Dimethylamylamine: A Drug Causing Positive Immunoassay Results for Amphetamines*

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Abstract
The Department of Defense (DoD) operates six forensic urine drug-testing laboratories that screen close to 5 million urine samples for amphetamines yearly. Recently, the DoD laboratories have observed a significant decrease in the confirmation rates for amphetamines because of specimens screening positive by two separate immunoassays and confirming negative by gas chromatography–mass spectrometry (GC–MS). Previous studies conducted by the Division of Forensic Toxicology, Armed Force Institute of Pathology (AFIP) utilizing a GC–MS basic drug screen and a designer drug screen revealed no common compound or compound classes as to the cause of the immunoassay-positive results. Additional information obtained from an immunoassay vendor suggested the anorectic compound dimethylamylamine (DMAA) may be the cause of the false-positive screens. An additional 134 false-positive samples were received and analyzed using liquid chromatography–tandem mass spectrometry (LC–MS–MS) for DMAA. LC–MS–MS analysis revealed the presence of DMAA in 92.3% of the false-positive samples at a concentration of approximately 6.0 mg/L DMAA, causing a positive screen on both immunoassay kits.

Introduction
Dimethylamylamine (DMAA) is a straight chain aliphatic amine (Figure 1) found naturally in geranium flowers. It is also referred to as forthane, methylhexaneamine, 1,3-dimethylpentylamine, and geranamine. DMAA was originally used as a nasal decongestant for its vasoconstrictor action on the nasal mucosa (1). Today, it can be found in nutritional and body-building energy supplements such as Jack3d™ and OxyELITE Pro™ that are available online and at health supplement suppliers such as General Nutrition Center (GNC). One manufacturer refers to DMAA as being a low-side-effect alternative to ephedrine (2). The nutritional supplements may list DMAA in the ingredients as any of the previously mentioned names, or as geranium oil extract, or as a “proprietary blend”. DMAA is available in over-the-counter party pills sold in New Zealand, and in November 2009, the government moved to restrict sales of those pills. Their use as a drug of abuse became prevalent in New Zealand after 1-benzylpiperazine (BZP) became a scheduled drug (3).

The Department of Defense (DoD) currently employs a three test system to report a positive result for a urine specimen. The tests are composed of two qualitative immunoassays and one confirmatory test, usually gas chromatography–mass spectrometry (GC–MS). For the amphetamines class, the DoD uses two different immunoassay reagents to improve selectivity and decrease over-the-counter medication positives from being ex-

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trated for confirmation. In July 2009, the confirmation rates began to decrease at several laboratories. The confirmation rates at DoD drug-testing laboratories for amphetamines were 82.3 and 81.2%, respectively, for fiscal years 2008 and 2009. To date (through June 2010), the confirmation rate for amphetamines in fiscal year 2010 is 50.4%. One laboratory reported confirmation rates as low as 23%. Some laboratories have implemented a third screening assay, which has shown improvement in confirmation rates (all three immunoassays must be positive for confirmation analysis to proceed). Low confirmation rates cost the DoD laboratories time, money, and material, as well as challenge DoD turnaround time requirements.

Testing at the Division of Forensic Toxicology (DFT), The Armed Forces Medical Examiner System (AFMES) was conducted in late 2009 on 52 specimens that screened positive and confirmed negative for amphetamines. The specimens were analyzed by an alkaloid drug screen and a designer drug screen. The results did not indicate a common denominator as to the cause of the positive immunoassay results.

At the annual DoD drug-testing program meeting, a representative from Siemens stated DMAA may be the cause of the positive immunoassay results. The DFT requested additional specimens that screened positive and confirmed negative to analyze specifically for DMAA.

**Experimental**

**Reagents and materials**

All organic solvents were high-performance liquid chromatography (HPLC) grade and purchased from Fisher Scientific (Pittsburgh, PA). Potassium hydroxide pellets were also purchased from Fisher Scientific. Formic acid and DMAA were purchased from Aldrich (Milwaukee, WI). A methanolic standard of amphetamine-d₈ was purchased from Cerilliant (Round Rock, TX).

**Immunoassay**

A Roche/Hitachi Modular P automated screening instrument (Indianapolis, IN) was used to screen urine samples for amphetamines. The kits used were Roche Amphetamines KIMS assay and Siemens Syva® EMIT® II Plus Amphetamines assay (Newark, DE). Each kit was calibrated on the Modular P analyzer using d-amphetamine spiked at 500 ng/mL with certified standards purchased from Cerilliant. Negative (75% cutoff concentration) and positive (125% cutoff concentration) controls were included in the initial calibration.

**Standards and calibrators preparation**

A stock solution of DMAA was prepared at target concentration of 1.0 mg/mL in ethanol and stored at ≤ –20°C. A stock solution of the internal standard amphetamine-d₈ was prepared in amber glass at a target concentration of 0.001 mg/mL and refrigerated. Working solutions of DMAA were prepared by serial dilution with ethanol at concentrations of 0.01 and 0.001 mg/mL. Calibrators for DMAA were spiked into certified drug-free negative urine at 25, 50, 100, 250, and 500 ng/mL.

**DMAA sample preparation and extraction**

To 1 mL of urine, 100 μL of the stock internal standard solution was added for a final concentration of 100 ng/mL. 3 drops of concentrated potassium hydroxide and 3 mL of ethyl acetate were also added. The samples were mixed for 5 min and centrifuged for 5 min at 3000 rpm. The upper organic layer was transferred to clean conical tubes and evaporated at 40°C under nitrogen at 5 psi after the addition of 25 μL of 10% methanolic HCl. The samples were reconstituted in 200 μL mobile phase (3:2 0.1% formic acid/methanol), transferred to properly labeled autosampler vials, and capped.

**DMAA instrumental analysis**

The LC–MS–MS analysis of DMAA was performed using an Agilent 1100 series HPLC system (Palo Alto, CA) coupled with an Applied Biosystems/MDS SCIEX 3200 QTRAP (Foster City, CA) equipped with a Turbo V™ source. Analyst 1.5 software was used for data acquisition and analysis.

Chromatographic separation was performed on an Agilent Zorbas XDB C₁₈ column (4.6 × 75 mm, 3.5 μm). The column compartment was maintained at 35°C, and the injection volume was set at 2 μL. The mobile phase was set at a constant flow of 800 μL/min and consisted of 0.1% formic acid in deionized water (A) and methanol with 5% acetonitrile and 0.1% formic acid (B). A gradient elution was used as follows: pre-injection equilibration with 65% A for 3.0 min, hold at 65% A for 2.0 min after injection, ramp to 40% A over 4.0 min, and hold until 6.0 min.

The MS was operated in positive electrospray ionization mode (+ESI). The analysis of DMAA and amphetamine-d₈ (ISTD) was operated in multiple reactions monitoring (MRM) acquisition mode. Two MRM transitions (* denotes quantitation transition) were monitored for both DMAA (m/z 116.1/57.0*, m/z 116.1/99.1) and amphetamine-d₈ (m/z 144.2/97.1*, m/z 144.2/127.2).

The source-dependent parameters for the MS–MS analysis of DMAA were determined by the flow injection analysis (FIA). The optimized source-dependent parameters for the analysis were as follows: GS1 gas (nebulizer) was set to 60 psi, GS2 gas

<table>
<thead>
<tr>
<th>Compound</th>
<th>MRM Transition (Da/Da)</th>
<th>Compound-Dependent Parameter (volts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMAA</td>
<td>116.1/57.0</td>
<td>DP 20.0, EP 6.5, CEP 12.0, CE 14.0, CXP 4.0</td>
</tr>
<tr>
<td>ISTD</td>
<td>144.2/97.1</td>
<td>DP 30.0, EP 4.0, CEP 12.0, CE 21.0, CXP 4.0</td>
</tr>
</tbody>
</table>

Table I. Compound-Dependent MS–MS Parameters
(turbo) was set to 70 psi, CUR (curtain gas) was set to 40 psi, TIS (TurboIonSpray® voltage) was set to 1500V, and the source temperature was set at 550°C.

The compound-dependent parameters for the MS–MS analysis of DMAA were determined by direct infusion. An integrated infusion pump delivered a 10 mg/L standard solution at a constant flow (10 μL/min) directly into the TIS source. The auto-optimization process determined the optimal parameters for each MRM transition. The following parameters were optimized during the process: DP (declustering potential), EP (entrance potential), CEP (collision cell entrance potential), CE (collision energy), and CXP (collision cell exit potential). Table I lists the optimal compound-dependent parameters for DMAA and internal standard.

Identification of DMAA in the random specimens was based on the ratio of MRM2/MRM1 transitions being within 20% of the average ratios and the relative retention time being within ±2% of the averages measured from the calibrators.

Results and Discussion

The screening results are presented in Table II. Most species...
mens screened positive with both immunoassays, although some did not, which was probably due to sample degradation. The confirmation results for DMAA are also presented in Table II. The confirmation method was developed solely to detect and quantitate DMAA. Overall, 92.3% of the specimens contained DMAA at or above 2.5 mg/L. Figure 2 illustrates the screening results from the two immunoassay kits. The charts plot DMAA concentrations versus immunoassay screening response for each immunoassay.

It was of interest to determine what concentration of DMAA alone would cause a positive immunoassay result. Certified negative drug-free urine was spiked with DMAA to determine the lowest concentration that yields a positive immunoassay result. The Roche Amphetamines KIMS assay and Siemens Syva EMIT II Plus Amphetamines assay gave positive responses at 7500 and 3125 ng/mL, respectively. The concentrations determined experimentally correlate fairly well to the real-life specimens analyzed in the study. The DMAA concentrations in the analyzed specimens range from 2.5 to 67.0 mg/L with 6.9 mg/L being the lowest concentration to give two positive immunoassay results using real urine samples. The specimens that confirmed negative for DMAA were subjected to a basic drug screen analysis to determine if there were any drugs present that could explain the positive immunoassay results. Each of the nine urine samples that confirmed negative for DMAA contained compounds known to cross-react with amphetamine immunoassays used by the DoD laboratories. Specifically, six of the negative DMAA urine samples contained phentermine, two contained bupropion and its metabolites, and one contained a high concentration of pseudoephedrine.

The cross-reactivity of immunoassay kits is affected by the specific coupling sites for the protein used in the assay. For amphetamines, the protein could be coupled to either the aromatic ring or the nitrogen, or a combination of both. The Siemens and Roche kits both target \(d\)-methamphetamine and \(d\)-amphetamine with some cross-reactivity to MDMA and MDA. Figure 1 illustrates the structural similarity between DMAA and the sympathomimetic amines that the immunoassay kits are designed to detect. Because DMAA is a small molecule with structural similarities to the amphetamines, it is likely to cross-react with any immunoassay targeting amphetamine-type compounds. Previous studies conducted at AFIP, DFT using GC–MS full scan analysis failed to detect DMAA. The

![Figure 2. Comparison of the screening responses for the two immunoassay kits used in the study. The charts plot the concentration of DMAA (ng/mL) versus the immunoassay response with 100 as the cutoff response. All samples that are plotted above the cutoff line are false-positive amphetamine screens.](Image)

![Figure 3. GC–MS splitless injection of a DMAA reference standard. DMAA is not detected because it is buried in the solvent front (A). GC–MS split injection (100:1) of a DMAA reference standard. DMAA is detected as a double peak because it is a racemic mixture (B). LC–MS–MS of DMAA and the amp-\(d_8\) internal standard. DMAA is detected as a double peak (C).](Image)
standard alkaline full scan GC–MS drug screen has a 4.0-min solvent delay to protect the life of the filament and electron multiplier. However, it was discovered that DMAA eluted in 2.0 min along with the solvent peak. A study was performed varying the GC inlet and oven parameters to determine the effects on DMAA detection. A series of injections performed while varying the inlet temperature from 120 to 270°C revealed no discernable thermal degradation of DMAA in the injection port. On a J&W DB-5MS column (20 m × 0.18 mm × 0.18 μm), DMAA is only retained 2.0 min at 50°C. In order to perform a GC–MS analysis, the solvent delay must be set before 2.0 min, and the split vent must be greater than 50:1. This eliminates most of the solvent before it gets onto the column and will allow detection of the DMAA peak. GC–MS analysis is feasible, but great care must be taken during the method development process for the initial temperature, injection parameters, and the solvent delay.

The GC–MS and LC–MS–MS analyses of DMAA result in a double chromatographic peak (Figure 3). DMAA has two chiral centers, which will result in four possible stereoisomers: \((R,S)\), \((S,R)\), \((S,S)\), and \((R,R)\)-1,3-dimethylpentylamine. The \((S,S)\) and \((R,R)\) stereoisomers are enantiomers (optical isomers) that have the same chemical and physical properties and cannot be separated. The same is true for \((R,S)\) and \((S,R)\). The \([(S,S),(R,R)]\) and \([(R,S),(S,R)]\) isomers are diastereomers that differ in some physical properties and can be separated. The smaller the distance between the optical centers, the better the chromatographic separation. For DMAA, the two methyl groups are at the C1 and C3 carbon positions, which result in a double chromatographic peak that is almost baseline resolved. The first peak is from \((S,S),(R,R)]\) and the second peak is from \([(R,S),(S,R)]\) isomers (4–7). Both the reference standard and the positive urine specimens had the double peak, indicating that DMAA is made and sold as a racemic compound. The quantitative results were calculated on the total area of both peaks as compared to a standard curve for DMAA.

Conclusions

Products containing DMAA have become an issue for the DoD drug-testing program. The cost for confirmation testing of the specimens containing DMAA can and has become quite substantial. DMAA is a straight chain amine with a fairly simple structure that shows reactivity with the antibodies currently employed in some commercially available immuno assays. The overall safety of DMAA should be explored to determine if this is a safer alternative to other sympathomimetic amines. High-throughput urine drug-screening laboratories need to be aware of the impact DMAA can have on their testing efficiency.

Acknowledgments

This work was funded in part by the American Registry of Pathology, Washington, D.C. 20306-6000. The authors would like to acknowledge the help of Josh Seither, Jeff Chmiel, and Laura Regester. The authors would also like to thank Mr. Robert Privon of Siemens for suggesting DMAA as a potential source of the screen-positive results. Additionally, the authors acknowledge the DoD drug-testing laboratories United States Army Forensic Toxicology Drug Testing Laboratory Tripler, Navy Drug Screening Laboratory Great Lakes, and Air Force Drug Testing Laboratory Brooks for their assistance in obtaining the 134 urine specimens.

References

6. B.D. Paul, J. Jermoni, D. Lesser, A. Jacobs, and D.A. Searles, Enantiomeric separation and quantitation of \((\pm\text{-amphetamine, (\pm-methamphetamine, (\pm-MDA, (\pm-MDMA, and (\pm-MDEA in urine specimens by GC–EI-MS after derivatization with \((R)-\text{-o-}\) or \((S)-\text{-o-}\) \((\pm\text{-methoxy-\alpha-(trifluoromethyl)phenylacetyl} chloride (MTPA). J. Anal. Toxicol. 28(6): 449–455 (2004).

Manuscript received September 13, 2010; revision received October 4, 2010.