GC–MS Analysis of Methamphetamine Impurities: Reactivity of (+)- or (−)-Chloroephedrine and cis- or trans-1,2-Dimethyl-3-phenylaziridine

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Abstract

S-(+)-Methamphetamine is frequently found as the only isomer in urine specimens from methamphetamine abusers. Enantiomerically pure S-(+)-methamphetamine can be synthesized from ephedrine or pseudoephedrine via chloroephedrine intermediates. These intermediates are unstable and capable of cyclizing to form cis- and trans-1,2-dimethyl-3-phenyl aziridine. Studies were done to determine if these intermediates could be detected when using a common gas chromatographic–mass spectrometric analytical method (derivatization with heptafluorobutyric anhydride, HFBA) for toxicological screening of methamphetamine. Analysis of (+)- or (−)-chloroephedrine after extraction into hexane and derivatization with HFBA indicated that both pseudoephedrine and ephedrine were the major compounds detected. Direct derivatization of a hexane solution of cis-1,2-dimethyl-3-phenyl aziridine yielded only the derivatives of ephedrine and pseudoephedrine, indicating that the aziridine intermediate is responsible for the formation of the ephedrine or pseudoephedrine. These studies indicate that the aziridine intermediates would not be detected in methamphetamine samples following HFBA derivatization.

Introduction

The occurrence of MPTP, a potent neurotoxin that was a synthetic impurity present in an illicitly synthesized meperidine analogue, was a dramatic example of the dangers associated with drug impurities present in clandestinely synthesized drugs. Methamphetamine ("speed") has developed into a major problem in the United States. Prior to the early 1970s, pharmaceutical-grade methamphetamine was readily available. Currently, clandestinely synthesized methamphetamine is used almost exclusively and ranks among the top 20 drugs in emergency room and medical examiner mentions. The acute lethal dose of amphetamines is generally thought to be in the range of 20–25 mg/kg. However, as tolerance develops, dosages as high as 800 mg have been given without significant effect (1). Chronic abusers may use as much as 15,000 mg per day (2). The concentration of methamphetamine in blood and tissues in fatalities associated with methamphetamine is highly variable (3). Methamphetamine concentrations in fatal cases have ranged from less than 1 mg/L to over 14 mg/L (4–7); however, there does not appear to be any correlation between the levels of methamphetamine and amphetamine in blood to methamphetamine-caused deaths (8,9). Often, low levels of amphetamines are thought to be incidental findings, but very low concentrations of methamphetamine (0.7 mg/L) have been observed in patients dying of what is now described as "classic stimulant toxicity" with agitation, hypertension, tachycardia, and hyperthermia (10).

Surprisingly, there is very little information on the occurrence of synthetic impurities in biological samples associated with methamphetamine. The only report to date was the occurrence of α-benzyl-N-methylphenethylamine that was present in 2 of 80 methamphetamine-positive urine samples (3,11). This impurity occurs during the synthesis of phenyl-2-propanone used in the reductive amination of methamphetamine. According to the DEA, this synthetic route occurred in only 16% of the clandestine laboratories seized in 1993. In the same study, the ephedrine reduction method was used in 81% of the laboratories (12). Although multiple recipes have been reported for the conversion of (−)-ephedrine or (+)-pseudoephedrine, the synthetic routes depicted in Figure 1 for the conversion of (−)-ephedrine to S-(+)-methamphetamine are often used. When using either PCl3, SOCl2, HCl (red phosphorus), or I2/NaI, the common product or intermediate from the halogenated intermediates is cis- or trans-1,2-dimethyl-3-phenylaziridines (13–15). The presence of these intermediates and impurities (including the aziridines) have been detected at varying levels in clandestinely prepared methamphetamine (13–16), and there is an anecdotal report that the halogenated and aziridine impurities "ruin the finer aspects of the meth [sic] high" (17).
Analytical methods for qualitative identification of (+)- or (-)-chloroephedrine or cis- or trans-1,2-dimethyl-3-phenylaziridine have been described for the analysis of drug formulations (14,16). Methods for detecting these impurities in biological samples have not been reported. A common approach used for the identification of methamphetamine in plasma or urine requires extraction and derivatization with an anhydride (e.g., heptfluorobutyric anhydride, HFBA) followed by analysis by gas chromatography–mass spectrometry (GC–MS) (18,19). This derivatization procedure was evaluated to determine if it could be used to detect (+)- and (-)-chloroephedrine or cis- and trans-1,2-dimethyl-3-phenylaziridine in aqueous samples during the analysis of methamphetamine. Unexpectedly, it was observed during these studies that all of these synthetic intermediates were primarily identified as HFBA derivatives of ephedrine and pseudoephedrine.

Experimental

Reagents and chemicals

(-)-Ephedrine and (+)-pseudoephedrine as the free base were purchased from Aldrich Chemical Co. (Milwaukee, WI). The (-)-ephe- drine.HCl and (+)-pseudoephedrine.HCl were purchased from Spectrum Health Care Products (New Brunswick, NJ). The other chemicals and reagents were high-performance liquid chromatography or reagent grade. Melting points were determined on a Thomas Hoover melting point apparatus. Proton nuclear magnetic resonance spectra (1H NMR) were obtained on a Varian Gemini 300 MHz spectrometer.

Table 1. Retention Times and Mass Spectral Data for Amphetamine, Methamphetamine, and Common Synthetic Impurities as their HFBA Derivatives

<table>
<thead>
<tr>
<th>Compound</th>
<th>tR (min)</th>
<th>Base m/z</th>
<th>M+ m/z (%)</th>
<th>M+H+ m/z (%)</th>
<th>Other ions, m/z (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)-Amphetamine</td>
<td>7.36</td>
<td>91</td>
<td>331 ND</td>
<td>240 168 192 117 118</td>
<td></td>
</tr>
<tr>
<td>(+)-Methamphetamine</td>
<td>9.55</td>
<td>254</td>
<td>345 ND</td>
<td>210 91 118 117 169</td>
<td></td>
</tr>
<tr>
<td>(+)-Chloroephedrine</td>
<td>7.28</td>
<td>254 ND</td>
<td>ND</td>
<td>210 117 174 153 380</td>
<td></td>
</tr>
<tr>
<td>(-)-Chloroephedrine</td>
<td>6.61</td>
<td>254 ND</td>
<td>ND</td>
<td>210 117 147 153 381</td>
<td></td>
</tr>
<tr>
<td>(-)-Ephedrine</td>
<td>9.98</td>
<td>254 ND</td>
<td>ND</td>
<td>210 144 117 117 115</td>
<td></td>
</tr>
<tr>
<td>(+)-Pseudoephedrine</td>
<td>11.07</td>
<td>254 ND</td>
<td>ND</td>
<td>210 144 117 117 115</td>
<td></td>
</tr>
</tbody>
</table>

Sample preparation for HFBA derivatization

(+)- and (-)-chloroephedrine-HCl and cis-1,2-dimethyl-3-phenylaziridine stock standard solu-
tions (2 mg/mL) were prepared in deionized water. Standard solutions were prepared fresh just prior to analysis. Two milliliters of standard solution was alkalinized with 300 μL of 12 N sodium hydroxide, and the analytes were extracted into 2 mL of hexane. The hexane layer was transferred into a 13 × 100-mm screw-capped test tube; 100 μL of HFBA was added, and the tube was capped. The mixture was incubated at 75°C for 20 min, then evaporated under a stream of air at 40°C. The resultant residue was dissolved in 50 μL of hexane, and 2 μL was injected into the GC-MS.

**GC-MS analysis**

GC-MS analysis was performed on the Varian Star 3400Cx (Varian, Sugar Land, TX) ion-trap GC-MS equipped with a 30-m × 0.25-mm i.d. capillary column with a 0.25-μm film thickness of 5% phenyl 95% methylpolysiloxane cross-linked (DB-5, J&W Scientific, Folsom, CA). The GC was operated in the splitless mode with a helium carrier gas linear velocity of 20 mL/min. The transfer line was set at 280°C. The GC parameters for the direct analysis of the amines used temperature programming, 60°C to 240°C at 10°C/min (injection port, 230°C). The GC parameters for the HFBA derivatives used temperature programming, 70°C to 240°C at 10°C/min (injection port, 190°C). The ion-trap MS was operated in the electron impact mode at 70 eV with ion control on Auto. Mass ranged from m/z 40 to 600 with a scan time of 0.7 s.

**Results and Discussion**

The GC-MS method was evaluated for the analysis of (+)- and (-)-chloroephedrine and cis- and trans-1,2-dimethyl-3-phenylaziridine after derivatization with HFBA. After derivatization of (+)-chloroephedrine, the HFBA derivative of (+)-chloroephedrine eluted at 7.28 min and accounted for 60% of the material injected (based on relative peak heights) along with ephedrine (5.51 min, 9%) and pseudoephedrine (6.03 min, 32%). After derivatization of (-)-chloroephedrine, the HFBA derivatives of (-)-chloroephedrine was not observed. Instead, the HFBA derivative of (+)-chloroephedrine (1%), ephedrine (74%), and pseudoephedrine (23%) were observed. The HFBA derivative of (-)-chloroephedrine could be formed by derivatization of a CH₂Cl₂ solution of (-)-chloroephedrine·HCl, and it eluted at 6.61 min. No peak at 6.61 min was detected after a basic extraction of (-)-chloroephedrine with hexane. After extraction of cis-1,2-dimethyl-3-phenylaziridine from a basic solution and derivatization with HFBA, the products detected were ephedrine (12%), pseudoephedrine (80%), and an unidentified material eluting at 5.46 min (Rₘ = 0.76, 8%).

The ions associated with the HFBA derivatives are listed in Table I. No chloro substituted ions were observed for the HFBA derivatives of (+)- or (-)-chloroephedrine gave mass spectra comparable to HFBA derivatives of ephedrine or pseudoephedrine. Therefore, the differentiation of these compounds is based upon their different retention times. It would be expected that the free base form of the chloroephedrine isomers cyclized only to their respective aziridine products during the analysis. In contrast, during the HFBA derivatization of (+)- or (-)-chloroephedrine, both HFBA derivatives of ephedrine and pseudoephedrine were detected. This indicates that during the derivatization procedure a carbonium ion is formed at the benzylic position once the aziridines are formed allowing epimerization to occur. Therefore, the ratio of ephedrine and pseudoephedrine detected after analysis cannot be used to determine the ratio of (+)- or (-)-chloroephedrine to cis- or trans-1,2-dimethyl-3-phenylaziridine before analysis.

It appears that if the chloroephedrines or aziridines were carried through an extraction and acyl derivatization sequence comparable to that used for methamphetamine, the chloroephedrines or aziridines did not give derivatives or mass spectra consistent with their structures. The observations in this study may explain why these halogenated intermediates and aziridine impurities have not been previously detected or reported by forensic toxicologists. Also, if methamphetamine is detected in a urine or blood specimen and ephedrine or pseudoephedrine is also identified, it cannot be assumed that ephedrine or pseudoephedrine were co-administered with methamphetamine. They may be present as artifacts of illicit methamphetamine synthesis.

No pharmacological or toxicological studies on
the intermediates described in this study have been done. Whether these impurities would present an acute or chronic problem in humans when co-administered with methamphetamine is unknown.

References


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