TECHNICAL NOTE

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Capillary Gas-Liquid Chromatography Separation of Phenethylamines in Amphetamine-Positive Urine Samples

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ABSTRACT: Good gas chromatography (GC) separation of molecules is essential for clean gas chromatography/mass spectrometry (GC/MS) confirmation of compounds. The trifluoro derivatives of ephedrine (E) and methamphetamine (MA) coelute on dimethyl silicone capillary columns, such as DB-1, which are most commonly used by chromatographers. Methods are described to separate E and MA to aid GC/MS confirmations of methamphetamine, ephedrine, or both E and MA together, whichever may be present in Enzyme Immunoassay (EIA)-analyzed amphetamine-positive urine samples. The use of the heptafluoro derivatives of E and MA on a DB-1 column, or the trifluoro derivatives of E and MA on a DB-17 column, is suggested for good gas chromatographic separation.

KEYWORDS: toxicology, urine, ephedrine, methamphetamine, immunoassay, chromatographic separation

Amphetamine abuse testing has been difficult and confusing for several reasons. The phenethylamines are numerous structurally related compounds and they all cross-react with immunoassay screening procedures [*1*]. The consequent problem is that some of the phenethylamines, such as ephedrine and phenylpropanolamine, are legally available in over-the-counter preparations, while others, such as *d*-amphetamine and methamphetamine, are scheduled by the Controlled Substances Act. To complicate things further, the separation of phenethylamines has produced a major confirmation problem in the past when using packed gas-liquid chromatography (GLC) columns [*2,3*]. Recently, capillary GLC with nitrogen-phosphorus detection (NPD) has yielded better resolution of specific molecules. However, when one of the most popular capillary columns (DB-1) is

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used, the trifluoro ephedrine and methamphetamine derivatives elute from the gas chromatograph at nearly the same time, 2.50 and 2.51 min, respectively (Fig. 1, *B* and *C*). Good GLC separation, even when gas chromatography/mass spectrometry (GC/MS) is used as the detector, is important because the phenethylamines are structurally similar and have similar ion fragments. Two alternative procedures are described in this paper which provide baseline separation of ephedrine and methamphetamine by capillary GLC.

Methods

Urine Extraction

To 0.8 mL of water (including 200 ng of phendimetrazine bitartrate as the internal standard), 0.2 mL of urine and 50 μL of 50% sodium hydroxide were added. After vortexing, 5 mL of *n*-butyl chloride was added and the tubes were placed on a mechanical shaker for 10 min. Following centrifugation, approximately 4 mL of the solvent was transferred to a conical test tube.

Derivatization

Twenty-five microlitres of trifluoroacetic anhydride was added to 4 mL of *n*-butyl chloride and mixed. Evaporation was performed on a N-Evap, at 50°C with a gentle flow

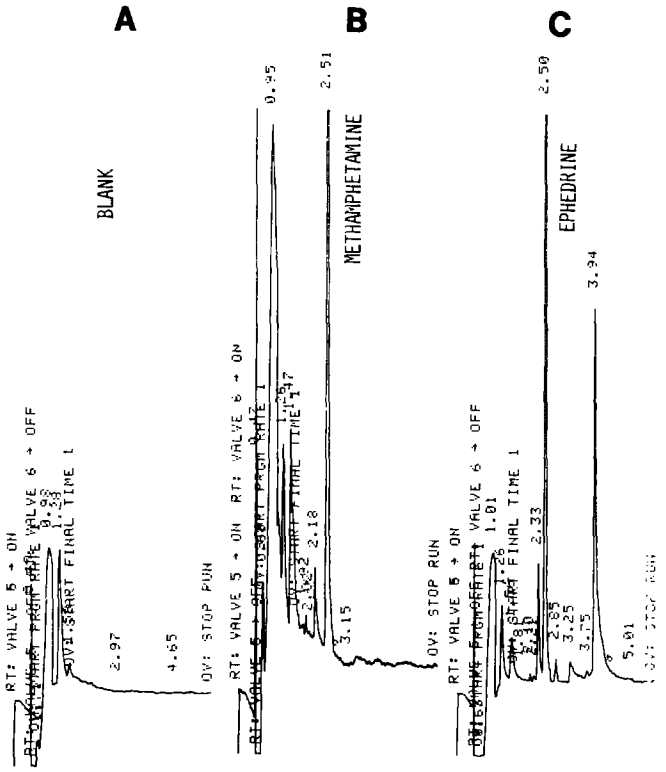


FIG. 1—*Chromatograms of urine extracts containing methamphetamine (MA) and ephedrine (E), using trifluoroacetic anhydride derivatization on a 15-m, DB-1 capillary column:* (A) = *blank urine;* (B) = *urine sample that was confirmed positive for MA by GC/MS (peak, 2.51 min);* (C) = *urine from a subject who ingested an ephedrine-containing medication (peak, 2.50 min).*

of nitrogen. This derivatization procedure was adequate for qualitative confirmation, but for quantitation, heating the capped tubes, at 70°C for 15 min prior to evaporation, is recommended. The dried samples were reconstituted in 50 μL of ethyl acetate, and 1 μL of the reconstituted sample was injected in the splitless mode. The same derivatizing procedure was used successfully with heptafluorobutyric anhydride.

Chromatography

A Hewlett-Packard 5880 A gas chromatograph, with two capillary column injection ports and two NPD detectors, was used. The columns were J & W DB-1 and DB-17, 15 m by 0.25 mm, with a 0.25-μm coating. The injection and detector port temperatures were 190°C and 300°C, respectively. The initial oven temperature was 110°C, which was held for 0.7 min. The program rate was 30°C/min, to reach 140°C, which was held for 4 min. When urine extracts were injected, a postrun heating cycle of 200°C for 3 min was used.

Results

Use of a DB-17 capillary column separates the trifluoro derivatives of methamphetamine and ephedrine by a significant margin (Fig. 2, *B* and *C*) with retention times of 3.20 and 2.42 min, respectively. Figure 3 shows the separation of trifluorophenethylamine

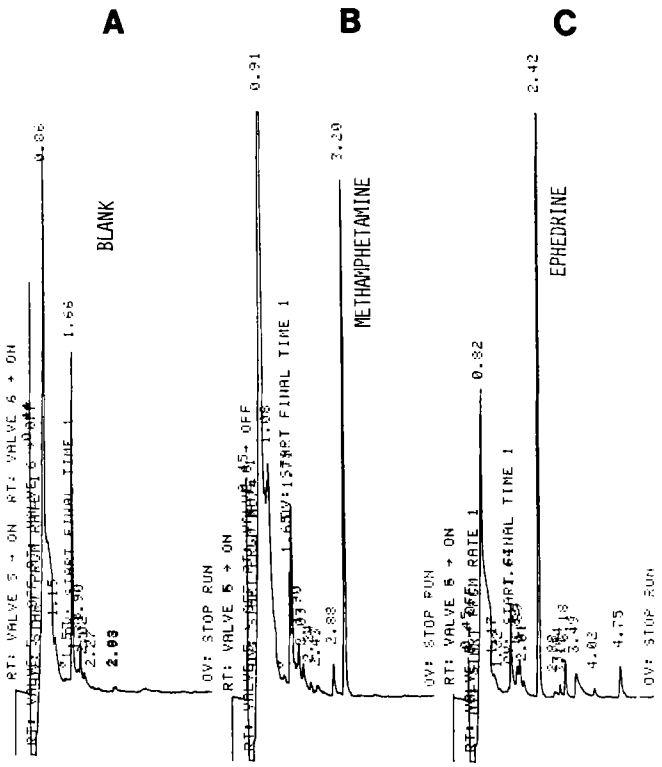
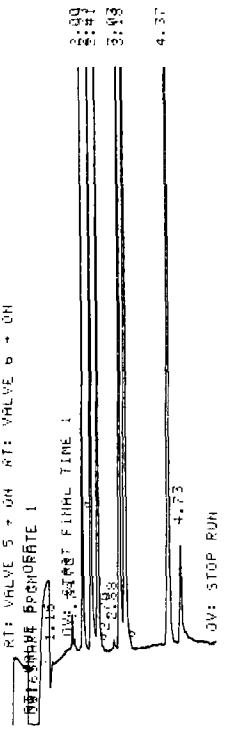


FIG. 2—*Chromatograms of urine extracts containing methamphetamine (MA) and ephedrine (E) using trifluoroacetic anhydride derivatization on a 15-m DB-17 capillary column:* (A) = *blank urine;* (B) = *urine sample that was confirmed positive for MA by GC/MS (MA = 3.20 min);* (C) = *urine sample from a subject who ingested an ephedrine-containing medication* (E = *2.42 min).*



3—Phenethylamine standards as trifluoro derivatives chromatographed on a 15-m DB-17 capillary column: phenylpropanolamine (2.00 min), amphetamine (2.23 min), ephedrine (2.41 min), pseudoephedrine (3.03 min), methamphetamine (3.18 min), and the internal standard, phendimetrazine (4.37 min).

derivatives on a DB-17 column with the internal standard, phendimetrazine. The following phenethylamine standards are shown in Fig. 3 in order of elution: phenylpropanolamine (2.00 min), amphetamine (2.23 min), ephedrine (2.41 min), methamphetamine (3.18 min), and the internal standard, phendimetrazine (4.37 min). The GC retention times for the trifluoro derivatives of phenethylamines are shown in Table 1.

Use of the DB-17 column and the trifluoro derivatives of phenethylamines is practical, providing baseline separation of the different substances. Yet, several laboratories rely on the use of DB-1 columns for confirmation of other drugs. Thus, it would be counterproductive to change capillary columns specifically for identification of specific sub-

TABLE 1—*GC retention times for trifluoro derivatives.*

	Retention Time, min	
Trifluoro Derivatives	DB-1	DB-17
Amphetamine	1.92	2.23
Methamphetamine	2.51	3.18
Ephedrine	2.50	2.41
Pseudoephedrine	2.85	3.03
Phenylpropanolamine	2.10	2.00

stances in amphetamine-positive urines. Therefore, one of us (M.E.) suggested the use of heptafluorobutyric anhydride to derivatize samples positive by the enzyme multiplied immunoassay technique (EMIT) amphetamine assay. The heptafluoro derivatives of ephedrine and methamphetamine separate well on DB-1 columns with the retention times of 3.26 and 2.82 min, respectively.

Conclusions

Ephedrine is present in many prescription and over-the-counter drug preparations used for the amelioration of asthma, allergy, and cold symptoms. The EMIT amphetamine assay is known to cross-react with closely related substances such as ephedrine and its major metabolite, phenylpropanolamine. Therefore, EMIT amphetamine-positive urine samples must be confirmed by a scientifically alternative method, such as GC/MS. Good gas chromatographic separation and identification are necessary to prevent false accusation of subjects using over-the-counter preparations containing ephedrine. The method in this technical note helps to separate phenethylamines in amphetamine-positive urine samples. Baseline resolution helps to achieve clean mass spectrometry fragmentation when this method is applied to GC/MS analysis of phenethylamines.

References

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