TECHNICAL NOTE

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Distinguishing Amphetamine, Methamphetamine and 3,4-Methylenedioxymethamphetamine from Other Sympathomimetic Amines After Rapid Derivatization with Propyl Chloroformate 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 gas chromatography as well as very similar mass spectral fragmentation patterns in the conventional electron impact mode of analysis. Recently, a new derivatization of amphetamine and methamphetamine has been described using propyl chloroformate. The derivatization is easily accomplished at room temperature by adding the derivatizing reagent in the extraction solvent because the reagent is stable in the presence of water. The electron impact mass spectrum of derivatized methamphetamine (base peak, m/z 144, other peaks at m/z 102, 58) is similar to the electron impact mass spectrum of both derivatized pseudoephedrine (base peak, m/z 144, other peaks, m/z 102, 58), and ephedrine (base peak, m/z 144, other peaks, m/z 102, 58). Therefore, misidentification of ephedrine and pseudoephedrine as methamphetamine is possible even if this new derivatization technique is used with conventional gas chromatography/electron impact mass spectrometry. We demonstrated that by using chemical ionization mass spectrometry, this problem can be eliminated. In the chemical ionization, using methane as a reagent gas, derivatized methamphetamine showed a protonated molecular ion as a base peak at m/z 236 and other strong peaks at m/z 144 and 119, both derivatized ephedrine and pseudoephedrine showed a base peak at m/z 192 and another strong peak at m/z 148, thus differentiating them clearly from methamphetamine. Amphetamine also showed a protonated molecular ion at m/z 222 and other strong peaks at m/z 130 and 119, whereas phenylpropanolamine after derivatization with propyl chloroformate showed a base peak at m/z 220 and another strong peak at m/z 238, thus differentiating it from amphetamine. The designer drug 3,4-methylenedioxymethamphetamine (MDMA) showed a molecular ion at m/z 279 using electron impact, after derivatization with propyl chloroformate. Using chemical ionization, a relatively stronger protonated molecular ion at m/z 280 was observed. We conclude that using chemical ionization instead of conventional electron impact and propyl chloroformate derivatization, misidentification of ephedrine or pseudoephedrine as methamphetamine or phenylpropanolamine as amphetamine can be eliminated.

KEYWORDS: forensic science, forensic toxicology, methamphetamine, ephedrine, pseudoephedrine, N-methyl-3,4-methylenedioxy amphetamine (MDMA), phenylpropanolamine, propyl chloroformate, chemical ionization

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) are commonly abused and may result in death from drug overdose (3). Because of widespread abuse of amphetamine and methamphetamine, drug testing for amphetamines is routinely done in forensic toxicology laboratories. Most gas chromatography-mass spectrometric (GC/MS) confirmation methods for amphetamines include a derivatization step. Commonly used derivatization reagents are trifluoroacetic anhydride, pentafluoropropionic anhydride, heptafluorobutyric anhydride, perfluorooctanoyl chloride, trichloroacetic anhydride, and 4-carbethoxyhexafluorobutyryl chloride (4–8).

Ephedrine and pseudoephedrine are widely used in many over the counter cold medications. Both ephedrine and pseudoephedrine can be misidentified as methamphetamine in the GC/MS confirmation of amphetamines using conventional electron impact, causing serious medical or legal problems. Ephedrine and pseudoephedrine are enantiomers and differ from methamphetamine by only the substitution of a hydroxyl group for hydrogen on the alpha carbon atom. 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 specimen (9–11). Pseudoephedrine can also cause false positive confirmation of methamphetamine by GC/MS due to a close retention time with methamphetamine as well as a similar mass spectral

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fragmentation pattern in the conventional electron impact mass spectrometric analysis (12).

Recently, Meatherall described a novel derivatization of amphetamines using propyl chloroformate (13). This new derivatization method is rapid compared to the conventional derivatizations of amphetamines which require 15 to 30 min of heating of the concentrated extract at various temperatures with the appropriate derivatizing reagents. Propyl chloroformate can be added directly to the extraction solvent for amphetamines because the derivatizing agent is stable in the presence of water in contrast to the commonly used derivatizing agents. Moreover, the reaction of amphetamines with propyl chloroformate is rapid and complete at room temperature (13). However, the mass spectral fragmentation patterns of methamphetamine and the interfering sympathomimetic amines ephedrine and pseudoephedrine are very similar after derivatization with propyl chloroformate in the electron impact mode. The author did not study the chemical ionization mass spectra of this novel derivative of amphetamines. Recently, bench top GC/MS have become available with chemical ionization capacity. We reported previously that the chemical ionization mass spectrum of methamphetamine is distinctively different from the mass spectra of both ephedrine and pseudoephedrine after various fluoro acyl derivatizations (14). Now, we report the chemical ionization mass spectra of propyl carbamate derivatives of amphetamines and show that the mass spectrum of the derivatized methamphetamine is again distinctly different from the mass spectra of both ephedrine and pseudoephedrine after derivatization.

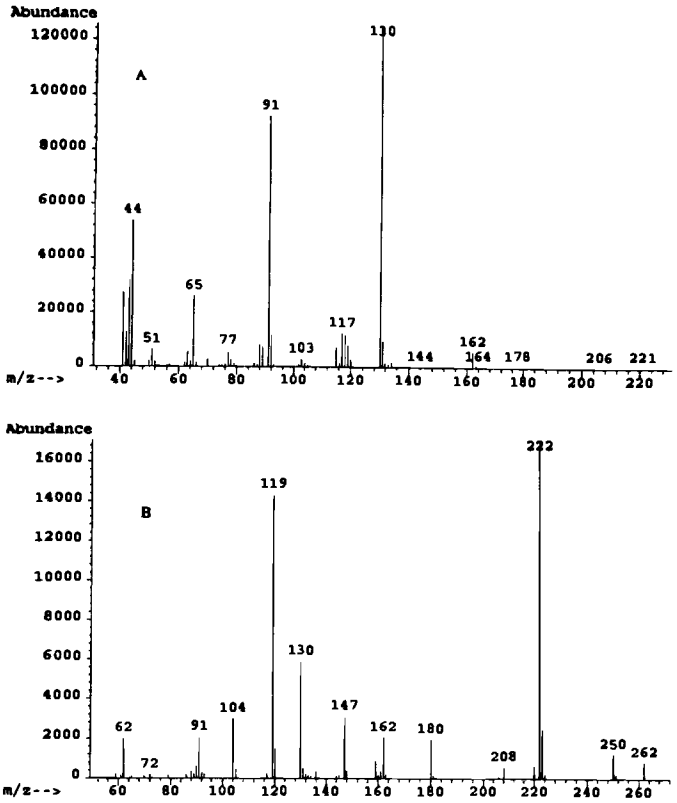

FIG. 1—*Mass spectra of amphetamine, (A) electron impact, (B) chemical ionization after derivatization with propyl chloroformate.*

Materials and Methods

Amphetamine, methamphetamine, ephedrine, and pseudoephedrine were purchased from Alltech (State College, PA) and MDMA was kindly provided by the US Drug Enforcement Agency (Dallas, TX). Propyl chloroformate was obtained from Aldrich Chemical Company (Milwaukee, WI). The initial screening of urines was done using an immunoassay technique (EMIT, Syva, San Jose, CA) and Monarch analyzer (Instrumentation Laboratory, Lexington, MA). Amphetamine, methamphetamine, MDMA, and other sympathomimetic amines (either EMIT positive urine or negative urine supplemented with them) along with the internal standard (D_8-methamphetamine, 1000 ng/mL) were extracted with hexane/chloroform (3:1 by vol) after alkalinization with 1 mL of carbonate buffer (pH 9.0) and 1 mL of 1 N sodium hydroxide. After extraction, 50 μL of propyl chloroformate were added to the organic phase and allowed to stand at room temperature for 10 min. The organic phase was concentrated to approximately 50 μL, and 2 μL was injected into a GC/MS.

The GC/MS analysis 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 operated in the positive chemical ionization mode using methane as the reagent gas (scan 50–700 m/z) or in the electron impact mode with a scan range of 40–800 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). The initial oven temperature of the gas chromatograph was 130°C. The oven temperature was increased at a rate of 10°C/min to 200°C. Then the oven temperature was increased at a rate of 20°C/min to 290°C. The final oven temperature was maintained for an additional 3 min. The solvent delay for the mass spectrometer was 6 min and the total run time was 14.5 min. The injector port temperature was 180°C. Splitless injection was used for all analyses.

Results and Discussion

In contrast to electron impact mass spectra which showed very weak molecular ion, methane chemical ionization yielded the protonated molecular ions as the base peak for both amphetamine and methamphetamine after derivatization with propyl chloroformate. The end product of the reaction is a carbamate. For example, amphetamine propyl carbamate showed a base peak at m/z 130 in the electron impact mode of analysis with other strong peaks at m/z 91 (relative abundance: 73%) and 44 (relative abundance: 42%). A very weak molecular ion was also observed at m/z 221. In contrast, the protonated molecular ion was the base peak at m/z 222 in the chemical ionization mode using methane as a reagent gas. Other strong peaks were also present at m/z 119 (relative abundance: 84%) and 130 (relative abundance; 35%) (Fig. 1). Phenylpropanolamine which can potentially interfere with the confirmation of amphetamine, showed a strong peak at m/z 130 (relative abundance 55%) and a base peak at m/z 44 in the electron impact mode after derivatization with propyl chloroformate. Therefore, the electron impact mass spectrum has some similarity with the electron impact mass spectrum of propyl amphetamine carbamate. On the other hand, phenylpropanolamine propyl carbamate showed a base peak at m/z 220 in the chemical ionization mode, thus distinguishing the compound from amphetamine propyl carbamate which showed a base peak at m/z 222. Moreover, the phenylpropanolamine propyl carbamate also showed a strong protonated molecular ion at m/z 238 (relative abundance: 24%) and other strong peaks at m/z 178 (relative abundance: 33%) and m/z 134 (relative abundance: 71%), thus further distinguishing it from

amphetamine propyl carbamate (Fig. 2). Similarly, phentermine propyl carbamate which was eluted from the column after amphetamine but before methamphetamine showed a base peak at m/z 144 in the electron impact mode, the same base peak as methamphetamine propyl carbamate. However, in the chemical ionization mode, we observed differences between mass spectra of amphetamine, methamphetamine, and phentermine after derivatization with propyl chloroformate. In the chemical ionization mode, the phentermine propyl carbamate showed a strong protonated molecular ion peak at m/z 236 (relative abundance: 67%). The base peak was observed at m/z 104 (Fig. 2).

In the electron impact mode, methamphetamine propyl carbamate showed a base peak at m/z 144 and other strong peaks at m/z 102 (relative abundance 28%) and 58 (relative abundance 42%). Meatherall observed a very weak protonated molecular ion at m/z 236 (13), but we did not observe any molecular ion in the electron impact mode (Fig. 3). The internal standard D_8-methamphetamine showed a base peak at m/z 65 and a very strong peak at m/z 151 (relative abundance: 93%). Another strong peak was also observed at m/z 109 (relative abundance: 41%). The ephedrine propyl carbamate which eluted from the column shortly after derivatized methamphetamine, showed a base peak at m/z 144 and other strong peaks at m/z 102 (relative abundance: 25%) and m/z 58 with a relative abundance of 47% (Fig. 4). Similarly, pseudoephedrine propyl carbamate also showed a base peak at m/z 144 in the electron impact mode (Fig. 5). Therefore, mass spectra of methamphetamine, ephedrine and pseudoephedrine after derivatization with propyl chloroformate all showed the same base peak at m/z 144 in the electron impact mode of analysis. Other

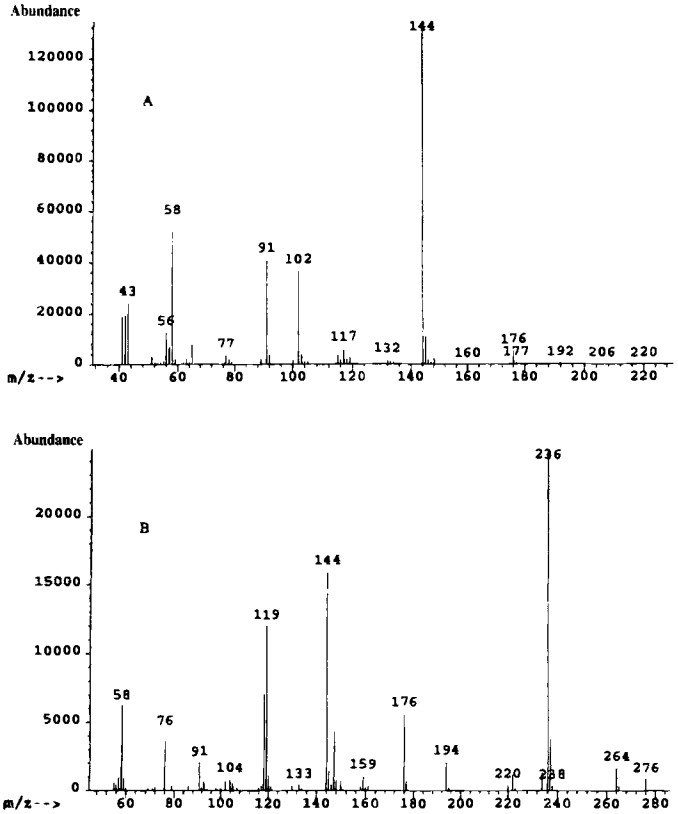

FIG. 3—*Mass spectra of methamphetamine, (A) electron impact, (B) chemical ionization after derivatization with propyl chloroformate.*

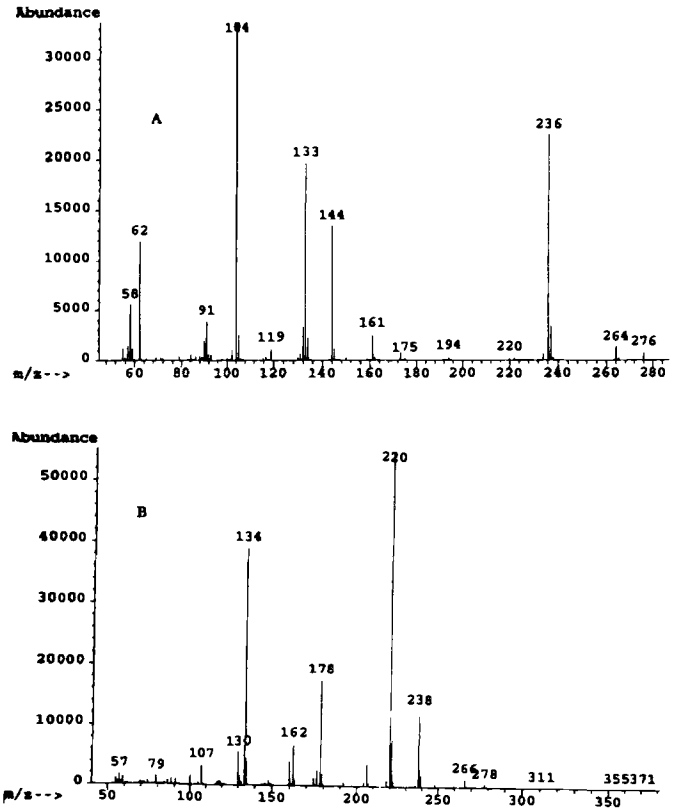

FIG. 2—*Chemical ionization mass spectra of, (A) phentermine, (B) phenylpropanolamine after derivatization with propyl chloroformate.*

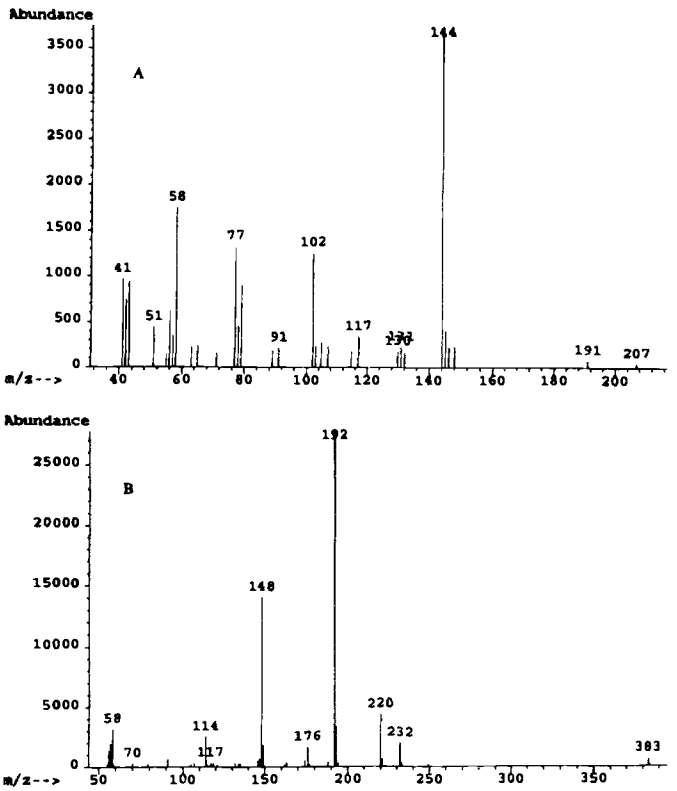

FIG. 4—*Mass spectra of ephedrine, (A) electron impact, (B) chemical ionization after derivatization with propyl chloroformate.*

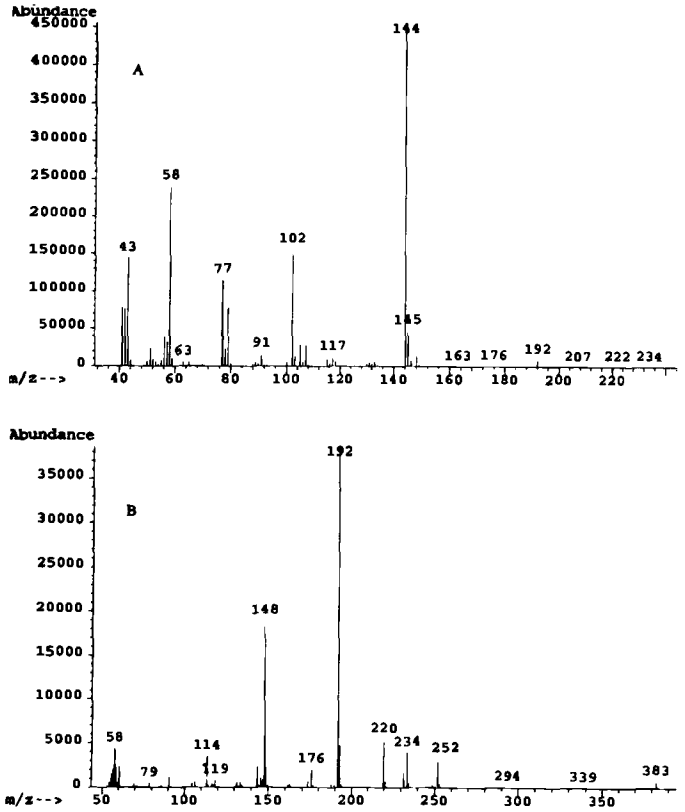

FIG. 5—*Mass spectra of pseudoephedrine, (A) electron impact, (B) chemical ionization after derivatization with propyl chloroformate.*

strong peaks at m/z 58 and 102 were also present in the electron impact mass spectra of methamphetamine, ephedrine, and pseudoephedrine after derivatization with propyl chloroformate. The only difference is the presence of a relatively strong peak at m/z 91 in methamphetamine propyl carbamate. Our observations are in agreement with Meatherall. In sharp contrast, in the chemical ionization mode using methane as a reagent gas, the mass spectrum of methamphetamine propyl carbamate is very different from the mass spectrum of ephedrine propyl carbamate as well as pseudoephedrine propyl carbamate. The mass spectrum of methamphetamine propyl carbamate showed a protonated molecular ion as the base peak at m/z 236. Other strong peaks were observed at m/z 144 (relative abundance: 64%) and m/z 119 (relative abundance 48%) (Fig. 3). As expected, the internal standard, D_8-methamphetamine also showed a protonated molecular ion as the base peak at m/z 244 and another strong peak at m/z 124. In sharp contrast, the ephedrine propyl carbamate and pseudoephedrine propyl carbamate both showed base peak at m/z 192 (Figs. 4,5). We also observed a strong peak at m/z 148 for both compounds in the chemical ionization mode, thus further differentiating them from methamphetamine propyl carbamate.

3,4-Methylenedioxymethamphetamine (MDMA), also called "Ecstasy" is a designer drug which is also abused and has resulted in deaths. MDMA can cross react with the amphetamine antibody causing a positive EMIT screen for amphetamines if present in the urine. We decided to study the mass spectral characteristics of the propyl chloroformate derivative of MDMA in both electron impact and chemical ionization modes. In the electron impact mode, the propyl chloroformate derivative of MDMA showed a base peak at m/z 144 and a weak molecular ion at m/z 279 (relative abundance 9%). We also observed another strong peak at m/z 102 (relative abundance 34%). The chemical ionization mass spectrum of propyl chloroformate derivative of MDMA showed a base peak at m/z 163. We also observed a distinct protonated molecular ion at m/z 280 (relative abundance 23%). Another strong peak was also observed at m/z 144 (relative abundance 36%) (Fig. 6).

Injector port temperature is critical in the analysis of amphetamines. Hornbeck et al. reported the appearance of a methamphetamine artifact peak from pseudoephedrine when the injector port temperature was 300°C, probably due to thermal dehydration. However, no such peak was observed when the injector port temperature was 185°C (10). We used an injector port temperature of 180°C and observed no artifact peak of methamphetamine when negative urine was supplemented with a high concentration of ephedrine or pseudoephedrine.

Chemical ionization mass spectral characterization of amphetamines has not been extensively 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 (15). We also reported a comprehensive study on the chemical ionization mass spectrometric behavior of common fluro acyl derivatives of amphetamine, methamphetamine, MDMA and other interfering sympathomimetic amines and demonstrated that chemical ionization

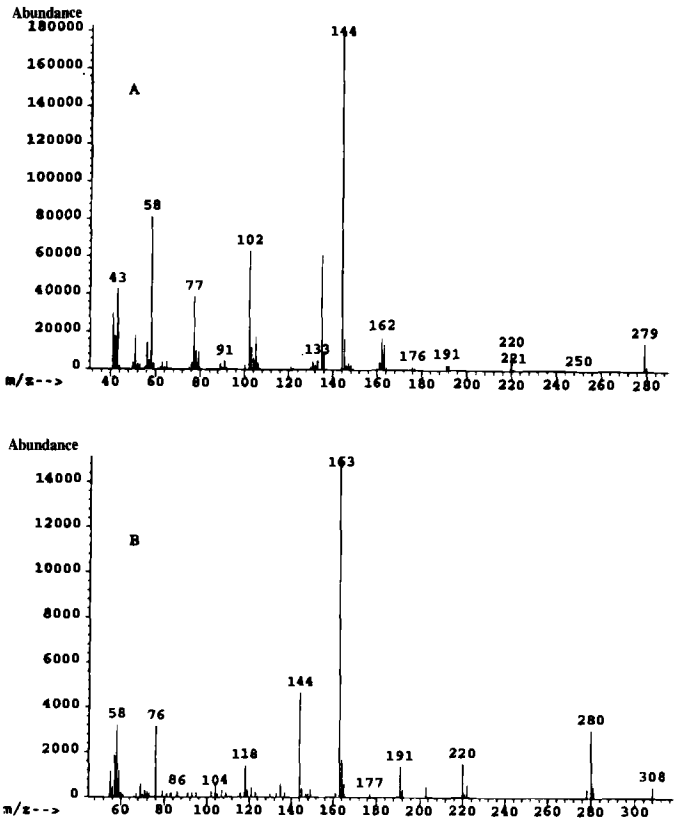

FIG. 6—*Mass spectra of MDMA, (A) electron impact, (B) chemical ionization after derivatization with propyl chloroformate.*

mass spectra are superior and have more differentiating characteristics than the conventional electron impact mass spectra of these compounds (14). The recently described propyl chloroformate derivative of amphetamines is superior to the conventional derivatives of amphetamines because the technique is rapid and does not require a separate incubation time for the formation of derivatives. Although, analysis time can be improved, the conventional electron impact mass spectrum of methamphetamine is again similar to both ephedrine and pseudoephedrine after derivatization with propyl chloroformate. We demonstrated that the chemical ionization mass spectra of these compounds are very different and more useful in differentiating methamphetamine from interfering amines, ephedrine, and pseudoephedrine as well as differentiating phentermine and phenylpropanolamine from amphetamine.

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