TECHNICAL NOTE

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Formation of Trifluoroacetylated Ephedrine During the Analysis of a Pseudoephedrine-Formaldehyde Adduct by TFAA Derivatization Followed by GC-MS

ABSTRACT: (+)-Pseudoephedrine reacts with formaldehyde to form (4*S*,5*S*)-3,4-dimethyl-5-phenyloxazolidine. Gas chromatography–mass spectrometry (GC-MS) analysis after the reaction of this oxazolidine with excess trifluoroacetic acid anhydride (TFAA) shows predominantly *N,O*-bis(trifluoroacetyl)pseudoephedrine with some of the monotrifluoroacetylated derivative. In addition, variable amounts of *N,O*-bis(trifluoroacetyl)ephedrine were detected by GC-MS. *N,O*-bis(trifluoroacetyl)ephedrine was not detected upon trifluoroacetylation of the source (+)-pseudoephedrine, and nuclear magnetic resonance analysis of the (4*S*,5*S*)-3,4-dimethyl-5-phenyloxazolidine showed no evidence of the (4*R*,5*S*) isomer. This suggests that the *N*,O-bis(trifluoroacetyl)ephedrine is formed by epimerization during the TFAA derivatization and GC-MS analysis of the pseudoephedrine-formaldehyde adduct.

KEYWORDS: forensic science, gas chromatography–mass spectrometry, pseudoephedrine, ephedrine, trifluoroacetylation, artifact

Ephedrine and pseudoephedrine are diastereoisomers that are common precursors for the preparation of *S*-methamphetamine in a clandestine methamphetamine laboratory (Fig. 1) (1,2). Gas chromatography–mass spectrometry (GC-MS) analysis of organic material at a clandestine laboratory does not just determine the presence of these drugs but can also indicate the potential source of the precursors and the route of methamphetamine synthesis being used. Methamphetamine and pseudoephedrine levels can indicate the degree of surface contamination or clean-up level during the remediation of a clandestine laboratory (3,4).

Derivatisation of pseudoephedrine prior to trace analysis by GC-MS is desirable for two reasons. First, underivatized pseudoephedrine (in either its free base or hydrochloride form) gives tailing peaks on common GC-MS columns, making peak integration difficult, particularly at low concentrations (5). This leads to a high limit of detection for underivatized pseudoephedrine. Second, the base peak in the mass spectrum of pseudoephedrine is at m/z 58 with no major peaks at m/z values >91, a pattern which is also seen for other amines such as methamphetamine and phentermine (6,7). Appropriate derivatization can provide improved separation and more discriminatory mass spectra (7–10). Perfluoro acid anhydrides such as trifluoroacetic acid anhydride (TFAA) are commonly used to derivatize basic drugs, including pseudoephedrine, prior to GC-MS analysis (10–13). As pseudoephedrine has an amine and a hydroxyl group, derivatization with excess TFAA forms *N,O*-bis-(trifluoroacetyl)pseudoephedrine while the *N*-mono-trifluoroacetyl derivative can form under incomplete reaction conditions (12). Excess perfluoro acid anhydride can cause high background levels in GC-MS, and can contaminate or degrade the stationary phase of

the column. Removal of the excess derivatizing agent can be accomplished either by drying the samples thoroughly prior to adding the GC-MS solvent or washing the samples with a mild base solution.

Several articles have reported that ephedrine or pseudoephedrine can react with aldehydes or ketones to form oxazolidines (14–17) which can be detected when underivatized pseudoephedrine or ephedrine are analyzed by GC-MS. The oxazolidines have almost identical retention times to the parent drugs, but their mass spectra show a base ion at m/z 71 rather than the m/z 58 characteristic of the parent drugs (15,16). This altered GC-MS behavior was reported by Lambert et al. (16) to have led to the misidentification of ephedrine as phenmetrazine. Those authors suspected that solvent contamination by formaldehyde could have led to the production of the oxazolidine (16). Similarly, Lewis et al. (15) suggested that the presence of formaldehyde in solvents or specimens during pseudoephedrine urinalysis leads to oxazolidine formation. Formaldehyde is a ubiquitous contaminant, and so samples of pseudoephedrine recovered from surfaces within a clandestine laboratory may include the formaldehyde adduct.

We now report that under some circumstances, GC-MS analysis of this pseudoephedrine-formaldehyde adduct ([4*S*,5*S*]-3,4-dimethyl-5-phenyloxazolidine) following TFAA derivatization shows the presence of *N,O*-bis(trifluoroacetyl)ephedrine in addition to the expected *N,O*-bis(trifluoroacetyl)pseudoephedrine.

Materials and Methods

All the solvents used were of at least either analytical or HPLC grade. The nonrestricted chemicals were obtained from Aldrich (Milwaukee, WI), Scharlau (Barcelona, Spain), and Supelco (Bellefonte, PA), and were 98% purity or better. *S,R*-Pseudoephedrine hydrochloride (99% purity) and *S,S*-ephedrine hydrochloride (99% purity) were obtained from ESR Ltd (Auckland, New Zealand).

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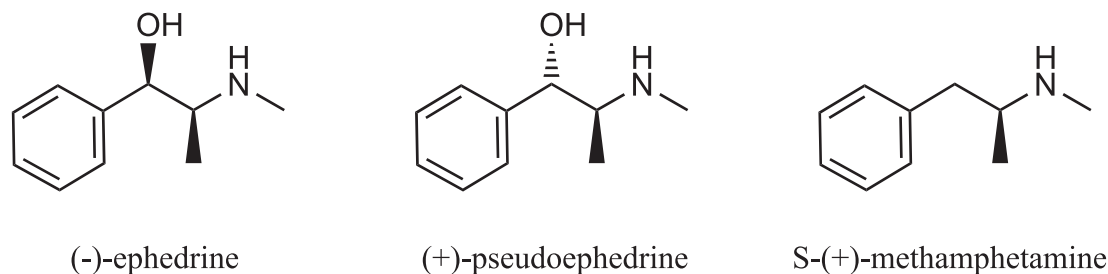
FIG. 1—*Structures of (−)-ephedrine, (+)-pseudoephedrine, and S-(+)-methamphetamine.*

A solution containing 20 μL n-tetradecane, C-14 (Supelco) in 50 mL n-heptane was used as internal standard.

The pseudoephedrine-formaldehyde derivative ([4S,5S]-3,4 dimethyl-5-phenyloxazolidine) was prepared based on the method reported by Lewis et al. (15). (+)-Pseudoephedrine hydrochloride (0.20 g) and formaldehyde (0.3 mL of 37% solution) were added to methanol (5 mL, HPLC grade) in a 10-mL round bottom flask, then the flask was capped and the mixture stirred for 15 min at room temperature. The volume of the solution was carefully reduced to about 0.3 mL under a stream of nitrogen at 30°C. To this solution, 0.5 mL milli-Q water was added, then the solution was extracted three times with 1 mL aliquots of dichloromethane (HPLC grade). The dichloromethane extract was dried over anhydrous sodium sulfate, then evaporated at 26°C in a heating block using a stream of nitrogen to give a white solid, which was recrystallized by vapor diffusion of diethyl ether into a methanol solution. The white crystalline hydrochloride salt which formed was filtered, and then dried under vacuum. Elemental analysis (Campbell Microanalysis Laboratory, University of Otago): calculated % for $C_{11}H_{16}ClNO$: C: 61.82; H: 7.55; N: 6.55. Found %: C: 62.01, 62.09; H: 7.55, 7.89; N: 6.54, 6.55. Nuclear magnetic resonance (NMR Centre, University of Auckland): ^1H NMR (in d^6-dimethylsulfoxide [d^6-DMSO], given as chemical shift, no. of H, multiplicity, coupling constants, identity): δ11.5, 1H, broad, NH; δ7.4–7.3, 5H, multiplet, phenyl; δ5.08 and 4.90 2H, broad, $H_{c,d}$; δ4.83 1H, doublet, 12 Hz, H_a; δ3.44, 1H, multiplet, H_b; δ2.89, 3H, broad, N-CH_3; δ1.37, 3H, doublet, 8.6 Hz, CH_3 (see Fig. 2).

The ephedrine analog ([4R,5S]-3,4-dimethyl-5-phenyloxazolidine) was prepared in a similar manner. However, it was more susceptible to hydrolysis, so was only characterized by ^1H NMR and GC-MS. ^1H NMR (d^6-DMSO): δ11.1, 1H, broad, NH; δ7.4–7.3, 5H, multiplet, phenyl; δ5.08, 1H, doublet, 7 Hz, H_a; δ5.3 and 4.4, 2H, broad, $H_{c,d}$; δ2.9, 3H, singlet, N-CH_3; δ0.88, 3H, doublet, 6.6 Hz, H_b.

A small amount of pseudoephedrine or (4S,5S)-3,4-dimethyl-5-phenyloxazolidine dissolved in 10 μL of dichloromethane was transferred to a GC vial, followed by 50 μL of ethyl acetate and 25 μL TFAA, then the vial was capped and incubated at 38°C for 1 h. The solvent and excess TFAA were evaporated off, then the sample was reconstituted with 1 mL ethyl acetate and 20 μL of C-14 in n-heptane was added as internal standard. The sample was shaken for 5 min and flushed with nitrogen prior to GC-MS analysis. N-trifluoroacetylmethamphetamine was prepared in an analogous manner.

GC-MS analysis was carried out on a HP 6890 GC equipped with a HP5973 mass selective detector (MSD), HP 7683B automatic sampler, and HP-5MS capillary column (30.0 m × 0.25 mm × 0.25-μm film thickness; Agilent Technologies, Inc., Santa Clara, CA). Helium was used as carrier gas with a flow rate of 1 mL/min. The injector temperature was set at 250°C and the interface temperature was set at 280°C. The oven temperature was programmed as

follows: an initial temperature of 70°C was held for 2 min, followed by an increase of 20°C/min to 280°C which was held for 2 min. The samples (1 μL) were introduced in splitless mode. Mass spectra were acquired in scan mode from m/z 41–500.

Results and Discussion

GC-MS analysis of underivatized pseudoephedrine gave a single peak with a retention time of 7.82 min under our analytical conditions. The mass spectrum of this peak is dominated by an ion at m/z 58 as a result of alpha-cleavage of the pseudoephedrine molecular ion. This base peak is characteristic of molecules such as pseudoephedrine, ephedrine, methcathinone, and methamphetamine which share a similar structure around the nitrogen atom (6).

The GC-MS chromatogram of a sample of pseudoephedrine which had been exposed to formaldehyde showed a peak at a retention time of 7.78 min, which is extremely close to the retention time of unaltered pseudoephedrine. However, this peak is much sharper and more symmetrical than that for pseudoephedrine, and the base peak in the mass spectrum is at m/z 71, with the next most intense peak at m/z 56 (15,16). As noted by earlier authors, this chromatographic peak was because of the compound (4S,5S)-3,4-dimethyl-5-phenyloxazolidine, which is the cyclic product formed from pseudoephedrine and formaldehyde (15,16). The reaction pathway for the formation of this pseudoephedrine-formaldehyde adduct is shown in Fig. 3. We have found that this formaldehyde adduct is often detected when wipe-sampling very low surface concentrations (<100 μg/100 cm^2) of pseudoephedrine. The presence of the oxazolidine may be due to the trace pseudoephedrine reacting with formaldehyde in the air, it may occur because of formaldehyde contamination in the wiping media or solvents, or it can also form by reaction with methanol in the injector port of the GC-MS if this is used as the injection solvent (5). The ephedrine-formaldehyde adduct, (4R,5S)-3,4-dimethyl-5-phenyloxazolidine, gave a similar
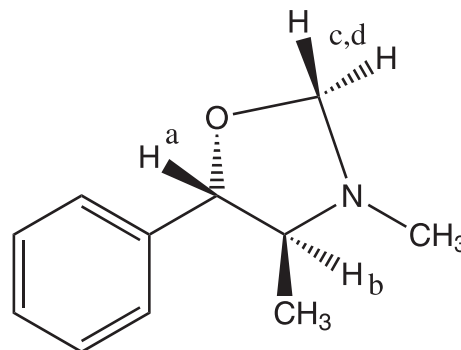

FIG. 2—*Nuclear magnetic resonance atom numbering for 3,4–dimethyl-5-phenyl-1,3-oxazolidine.*
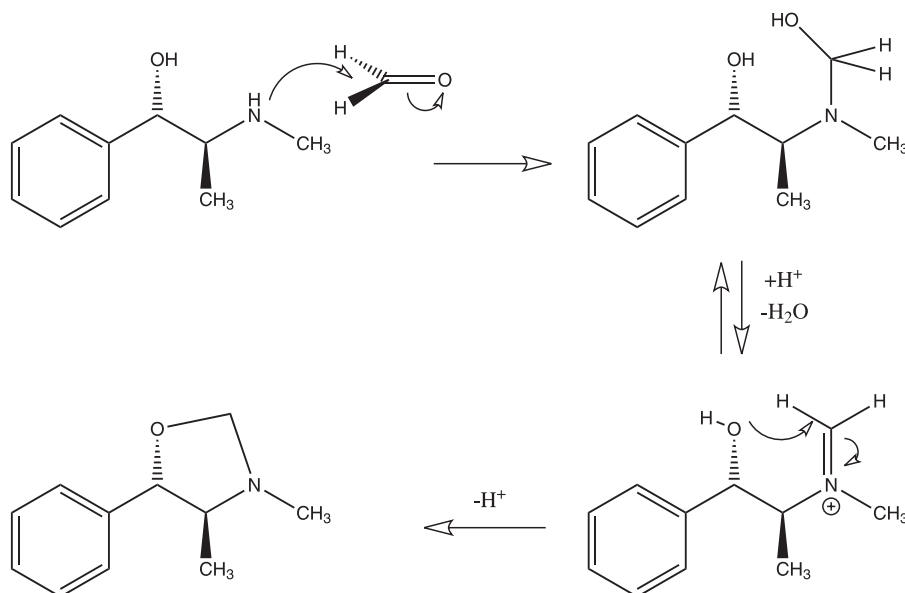
FIG. 3—*Reaction pathway for formation of 3,4-dimethyl-5-phenyloxazolidine from pseudoephedrine and formaldehyde.*

GC-MS retention time and mass spectrum as that seen for the pseudoephedrine-formaldehyde adduct.

A common strategy to improve the GC-MS elution, detection, and identification of basic drugs is to derivatize them with TFAA. When pseudoephedrine was reacted with excess TFAA in ethyl acetate at 38°C for 1 h, it formed *N,O*-bis(trifluoroacetyl)pseudo-ephedrine which gave a sharp GC-MS peak at 8.24 min, with no evidence for monotrifluoroacetylated pseudoephedrine or ephedrine derivatives. The mass spectrum of this compound had a base ion at m/z 154 resulting from the β-cleavage between C-1 and C-2 of the *N*-trifluoroacetylated pseudoephedrine side chain (12). The molecular ion at m/z 338 was only present in low abundance, with other ions observed at higher abundance being at m/z 110, 69, and 244 (12,18). Some *N*-(trifluoroacetyl)pseudoephedrine (the only detected monosubstituted derivative) could be observed under conditions where the derivatization of pseudoephedrine did not go to completion, such as by using less TFAA, a lower temperature, or a shorter derivatization time. This compound had a retention time of 9.10 min, and had a base peak in its mass spectrum at m/z 155.

As part of a study on the analysis of trace levels of pseudoephedrine, we performed the TFAA derivatization and GC-MS analysis on a sample of pseudoephedrine that had been exposed to formaldehyde. Investigations showed several potential sources of formaldehyde, including the laboratory air and the filter paper used to collect the sample (5). This sample gave an additional peak at 7.89 min as well as the expected peaks noted above. We therefore synthesized the pseudoephedrine-formaldehyde adduct, (4*S*,5*S*)-3, 4-dimethyl-5-phenyloxazolidine, to help identify the source of this additional peak. The oxazolidine was prepared according to the literature, and isolated as a crystalline solid that was characterized by elemental analysis and NMR. Examination of the ¹H NMR for a d⁶-DMSO solution of (4*S*,5*S*)-3,4-dimethyl-5-phenyloxazolidine in the region characteristic of the benzylic proton (H$_a$) showed no signal attributable to the *R,S*-isomer at 5.1 ppm and similarly, no signal attributable to the benzylic hydrogen of the *S,S* isomer (at 4.8 ppm) was observed for the (4*R*,5*S*)-3,4-dimethyl-5-phenyloxazolidine prepared from ephedrine. The methyl and ring methylene protons of the diastereoisomers are also distinctive, and showed that the ring formation occurs with retention of stereochemistry at the chiral carbons.

When (4*S*,5*S*)-3, 4-dimethyl-5-phenyloxazolidine was reacted with TFAA for 1 h at 38 or 65°C, followed by solvent removal and reconstitution in ethyl acetate, the major peaks seen in the GC-MS chromatogram (Fig. 4) were at 7.89 min, 8.24 min (*N,O*-bis[trifluoroacetyl]pseudoephedrine), and 9.10 min (*N*-trifluoroacetyl pseudoephedrine), with no evidence of unreacted (4*S*,5*S*)-3, 4-dimethyl-5-phenyloxazolidine (7.78 min) (12,19). The presence of monotrifluoroacetylated pseudoephedrine in the chromatogram, even when the oxazolidine was allowed to react with excess TFAA for 24 h at 38°C, shows that the conversion to *N,O* di-trifluoro-acetyl pseudoephedrine does not go to completion.

The mass spectrum of the compound with retention time 7.89 min (Fig. 5) was almost identical to that for *N,O*-bis(trifluoro-acetyl) pseudoephedrine. The base ion was at m/z 154, which as noted earlier is characteristic of *N*-trifluoroacetylation (12,19). The mass spectrum also has a small peak at m/z 244 which is a characteristic of *O*-trifluoroacetylation of pseudoephedrine-like compounds (12,19).

The retention time of 7.89 min is slightly less than that for *N,O*-bis(trifluoroacetyl)pseudoephedrine (8.24 min) and much less than that for *N*-mono-(trifluoroacetyl)pseudoephedrine (9.10 min) suggesting that the compound does not have a free OH, and that it is more similar to *N,O*-bis(trifluoroacetyl)pseudoephedrine. The retention time is also very slightly earlier than *N*-trifluoroacetylmetham-phetamine (7.95 min). Based on the mass spectral evidence and the relative elution behavior of TFAA derivatives of methamphetamine and ephedrine reported by Lin and Lua (19), we hypothesized that this peak was due to *N,O*-bis(trifluoroacetyl)ephedrine. Epimerization of *N*-trifluoroacetylated derivatives of norephedrine have been reported (20), which provides precedent for this proposal.

When a genuine sample of ephedrine was derivatized with TFAA and analyzed by GC-MS, *N,O*-bis(trifluoroacetyl)ephedrine eluted with a retention time of 7.89 min, supporting this hypothesis. We then confirmed that *N,O*-bis(trifluoroacetyl)ephedrine was not observed in the GC-MS chromatogram of a TFAA-derivatized sample of the pseudoephedrine used to prepare the formaldehyde adduct. As noted earlier, ¹H NMR of the pseudoephedrine-formaldehyde adduct ([4*S*,5*S*]-3,4-dimethyl-5-phenyloxazolidine) showed no evidence for the ephedrine-formaldehyde adduct (4*R*,5*S* isomer).
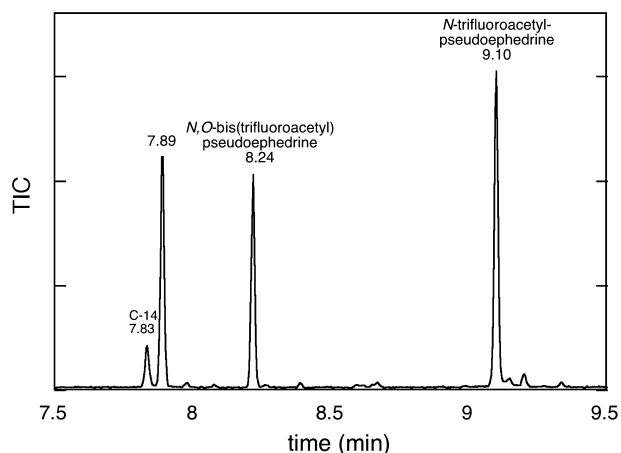
FIG. 4—*GC-MS chromatogram of the solution obtained after TFAA derivatization of (S,S)-3, 4-dimethyl-5-phenyloxazolidine.*
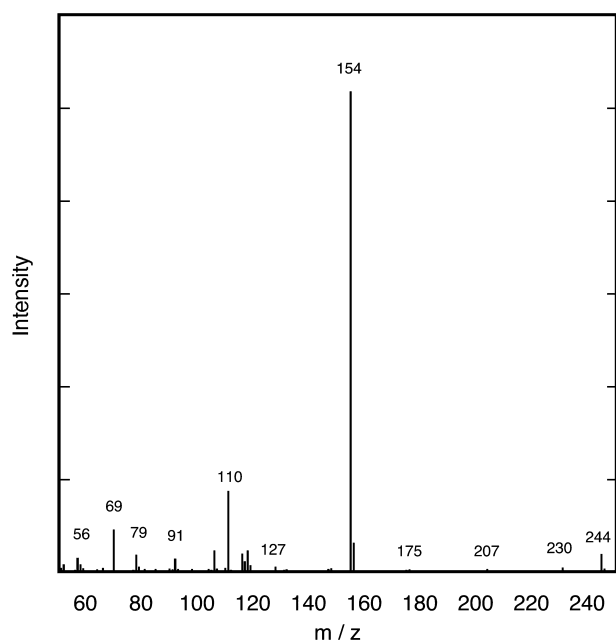

FIG. 5—*Mass spectrum of the peak eluting at 7.89 min during GC-MS analysis of the products from the reaction of (S,S)-3,4-dimethyl-5-phenyloxazolidine with TFAA.*

Therefore, the presence of N,O-bis(trifluoroacetyl)ephedrine in a TFAA-derivatized sample of the pseudoephedrine-formaldehyde adduct must result from epimerization during the derivatization and GC-MS analysis.

Conclusion

Analysis of underivatized pseudoephedrine and pseudoephedrine is possible for bulk samples, however it is not suitable for trace samples since it is too insensitive and is susceptible to formation of impurities such as the oxazolidines formed from pseudoephedrine in the presence of carbonyl compounds. TFAA derivatization of pseudoephedrine forms N,O-bis(trifluoroacetyl) pseudoephedrine, and also N-mono-trifluoroacetyl pseudoephedrine in the case of incomplete derivatization. In the presence of

formaldehyde, pseudoephedrine forms (4S,5S)-3, 4-dimethyl-5-phenyloxazolidine. Upon reaction with TFAA, this compound forms N,O-bis(trifluoroacetyl)pseudoephedrine, but N-mono-trifluoroacetyl pseudoephedrine is also detected even when TFAA is in excess and extended reaction times are used. In addition, variable amounts of N,O-bis(trifluoroacetyl)ephedrine are also observed which results from epimerization during the derivatization and analysis process. The reverse epimerization is also possible, so that pseudoephedrine could appear as an artifact in an ephedrine sample. Therefore, care must be taken when interpreting TFAA-derivatized pseudoephedrine or ephedrine GC-MS chromatograms if formaldehyde contamination is possible.

References

1. Skinner HF. Methamphetamine synthesis via HI / red phosphorus reduction of ephedrine. Forensic Sci Int 1990;48:123–34.
2. Barker WD, Antia A. A study of the use of Ephedra in the manufacture of methamphetamine. Forensic Sci Int 2007;166:102–9.
3. Sacramento County Environmental Management Department. Criteria for the assessment and remediation of clandestine methamphetamine laboratories. http://www.emd.saccounty.net/Documents/Info/SAC%20-%20 Criteria%20for%20Clan%20Lab%20Remediation.pdf. Accessed December 18, 2008.
4. Minnesota Department of Health. Clandestine drug lab general cleanup guidance. http://www.health.state.mn.us/divs/eh/meth/lab/guidance0407.pdf. Accessed December 18, 2008.
5. Abdullah AFL. Quantitation of trace residues in clandestine laboratory investigation and remediation. [dissertation]. Auckland (New Zealand): The University of Auckland, 2008.
6. Smith RM. Understanding mass spectra: a basic approach, 2nd edn. Hoboken: Wiley Interscience, 2005.
7. Dasgupta A, Gardner C. Distinguishing amphetamine and methamphetamine from other interfering sympathomimetic amines after various fluoro derivatization and analysis by gas chromatography–chemical ionization mass spectrometry. J Forensic Sci 1995;40:1077–81.
8. Rohrich J, Kauert G. Determination of amphetamine and methylenedioxy-amphetamine derivatives in hair. Forensic Sci Int 1997;84:179–88.
9. Karl B, Halket JM. Handbook of derivatives for chromatography, 2nd edn. Chichester: John Wiley & Sons, 1993.
10. Kronstrand R, Jones AW. Drugs of abuse—analysis. In: Siegel JA, editor. Encyclopedia of forensic sciences. London: Academic Press, 2000;598–610.
11. Brettell TA. Analysis of N-mono-trifluoroacetyl derivatives of amphetamine analogues by gas chromatography and mass spectrometry. J Chromatogr 1983;257:45–52.
12. Coutts RT, Dawe R, Jones GR, Liu SF, Midha KK. Analysis of perfluoroacyl derivatives of ephedrine, pseudoephedrine and analogues by gas chromatography and mass spectrometry. J Chromatogr 1980;190:53–65.
13. Jones JB, Mell LD. A simple wash procedure for improving chromatography of HFAA derivatized amphetamine extracts for GC/MS analysis. J Anal Toxicol 1993;17:447.
14. Neelakantan L. Asymmetric synthesis II. Synthesis and absolute configuration of oxazolidines derived from (−)ephedrine and aromatic aldehydes. J Org Chem 1971;36:2256–60.
15. Lewis R. Formation of an interfering substance, 3,4-dimethyl-5-phenyl-1,3-oxazolidine, during a pseudoephedrine urinalysis. J Forensic Sci 2000;45:898–901.
16. Lambert EEW. Phenmetrazine or ephedrine? Fooled by library search. J Chromatogr A 2004;1045:259–62.
17. Beckett AH, Jones GR, Hollingsbee DA. Degradation of (−)-ephedrine in solution and during extraction with diethyl ether. J Pharm Pharmacol 1978;30:15–9.

18. Pfleger K, Maurer HH, Weber A. Mass spectral and GC data of drugs, poisons, pesticides, pollutants and their metabolites, 2nd edn. New York: VCH, 1992.
19. Lin HR, Lua AC. Simultaneous determination of amphetamines and ketamines in urine by gas chromatography–mass spectrometry. Rapid Commun Mass Spectrom 2006;20:1724–30.
20. Dufrasne F, Neve J. Synthesis of 1,2-diamino-1-phenylpropane diastereoisomers from u-N-trifluoroacetyl-2-amino-1-phenylpropan-1-ol. Monatsh Chem 2005;136:739–46.

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