µL of ethyl acetate and 50 µL of heptafluorobutrylic acid anhydride to dried extracts. Vials were capped and derivatized at 70 °C for 10 min.

After the samples cooled, 25 µL of 1% hydrochloric acid in acetonitrile were added, and derivatized extracts were evaporated to dryness. Extracts were reconstituted with 75 µL of heptane, vortex-mixed, centrifuged at 1850g for 5 min, and transferred to autosampler vials.

**GC-MS ANALYSIS**

GC/MS analysis was performed with an Agilent 6890 GC interfaced to an Agilent 5973 mass-selective detector. The GC was equipped with a DB-35MS capillary column (15 m × 0.32 mm, internal diameter × 0.25 µm film thickness). Helium was used as carrier gas at a constant pressure of 2.0 psi; 1 µL of derivatized urinary extract was injected (split ratio 10:1). Injection port and transfer line temperatures were 250 °C and 280 °C, respectively. The initial column temperature of 70 °C was held for 2 min, followed by temperature ramps of 20 °C/min to 160 °C, hold for 2 min; 15 °C/min to 200 °C, with a 1-min postrun hold at 300 °C. Total separation run time was 12.2 min. The ion source was maintained at 230 °C and quadrupole at 150 °C. Selected ion monitoring mode was used with a dwell time of 10 ms. Three ions for each analyte and 2 for
each deuterated internal standard were monitored (quantification ions are italic): HMA-d$_5$, m/z 240, 360, 333; MDA-d$_5$, m/z 167, 244; MDA-d$_{10}$, m/z 162, 135, 375; HMMA-d$_5$, m/z 360, 254, 210; MDMA-d$_5$, m/z 258, 213; MDMA-d$_{10}$, m/z 254, 210, 162, MDEA-d$_5$, m/z 268, 240, 162; and MDEA-d$_{10}$, m/z 274, 244.

Choice of monitored ions for MDMA, MDA, and MDEA were reported previously by Stout et al. (27). Moreover, HMA and HMMA were derivatized on both amine and hydroxyl functional groups corresponding to m/z 268, and 210; MDMA - d$_0$, m/z 258, 213, and MDEA-d$_0$, m/z 268, 240, 162; and MDEA-d$_{10}$, m/z 274, 244.

Deuterated HMA and HMMA are not yet commercially available; therefore, MDA-d$_5$ was chosen as the internal standard for HMA, MDA, and HMMA.

**DATA ANALYSIS**

Calibration with internal standardization was accomplished by linear regression analysis curve fitting with a weighting factor of 1/x. Peak area ratios of target analytes and internal standards were calculated by MSD Chemstation software (v D.00.00). Working calibration standards were prepared daily at 10, 25, 50, 100, 250, 500, and 10 000 μg/L.

**VALIDATION EXPERIMENTS AND ACCEPTANCE CRITERIA**

Method linearity was investigated by evaluating of the regression line and produced by the squared correlation coefficient ($r^2$). Six calibrators were included in each curve and were required to meet all qualitative identification and quantification criteria. Each calibrator was calculated against the linear regression curve from 6 calibrators; quantitative accuracy was required within 20% of target. Linearity was achieved with a minimal $r^2$ of 0.99.

Method sensitivity was evaluated by determining limit of detection (LOD) and LOQ for each compound. LOD was defined as lowest concentration with ion signal-to-noise ratio (determined by peak height) ≥3:1 with satisfactory chromatography (peak shape and resolution), acceptable retention time (~2% of average calibrator retention time), and qualifier ion ratios within 20% of the average of 6 calibrators. LOQ was defined as the lowest concentration that met LOD criteria with measured concentration within 20% of target concentration in 6 replicates.

Recovery for each analyte was assessed by adding internal standard to 1 set of low, medium, and high control solutions (30, 300, and 3000 μg/L) before solid phase extraction and to a second set after extraction, but before evaporation. Samples were derivatized and analyzed. Another set was prepared with undiluted analyte and internal standard solutions that were evaporated, derivatized, and analyzed. Recovery (percentage) was calculated by comparing the peak area ratios of analyte to internal standard for extracted and unextracted samples.

Validation samples at 3 concentrations (30, 300, and 3000 μg/L) were prepared from stock solutions and analyzed (n = 6) with independent calibration curves on 6 assays. Method recovery, produced as a percentage, was calculated by comparing mean calculated concentrations of HMA, MDA, HMMA, MDMA, and MDEA in validation samples to target concentrations. Interassay precision was assessed by calculating percent relative standard deviation (%RSD) of 6 replicates over 5 independently calibrated assays (n = 30). Intraassay precision was assessed by calculation of %RSD from one run (n = 6). Intraassay data are presented for the day with greatest variability. Intraassay and interassay precision were calculated at 3 concentrations across the assay’s dynamic range.

To evaluate interference and method selectivity, 8 blank (no analyte or IS added) urine specimens were evaluated for coeluting chromatographic peaks that might interfere with detection of analytes of interest or deuterated internal standards. Internal standard materials also were tested for the presence of native analyte ions. To assess possible interference from other commonly available drugs, low concentration quality control (QC) samples were enriched individually to contain 10 000 μg/L of 6-acetylmorphine, acetylsalicylic acid, anhydroecgonine methyl estermsylate, benzoylecgonine, brompheniramine, caffeine, cannabinol, clonidine, chlorpheniramine, diphenhydramine, dextromethorphan, ephedrine ethyl ester, ephedrine methyl ester, epinfluramine, GHB, hydrocodone, hydromorphone, 11-hydroxy-tetrahydrocannabinol, ibuprofen, m-hydroxybenzoylecgonine, m-hydroxybenzoylecgonine, m-hydroxycocaine, methadone, morphine, nicotine, norcocaine, norcocaethylene, norpseudoephedrine, oxymorphone, p-hydroxybenzoylecgonine, p-hydroxycocaine, p-methoxyamphetamine, p-methoxymethamphetamine, pentazocine, phentermine, phenylpropanolamine, and pseudoephedrine.

Moreover, to evaluate interference with high concentrations of ephedrine, pseudoephedrine, and propanolamine, low concentration QC samples were enriched individually to contain 1 g/L of ephedrine, pseudoephedrine, and propanolamine. Acceptance criteria required adequate resolution and peak shape and quantification within 20% of target concentrations.

Several studies were performed to determine stability of HMA, MDA, HMMA, MDMA, and MDEA under different conditions. Temperature stability studies were performed on 4 sets of control urine samples enriched at 30, 300, and 3000 μg/L for each analyte. One set of control samples (n = 6) was subjected to 3 freeze/thaw cycles. The 2nd set of samples (n = 6) was maintained at room temperature for 14 h before analysis, the 3rd set (n = 6) was refrigerated for 72 h before analysis, and the 4th set (n = 3) was maintained at room temperature for 72 h after derivatization on the autosampler. Concentrations of analytes in these samples were calculated and compared with freshly prepared control samples.
Clinical specimens
A within-subject, placebo-controlled, double-blind, randomized MDMA administration study was approved by the National Institute on Drug Abuse Institutional Review Board, and participants provided written informed consent. Individuals with a history of MDMA use, substantiated with a positive biological test, received 1.6 mg/kg oral MDMA and resided on the secure clinical research unit. Urine specimen was collected 1.2 h after MDMA administration and was stored at −20 °C until analysis.

Results
As shown in Fig. 1, the retention times of internal standards, MDA-d₅, MDMA-d₅, and MDEA-d₆ added into blank urine were 8.74, 10.18, and 10.34 min, respectively. Retention times of HMA, MDA, HMMA, MDMA, and MDEA were 7.94, 8.78, 9.09, 10.22, and 10.39 min, respectively. Total GC-MS analysis time was 12.2 min per sample. Selected ion monitoring chromatograms of target ions of HMA, MDA, HMMA, MDMA, and MDEA in urine are depicted in Fig. 1. To document method applicability, a urine specimen collected 1.2 h after controlled administration of 1.6 mg/kg oral MDMA was analyzed (Fig. 2). Quantification of this urine specimen revealed total drug concentrations of 1462.4 μg/L MDMA, 29.3 μg/L HMA, 46.7 μg/L MDA, and 4261.8 μg/L HMMA.

Method validation
Linearity. Regression analysis of calibration data achieved satisfactory linearity over the concentration range investigated. Squared correlation coefficients ($r^2$) were >0.99, indicating a linear relationship from 25–5000 μg/L for HMA, MDA, HMMA, MDMA, and MDEA. Slopes, intercepts, and squared coefficients of correlation are presented in Table 1.

Sensitivity. LODs were established by extraction of urine samples containing decreasing concentrations of HMA, MDA, HMMA, MDMA, and MDEA. The LOD was 10 μg/L, and LOQ was 25 μg/L for all analytes (Table 1).

Extraction recovery. Mean extraction efficiencies of HMA, MDA, HMMA, MDMA, and MDEA were calculated from 6 replicates of 30, 300, and 3000 μg/L QC samples. Recoveries for all analytes at all concentrations were >85.5%.

Precision and recovery. To evaluate precision and recovery, we analyzed QC samples containing 30, 300, and 3000 μg/L of HMA, MDA, HMMA, MDMA, and MDEA. Results are summarized in Tables 2 and 3. To determine intraassay recovery and precision, we performed 6 replicate analyses at each concentration. Within-run CVs for all compounds were <6%. Recovery, calculated as the percentage of target concentration, was 90%–117% (Table 2).

Fig. 1. Extracted ion chromatograms of the 50 μg/L HMA (240 m/z, peak 1), MDA-d₅ (167 m/z, peak 2), MDA (162 m/z, peak 3), HMMA (360 m/z, peak 4), MDMA-d₅ (258 m/z, peak 5), MDMA (254 m/z, peak 6), MDEA-d₆ (274 m/z, peak 7), and MDEA (268 m/z, peak 8) urine calibrator.
Interassay precision and recovery, determined at the same concentrations, for 6 batches and 6 replicates per batch, were 3.5%–12.9% and 90%–112% respectively, for all analytes at all concentrations. Interassay precision is shown in Table 3.

Specificity. Eight blank urine specimens from different individuals were analyzed to evaluate chromatographic interference. No interference peaks were detected. The absence of analyte ions in blank urines fortified with internal standards demonstrated that internal standards did not contain relevant amounts of native drug.

Interference study. No interference was detected in the drugs tested up to 10,000 μg/L with HMA, MDA, HMMA, MDMA, and MDEA. Moreover no interference was detected with ephedrine, pseudoephedrine, and propanolamine up to 1 g/L.

Investigation of sample storage/stability. Stability of HMA, MDA, HMMA, MDMA, and MDEA at low-, medium-, and high-QC concentrations (n = 6) was evaluated under several conditions; after 3 freeze/thaw cycles, 72 h at 4 °C, and 14 h at room temperature (see Table 1 in the online Data Supplement). Quantification of all QC samples was acceptable (within 15% of target) for all stability challenges. Studies also were conducted to evaluate stability of analytes after storage at 72 h at ambient temperature on the autosampler. Concentrations of samples analyzed immediately and after 72 h differed by <20% (see Table 1 in the online Data Supplement).

Discussion

Selection of deuterated internal standards
MDMA-d₅, MDA-d₅, and MDEA-d₆ were included as internal standards for their respective d₀-compounds. Deuterated analogs are not commercially available for HMA and HMMA. MDA-d₅ and MDMA-d₅ were evaluated as internal standards for these analytes because of their similar chemical structure (see Fig. 1 in the online Data Supplement). MDA-d₅ has a primary amine function (similar to HMA), and MDMA-d₅ has a secondary amine function (similar to HMMA). Both compounds produced equivalent quantitative recovery and limits of quantification for HMA and HMMA. Because of the fact that MDA-d₅ has a retention time (8.86 min) closer to HMA (8.04 min) and HMMA (9.20 min) than MDMA-d₅ (10.28 min) (Fig. 1), MDA-d₅ was selected as the internal standard for HMA and HMMA.

Isolation of the compounds
Our goal was to develop an analytical method that is robust and highly sensitive to effectively characterize terminal elimination half-lives. In addition, it was important to monitor total metabolite excretion and thus, the value of the hydrolysis step to liberate glucuronide conjugates. This required optimization of hydrolysis before extraction. Furthermore, as addressed by Clauwaert et al. (16), up to 50% of free MDA and MDMA may be lost during extraction when the organic phase containing the compounds is evaporated to dryness under a stream of

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Internal Standard</th>
<th>LOD (μg/L)</th>
<th>LOQ (μg/L)</th>
<th>Equation&lt;sup&gt;a&lt;/sup&gt;</th>
<th>R²</th>
</tr>
</thead>
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<tr>
<td>HMA</td>
<td>d₅-MDA</td>
<td>10</td>
<td>25</td>
<td>Y = 0.0705 (0.00693) X - 0.7628 (0.2236)</td>
<td>0.998</td>
</tr>
<tr>
<td>MDA</td>
<td>d₅-MDA</td>
<td>10</td>
<td>25</td>
<td>Y = 0.0421 (0.00408) X - 0.2595 (0.1875)</td>
<td>0.998</td>
</tr>
<tr>
<td>HMMA</td>
<td>d₅-MDA</td>
<td>10</td>
<td>25</td>
<td>Y = 0.1605 (0.2513) X - 0.6274 (0.1536)</td>
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<tr>
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<td>d₅-MDA</td>
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<td>25</td>
<td>Y = 0.0475 (0.1115) X - 0.1429 (0.0978)</td>
<td>0.999</td>
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<tr>
<td>MDEA</td>
<td>d₆-MDEA</td>
<td>10</td>
<td>25</td>
<td>Y = 0.0270 (0.0007) X - 0.1632 (0.0752)</td>
<td>0.999</td>
</tr>
</tbody>
</table>

<sup>a</sup> n = 5.

<sup>b</sup> Data are mean (SD).
Table 2. Intraassay precision and recovery for determination of HMA, MDA, HMMA, MDMA, and MDEA in urine.a

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Expected Concentration, µg/L</th>
<th>Observed Concentration, µg/L</th>
<th>Precision, %</th>
<th>Recovery, %</th>
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<td>HMA</td>
<td>30 33.4 (0.7)</td>
<td>32.4 (0.7)</td>
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<td>111.3</td>
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<tr>
<td>MDA</td>
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<td>284.6 (16.7)</td>
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<td>95.5</td>
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<td>MDEA</td>
<td>30 33.9 (0.6)</td>
<td>33.6 (0.6)</td>
<td>1.8</td>
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</tr>
<tr>
<td>MDA</td>
<td>300 273.8 (9.9)</td>
<td>273.6 (9.9)</td>
<td>3.6</td>
<td>91.3</td>
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<tr>
<td>3000</td>
<td>3044.6 (105.3)</td>
<td>3041.6 (105.3)</td>
<td>3.5</td>
<td>101.5</td>
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<td>2.8</td>
<td>117.0</td>
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<td>MDEA</td>
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<td>33.3 (0.7)</td>
<td>2.2</td>
<td>111.7</td>
</tr>
<tr>
<td>3000</td>
<td>3118.8 (126.3)</td>
<td>3115.8 (126.3)</td>
<td>4.0</td>
<td>104.0</td>
</tr>
</tbody>
</table>

a n = 5.

b Data are mean (SD).

Table 3. Interassay precision and recovery for determination of HMA, MDA, HMMA, MDMA, and MDEA in urine.a

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Expected Concentration, µg/L</th>
<th>Observed Concentration, µg/L</th>
<th>Precision, %</th>
<th>Recovery, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMA</td>
<td>30 31.8 (3.6)</td>
<td>31.6 (3.6)</td>
<td>11.2</td>
<td>106.0</td>
</tr>
<tr>
<td>MDA</td>
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<td>285.5 (13.2)</td>
<td>4.6</td>
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<td>9.6</td>
<td>103.7</td>
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<tr>
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<td>6.2</td>
<td>98.3</td>
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</table>

a n = 5.

b Data are mean (SD).

symmetric peaks in <12 min. Attempts to use a different chromatographic column (HP 5MS 30 m × 0.25 µm × 0.25 mm) were unsatisfactory because of longer retention times and lack of resolution between MDMA and MDEA.

A split ratio of 10:1 was selected for injection onto the GC-MS. Splitless injection and a split ratio of 1:5 improved LOQs, but column overload and poorer peak resolution occurred at higher analyte concentrations. The linear dynamic range of the assay was much greater with the 10:1 split injection (Table 1).

METHOD VALIDATION

The purpose of this investigation was to develop a specific and sensitive assay for the simultaneous determination of HMA, MDA, HMMA, MDMA, and MDEA. Three previously published studies investigated the recovery of HMA and HMMA from urine after hydrolysis (12, 28, 29). Our validated hydrolysis is faster and logistically easier than autoclave hydrolysis (12) and notably more cost-effective than enzyme hydrolysis (28, 29). Moreover, our validated SPE procedure followed by optimized derivatization yielded higher average recoveries, especially for HMA, than previously published methods.

In addition, the other methods for HMA and HMMA showed higher LOQ and smaller dynamic ranges (28, 29). Although Helmlin et al. investigated HMA and HMMA in combination with MDMA, no validation data were presented (12). Other studies by Hensley et al. (30) showed a wide dynamic range (5–1000 µg/L) for MDMA, MDA, and MDEA but did not investigate MDMA metabolites, HMA, and HMMA.

Intra- and interassay results also were satisfactory (CVs <15%). Quantification of HMA, MDA, HMMA, MDMA, and MDEA was acceptable (within 20% of target) for all stability challenges. Our study demonstrated no considerable loss of MDEA, MDMA, or metabolites, HMA, MDA, and HMMA in urine at any of the investigated temperatures and times. The same results were observed by Clauwaert et al. (31), who performed extended studies of stability of MDA, MDMA, and MDEA and who furthermore demonstrated stability of these drugs at –20, 4, or 20 °C in urine for 21 weeks.

A robust, rapid, sensitive, and relatively inexpensive gas chromatography-mass spectrometry assay for simultaneous quantification of urinary MDEA, MDMA, MDA, HMMA, and HMA is presented. The method is suitable for pharmacokinetic studies, specimen batches with various drug concentrations, and for identification and quantification of MDEA, MDMA, and MDA in postmortem, emergency, and workplace toxicology.

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References


