TECHNICAL NOTE

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A Convenient Derivatization Method for Gas Chromatography/ Mass Spectrometric Determination of Phenmetrazine in Urine Using 2,2,2-Trichloroethyl Chloroformate

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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 2,2,2-trichloroethyl chloroformate. Quantitation of urinary phenmetrazine can be easily achieved by using N-propylamphetamine as an internal standard. The phenmetrazine 2,2,2-trichloroethyl carbamate showed a molecular ion isotope cluster at m/z 351, 353, 355, and 357 (isotope effect of three chlorine atoms in the derivatized molecule) and other peaks at m/z 247, 245, 204, 114, and 70 in the electron ionization mass spectrometry, thus aiding in unambiguous identification. The underivatized phenmetrazine showed a relatively weaker molecular ion at m/z 177 and a base peak at m/z 71. The N-propylamphetamine 2,2,2-trichloroethyl carbamate (internal standard) showed a very weak molecular ion at m/z 351 and a base peak at m/z 260. Another strong characteristic peak at m/z 91 was also observed. The retention time of derivatized phenmetrazine (9.5 min) was substantially longer than the retention time of the underivatized molecule (2.5 min). Moreover, underivatized phenmetrazine showed poor peak shape (substantial tailing) while derivatized phenmetrazine had excellent chromatographic property. The within-run and between-run precisions of the assay were 1.9% and 3.2% at a urinary phenmetrazine concentration of 20 mg/mL. The assay was linear for urinary phenmetrazine concentration of 1 mg/mL to 100 mg/mL with a detection limit of 0.5 mg/mL.

KEYWORDS: forensic science, phenmetrazine, gas chromatography-mass spectrometry, 2,2,2-trichloroethyl chloroformate

Phenmetrazine (Preludin) is the N-desmethyl analogue of phendimetrazine. Phenmetrazine is 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 The drug has a high potential for abuse and it has been withdrawn from the market in Sweden.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 mg/mL in a fatal case caused by intravenous use of the drug (2). Gottschalk reported an average urinary phenmetrazine concentration of 21 mg/mL in 12 fatalities (personal communication, 1977). The highest reported concentration in that communication was 90 mg/mL of urinary phenmetrazine. Cravey reported a urinary phenmetrazine concentration of 2.5 mg/mL in a person who committed suicide by gunfire (1). In another report the urinary phenmetrazine concentration varied between 56 and 290 mg/mL in seven drug users (3).

Phenmetrazine cross-reacts with the EMIT assay for urinary amphetamines only at higher concentrations and, therefore, an initial screen of urine using EMIT dau kit may be negative even for significant concentration of urinary phenmetrazine. 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 underivatized drug (4). However, the peak suffered from tailing. Therefore, 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, but the derivatization process required heating of the reaction mixture at 80°C for 30 min (8). Therefore, we continued our search for a more suitable derivative that can be prepared more rapidly. We studied the possibility of using 2,2,2-trichloroethyl chloroformate as the derivatizing agent for phenmetrazine. Because of the presence of three chlorine atoms in 2,2,2-trichloroethyl chloroformate the reaction with phenmetrazine could be rapid and quantitative at room temperature. The molecular weight of phenmetrazine is 177, while the molecular weights of the phenmetrazine 2,2,2-trichloroethyl carbamate are 351, 353, 355, and 357. Therefore, this

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new derivative of phenmetrazine may produce more characteristic ions at a higher mass range for unambiguous confirmation, and lesser volatility of the derivative may aid in avoiding interferences from more volatile components of the urinary matrix. Here we describe GC/MS confirmation and quantitation of urinary phenmetrazine after derivatization with 2,2,2-trichloroethyl chloroformate. The derivatization can be carried out at room temperature in 10 min.

Materials and Method

Phenmetrazine, the internal standard N-propylamphetamine, amphetamine, and methamphetamine were obtained from Alltech Applied Science (College Park, PA). The derivatizing agent 2,2,2-trichloroethyl chloroformate, HPLC grade 1-chlorobutane (extraction solvent), sodium tetraborate decahydrate and sodium hydroxide were purchased from Aldrich (Milwaukee, WI). The stock solution of internal standard (propyl amphetamine) was prepared in methanol (1 mg/mL). The EMIT assay kit for urinary methamphetamine was obtained from Behring diagnostics (San Jose, CA) and the assays for determining cross reactivity of urinary phenmetrazine with amphetamine were run on the SYVA-30 R automated analyzer also obtained from Behring diagnostics.

To extract phenmetrazine from urine, a 2 mL aliquot of urine was supplemented with 20 mL of the internal standard solution (the final concentration of the internal standard in urine was 10 mg/mL), followed by the addition of 1 mL borate buffer (pH 9.8) and 1 mL 1N 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 mL of organic phase remained. Then 50 mL of the derivatizing agent (2,2,2-trichloroethyl chloroformate) were added to the remaining organic phase. The reaction mixture was allowed to stand at room temperature for 10 min. Then 100 mL of ethyl alcohol (200 proof dehydrated alcohol, Quantum Chemical, Tuscola, IL) was added to the reaction mixture to destroy excess derivatizing agent. The organic phase was further concentrated under air to approximately 50 mL and then 2 mL were injected into the GC/MS.

The gas chromatography/mass spectrometric analysis was carried out using a model 5890 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 mm 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 290°C. The final temperature was maintained for an additional 1 min. The run time was 11.75 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 (scanning range: m/z 50 to 500).

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-propylamphetamine. The spectra were obtained using the scan mode.

Results and Discussion

Chromatographic Properties of Derivatized Phenmetrazine

We observed baseline separation between the derivatized internal standard and phenmetrazine. The chemical structures of the derivatives are given in Fig. 1. The N-propylamphetamine 2,2,2-trichloroethyl carbamate (internal standard) eluted at 8.6 min while phenmetrazine 2,2,2-trichloroethyl carbamate eluted at 9.5 min. The typical total ion chromatogram of a urine specimen supplemented with 20 mg/mL of phenmetrazine is given in Fig. 2. Underivatized phenmetrazine showed a short retention time (2.5 min) and the peak shape was poor with substantial tailing (data not shown).

Mass Spectral Characteristics of Derivatized Phenmetrazine and the Internal Standard

Phenmetrazine is a small molecule with a molecular weight of 177. The underivatized phenmetrazine showed a molecular ion at m/z 177 (relative abundance 9.3%) and a base peak at m/z 71 (Fig. 3). The molecular weight of phenmetrazine 2,2,2-trichloroethyl carbamate was m/z 351, with peaks at m/z 353, 355, and 357 due to the presence of three chlorine atoms (isotopes, 35 and 37) (Fig. 4). The presence of the strong molecular ion isotope peaks at m/z 351 and 353 aid in unambiguous identification of phenmetrazine. The strong peaks at m/z 245 (relative abundance 25.3%) and m/z 247 (relative abundance 24.1%) were due to fragmentation of the morpholine ring while retaining the derivatized part of the molecule. Another strong characteristic peak at m/z 204 [M − OCH_2CCl_3] with a relative abundance of 20.2% was also observed. The base peak was observed at m/z 114 and another strong peak was observed at m/z 70.

The internal standard, N-propylamphetamine 2,2,2-trichloroethyl carbamate, gave a weak molecular ion at m/z 351 with a relative abundance of 0.03% (Fig. 5). The strong characteristic peaks at m/z 260 (base peak), 262 (relative abundance 94.1%), 264 (relative abundance 30.6%) and a relatively weak peak at m/z 266 (relative abundance 3.3%) were attributable to the isotopic effect of chlorine in the derivatized N-propyl amphetamine molecule after the loss of $C_6H_5CH_2$ fragment. Another strong characteristic peak at m/z 91 (relative abundance 78.3%) was also observed.

Precision, Linearity and Detection Limit

The within-run and between-run precision at a urinary phenmetrazine concentration of 20 mg/mL were 1.9% (mean = 19.2, standard deviation (SD) = 0.37 mg/mL, n = 8) and 3.2% (mean = 19.3, SD = 0.61 mg/mL, n = 8), respectively. The assay was

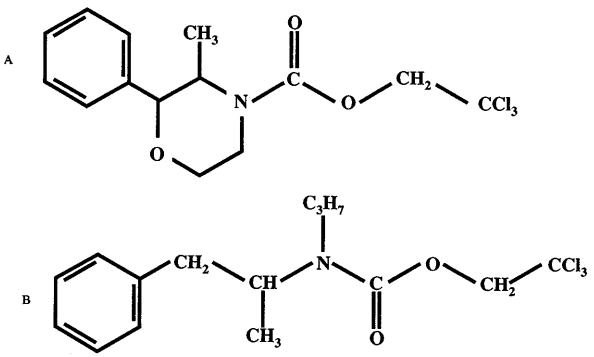

FIG. 1—*Chemical structure of (*A*) phenmetrazine 2,2,2-trichloroethyl carbamate and (*B*) N-propyl amphetamine 2,2,2-trichloroethyl carbamate.*

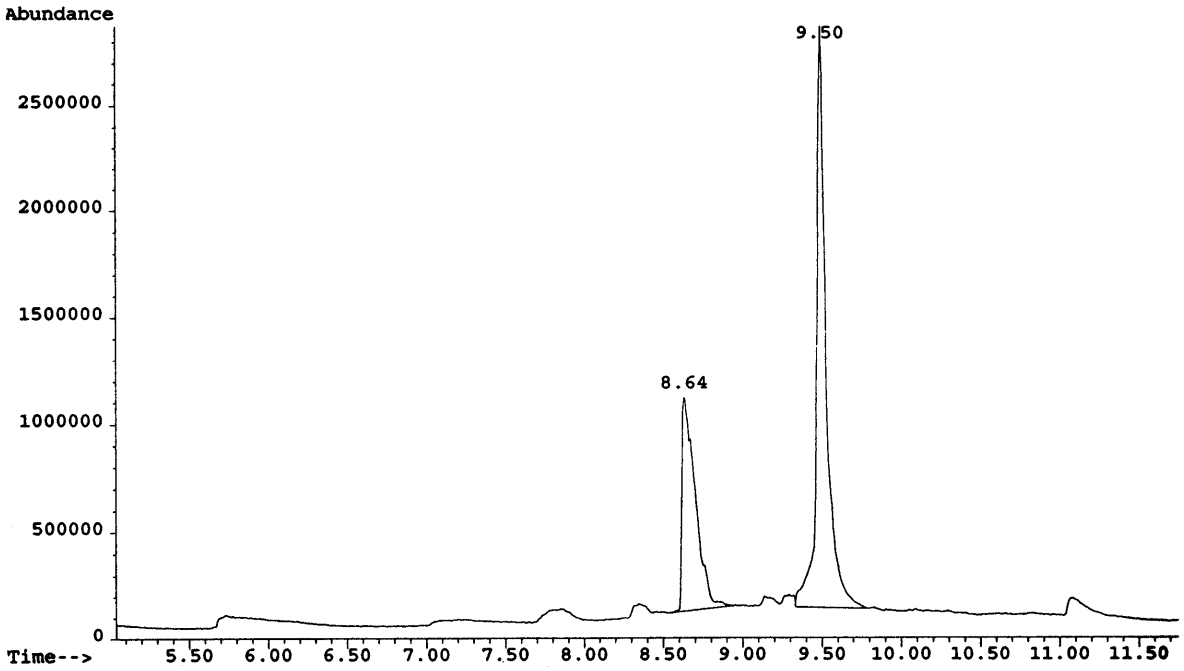

FIG. 2—*Total ion chromatogram of a typical urinary extract showing the separation between derivatives of N-propylamphetamine (internal standard) and phenmetrazine. The peak at retention time of 8.64 is N-propyl amphetamine 2,2,2-trichloroethyl carbamate and the peak with a retention time of 9.50 min is phenmetrazine 2,2,2-trichloroethyl carbamate. The concentration of phenmetrazine was 20 mg/mL while the concentration of the internal standard was 10 mg/mL.*

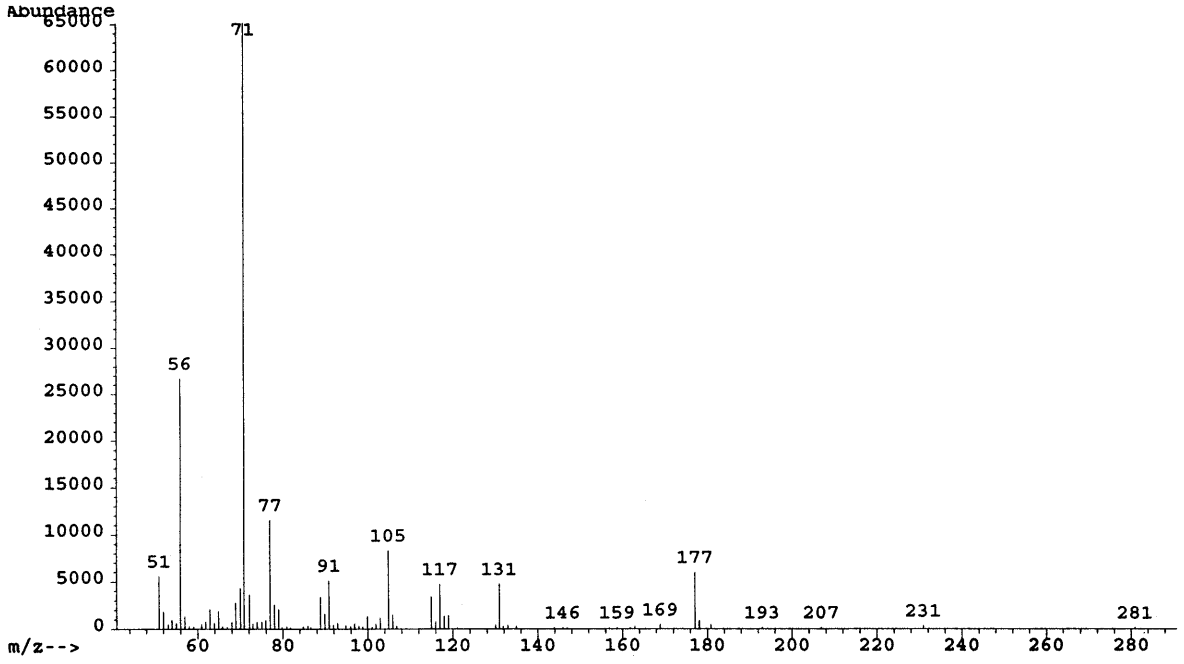

FIG. 3—*Electron ionization mass spectrum of underivatized phenmetrazine.*

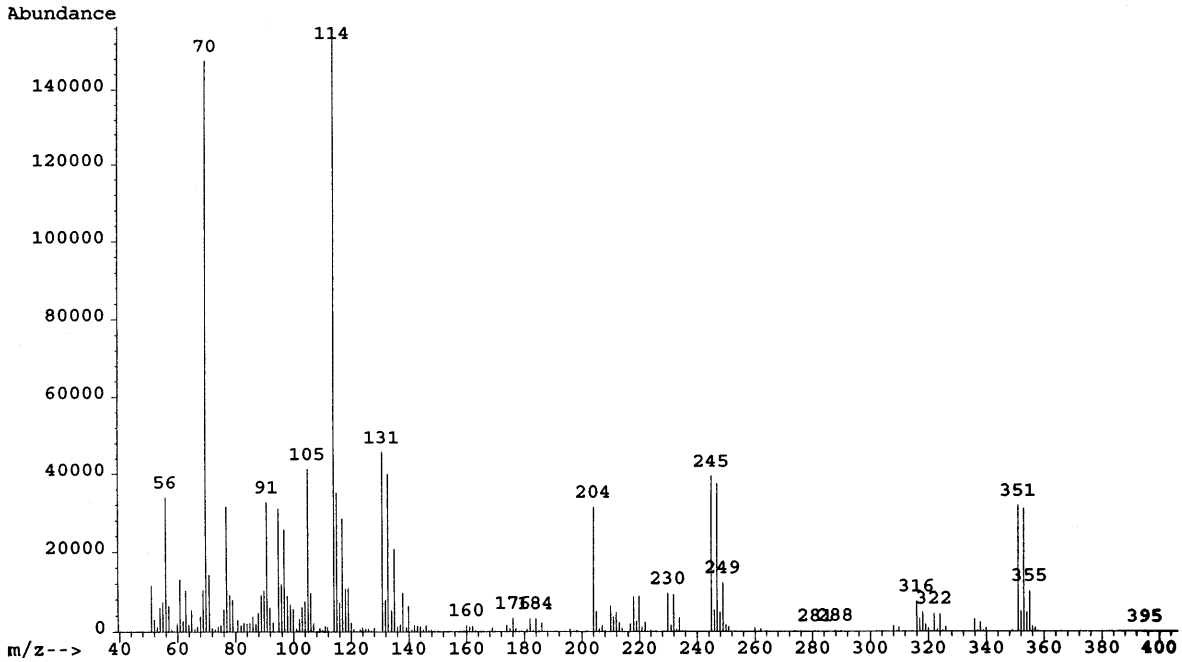

FIG. 4—*Electron ionization mass spectrum of phenmetrazine 2,2,2-trichloroethyl carbamate.*

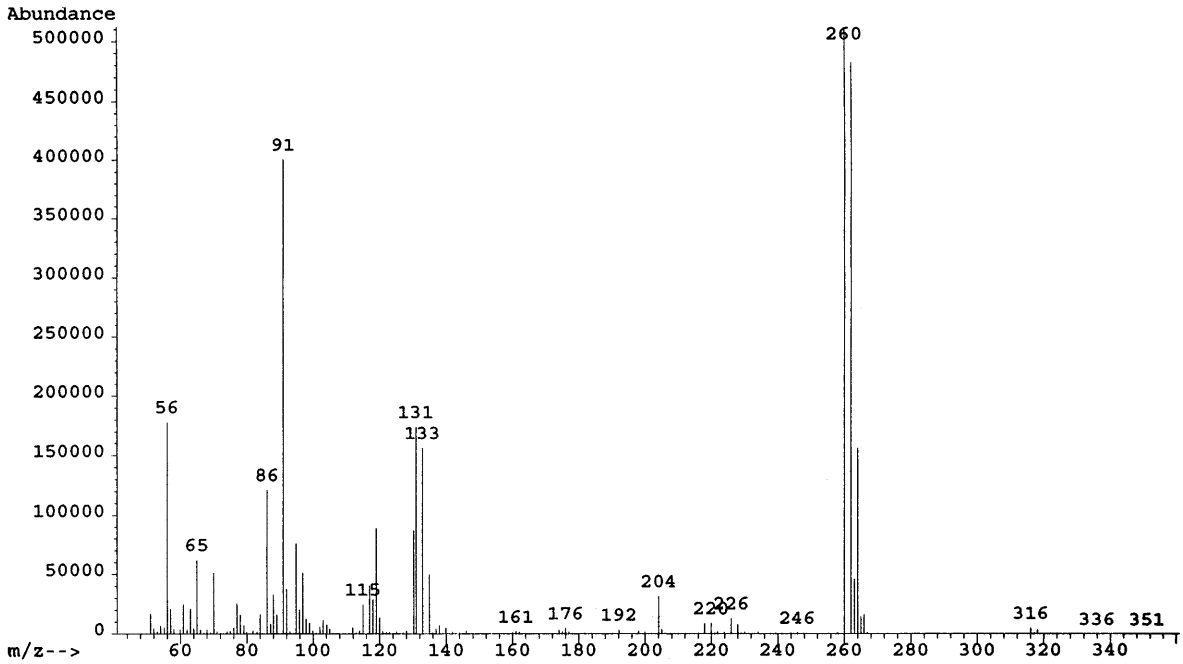

FIG. 5—*Electron ionization mass spectrum of N-propylamphetamine 2,2,2-trichloroethyl carbamate (internal standard).*

linear for a urinary phenmetrazine concentration of 1 mg/mL to 100 mg/mL. Using the *x*-axis as the target concentration and the *y*-axis as the observed concentration in the linearity study, we derived the regression equation:

$$y = 0.94x - 0.5 (r = 0.99)$$

The detection limit was 0.5 mg/mL urinary phenmetrazine.

Both derivatized phenmetrazine and the internal standard eluted at high temperatures. Therefore, we did not observe any coeluting peak or significant background noise. Moreover, in each analysis derivatized phenmetrazine and the internal standard were identified from mass spectral characteristics.

Interferences Study

Amphetamine and methamphetamine derivatized with 2,2,2-trichloroethyl chloroformate appeared at shorter retention times than the internal standard. Moreover, the mass spectral fragmentation patterns were also different. Amphetamine 2,2,2-trichloro ethyl carbamate showed a weak molecular ion at m/z 309 (relative abundance 0.7%) with a chlorine isotope peak at m/z 311 (relative abundance: 0.6%). The base peak was observed at m/z 218 due to the loss of $C_6H_5CH_2$ fragment from amphetamine 2,2,2-trichloroethyl carbamate. Another strong peak was observed at m/z 220 (relative abundance 96.1%) due to isotopic effect of chlorine atoms. Another strong characteristic peak at m/z 91 (relative abundance 87.4%) was also observed. The electron ionization mass spectrum of methamphetamine 2,2,2-trichloroethyl carbamate also showed very weak molecular ion cluster at m/z 323 (relative abundance 0.1%) and 325 (0.08%). The base peak was shifted to m/z 232 as expected. Another strong peak at m/z 234 (relative abundance 93.4%) was observed due to isotopic effect of chlorine. Two other characteristic peaks were observed at m/z 91 (relative abundance: 75.6%) and m/z 58 (relative abundance 52.6%) as expected. Ephedrine, pseudoephedrine, phentermine and phenylpropanolamine did not interfere with the assay. Pseudoephedrine showed a base peak at m/z 232 (the same base peak as methamphetamine) and another strong peak at m/z 234 (relative abundance 96.1%). Another strong peak was observed at m/z 121. Phentermine also showed a base peak at m/z 232 and another strong peak at m/z 234 (relative abundance 94.3%). Another strong peak was observed at m/z 91 (relative abundance 71.1%).

Analysis of Phenmetrazine in Blood

This assay can also be used to determine the concentration of phenmetrazine in blood. We supplemented one serum specimen obtained from a local blood bank with 10 mg/mL of phenmetrazine. The observed phenmetrazine concentration was 9.6 mg/mL. Because many postmortem serum specimens are highly hemolyzed, we hemolyzed whole blood and then obtained grossly hemolyzed serum specimens for supplementation with phenmetrazine. We also supplemented one postmortem serum with 25.0 mg/mL of phenmetrazine. In all cases we observed good correlation between the target and the observed concentrations (Table 1).

Application of the Assay

Phenmetrazine at the concentration of 1 mg/mL did not cross-react with the amphetamine EMIT immunoassay. We observed cross-reactivity with EMIT immunoassay at a urinary phenmetrazine concentration of 6.6 mg/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 volatile components of urinary matrix may occur. Derivatized phenmetrazine showed a longer retention time and good chromatographic property. The phenmetrazine methyl carbamate (derivatization with methyl chloroformate) showed a molecular ion with a relative abundance of 10% at m/z 235 (6). In our new derivatization protocol using 2,2,2-trichloro ethyl chloroformate, phenmetrazine 2,2,2-trichloroethyl carbamate showed a relatively strong molecular ion at a higher amm (m/z 351, relative abundance 20.5%), thus aiding in unambiguous identification and quantitation of urinary phenmetrazine. In addition, we also observed another strong molecular ion at m/z 353 and two other relatively weak molecular ions at m/z 355 and 357, due to isotopic abundance of chlorine. Moreover, phenmetrazine 2,2,2-trichloroethyl carbamate eluted at a higher temperature than phenmetrazine methyl carbamate. Therefore, our assay is free from interference from more volatile components of urine. The derivatization with 2,2,2-trichloroethyl chloroformate also can be carried out at room temperature in 10 min, similar to derivatization with methyl chloroformate.

TABLE 1—*Target and observed phenmetrazine concentrations in sera supplemented with various concentrations of phenmetrazine.*

Specimen	Phenmetrazine, mg/mL	
	Target	Observed
Serum	10.0	9.6
Hemolyzed serum 1	15.0	16.8
Hemolyzed serum 2	20.0	19.1
Postmortem serum	25.0	26.6

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