TECHNICAL NOTE

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Distinguishing Amphetamine and Methamphetamine from Other Interfering Sympathomimetic Amines After Various Fluoro Derivatization and Analysis by Gas Chromatography-Chemical Ionization Mass Spectrometry

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ABSTRACT: Misidentification of ephedrine and pseudoephedrine as methamphetamine has been reported because of similar retention times of their derivatives in the gas chromatograph as well as very similar mass fragmentation pattern. This problem of misidentification is avoided by chemical ionization mass spectrometry using methane as the reagent gas. Chemical ionization mass spectral patterns of trifluoroacetyl, pentafluoropropionyl, heptafluorobutyryl and perfluorooctanoyl derivatives of amphetamine, methamphetamine, the internal standard d8-methamphetamine and 3,4-methylenedioxy methamphetamine were studied after extraction from human urine. The mass spectral patterns of all these drugs are distinctively different in chemical ionization mode from commonly interfering sympathomimetic amines, ephedrine, pseudoephedrine, phentermine and phenylpropanolamine.

KEYWORDS: forensic science, forensic toxicology, street drugs, amphetamine, methamphetamine

Amphetamines are central nervous system stimulants that produce alertness, wakefulness, increased energy and reduced hunger [*1,2*]. Amphetamine, methamphetamine and the designer drug 3, 4-methylenedioxymethamphetamine (MDMA) also called "Ecstasy" are commonly abused and have resulted in many deaths from overdoses [*3*]. Because amphetamines are abused widely, forensic drug testing for amphetamines is commonly done in both public and private sectors. The identification of those drugs is usually accomplished by basic extraction from urine, derivatization and Gas Chromatography/Mass Spectrometric (GC/MS) analysis. Unfortunately, other sympathomimetic amines which are widely used in many over the counter cold medications can be misidentified as amphetamine or methamphetamine causing serious medical or legal problems. Ephedrine and pseudoephedrine differ from methamphetamine by only the substitution of a hydroxyl group for the hydrogen on the alpha carbon atom while phentermine and methamphetamine are structural isomers and their underivatized mass spectra in the electron impact mode are almost identical. NIDA recently suspended the license of a certified laboratory for misidentification of methamphetamine. The interference may have been caused by the presence of ephedrine in the urine specimens [*4–6*]. Other sympathomimetic amines like pseudoephedrine, phentermine and phenylpropanolamine may cause false positive results in the GC/MS analysis of amphetamines in the conventional electron impact mode [*7*]. Recently, bench top models of GC/MS are available with chemical ionization capacity, a soft ionization technique. Now we report the elimination of misidentification of sympathomimetic amines as amphetamines through the use of chemical ionization mass spectra.

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Materials and Methods

Amphetamine and methamphetamine were purchased from Altech (State College, PA), and MDMA was kindly provided by the US Drug Enforcement Agency (Dallas, TX). Trifluoroacetic anhydride, pentafluoropropionic anhydride and heptafluorobutyric anhydride were obtained from Pierce (Rockford, IL) and perfluorooctanoyl chloride from PCR Incorporated (Gainesville, FL). To extract amphetamine, methamphetamine, MDMA and interfering sympathomimetic amines from urine, 5 mL of urine was supplemented with 50 μL of internal standard (d8 methamphetamine, 100 μg/mL) and was alkalinized with 1 mL of carbonate buffer (pH 9.0) and 1 mL of 1 N sodium hydroxide. After adding 4 mL of 1-chlorobutane, the sample was vortex-mixed for 1 min and then mixed for an additional 10 min in a rotating mixer. After centrifuging at 1500 g, the upper organic layer was transferred to a disposable 5 mL screw capped conical test tube. The solvent was evaporated under air at room temperature to approximately 100 μL volume. Then 100 μL of the appropriate derivatizing agent was added followed by incubation at 60°C for 15–30 min. For derivatization with perfluorooctanoyl chloride, amphetamines were extracted in cyclohexane instead of 1-chlorobutane as recom-

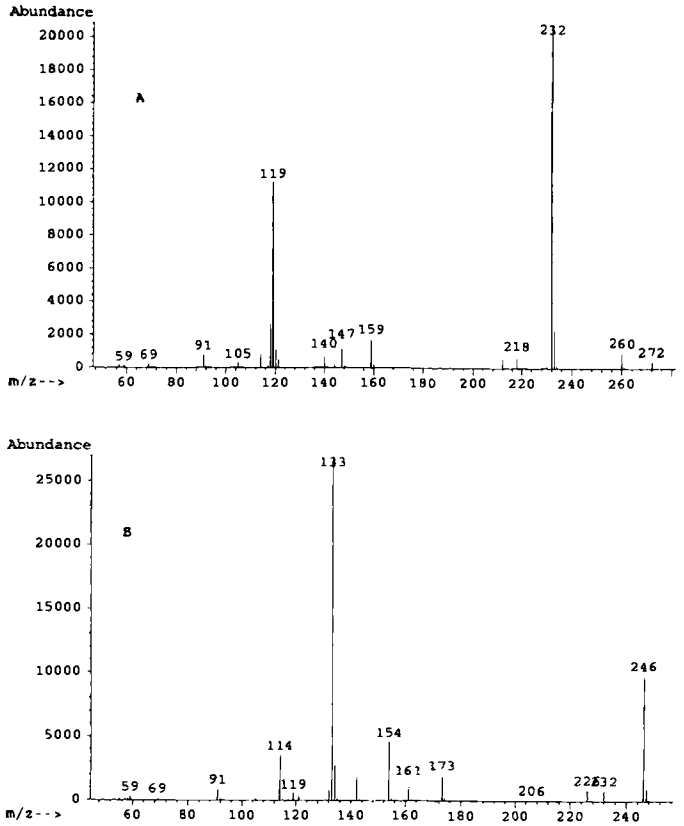

FIG. 1—*Chemical ionization mass spectral fragmentation pattern of trifluoroacetyl derivative of* (A) *amphetamine,* (B) *phentermine after extraction from human urine.*

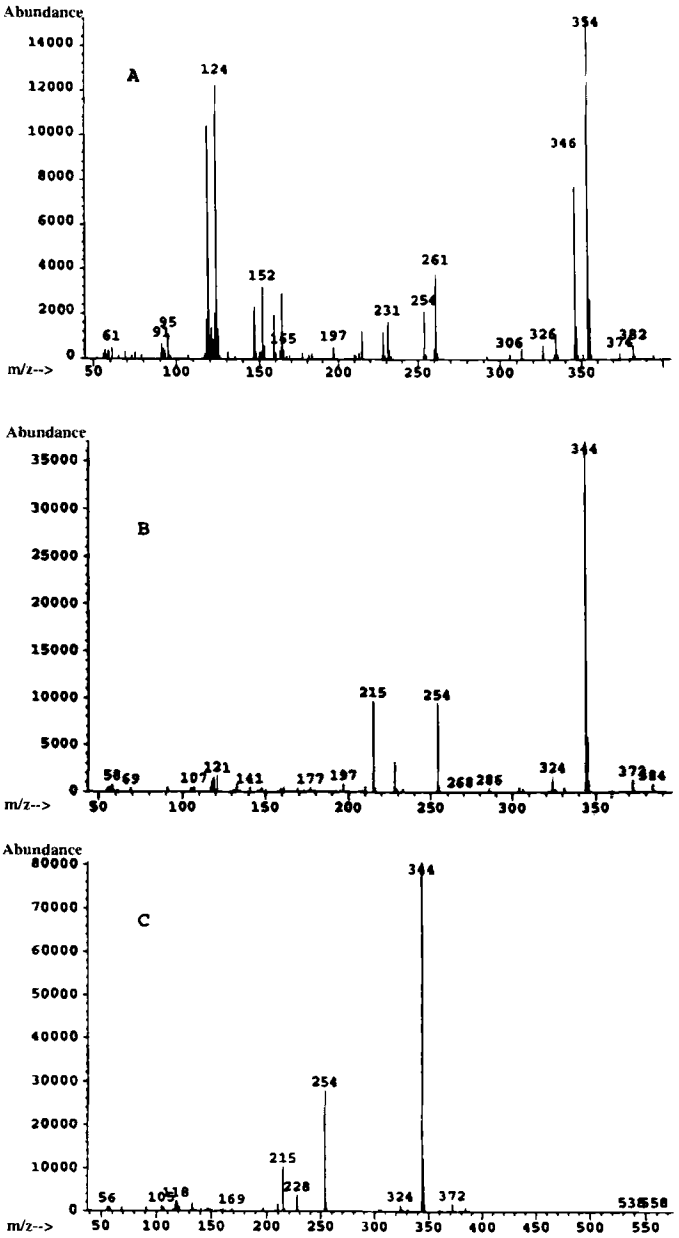

FIG. 2—*Chemical ionization mass spectral fragmentation pattern of heptafluorobutyryl derivatives of* (A) *methamphetamine along with internal standard* (B) *ephedrine* (C) *pseudoephedrine after extraction from human urine.*

mended by the authors [*8*]. For derivatization, 100 μL of perfluorooctanoyl chloride was added to the concentrated extract and the reaction mixture was incubated at 60°C for 30 min. After the reaction, excess derivatizing agent was evaporated to dryness, and the dry residue was reconstituted to 50 μL of methanol to destroy any remaining traces of derivatizing agent. Two microliter was injected into the GC/MS.

The Gas Chromatography-Mass Spectrometric analysis (GC/MS) was carried out by using a Model 5890 series II Gas Chromatograph coupled with a 5972 series Mass Selective Detector (Hewlett Packard, Palo Alto, CA). The mass spectrometer was used in the positive chemical ionization mode using methane as a reagent gas (scan 50–700 m/z). The capillary column used was an Ultra-2 also available from Hewlett Packard. The 25-m column with an internal diameter of 0.20 mm was coated with phenyl methylsilicone (0.33 μm thickness). For the analysis of trifluoroacetyl, pentafluoropropionyl and heptafluorobutyryl derivatives of amphetamines, the initial oven temperature was 100°C. After maintaining that temperature for 5 min, the oven temperature was raised at a rate of 10°C/min to reach an oven temperature of 170°C. Then the oven temperature was raised at a rate of 20°C/min to 290°C which was maintained for an additional 1 min. For the analysis of perfluorooctanoyl derivatives of amphetamines, the initial oven temperature was 140°C. After maintaining that temperature for 5 min, the oven temperature was increased at a rate of 10°C/min to 220°C. Then the oven temperature was raised at a rate of 20°C/min to reach a final temperature of 290°C. In both analyses, the solvent delay was 6 min and the injection port temperature was 180°C. We used splitless injection for all analysis.

Results and Discussions

In contrast to the electron impact spectra which showed very weak molecular ion peaks (relative abundances < 1%) for trifluoroacetyl, pentafluoropropionyl and heptafluorobutyryl derivatives of amphetamines and methamphetamine, chemical ionization spectra using methane as a reagent gas showed the protonated molecular ion as the base peaks (relative abundance 100%) for all three fluoro derivatives of amphetamine and methamphetamine. As expected, the internal standard, d8-methamphetamine, also showed the protonated molecular ion as the base peak which was 8 amμ higher

than methamphetamine. This dramatic change in the mass spectral fragmentation pattern in the chemical ionization mode results in unambiguous identification of amphetamine and methamphetamine extracted from human urine. We used the scan mode for our chemical ionization mass spectral analysis. Using 5 mL of urine, we obtained excellent mass spectral quality in the scan mode for amphetamine concentration as low as 300 ng/mL. We considered a peak as amphetamine or methamphetamine if the retention time matched within 10% of the reference peak and the spectrum of the analyte matched with the reference spectrum of the amphetamine or methamphetamine (matching quality of 8000 or higher where 10,000 is the ideal match).

Trifluoroacetyl Derivatives

The trifluoroacetyl derivative of amphetamine showed a protonated molecular ion at m/z 232 as the base peak and a strong peak at m/z 119 (Relative abundance 54.0%). The interfering compound phentermine, which was also derivatized under that condition and eluted immediately after amphetamine, however showed a base peak at m/z 133 and a strong protonated molecular ion at m/z 246 (Relative abundance 48.2%), thus eliminating the possibility of any misidentification. The trifluoroacetyl derivative of methamphetamine showed a protonated molecular ion at m/z 246 and a strong peak at m/z 119 (Relative abundance 46.8%). The trifluoroacetyl derivative of d8-methamphetamine showed a protonated molecular ion as the base peak at m/z 254 and a strong peak at m/z 124 as expected. When urine specimens supplemented with amphetamine, methamphetamine, MDMA, interfering sympathomimetic amine and internal standard were extracted and derivatized, methamphetamine and d8-methamphetamine were eluted together and both m/z 246 and m/z 254 peaks were present. The relative abundance of m/z 246 peak was roughly 50% of the m/z 254 peak when the urine was supplemented with 500 ng/mL of methamphetamine because the concentration of internal standard was 1000 ng/mL. Quantitation can be easily done by comparing abundance of m/z 254 peak of the internal standard with m/z 246 peak of methamphetamine in the average spectrum of the entire peak. The trifluoroacetyl derivatives of ephedrine and pseudoephedrine both showed a base peak at m/z 244 thus easily differentiating them from methamphetamine. Moreover, the peak at m/z 119 was relatively weak (Relative abundances 2.1% and 3.3%, respectively) and pseudoephedrine also showed peaks at m/z 256 and 276 further aiding in distinguishing them from methamphetamine (Table 1). In electron impact mode methamphetamine, ephedrine and pseudoephedrine all showed base peaks at m/z 154 and other similar mass spectral fragmentation patterns.

The trifluoroacetyl derivative of MDMA showed a protonated molecular ion peak at m/z 290 (Relative abundance 20.2%) and a base peak at m/z 163. The trifluoroacetyl derivative of phenylpropanolamine did not show any molecular ion peak but showed a base peak at m/z 230.

Pentafluoropropionyl Derivatives

As expected, pentafluoropropionyl derivative of amphetamine showed a protonated molecular ion peak as the base peak at m/z 282, which was 50 amμ more than the corresponding trifluoroacetyl derivative. The pentafluoropropionyl derivative of methamphetamine showed a protonated molecular ion peak as the base peak at m/z 296. The pentafluoropropionyl derivative of ephedrine and pseudoephedrine, both showed a base peak at m/z 294 again

TABLE 1—*Chemical ionization mass spectral characteristics of fluoro derivatives of amphetamine, methamphetamine, MDMA and sympathomimetic amines.*

	M + 1	Base	Other Peaks (Relative abundance)		
Trifluoroacetyl					
Amphetamine	232	232	140	119	91
	(100)	(100)	(4.5)	(54.0)	(4.6)
Methamphetamine	246	246	154	119	91
	(100)	(100)	(15.9)	(46.8)	(3.5)
MDMA	290	163	154	128	91
	(20.2)	(100)	(5.3)	(3.6)	(0.2)
Phentermine	246	133	173	154	114
	(48.2)	(100)	(7.6)	(18.3)	(9.6)
Ephedrine	—	244	276	256	148
		(100)	(31.1)	(25.5)	(36.1)
Pseudoephedrine	358	244	276	154	110
	(2.0)	(100)	(8.3)	(13.1)	(1.2)
Phenylpropanolamine	—	230	258	140	115
		(100)	(6.3)	(10.1)	(25.9)
Pentafluoropropionyl					
Amphetamine	282	282	190	119	91
	(100)	(100)	(2.5)	(44.2)	(2.5)
Methamphetamine	296	296	204	119	91
	(100)	(100)	(21.1)	(90.7)	(5.6)
MDMA	340	163	204	119	91
	(12.0)	(100)	(6.3)	(0.3)	(0.3)
Phentermine	296	133	204	164	121
	(20.8)	(100)	(9.4)	(32.0)	(28.3)
Ephedrine	—	294	326	306	204
		(100)	(9.1)	(6.6)	(4.0)
Pseudoephedrine	458	294	204	165	118
	(1.0)	(100)	(21.0)	(11.7)	(2.2)
Phenylpropanolamine	—	280	190	165	121
		(100)	(8.1)	(21.5)	(11.8)
Heptafluorobutyryl					
Amphetamine	332	332	240	119	91
	(100)	(100)	(6.0)	(69.8)	(6.2)
Methamphetamine	346	346	254	119	91
	(100)	(100)	(34.7)	(98.1)	(5.6)
MDMA	390	163	254	203	191
	(5.4)	(100)	(1.4)	(6.2)	(4.6)
Phentermine	346	133	254	214	121
	(20.3)	(100)	(14.6)	(19.4)	(12.8)
Ephedrine	—	344	254	119	95
		(100)	(27.3)	(2.9)	(2.7)
Pseudoephedrine	558	344	254	215	118
	(0.3)	(100)	(29.3)	(12.9)	(2.2)
Phenylpropanolamine	—	330	240	215	121
		(100)	(11.3)	(36.5)	(13.9)
Perfluorooctanoyl					
Amphetamine	532	119	440	118	91
	(28.0)	(100)	(1.9)	(82.5)	(13.7)
Methamphetamine	546	119	397	118	91
	(13.8)	(100)	(15.8)	(26.0)	(20.0)
MDMA	590	163	454	415	203
	(0.7)	(100)	(0.5)	(0.5)	(6.5)
Phentermine	546	133	454	414	91
	(5.6)	(100)	(3.0)	(2.1)	(2.7)
Ephedrine	—	121	544	415	149
		(100)	(12.7)	(10.0)	(47.4)
Pseudoephedrine	—	544	454	415	119
		(100)	(38.6)	(52.6)	(18.6)
Phenylpropanolamine	—	530	440	415	91
		(100)	(5.8)	(12.9)	(8.1)

differentiating them from methamphetamine. The pentafluoropropionyl derivative of phentermine showed a base peak at m/z 133 while pentafluoropropionyl derivative of phenylpropanolamine showed a base peak at m/z 280. The pentafluoropropionyl derivative of MDMA showed a protonated molecular ion peak at m/z 340 (Relative abundance 12.0%) and a base peak at m/z 163 (Table 1).

Heptafluorobutyryl Derivatives

The heptafluorobutyryl derivatives of methamphetamine, ephedrine and pseudoephedrine all showed base peaks at m/z 254 in the electron impact mode and may cause misidentification. By contrast, using the chemical ionization, the heptafluorobutyryl derivative of methamphetamine showed a protonated molecular ion as the base peak at m/z 346 and a very strong peak at m/z 119, while both ephedrine and pseudoephedrine showed a base peak at m/z 344 and a very weak peak at m/z 119. The heptafluorobutyryl derivative of phentermine showed a base peak at m/z 133 and a protonated molecular ion peak at m/z 346 (Relative abundance 20.3%). The heptafluorobutyryl derivative of MDMA showed a protonated molecular ion peak at m/z 390 (Relative abundance 5.4%) and a base peak at m/z 163.

Phenylpropanolamine, which causes a false positive result for amphetamine and methamphetamine in some immunoassays [*9*] was also derivatized by our reaction condition. However, phenylpropanolamine was clearly separated from both amphetamine and methamphetamine and showed a base peak at m/z 330.

Perfluorooctanoyl Derivatives

The recently described perfluorooctanoyl derivatives of amphetamine and methamphetamine had the advantage of relatively lower volatility and higher molecular weight [*8*]. However, perfluorooctanoyl derivatives of amphetamine and phentermine eluted together from the gas chromatographic column and the separation between ephedrine and methamphetamine was also poor. From the mass spectral characterization point of view, in the conventional electron impact mode, neither amphetamine nor methamphetamine showed a molecular ion peak and the base peaks were observed at m/z 440 for amphetamine and m/z 454 for methamphetamine. In the chemical ionization mode, amphetamine showed a protonated molecular ion peak at m/z 532 (Relative abundance 28.0%) and a base peak at m/z 118 while methamphetamine showed a protonated molecular ion peak at m/z 546 (Relative abundance 13.8%) and a base peak at m/z 119. We also observed a weak protonated molecular ion peak at m/z 590 (Relative abundance 0.7%) for MDMA. The perfluorooctanoyl derivative of phentermine showed a base peak at m/z 133 and a protonated molecular ion peak at m/z 546 (Relative abundance 5.6%), a mass spectral characteristic different from derivatized amphetamine and both ephedrine and pseudoephedrine showed a peak at m/z 544, thus differentiating them from methamphetamine.

Misidentification Problem of Ephedrine or Pseudoephedrine As Methamphetamine

Recently, some laboratories participating in the National laboratory certification program reported the presence of methamphetamine in several urine specimens which contained only ephedrine or pseudoephedrine. Those laboratories used heptafluorobutyryl, pentafluoropropionyl or 4-carbethoxyhexafluorobutyryl derivatives. Thurman et al. developed a table of selected ions for amphetamines and interfering sympathomimetic amines to avoid misidentification in the conventional electron impact mode [*10*]. Elsohly et al. oxidized ephedrine, pseudoephedrine and phenylpropanolamine which are alpha hydroxyl amines with periodate while leaving amphetamine and methamphetamine intact in order to avoid misidentification [*11*]. However, their approach requires additional steps and is time consuming.

Another reason of misidentification of ephedrine or pseudoephedrine as methamphetamine is the conversion of those drugs to methamphetamine during analysis. Hornbeck et al. reported the appearance of a methamphetamine artifact peak from pseudoephedrine when the injector port temperature was 300°C. However, no such peak was observed when the injector port temperature was 185°C [*5*]. We used an injector port temperature of 180°C and we observed no artifact peak of methamphetamine when negative urine was supplemented with ephedrine and pseudoephedrine with a concentration upto 500 μg/mL and subsequently extracted, derivatized and analyzed using our protocol.

Chemical ionization mass spectral identification of amphetamines had been poorly studied in the past. Wu et al. compared chemical ionization mass spectra of underivatized amphetamine and methamphetamine using methane as a reagent gas with conventional electron impact mass spectra of heptafluorobutyryl and 4-carbethoxyhexafluorobutyryl derivatives of amphetamine and methamphetamine [*12*]. This study is the first comprehensive study on the chemical ionization mass spectral behavior of commonly used fluoro derivatives of amphetamine, methamphetamine, MDMA and commonly interfering sympathomimetic amines.

Our results clearly indicate that chemical ionization mode is superior to the conventional electron impact mode for unambiguous confirmation of amphetamine and methamphetamine in human urine. Our choice of d8-methamphetamine as an internal standard provided the additional advantage of the presence of another strong peak at 8 amμ more that the protonated molecular ion peak of methamphetamine thus providing an additional distinguishing feature in the mass spectra to further avoid misidentification of ephedrine or pseudoephedrine as methamphetamine. However, chemical ionization mass spectral analysis can not completely eliminate misidentification of ephedrine or pseudoephedrine if extraction, derivatization or GC/MS analytical step lead to conversion of those drugs to methamphetamine. The injector port temperature is critical for those conversions. Therefore, extraction, derivatization and GC/MS analysis should also be carefully controlled in order to avoid this misidentification problem.

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