Retrospective Study of Urinalysis for dl-Amphetamine and dl-Methamphetamine Analysis under Current Department of Defense Guidelines

Ronald L. Shippee and Donald J. Kippenberger
1U.S. Army Forensic Toxicology Drug Testing Laboratory, 2490 Wilson Street, Fort Meade, Maryland 20755 and
2Research Dynamics, Inc., 1355 Central Parkway South, Suite 100, San Antonio, Texas 78232

Abstract

Under current Department of Defense (DOD) directive, the laboratories certified to conduct urinalysis in support of the DOD Drug Deterrence Program are required to conduct dl-isomer analysis on all specimens that confirm at a concentration greater than 500 ng/mL methamphetamine (METH). Although the same cutoff concentration is required for amphetamine (AMP) reporting, there is no requirement for dl-isomer analysis of AMP-positive specimens. Of the 894,823 specimens screened by the Army Drug Testing Laboratory at Ft. Meade, MD during a 19-month period, 339 confirmed positive for METH. From this positive population, seven specimens failed to confirm at or above the DOD cutoff of > 20% d-isomer. One of the seven specimens contained 534 ng/mL l-AMP and was reported positive for AMP. Although 100% of the AMP was the l-isomer, under current DOD directive, this information was not passed along to the Medical Review Officers (MRO) to assist them during the interview process. Although this situation appears to be a rare event, consideration should be given to requiring dl-isomer analysis of AMP-positive specimens and forwarding this information to the MRO.

Materials

d-AMP, d-METH, dl-METH-d11, dl-AMP-d5, dl-MDMA-d5, dl-MDA-d5, and dl-MDEA-d6 were purchased from Radian Corp. l-AMP and l-METH were purchased from Alltech Corp. Optical purity of 98% or greater for these standards was certified by the manufacturer. Dimethylformamide, heptafluorobutyric anhydride and (S)-N-trifluoroacetyl-L-prolyl chloride were obtained from EM Science Industries, Inc., Sigma Corp., and Regis Technologies, respectively. Methanol, ethyl acetate, methylene chloride, isopropanol, and chloroform were Mallinckrodt high-performance liquid chromatography grade.
Procedures

After accessioning the specimens, an aliquot (approximately 2 mL) was poured from the specimen bottle and tested using an Olympus AU800 (Olympus America Inc., Melville, NY). The initial screening analysis was performed using a kinetic interaction of microparticles (Abuscreen Online, Roche Diagnostics Systems, Somerville, NJ). At the 500-ng/mL cutoff, the assay was designed to report as positive for urine samples containing either 500 ng/mL of AMP or 500 ng/mL METH. Additionally, 4.0 mL of a 0.4M sodium metaperiodate solution was added to each 85-ml bottle of microparticle reagent supplied by the manufacturer. The aliquot was obtained from the specimen bottle and a re-screen was performed using a homogenous enzyme immunoassay technique (Emit II, Syva Co., Cupertino, CA) on the AU800. This methodology has been shown to have a low cross-reactivity with prescription drugs (fenfluramine) commonly used for weight control.

Subsequent to a presumptive positive from the re-screen assays, a third aliquot was obtained and prepared for confirmation testing. AMP and METH were extracted using a liquid-liquid extraction and derivatization method described by Cody and Schwarzhoff (2). Briefly, 100 μL of AMP/METH internal standard (15,000 ng/mL dl-METH-d15/15,000 ng/mL dl-AMP-d5) was added to either 3.0-mL specimen or a diluted specimen to a volume of 3.0 mL. After the addition of 1.0 mL 1M NaOH, 20 mL of 5% (v/v) isopropanol in methylene chloride was added and shaken for 3 min. After aspirating the upper layer to waste, 2.0 mL of 0.1M H2SO4 was added. Tubes were shaken for 3 min, and the upper aqueous layer was transferred to a clean tube. Three drops of saturated KOH were added, followed by 2.0 mL methylene chloride. Tubes were vortex mixed for 1 min, and the upper layer was aspirated to waste. The lower layer was derivatized with 100 μL 5% dimethylformamide (1 mL of concentration HCl mixed with 99 mL methyl alcohol then added to dimethylformamide, 95:5), 35 μL of heptafluorobutyric anhydride, and 100 μL of ethyl acetate. Specimens were evaporated to dryness under nitrogen and reconstituted with 1 mL of ethyl acetate.

Results and Discussion

During the September 1997 to April 1999 timeframe, the laboratory screened a total of 894,823 active-duty military specimens for AMP. The testing of this population produced 339 screened and confirmed positives for METH at concentrations greater than the 500-ng/mL cutoff. Of the 339 confirmed positives for METH, 332 contained d-METH at a concentration greater than 20% d isomer. There were seven specimens that did not meet the requirements of d-METH analysis and were reported negative for METH (Table I).

Five of the seven negative specimens confirmed positive for METH analogues. The presence of METH in the urine was most likely due to “contamination” of the ingested analogue or produced from metabolism. Specimen D fits the profile of normal Vicks inhaler usage as described by Fitzgerald et al. (3). In this controlled study, three subjects were instructed to inhale the medication every 20 min for 6 h. Urine samples were collected over the subsequent 80 h and analyzed by GC–MS for l-METH and total AMP. One subject reached a peak concentration for l-METH of 6000 ng/mL. Levels of AMP ranged from 250 to 455 ng/mL with [AMP]/[METH] less than 0.08.

An additional explanation for these results could be explained by ingestion of a prescription drug such as selegiline. Selegiline is a monoamine oxidase inhibitor often prescribed to control Parkinsons Disease (4). Urinalyses of subjects taking selegiline

<table>
<thead>
<tr>
<th>Specimen</th>
<th>METH ng/mL</th>
<th>AMP %d %l</th>
<th>MDA ng/mL</th>
<th>MDMA ng/mL</th>
<th>THC ng/mL</th>
<th>COC ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>619</td>
<td>3.27</td>
<td>76.73</td>
<td>32</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>B</td>
<td>2949</td>
<td>2.92</td>
<td>97.08</td>
<td>143</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>C</td>
<td>1143</td>
<td>3.26</td>
<td>96.74</td>
<td>31</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>D</td>
<td>6061</td>
<td>3.02</td>
<td>96.98</td>
<td>158</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>E</td>
<td>1091</td>
<td>3.41</td>
<td>86.59</td>
<td>36</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>F</td>
<td>1104</td>
<td>3.68</td>
<td>96.14</td>
<td>51</td>
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<td>100</td>
</tr>
<tr>
<td>G</td>
<td>17,670</td>
<td>3.41</td>
<td>96.59</td>
<td>534</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

and Enantiomeric Analysis
showed excretion of only the \( l \) form of AMP and METH (5). Given the fact that the study involved only four specimens and provides no information concerning the dose ingested or urinalysis time after ingestion, \( l \)-METH concentrations ranged from 829 to 4690 ng/mL with \( [\text{AMP}] / [\text{METH}] \) ranging from 0.37 to 0.42.

Specimen H is more difficult to explain. The total METH concentration is above the normal concentration reported for routine use of Vicks inhaler. The \( [\text{AMP}] / [\text{METH}] \) of 0.03 would argue against selegiline ingestion. Of particular concern is the fact that although the \( d \)-METH analysis resulted in a negative report for METH, the laboratory reported the specimen positive for AMP based on current DOD criteria.

There is no provision under the current DOD directive to report the isomeric form of the AMP result. The laboratory reported specimen H positive for AMP and negative for METH. Thus, the MRO who had to interpret the result for specimen H was faced with a difficult task. This case is further complicated by the fact that the specimen was from a soldier stationed in a European country. It is generally accepted that in Europe, the illicit production of AMP far exceeds that of METH. Upon further investigation (personal communication), it was disclosed that the individual who provided specimen H admitted to illegal AMP use.

Although the occurrence of a specimen containing a high concentration of licit METH while confirming positive for \( l \)-AMP appears to be a rare event, consideration should be given to requiring \( dl \)-isomer analysis of AMP-positive specimens and forwarding this information to the MRO. This information would also assist the MRO when determining possible use of prescription drugs such as clobenzorex that metabolize to \( l \)-AMP.

References


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