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Formation of an Interfering Substance, 3,4-Dimethyl-5-Phenyl-1,3-Oxazolidine, During A Pseudoephedrine Urinalysis

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16. Abstract

During fatal aviation accident investigations, bio-samples from the victims are submitted to the FAA Civil Aeromedical Institute for drug analysis. In the process of one such analysis, an unknown substance was found in a urine sample. Its initial analyses by thin layer chromatography and by liquid-liquid extraction/gas chromatography (GC) disclosed the presence of pseudoephedrine. Subsequent analyses of the reaccessioned sample by solid phase separation/GC Fourier transform infrared/GC mass spectrometry indicated that the retention times of the unknown substance matched with those of pseudoephedrine. However, its infrared and mass spectra were different—the –OH and –NH– groups were missing, a C-O-C group was present, and the molar mass was 12 atomic mass units (amu) more than that of pseudoephedrine. A subsequent literature search suggested that ephedrine-like amines react with aldehydes to form oxazolidines. Therefore, the 12-amu increase could be accounted for by condensation of pseudoephedrine with formaldehyde. Since this aldehyde is present in various grades of methanol, and methanol was used during the solid phase separation, 3,4-dimethyl-5-phenyl-1,3-oxazolidine was synthesized by using (+)-pseudoephedrine·HCl and formaldehyde. The analytical and spectral findings of the synthesized compound were consistent with those of the unknown interfering substance, confirming that it was the oxazolidine. Aldehyde contaminants can transform the drug of interest and may result in misidentification of a compound not originally present in specimens. Therefore, chemicals used in analyses should be of the highest available purity, and a multi-analytical approach should be adopted to maintain a high degree of quality assurance.

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INTRODUCTION

In fatal aircraft accident investigations, postmortem samples collected from the accident victims at autopsy are submitted to the Federal Aviation Administration's (FAA's) Civil Aeromedical Institute (CAMI) for toxicological evaluation (Public Law, 1988). The submitted samples are analyzed for the presence of drugs, including sympathomimetic amines (SMAs), such as ephedrine, pseudoephedrine, and phenylpropanolamine. An unknown substance, which interfered with the analysis of pseudoephedrine, was found during the analysis of a urine sample from a pilot who died in an aviation accident. Under our gas chromatographic conditions, this substance had the same retention time as that of pseudoephedrine, but its infrared and mass spectra were different. The pseudoephedrine's characteristic O-H and N-H bond stretchings were missing in the infrared spectrum of the substance, and its molar mass was 12 atomic mass units (amu) more than pseudoephedrine.

It is known that ephedrine-like amines undergo cyclization with aliphatic, as well as aromatic, aldehydes, forming oxazolidines (Neelakantan, 1971; Beckett, Jones, and Hollingsbee, 1978; Nishiyama and Yamada, 1989; and Walker, Wood, Akmal, and Sharks, 1992). This cyclization reaction involves the condensation of the amines with an aldehyde, with a resulting loss of one water molecule (see Fig. 1). Therefore, the molar mass of the oxazolidine is the total of the masses of the amine plus the aldehyde, minus the mass of water. Since the molar mass of the unknown compound was only 12 amu more than that of pseudoephedrine, the increase was potentially accounted for by the addition of a carbon atom through the reaction between pseudoephedrine and formaldehyde.

The problem of aldehydic impurities in solvents is not new—for example, the presence of trace amounts of formaldehyde, acetaldehyde, and/or propionaldehyde has been reported in diethyl ether and ethyl acetate (Beckett et al., 1978; TOXI-NEWS, 1996). Formaldehyde is also present in some common grades of methanol. During the analysis of ephedrine, while using diethyl ether as an extraction solvent, as many as three peaks corresponding to oxazolidines of these three aldehydes were observed by Beckett et al. (1978). Similarly, acetaldehyde as a contaminant in ethyl acetate has been reported to potentially react with ephedrine, pseudoephedrine, and phenylpropanolamine, thereby potentially affecting their analyses (TOXI-NEWS, 1996). It appears that a chemical, such as methanol, containing

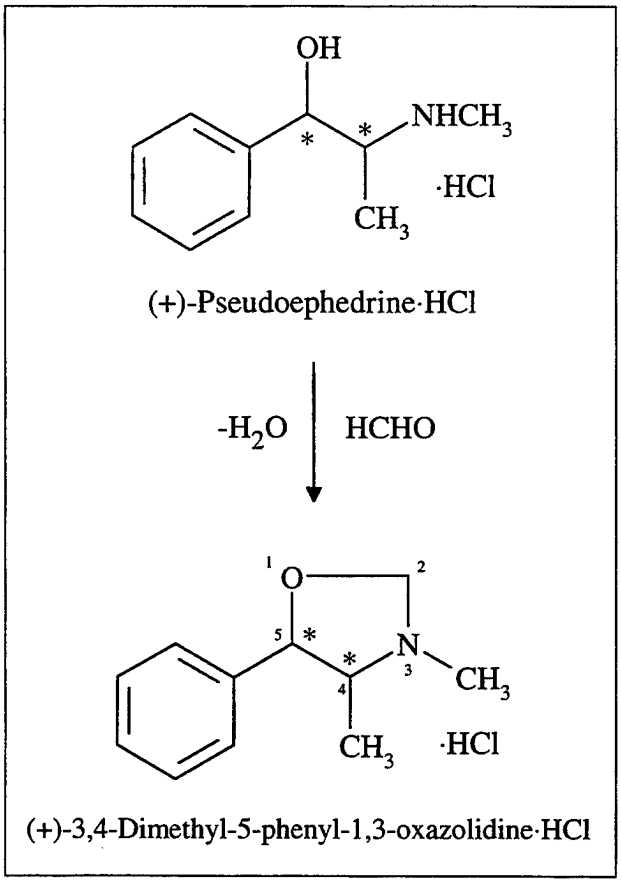

FIG. 1—The chemical scheme for the synthesis of the oxazolidine using (+)-pseudoephedrine·HCl and formaldehyde (HCHO). The asterisks indicate chiral centers (asymmetric carbons) in the molecules.

formaldehyde as an impurity might have been used during the pseudoephedrine urinalysis, causing the formation of the oxazolidine.

In view of the initial spectral findings and the potential aldehydic impurity, the oxazolidine was synthesized using pseudoephedrine and formaldehyde. Furthermore, analytical and chemical properties of the synthesized oxazolidine were compared with those of pseudoephedrine to elucidate the chemical structure of the unknown interfering substance.

MATERIALS AND METHODS

Materials

All reagents and solvents were of analytical grade and were of the highest available purity. These chemicals, standards, and other agents were obtained from commercial sources. (+)-Pseudoephedrine as hydrochloric acid salt was supplied by Sigma Chemical Co., St. Louis, MO. Supplies for the thin layer chromatography were provided by TOXI-LAB, Inc., Irvine, CA. CS ChemOffice software for the calculation of theoretical NMR spectra was supplied by CambridgeSoft Cambridge, MA.

Oxazolidine—(+)-3,4-Dimethyl-5-phenyl-1,3-oxazolidine·HCl was synthesized (Fig. 1) in our laboratory and characterized by its melting point, elemental analyses (Galbraith Laboratories, Inc., Knoxville, TN), and spectral analyses. To a 50 mL portion of methanol, 2.0 g (9.9 mmol) of (+)-pseudoephedrine·HCl and 1.2 g (40 mmol) of formaldehyde (3 mL of 37%) solution were added. This homogeneous mixture was then stirred for 15 min at the ambient temperature. After stirring, the volume of the mixture was reduced to 2-3 mL, using a 40°C water bath and a stream of nitrogen gas. To this concentrate, 2 mL of water was added, and the solution was extracted three times with 8 mL aliquots of chloroform. After drying over sodium sulfate, the chloroform extract was transferred and then evaporated to dryness. The white solid was recrystallized from a diethyl ether-methanol solvent system. The product was filtered and dried under vacuum at 40°C for 10 min, yielding a white crystalline powder (1.7 g; 82%) melting at 173-174°C (dec.).

Screening

Thin layer chromatography: The urine sample was screened for the presence of drugs following the TOXI-LAB's standard recommended procedure for the analysis of basic drugs.

Gas chromatography: A 5-mL portion of urine was mixed with 500 ng of the internal standard propylamphetamine and was then subjected to liquid-liquid basic extraction with ammonium hydroxide (>10.00 pH) followed by extraction with 5 mL of chloroform. The chloroform layer was transferred to another test tube and was washed with 5 mL of 0.1N hydrochloric acid. The acidic aqueous layer was transferred and made basic (>10.00 pH) with ammonium hydroxide. To this mixture, an equal volume of chloroform was added and gently mixed. The chloroform layer was removed and evaporated down to approximately 25 µL using a stream of nitrogen. One µL of the evaporate was injected into a 5890 Model Series II Hewlett Packard gas chromatograph (GC), functioning under our standard laboratory GC screening conditions. The GC was equipped with flame ionization and nitrogen phosphorus detectors (FID/NPD). A crosslinked 5% phenyl methyl silicone column (15 m x 0.25-mm i.d.; 0.25-mm film thickness) was used.

Confirmatory Analysis

A reaccessioned urine sample was subjected to solid phase separation, following the manufacturer's (Bond Elut Certify™, Varian Sample Preparation Products, Harbor City, CA) recommended procedure for isolation of basic drugs. During this procedure, methanol was used in various steps. The eluates were evaporated and subjected to analyses using a Hewlett Packard 5890 Series II Gas Chromatograph equipped with a HP 5965B infrared detector (GC/FTIR) and using a Hewlett Packard 5890 Series II Gas Chromatograph in combination with a 5989A Mass Spectrometer (GC/MS). Further analysis was performed on the extract using the GC/MS PCI technique.

The column used for the GC/FTIR analysis was a Hewlett Packard HP1 crosslinked 100% methyl siloxane column (15 m x 0.32-mm i.d.; 1-mm film thickness). For the GC/MS analysis, a Hewlett Packard ULTRA 1 crosslinked 100% methyl siloxane

column (12 m x 0.2-mm i.d.; 0.33-mm film thickness) was used. For both analyses, the injection volume was 1 µL in the splitless mode, with a purge time of 0.5 min. The GC oven temperature was increased from 70°C to 160°C at 15°C/min and then to 290°C at 40°C/min. The final temperature of 290°C was maintained for 1.75 min, totaling an 11-min run time. Helium was the carrier gas with a flow of 1 mL/min. The injector temperature was maintained at 250°C. The transfer line was set at 280°C. The retention time of the oxazolidine was 6.4 min under the GC/FTIR conditions, whereas it was 4.07 min under the GC/MS conditions.

RESULTS AND DISCUSSION

Initial screening of the urine specimen by TOXI-LAB revealed a spot consistent with the characteristic Rf value (≈ 0.14) and the color of pseudoephedrine/ephedrine. Based on the retention time, the GC/FID/NPD analysis of the liquid-liquid extract further concluded that pseudoephedrine was present in the specimen. The retention time for the analyte of interest from the solid-phase extract matched that of pseudoephedrine during the GC/FTIR and GC/MS analyses. Gas chromatography-Fourier transform infrared spectrometry (GC/FTIR) revealed the presence of characteristic absorptions of aromatic C–H, monosubstituted benzene, $-CH_2-$, $-CH_3$, and C–O–C groups. The characteristic pseudoephedrine O–H stretch at 3600 cm^{-1} and the N–H stretch at 3400 cm^{-1} were absent in the analyte spectrum (Figs. 2A & 2B). However, a strong absorbance associated with an ether (C-O-C) stretch at 1100 cm^{-1} was observed (Fig. 2A). The gas chromatography-mass spectrometry (GC/MS) electron ionization (EI) spectra disclosed a base peak of 71 amu and positive chemical ionization (PCI) gave a molar mass of 178 (M + 1). As is shown in Fig. 3A, the unknown substance has a mass spectrum with a base peak of 71 amu, whereas pseudoephedrine has a base peak at 58 amu (Fig. 3B). PCI mass spectrometry of the unknown substance exhibited a mass of 178 amu (M + 1), indicating its actual molar mass to be 177 amu (Fig. 4), which is 12 amu more than the molar mass of pseudoephedrine (165 amu). The synthesized compound had similar spectral characteristics as that of the unknown, interfering substance. The ^{1}H and ^{13}C nuclear magnetic resonance spectra obtained at the University of Southern Mississippi, Hattiesburg, MS, were consistent with the nine different peaks of protons and carbons corresponding to the oxazolidine molecule (Fig. 5). The calculated elemental analysis for the artifact, $C_{11}H_{16}ClNO$, is: C, 61.82%; H, 7.55%; N, 6.55% and the observed elemental analysis is: C, 61.44%; H, 7.61%; N, 6.55%. The Mass Spec for MS (70 eV) is M/Z (relative intensity): 71 (100%); 56 (26%); 91 (10%); 117 (7%).

Findings from this study revealed that the presence of formaldehyde as an impurity in the chemicals used for analysis has a potential to form a substance that could interfere with the pseudoephedrine analysis. Identified as 3,4-dimethyl-5-phenyl-1,3-oxazolidine (Fig. 1), the substance is formed by a reaction between formaldehyde and pseudoephedrine, involving the –OH and –NH– groups of pseudoephedrine. This type of reversible reaction has been reported with ephedrine-like amines and aldehydes, forming oxazolidines (Neelakantan, 1971; Beckett, Jones, and Hollingsbee., 1978; Nishiyama and Yamada, 1989; and Walker and Wood, 1992). The reaction is a condensation between an aldehyde and a secondary amine leading to the formation of an intermediate iminium ion, which subsequently reacts with an active hydrogen atom forming an oxazolidine. Since pseudoephedrine has an –NH– group (a secondary amine) as well as an –OH group (an active hydrogen atom), the reaction could be referred to as an intramolecular reaction, leading to the formation of the five-membered cyclic oxazolidine with a 12-amu increase (Fig. 1). In this reaction, the chirality of the pseudoephedrine's two asymmetric carbons does not change, suggesting that the stereochemistry of the formed compound remains the same as that of the starting material. In those SMAs—for example, amphetamine and methamphetamine—wherein the –OH group is absent, the formation of the oxazolidines is not possible.

Findings from this study clearly emphasize the importance of using high-quality reagents and of the identification of unknowns by multi-analytical approaches, including spectral analyses. The presence of contaminants in extraction solvents, or in any other chemical, can result in a false negative or false positive analytical finding. If the substance formed from the contaminant is a known drug, then the findings could be misleading. Therefore, it is prudent that chemicals of known purity be used during the drug analysis to maintain a high degree of quality assurance for a laboratory.

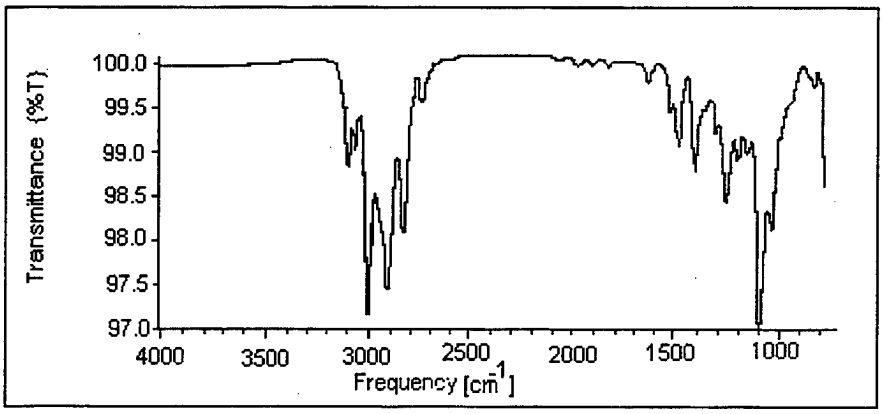

FIG. 2A—The infrared spectrum obtained in the process of the urine extract GC/FTIR analysis.

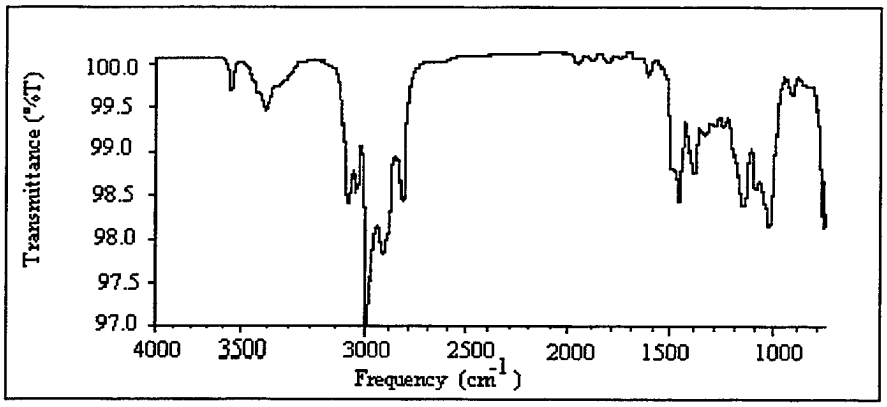

FIG. 2B—The infrared spectrum of (+)-pseudoephedrine subjected to the GC/FTIR analysis.

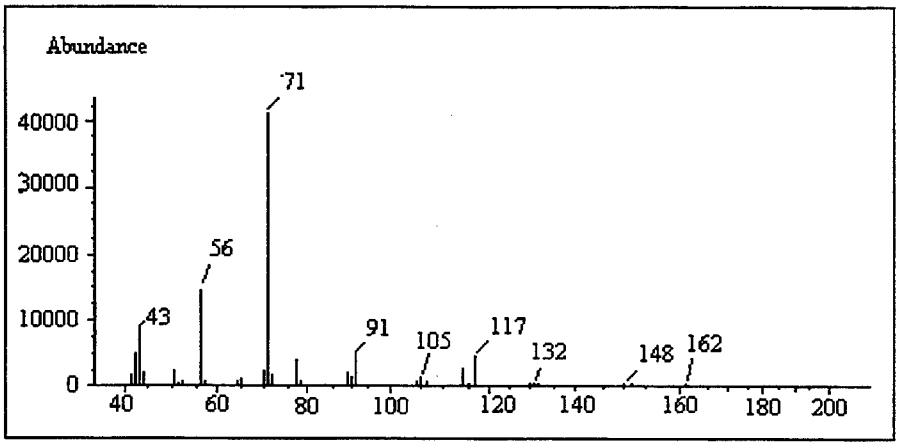

FIG. 3A—The mass spectrum obtained after the GC/MS analysis of the urine extract.

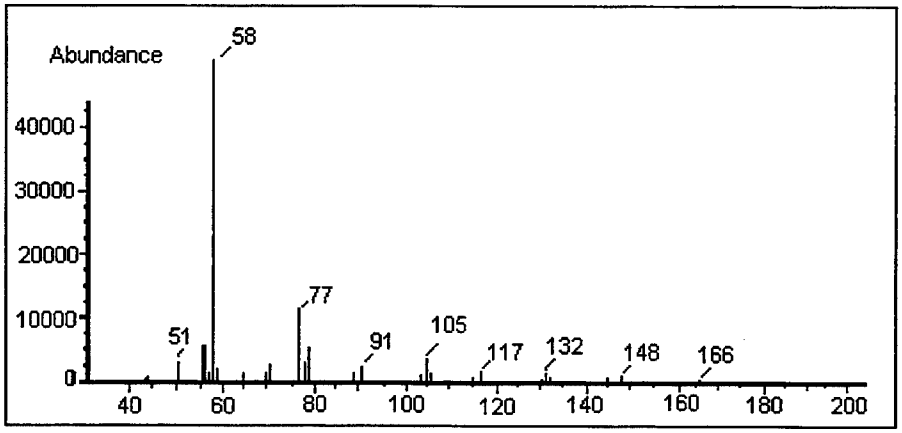

FIG. 3B—The mass spectrum of (+)-pseudoephedrine subjected to the GC/MS analysis.

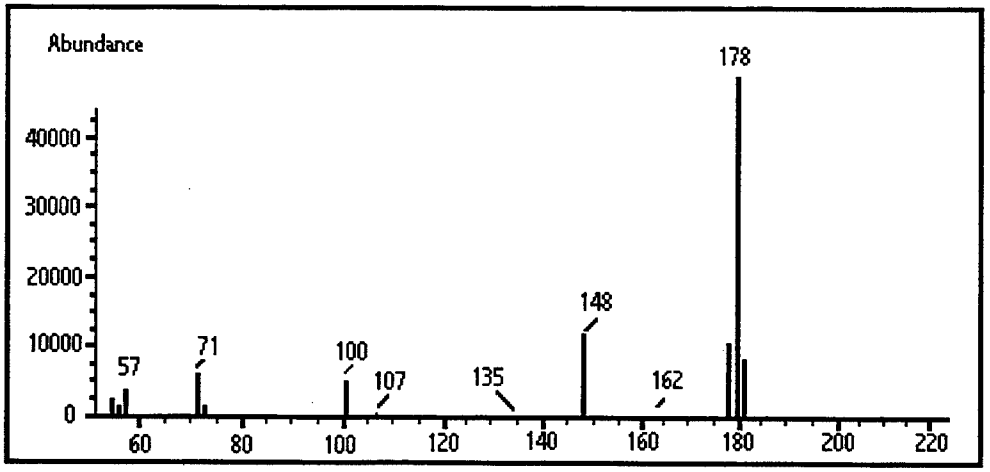

FIG. 4—The PCI-based GC mass spectrum obtained for the urine extract.

Carbon 13 NMR in DMSO

Atom	Observed (PPM)	Calculated (PPM)
C(3)	10.6	12.6
C(1)	35.6	32.8
C(2)	66.7	68.4
C(5)	84.0	85.6
C(4)	85.3	91.7
C(9)	127.1	125.8
C(7)	128.5	128.3
C(8)	129.2	128.6
C(6)	135.9	138.8

NMR in DMSO

Atom	Observed (PPM)	Calculated (PPM)
CH_3(3)	1.00	1.10
CH_3(1)	2.12	2.27
CH(2)	3.10	3.50
CH(5)	4.45	4.35
CH(4)	4.48	4.44
CH(9)	7.04	7.19
CH(7)	7.06	7.19
CH(8)	7.11	7.19

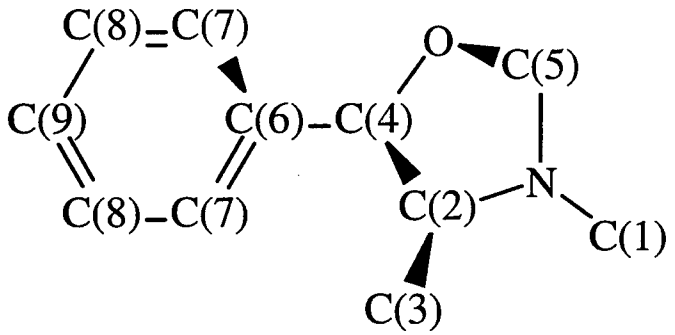

FIG. 5—Comparison of observed NMR spectra with calculated NMR spectra.

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