Identification of α-Phenylethylamine in Judicial Samples

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Abstract

α-Phenylethylamine was recently identified in samples from several judicial cases using chromatographic (high-performance liquid chromatography–diode-array detection, gas chromatography–mass spectrometry, and gas chromatography–Fourier transform infrared detection) and spectrometric (nuclear magnetic resonance) techniques. In the first case, 1 kg of a white powder was found in a basement laboratory. It contained caffeine and more than 15% α-phenylethylamine. In the second case, two white powders were seized from a female. One powder consisted of pure amphetamine, and the other was a mixture of caffeine, amphetamine, and α-phenylethylamine. Four months later, a couple, who were known drug users, were found dead in their apartment. Urine samples of both victims contained large amounts of amphetamine together with α-phenylethylamine. Recently, 0.13 kg of a white powder and 0.30 kg of an orange powder were seized during a law enforcement operation. Both powders were mixtures of caffeine, amphetamine, and α-phenylethylamine. The data presented demonstrate the recent and unrelated repetitive occurrence of α-phenylethylamine in the circuit of illicit drugs.

Introduction

Designer drugs have been synthesized by chemists in basement laboratories to create substances with specific effects but also to circumvent legal restrictions (1). Amphetamine derivatives like methamphetamine ("ice"), 3,4-methylenedioxyamphetamine (MDA, "love drug"), 3,4-methylenedioxymethamphetamine (MDMA, "Adam," or "XTC"), and 3,4-methylenedioxyethamphetamine (MDEA, "Eve") form a major class of commonly used designer drugs (2). The widespread abuse of MDMA and MDEA as recreational entactogens, especially, has recently become a problem in Belgium. This trend is reflected in the number of judicial samples, analyzed in our laboratory, that were found positive for these compounds.

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Since April 1994, however, another amphetamine-like compound, which had not previously been demonstrated in our laboratory nor reported in the context of drug abuse, was found in some samples. The detailed description of the investigated samples and the identification procedures for this compound are presented in this paper.

Experimental

Solvents and reagents

All solvents were of high-performance liquid chromatographic-grade purity. Heptafluorobutyric acid anhydride (HFBA) and racemic α-phenylethylamine were obtained from Sigma (St. Louis, MO). Amphetamine and caffeine were available from the standards collection at the Laboratory of Toxicology (University of Gent, Belgium).

Drug screening

Postmortem samples were analyzed following a previously described comprehensive screening using enzyme-multiplied immunoassay techniques (EMIT), radioimmunoassay (RIA), and chromatographic techniques such as high-performance liquid chromatography with diode-array detection (HPLC–DAD) and gas chromatography with mass spectrometric detection (GC–MS) (3,4).

For the analysis of the powder samples, the EMIT and RIA techniques were omitted.

Sample preparation

Powder samples were weighed and dissolved in methanol (at 1 mg/mL for HPLC and GC–MS) or acetonitrile (at 10 mg/mL for GC–Fourier transform infrared detection [FTIR]), vortex mixed, and injected after centrifugation. For the cleanup of urine samples, solid-phase extraction on Bond Elut Certify® columns (130 mg) (Varian, Sunnyvale, CA) was used.

The procedure recommended by the manufacturer for the extraction of amphetamine and other basic drugs in urine samples was applied with some small modifications. The sug-

gested internal standard (*N*-propylamphetamine) was not available, extractions were performed on 2-mL urine samples (instead of 5 mL) and diluted to a total volume of 5 mL with HPLC-grade water, and evaporation was performed at room temperature under a gentle flow of nitrogen with the addition of 50 µL of methanolic HCl during the final evaporation period.

All samples were also injected after derivatization with HFBA (50 µL) for 20 min at 70°C. The excess HFBA reagent was evaporated under nitrogen, and the sample was redissolved in 35 µL acetonitrile prior to injection on the GC.

Identification procedure

For the structural elucidation of the unknown compound, additional state-of-the-art analytical techniques (i.e., ion-trap GC–MS, GC–FTIR, and nuclear magnetic resonance [NMR]) were used.

For GC–MS analysis, a series 3400 Varian gas chromatograph was used in combination with a Finnigan MAT Magnum mass selective ion-trap detector (San José, CA). A J&W Scientific DB-5 MS capillary column (30 m × 0.25-mm i.d.; 0.25-µm film thickness) (Folsom, CA) was installed in the gas chromatograph. The injector and the transfer line were held at 280 and 270°C, respectively. The carrier gas was helium, and its flow rate was 0.8 mL/min. The initial oven temperature of 70°C was held for 1 min, then programmed to 100°C at 30°C/min and more slowly ramped to 270°C at 10°C/min. Samples (1 µL) were injected in the splitless mode.

For GC–FTIR analysis, a Perkin-Elmer AutoSystem gas chromatograph (Buckinghamshire, U.K.) was used in combination with a Perkin Elmer GC–IR System 2000 interface and an FTIR System 2000 detector. An HP Ultra-1 capillary column (25 m × 0.32-mm i.d.; 0.50-µm film thickness) (Hewlett-Packard, Palo Alto, CA) was installed in the gas chromatograph. The programmed temperature vaporization injector from Gerstel (Brielle, The Netherlands) was used in the splitless mode and programmed from 50 to 250°C at a rate of 12°C/s with a splitless time of 1 min. The initial oven temperature of 100°C was held for 1 min and then programmed to 270°C at 10°C/min. The light pipe was heated at a constant temperature of 225°C. The carrier gas was helium, and its flow rate was 0.95 mL/min.

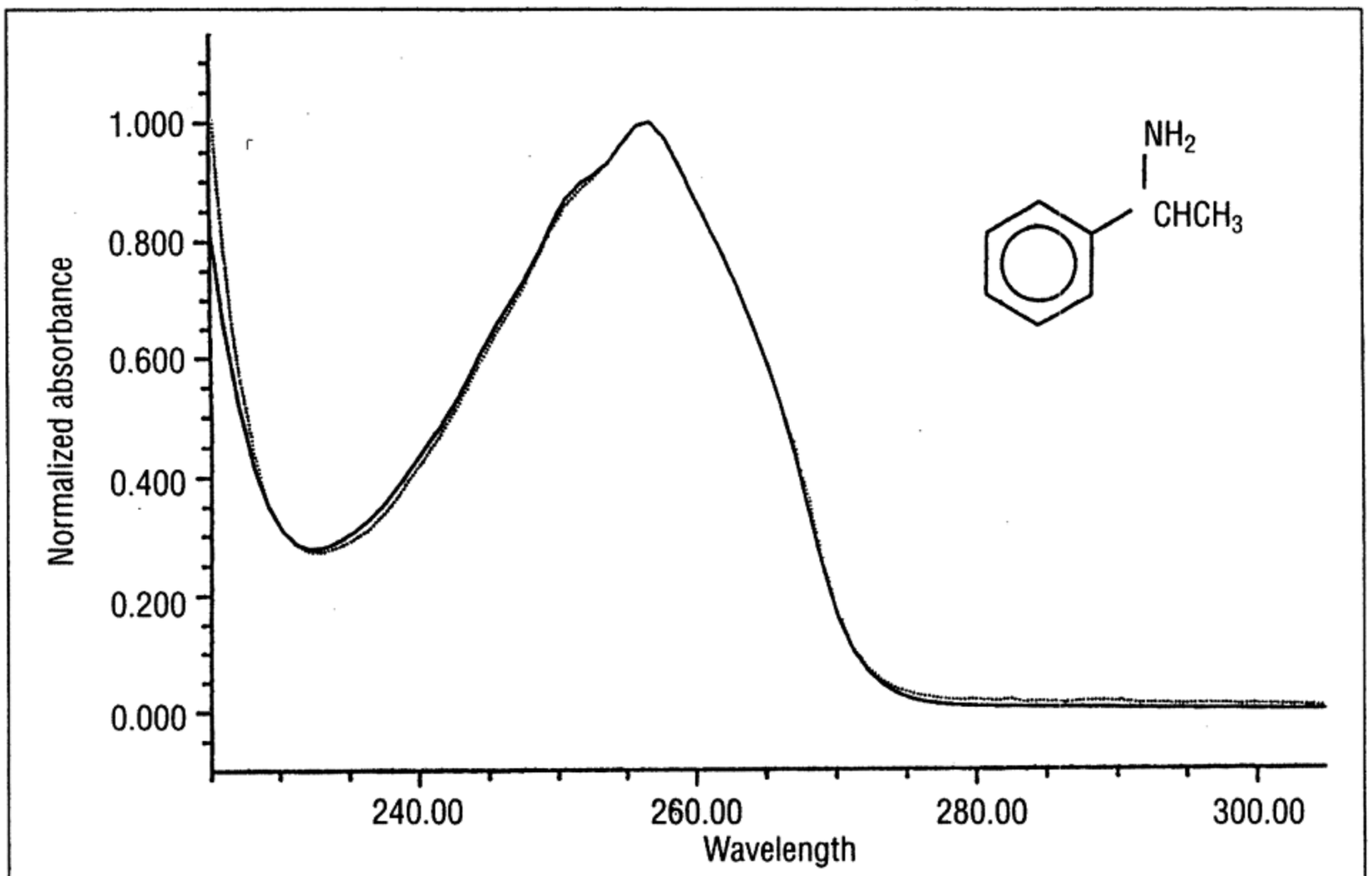

Figure 1. Structural formula of α-phenylethylamine ($C_8H_{11}N$; molecular weight = 121) and overlayed UV spectra of the standard (straight line) and the powder sample (dotted line) of Case 1.

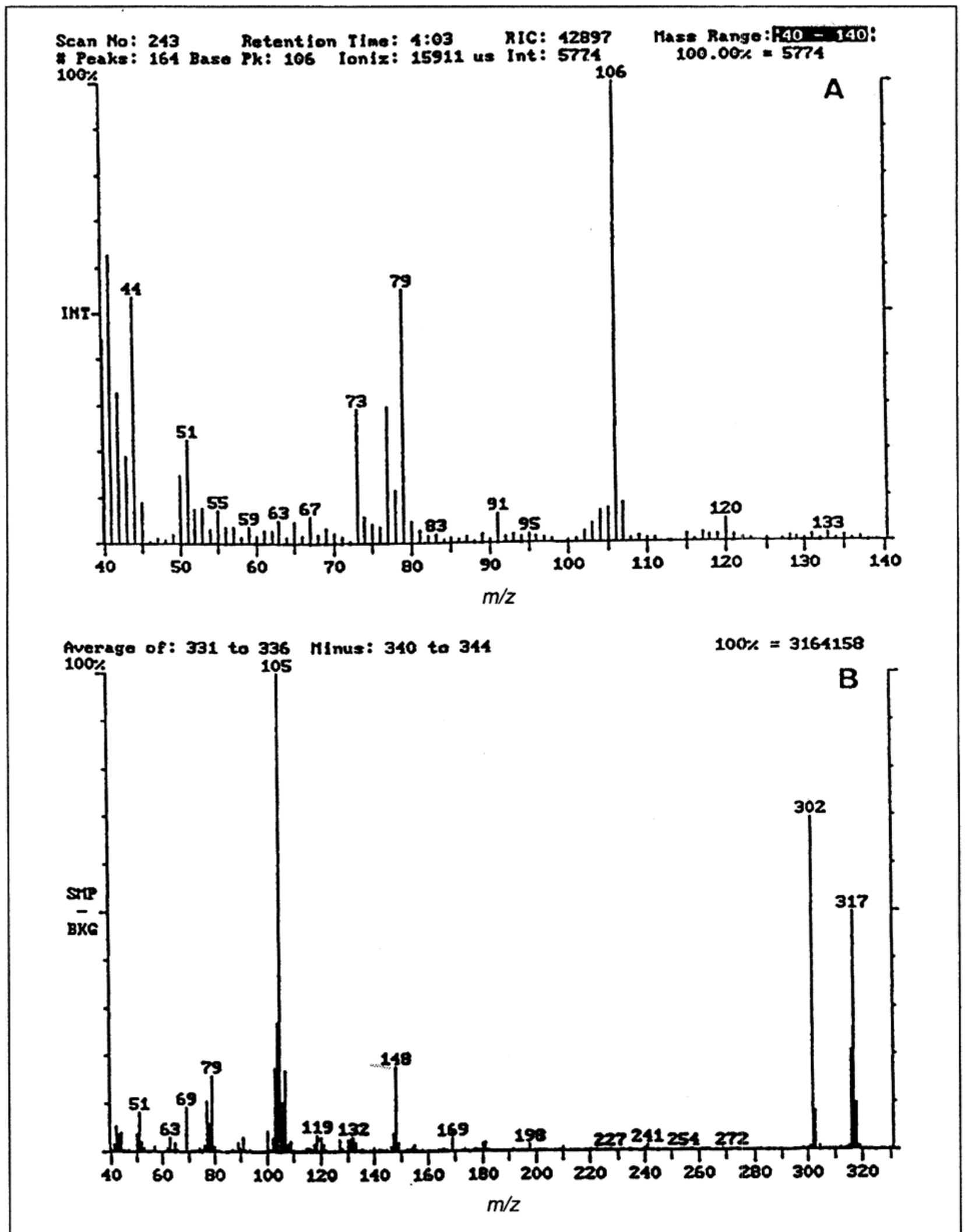

Figure 2. EI–MS spectra of α-phenylethylamine obtained from (A) powder sample B of Case 2 and (B) the HFB-derivatized urine A sample extract of Case 3.

The proton NMR spectra were obtained on a Brucker WH 360 instrument. A saturated solution of the powder samples in deuterated methanol was used to record chemical shifts relative to tetramethylsilane (in parts per million [ppm]).

Results and Discussion

The first relevant case dates from April 1994, when approximately 1 kg of a white powder was found in a basement laboratory together with 11 other pieces of evidence (powders and tablets).

As expected, the routine HPLC and GC–MS screening methods identified drugs such as cocaine, MDMA, MDEA, cannabinoids (marijuana), and associated compounds such as phentermine and caffeine in most of the seized powders and tablets. However, in the plastic bag containing the largest amount of powder (1001.7 g), a mixture of caffeine with an unknown compound was present.

On the HPLC system (3), the unknown compound eluted with a capacity factor (k') of 3.2. Both the compound and β-phenylethylamine showed a similar ultraviolet (UV) spectrum, displaying an absorption maximum at 257 nm. However, β-phenylethylamine eluted almost 2 min later ($k' = 4.9$). Our general screening procedure using GC–MS (4) only revealed the presence of caffeine. By using the low temperature screening conditions for amphetamines, the unknown compound was detected. It chromatographed at a retention time of 4.2 min. The GC–MS library search (National Institute of Standards and Technology) tentatively identified the compound as 1-methylbenzylamine or α-phenylethylamine, and its molecular weight was 121 (the structural formula is shown in Figure 1). This molecule clearly had a different retention behavior and mass spectrum compared with its known positional isomer, β-phenylethylamine (retention time [t_R] = 4.9 min). Both compounds have been classified as endogenous putrefactants in the literature (5–8). Nevertheless, only β-phenylethylamine had been demonstrated in our laboratory before, especially in putrefied forensic samples. Based on the obtained structural information, pure α-phenylethylamine standard was purchased, diluted at 1 µg/µL, and injected on both systems under the same chromatographic conditions as the sample. Coelution of the peak of the standard and of the unknown was twice observed, and both spectra showed good correlation, as shown in Figure 1, for the UV spectrum.

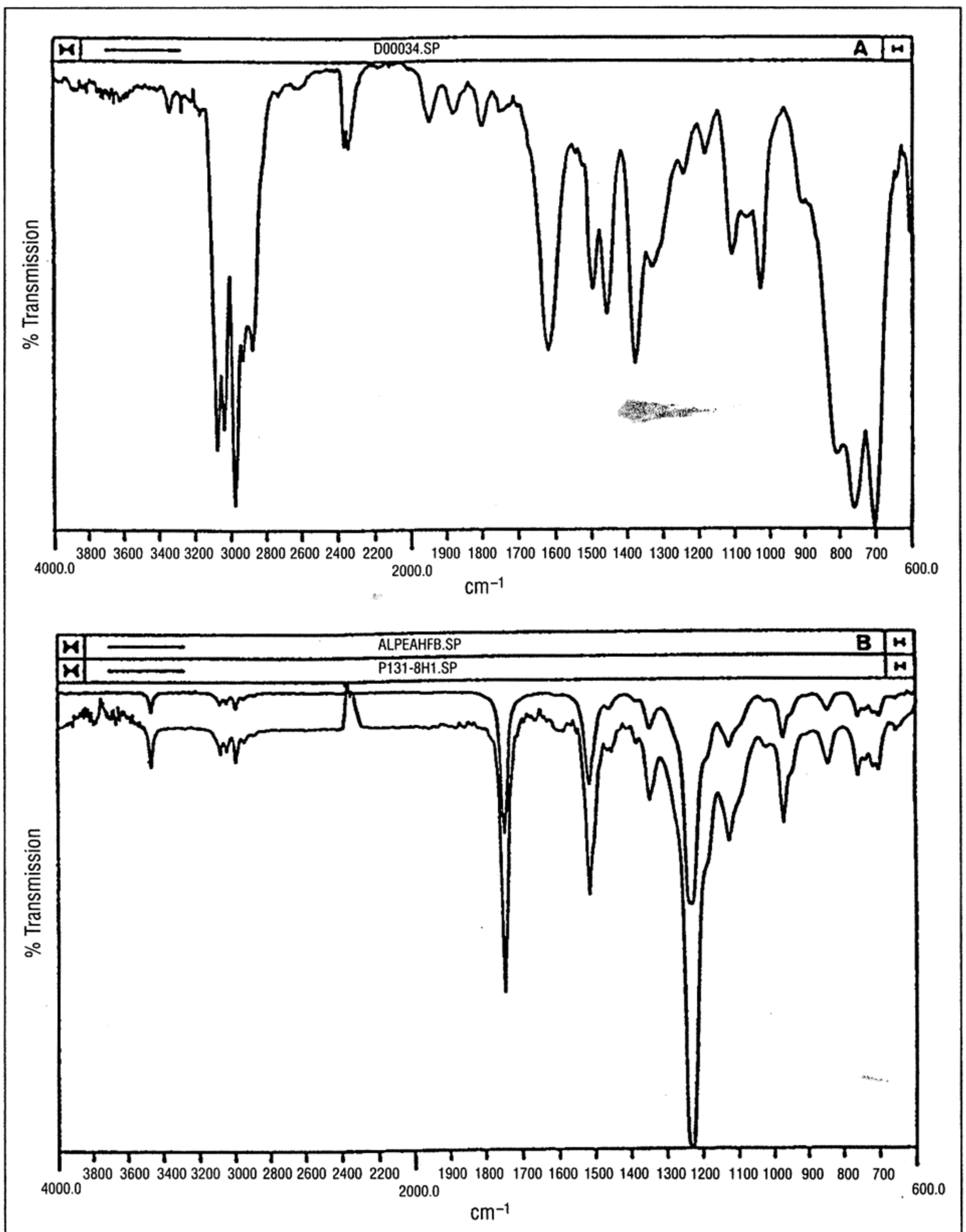

Figure 3. Vapor-phase FTIR spectra of α-phenylethylamine obtained from (A) the underivatized standard and (B) the HFB-derivatized standard and powder sample A of Case 4 (shown overlayed in B).

The electron-impact (EI) mass spectrum of α-phenylethylamine (Figure 2A) showed virtually no molecular ion (9). Instead, a peak at *m/z* 120 (M–1) was present, clearly differentiating the spectrum from that of β-phenylethylamine. The base peak ($[M–CH_3]^+$, *m/z* 106) resulted from the loss of a methyl fragment through α-cleavage, initiated by a radical nitrogen. Alternatively, a charged imine product ($[CH_3–CH=NH_2]^+$, *m/z* 44) was formed. Ionization in the π-electron system of the aromatic ring led to fragmentation in which the aromatic ring was part of the charged fragment. This process was responsible for two prominent ions at *m/z* 77 and 79 (with hydrogen rearrangement).

Further confirmation with complementary state-of-the-art analytical techniques was necessary for proper identification. Therefore, vapor-phase FTIR spectra of both the standard and the sample were recorded. Again, the unknown peak was identified as α-phenylethylamine based on its retention time (t_R = 4.3 min) and FTIR spectrum (Figure 3A). In this spectrum, bands at approximately 3000 cm^{-1} (aromatic C–H stretching), 1630 cm^{-1}, and 1350 cm^{-1} (primary amine) were observed.

Because the chromatographic properties of underivatized α-phenylethylamine were poor and the sensitivity obtained under these conditions was low, a derivatization step with HFBA was performed. This also generated an additional identification criterion. The HFB derivative eluted later (t_R = 7.0 min), and superior results (better peak shape and sensitivity) in comparison with the underivatized samples were obtained. In both the GC–FTIR and GC–MS experiments, coelution of the derivatized standard and sample peaks was observed, and the corresponding spectra proved to be identical. In the EI spectrum of the mono-HFB derivative of α-phenylethylamine (Figure 2B), three prominent ions were present: the molecular ion (*m/z* 317), the $[M–CH_3]^+$ ion (*m/z* 302), and the base peak (*m/z* 105), which corresponded to the $[Ph–CH=NH]^{+\cdot}$ ion. The FTIR spectrum of HFB-derivatized α-phenylethylamine (Figure 3B) was dominated by a large band at approximately 1230 cm^{-1}, characteristic for these derivatives. Sharp bands at approximately 1750 cm^{-1} and 1500 cm^{-1}, both from the amide, were clearly discernible.

Finally, an NMR spectrum was recorded from the α-phenylethylamine standard and a sample. This provided the final proof with respect to the identity of this compound (Figure 4). Assignments were simple and straightforward. Relevant signals for chemical shifts were observed at approximately 7.5, 4.5, and 1.5 ppm. They corresponded with the protons on the phenyl group (multiplet), the α-carbon (quadruplet), and the methyl group (duplet), respectively: ^1H NMR (360 MHz, CD_3OD), δ 7.42 (multiplet, 5H, Ph), 4.46 (quadruplet, 1H, $CHCH_3$), 1.65 (duplet, 3H, CH_3CH). A perfect match was found with a literature reference spectrum (10).

Since the first case, three other cases have followed in which α-phenylethylamine was involved. In the second case, small amounts of two white powders were found in the possession of a 20-year-old female and sent to us for analysis by a general practitioner. One powder contained only amphetamine, and the other was a mixture of caffeine, amphetamine, and α-phenylethylamine. As the two-carbon homologue of amphetamine, α-phenylethylamine eluted significantly earlier than amphetamine in the gas chromatographic systems. In the third case, a couple, who were known drug users, were found dead in their apartment. By using EMIT screening, urine samples of both the male and female were positive for amphetamines. Further investigation identified large amounts of amphetamine together with α-phenylethylamine. No other drugs were present. In the fourth case, 17 different pieces of evidence were seized during another law enforcement operation. As in Case 1, "traditional" drugs were found in most tablets and powders, but two powders, one white (0.13 kg) and the other orange (0.3 kg), were mixtures of caffeine, amphetamine, and α-phenylethylamine. An overview of the analyzed samples is given in Table I.

In conclusion, the described chromatographic and spectral data provided unequivocal proof with respect to the identity of the early-eluting substance as α-phenylethylamine.

The presented data only demonstrate the recent and unrelated repetitive occurrence of α-phenylethylamine in the circuit of illicit drugs. To our knowledge, only one parallel case is known, describing the two-carbon homologue of methamphetamine, *N*-methyl-α-phenylethylamine (11).

Several speculations can be put forward to explain the described phenomenon. α-Phenylethylamine might well be circulating accidentally, following an unintentional synthesis starting from a wrongly supplied precursor (i.e., acetophenone or phenylmethyl ketone instead of phenylacetone or benzyl methyl ketone) (12) by a fraudulous operator. Alternatively, two-carbon homologues of the existing series of illegal amphetamines are potentially interesting either to clandestine drug cooks to circumvent legal restrictions and/or because α-phenylethylamine is still cheaply available from several chemical suppliers in unlimited quantities.

However, it should be stressed that both the pharmacological activity and the toxicity of α-phenylethylamine are virtually unknown. Reports in the literature are scarce (13,14) and indicate only a weak central (nervous system) stimulant activity.

Nevertheless, basement chemists may again have complicated the diagnosis and management of drug intoxications.

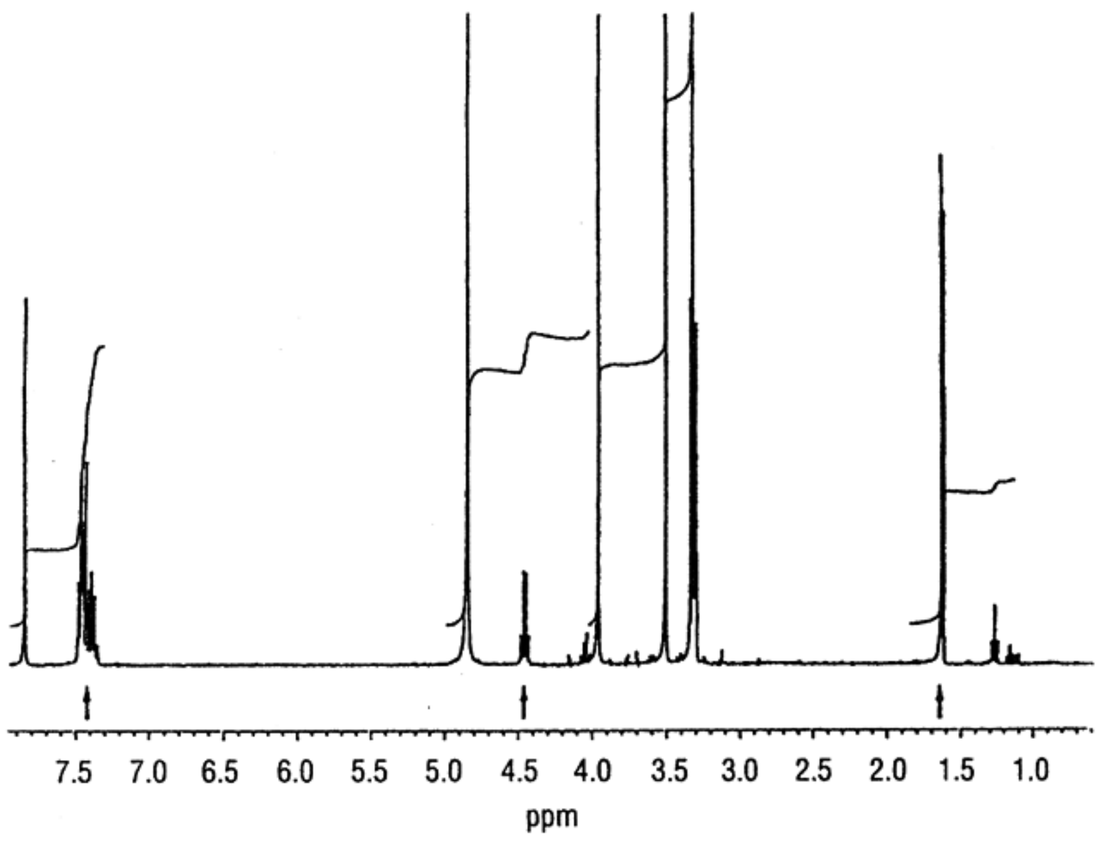

Figure 4. Proton NMR spectrum obtained from the powder sample of Case 1 (mixture with caffeine). Arrows indicate relevant chemical shifts.

Table I. Toxicologically Relevant Substances Identified in Four Different Cases

Samples	α-Phenylethylamine	Amphetamine	Caffeine
Case 1			
powder	+ (17%)	–	+
Case 2			
powder A	–	+	–
powder B	+	+	+
Case 3			
urine A	+ (12 µg/mL)	+	–
urine B	+ (28 µg/mL)	+	+
Case 4			
powder A	+ (25%)	+	+
powder B	+	–	+

* + = Present; – = not present.

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