**TECHNICAL NOTE**

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# Gas Chromatography-Electron Ionization and Chemical Ionization Mass Spectrometric Analysis of Urinary Phenmetrazine After Derivatization with 4-Carbethoxyhexafluorobutyryl Chloride—A New Derivative

**REFERENCE:** Dasgupta A, Hart A, Humphrey P, Blackwell W. Gas chromatography-electron ionization and chemical ionization mass spectrometric analysis of urinary phenmetrazine after derivatization with 4-carbethoxyhexafluorobutyryl chloride—a new derivative. J Forensic Sci 1998;43(3)636–640.

**ABSTRACT:** Phenmetrazine is a central nervous system stimulant currently used as an anorectic agent. The drug is abused and is reported to cause death from overdose. We describe a new derivatization method for phenmetrazine using 4-carbethoxyhexafluorobutyryl chloride. Quantitation of urinary phenmetrazine can be easily achieved by using N-ethyl amphetamine as an internal standard. The electron ionization mass spectrum of 4-carbethoxyhexafluorobutyryl derivative of phenmetrazine showed a molecular ion at m/z 427 and a base peak at m/z 70. In the methane chemical ionization mass spectrum, the base peak was observed at m/z 428 (protonated molecular ion). In the electron ionization mass spectrum of 4-carbethoxyhexafluorobutyryl derivative of the internal standard, N-ethyl amphetamine we did not observe a molecular ion. However, in the chemical ionization mass spectrum, the protonated molecular ion at m/z 414 was the base peak. The retention time of derivatized phenmetrazine (8.4 min) was substantially longer than the retention time of the underivatized molecule. Moreover, underivatized phenmetrazine showed poor peak shape (substantial tailing) while derivatized phenmetrazine had excellent chromatographic properties. The within-run and between-run precisions of the assay were 2.6% and 3.1% respectively at a urinary phenmetrazine concentration of 10 μg/mL. The assay was linear for urinary phenmetrazine concentration of 1 to 100 μg/mL with a detection limit of 0.2 μg/mL.

**KEYWORDS:** forensic science, phenmetrazine, gas chromatography-mass spectrometry, 4-carbethoxyhexafluorobutyryl

Phenmetrazine (Preludin) is the N-desmethyl analog of phendimetrazine and also the primary metabolite of phendimetrazine. The drug is used clinically as an anorectic agent and is available for oral use as a hydrochloride salt in a single dose of 25 mg and daily doses of up to 75 mg (1). The drug has a high potential for abuse and it has been withdrawn from the market in Sweden. Phenmetrazine overdose can cause dizziness, tremor, tachycardia, hypertension, cardiac arrhythmia, convulsion, coma, and circulatory collapse. Several deaths have been reported from phenmetrazine overdose. Norheim reported a urinary phenmetrazine concentration of 24 μg/mL in a fatal case caused by intravenous use of the drug (2).

Gottschalk reported an average urinary phenmetrazine concentration of 21 μg/mL in 12 fatalities (personal communication, 1977). The highest reported concentration in that communication was 90 μg/mL of urinary phenmetrazine. Cravey reported a urinary phenmetrazine concentration of 2.5 μg/mL in a person who committed suicide by gunfire (1). In another report the urinary phenmetrazine concentration varied between 56 and 290 μg/mL in seven drug users (3).

There are only a few reports in the literature dealing with gas chromatography/mass spectrometric (GC/MS) identification and quantitation of urinary phenmetrazine. Phenmetrazine can be analyzed without derivatization. Beckett et al. described a protocol for flame ionization gas chromatographic determination of the underivatized drug (4). However, the peak had substantial tailing. Other investigators derivatized phenmetrazine prior to analysis. Franklin et al. described an N-acetyl derivatization of the drug (5). Recently, Kronstrand et al. described a GC/MS protocol for the determination of phenmetrazine in urine after derivatization with methyl chloroformate (6). The authors took advantage of their previously described protocol for derivatization of amphetamines using methyl chloroformate (7) and used ethylamphetamine as an internal standard. We recently reported GC/MS identification of phenmetrazine after derivatization with perfluorooctanoyl chloride, (8). Now we would like to report another derivatization protocol for phenmetrazine using 4-carbethoxyhexafluorobutyryl chloride.

## Materials and Method

Phenmetrazine, the internal standard N-ethyl amphetamine, amphetamine, and methamphetamine were obtained from Alltech Applied Science (College Park, PA). The derivatizing agent 4-carbethoxyhexafluorobutyryl chloride was purchased from PCR

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Chemicals (Gainesville, FL), high performance liquid chromatography (HPLC) grade 1-chlorobutane (extraction solvent), sodium tetraborate decahydrate and sodium hydroxide were purchased from Aldrich (Milwaukee, WI). The stock solution of internal standard (N-ethyl amphetamine) was prepared in methanol (1 mg/mL).

To extract phenmetrazine from urine, a 2 mL aliquot of urine was supplemented with 20 μL of the internal standard solution (the final concentration of the internal standard in urine was 10 μg/mL), followed by the addition of 1 mL borate buffer (pH 9.8) and 1 mL 1 N sodium hydroxide. The borate buffer was prepared by dissolving 20 gm of sodium tetraborate decahydrate in 1 L of deionized water. Phenmetrazine and the internal standard were extracted from the alkaline urine using 10 mL of 1-chlorobutane. The sample was vortex mixed for 1 min and then further mixed in a rotating mixer for an additional 10 min. After centrifugation for 5 min at 1500 g, the upper organic layer was transferred to a conical test tube and the organic phase was evaporated under air until approximately 50 μL of organic phase remained. Then 50 μL of the derivatizing agent (4-carbethoxyhexafluorobutyryl chloride) were added to the remaining organic phase. The reaction mixture was heated at 80°C for 20 min. Then 50 μL of methanol was added to the reaction mixture to destroy any excess derivatizing agent. The organic phase was further concentrated under air to approximately 50 μL and then 2 μL were injected into the GC/MS.

The gas chromatography/mass spectrometric analysis was carried out using a model 5790 series II gas chromatograph coupled to a 5972 mass selective detector (Hewlett Packard, Palo Alto, CA). The chromatographic separation was achieved with an Ultra 2 capillary column (25 m × 0.2 mm), coated with cross-linked phenyl methyl silicone (0.33 μm film thickness). The initial oven temperature of the gas chromatograph was 175°C. After maintaining that temperature for 5 min, the oven temperature was increased at a rate of 20°C/min to reach an oven temperature of 300°C. The final temperature was maintained for an additional 1 min. The run time was 12.25 min with a solvent delay time of 5 min. Injections were in the splitless mode with an injector port temperature was 250°C. The mass spectrometer was operated in the electron ionization mode (selected ion monitoring: m/z 322, 321, 176, 118, 98, 91, and 70). For chemical ionization we used methane as a reagent gas.

The quantitation was done by comparing the area under the peak for derivatized phenmetrazine with the area under the peak of the derivatized internal standard, N-ethyl amphetamine. The spectra were obtained using electron ionization and selected ion monitoring mode.

## Results and Discussion

### *Chromatographic Properties of Derivatized Phenmetrazine*

We observed baseline separation between the derivatized internal standard and phenmetrazine. The derivatized phenmetrazine showed a retention time of 8.4 min while the derivatized internal standard eluted at 7.3 min. The typical total ion chromatogram of a urine specimen supplemented with 10 μg/mL of phenmetrazine is given in Fig. 1. Underivatized phenmetrazine showed a short retention time and the peak shape was poor with substantial tailing (data not shown).

### *Mass Spectral Characteristics of Derivatized Phenmetrazine and the Internal Standard*

In the electron ionization mass spectrum of 4-carbethoxyhexafluorobutyryl derivative of phenmetrazine, a weak molecular ion was observed at m/z 427 (relative abundance 1.0%). The base peak was observed at m/z 70. A strong peak was observed at m/z 321 (relative abundance 40%) due to the fragmentation in the lactone ring of the molecule which retained 4-carbethoxyhexafluorobutyryl group. Another peak was observed at m/z 176 (relative abundance 8.7%) due to the loss of 4-carbethoxyhexafluorobutyryl group (Fig. 2). In the chemical ionization mass spectrum using

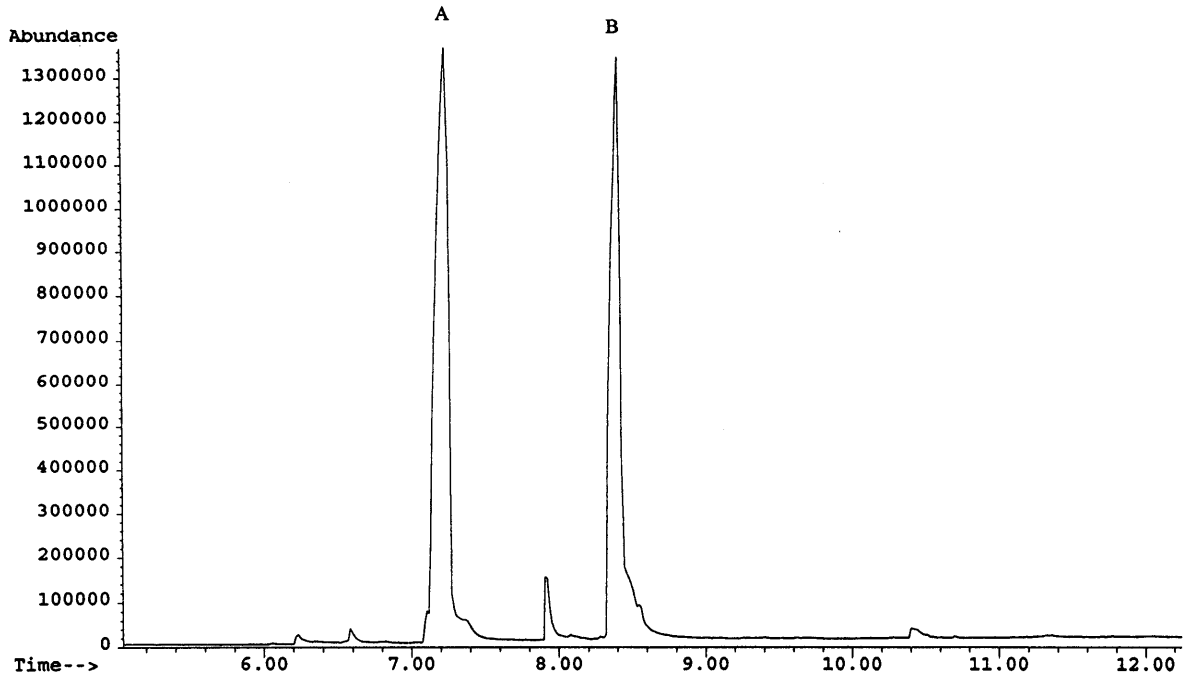


FIG. 1—*Total ion chromatogram of a typical urinary extract showing the separation between derivatives of N-ethyl amphetamine (internal standard) and phenmetrazine. Peak A is the derivatized, internal standard and peak B is the derivatized phenmetrazine. The concentration of phenmetrazine was 10 μg/mL.*

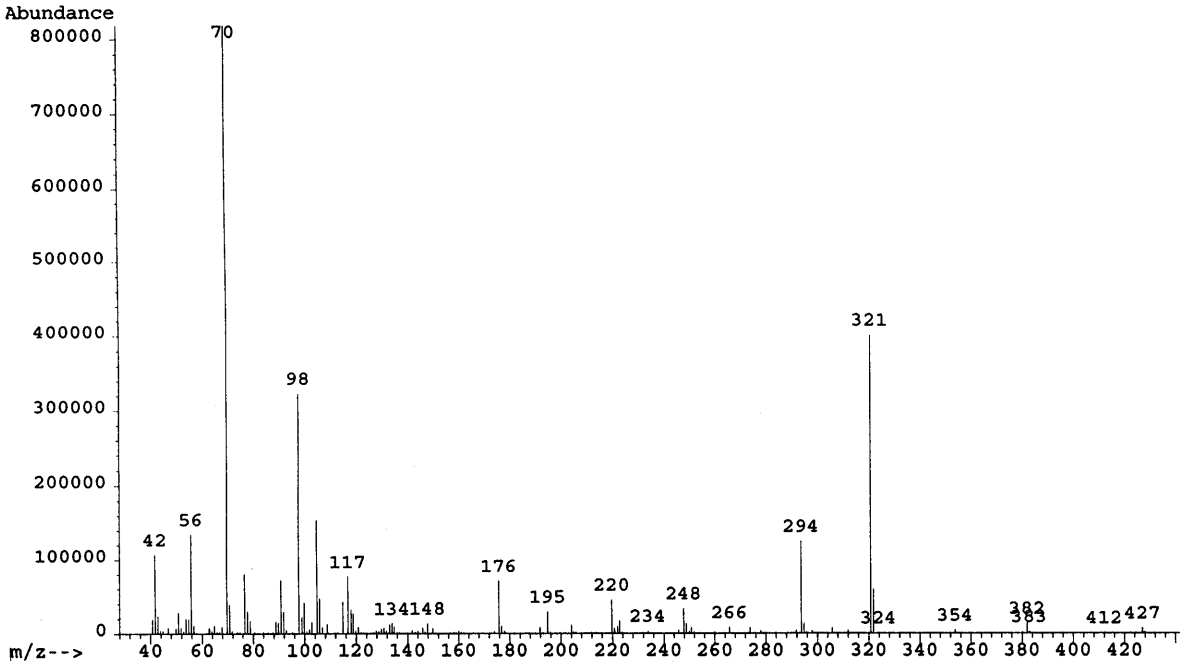


FIG. 2—*Electron ionization mass spectrum of 4-carbethoxyhexafluorobutyryl derivative of phenmetrazine.*

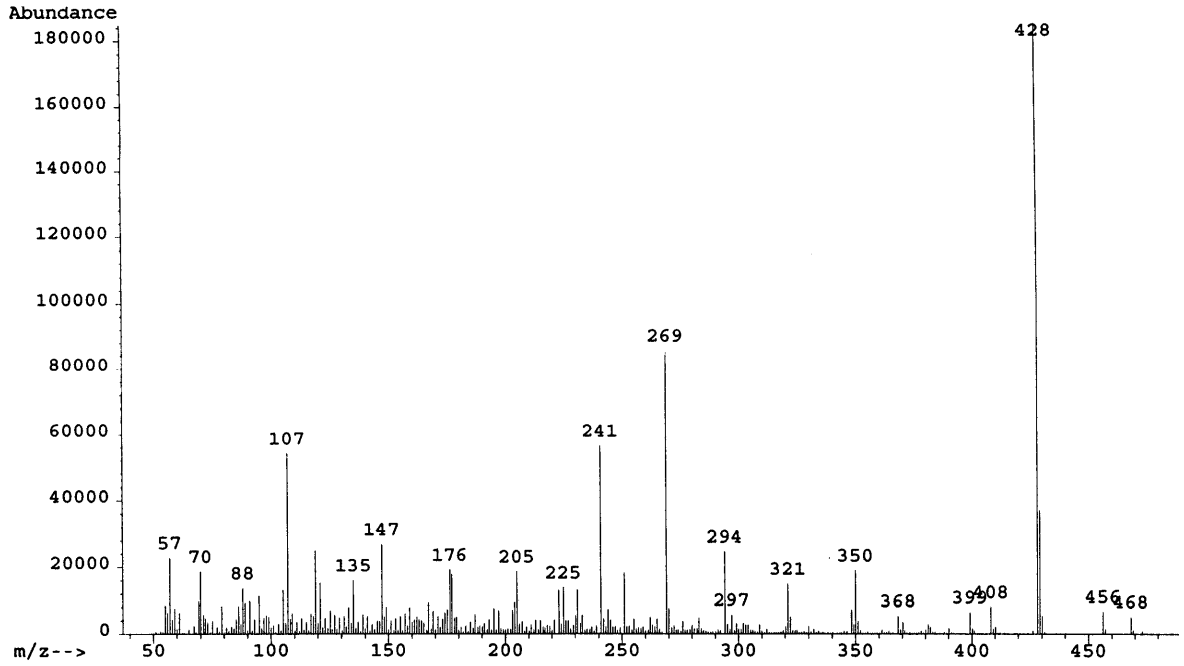


FIG. 3—*Chemical ionization mass spectrum of 4-carbethoxyhexafluorobutyryl derivative of phenmetrazine.*

methane as a reagent gas, the base peak at m/z 428 was the protonated molecular ion (Fig. 3).

In the electron ionization mass spectrum of 4-carbethoxyhexafluorobutyryl derivative of N-ethyl amphetamine (internal standard), we did not observe any molecular ion. The base peak was observed at m/z 322 due to the loss of $C_6H_5CH_2$ group from the derivatized molecule. Other characteristic peaks were observed at m/z 118 (relative abundance 14%) and 91 (relative abundance 24%) Fig. 4. In the chemical ionization mass spectrum, the protonated molecular ion at m/z 414 was the base peak (Fig. 5).

*Precision, Linearity and Detection Limit*

The within-run and between-run precision at a urinary phenmetrazine concentration of 10 μg/mL were 2.6% (mean = 10.1, standard deviation (SD) = 0.26 μg/mL, $n$ = 8) and 3.1% (mean = 10.2, SD = 0.32 μg/mL, $n$ = 8), respectively. The assay was linear for a urinary phenmetrazine concentration of 1 to 100 μg/mL. Using the $x$-axis as the target concentration and $y$-axis as the observed concentration in the linearity study, we derived the regression equation:

$$y = 1.08 \times -0.79 (r = 0.99)$$

The detection limit for urinary phenmetrazine was 0.2 μg/mL.

*Interference Study*

Amphetamine, methamphetamine, ephedrine, phenylpropanolamine, MDMA and phendimetrazine after derivatization with 4-carbethoxyhexafluorobutyryl chloride did not interfere with the analysis of phenmetrazine. Moreover, the mass spectral fragmentation patterns were different and positive identification of phenmetrazine can be easily achieved in the presence of these drugs.

*Application of the Assay*

Phenmetrazine at the concentration of 1 μg/mL did not cross-react with the amphetamine EMIT immunoassay. We observed cross reactivity with the EMIT immunoassay at a urinary phenmetrazine concentration of 6.6 μg/mL and higher. Therefore, if abuse of phenmetrazine is suspected, an unambiguous confirmation by mass spectrometry is essential. Phenmetrazine can be analyzed by GC/MS without derivatization but the retention time of underivatized phenmetrazine is relatively short and the peak shape is poor. Therefore, quantitation may be a problem and interference from

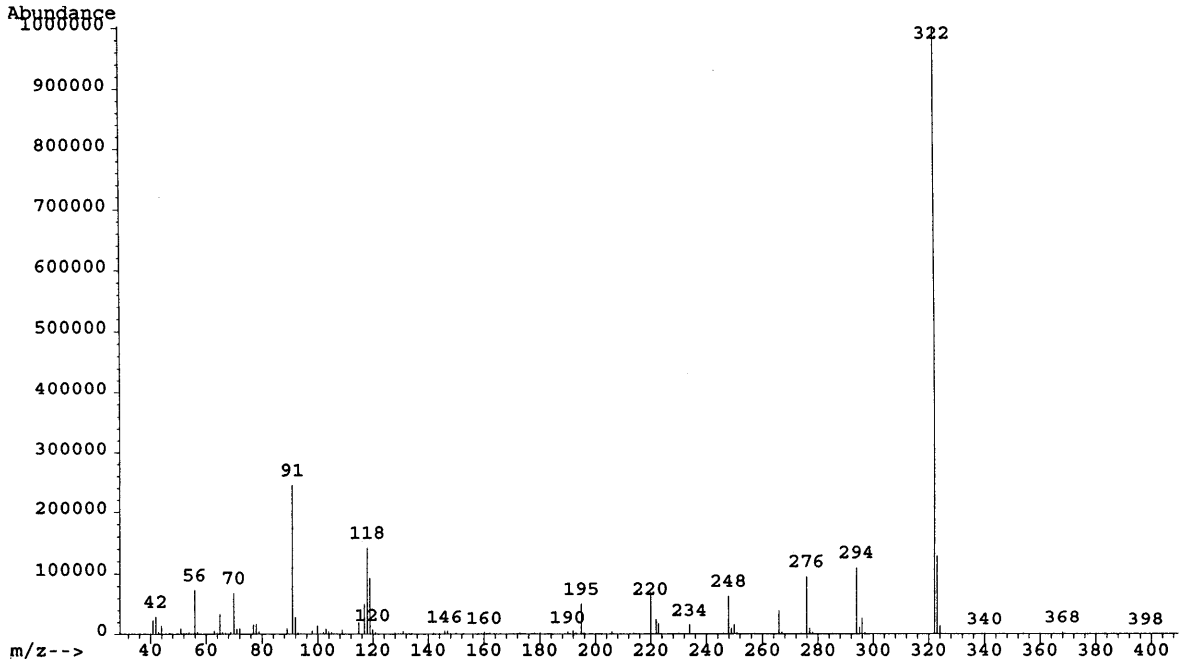


FIG. 4—*Electron ionization mass spectrum of 4-carbethoxyhexafluorobutyryl derivative of N-ethyl amphetamine (internal standard).*

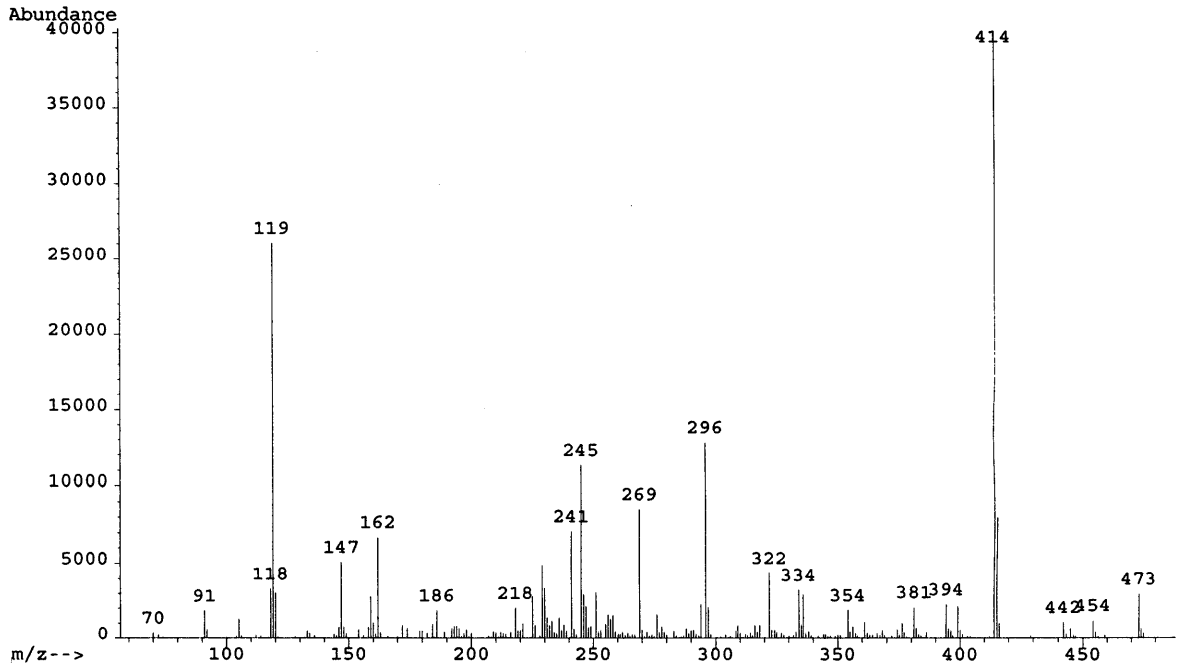


FIG. 5—*Chemical ionization mass spectrum of 4-carbethoxyhexafluorobutyryl derivative of N-ethyl amphetamine (internal standard).*

volatile components of urinary matrix may occur. Derivatized phenmetrazine showed a longer retention time and good chromatographic properties. The 4-carbethoxyhexafluorobutyryl derivative of phenmetrazine eluted even at a higher temperature than the previously described perfluorooctanoyl derivative and, therefore, this assay is free from interferences from more volatile components of the urinary matrix.

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