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Identification of famprofazone ingestion

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Abstract After a traffic accident a 32-year-old man was suspected of having previously taken an illegal drug. An immunochemical screening procedure revealed positive results for amphetamines in both urine and blood samples. The preliminary test was confirmed by GC/MS and both amphetamine and methamphetamine were found in both body fluids. However, the man denied any use of drugs but claimed to have taken four tablets of Gewodin. One of the ingredients, famprofazone, undergoes metabolic conversion to amphetamine and methamphetamine. Using GC/MS the ingestion of famprofazone was verified by identification of the unchanged parent compound in the urine sample.

Key words Famprofazone · Amphetamine · Methamphetamine · Drug abuse · GC/MS analysis

Introduction

The interpretation of amphetamine and methamphetamine positive urine or blood testing results is a challenge in forensic toxicology for several reasons. Because amphetamine and methamphetamine are also available by prescription, in such cases their use is legitimate. A number of different compounds have been shown to be metabolized to either amphetamine or methamphetamine. At least 14 compounds, under a wide variety of different names, have been shown to be precursors of methamphetamine and/or amphetamine [1, 2, 5]. Often identification of the unchanged precursor drug and/or specific metabolites is used to distinguish between an illegal or legitimate use. Also the enantiomeric form of the drug can be very helpful in determining the potential source of amphetamine or methamphetamine. In the United States the presence of *l*-methamphetamine alone is consistent with the use of a Vicks Nasal inhaler containing the less potent *l*-isomer of methamphetamine. Laboratories are requested to confirm positive methamphetamine results with a method that is capable of differentiating between the *d*- and *l*-isomer.

In the case presented here, after a traffic accident a 32-year-old man was suspected of having previously taken an illegal drug. Urine and blood were collected and an immunoassay was used as a preliminary test with positive results for amphetamines. With a quantitative GC/MS procedure amphetamine and methamphetamine were confirmed in the body fluids. However, the man denied any use of drugs but claimed to have taken four tablets of Gewodin in the last 6 h before the blood sample was taken (2×2; 6 h and 3 h before).

Gewodin is used as an antipyretic/analgesic in Germany and one tablet contains 250 mg paracetamol (acetaminophen), 75 mg propyphenazone (isopropylphenazone), 30 mg caffeine and 25 mg famprofazone (4-isopropyl-2-methyl-3-[[N-methyl-N-(α-methylphenylethyl) amino]methyl]-1-phenyl-3-pyrazolin-5-one). This multiingredient medication is available over-the-counter and it is recommended for headache, migraine, toothache and pain associated with rheumatism.

This drug has been demonstrated to be metabolized to methamphetamine and amphetamine following adminis-

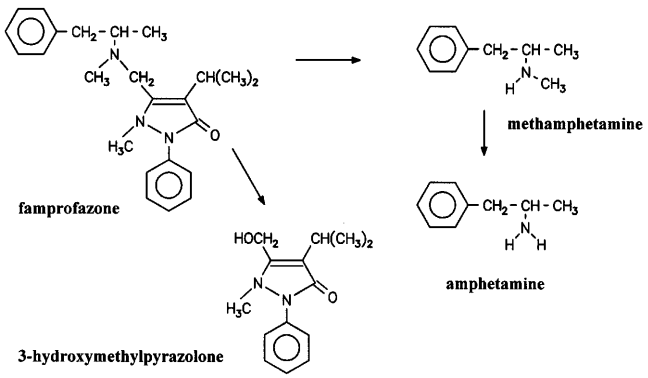

Fig. 1 Main metabolic pathway of famprofazone

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tration (Fig. 1) and has been shown to produce positive drug tests [12]. Therefore, famprofazone has been removed from the market in Korea [8].

Materials and methods

Chemicals

n-Hexane, n-butyl chloride, dichloromethane, methanol, ethyl acetate (Uvasol, Merck Darmstadt, Germany), water (HPLC grade, Baker Gross-Gerau, Germany), sodium hydroxide (p.a. Merck), S(-)-N-trifluoroacetyl-prolyl chloride (TPC; 0.1 mol/l in dichloromethane) (Aldrich, Steinheim Germany), N-methyl-bis-trifluoroacetamide (MBTFA) (Macherey-Nagel, Dueren Germany). Amphetamine, methamphetamine as well as d_3-amphetamine and d_3-methamphetamine were obtained commercially (Sigma, Deisenhofen Germany).

Instrumentation

A Model 5890 Series II Plus GC (Hewlett Packard) with a 5972 mass selective detector (MSD) was used for analysis. Data acquisition and analysis were performed using standard software supplied by the manufacturer. A fused silica capillary column HP-5MS (30 m × 0.25 I.D.; film thickness = 0.25 µm) was used.

Non-enantioselective determination of amphetamine and methamphetamine

Amphetamine and methamphetamine were determined as described previously [4]. After addition of internal standard (20 ng) and 30 µl 2 mol/l aqueous sodium hydroxide to 0.2 ml of serum or urine, samples were extracted with 0.5 ml *n*-hexane and 0.16 ml of the organic supernatant was transferred to a vial. To form the trifluoroacetyl derivatives, 40 µl MBTFA was added to the extract, the sample was mixed by vortexing and incubated at 90 °C for 15 min. A volume of 1 µl was injected into the GC/MS. Temperature programme 80 °C for 1 min, 10 °C/min up to 180 °C held for 4 min, 15 °C/min up to 290 °C held for 5 min; split-splitless injector at 250 °C. The mass fragments m/z 91, 118 and 140 were chosen as diagnostic ions for the TFA-derivative of amphetamine, masses m/z 91, 118 and 154 were used to indicate the presence of the TFA-derivative of methamphetamine.

Enantioselective determination of amphetamine and methamphetamine

Aliquots of 1 ml of serum or urine were alkalized by addition of 0.15 ml 0.1 mol/l aqueous sodium hydroxide and extracted with 5 ml *n*-butyl chloride. The whole organic layer was transferred to a vial and incubated at ambient temperature for 15 min after addition of 50 µl TPC. Following extraction with 2 ml 0.01 mol/l aqueous sodium chloride, the supernatant was transferred to a glass vial and dried under a stream of nitrogen at 50 °C. Following reconstitution in 200 µl ethyl acetate 1 µl was injected into the GC/MS. Temperature programme 140 °C for 1 min, 10 °C/min up to 250 °C held for 2 min, 10 °C/min up to 290 °C held for 5 min; split-splitless injector at 250 °C. Mass chromatography with the ion m/z 237 indicated the presence of S-trifluoroacetyl-prolyl-*R*,*S*-amphetamine, the characteristic mass fragment for *S*-trifluoroacetyl-prolyl-*R*,*S*-methamphetamine was m/z 251. Additionally the masses m/z 166 and 194 were detected as qualifier ions.

Standard extractions

A 5 ml portion of urine was refluxed with 1.5 ml of 37% hydrochloric acid for 15 min. Following hydrolysis, the sample was basified with 2 ml of 10 mol/l aqueous sodium hydroxide and the resulting solution was mixed with 2.5 ml of 2.3 mol/l aqueous ammonium sulphate to obtain a pH between 8 and 9. This solution was extracted with 5 ml of a dichloromethane-isopropanol-ethyl acetate mixture (1:1:3 v/v). After phase separation by centrifugation, the organic layer was transferred and evaporated to dryness. The residue was dissolved in 50 µl of methanol and 0.2 µl was injected into the gas chromatograph.

The serum sample (1 ml) was adjusted to pH 9 by addition of sodium hydrogen carbonate and extracted with 5 ml of a mixture of n-hexane-ethyl acetate-isoamylalcohol (7:3:0.1 v/v). The organic layer was transferred and 2 ml of 2 mol/l sulphuric acid was added. After centrifugation the aqueous phase was separated and alkalized to pH 9 by addition of a few drops of 10 mol/l sodium hydroxide and extracted with 3 ml chloroform. After phase separation by centrifugation, the organic layer was transferred and evaporated to dryness. The residue was dissolved in 100 µl of ethyl acetate and 1 µl was injected into the gas chromatograph. The temparature programme was 80 °C for 1 min, 10 °C/min up to 300 °C held for 5 min; split-splitless injector at 250 °C.

Results

The results of the serum and urine analysis are summarized in Table 1. Preliminary immunochemical tests (CEDIA, Boehringer Mannheim, Germany) of urine and serum samples revealed positive results for amphetamines. With confirmation using the non-enantioselective GC/MS method, methamphetamine was measured in the urine sample at a concentration of 2831 ng/ml and amphetamine was calculated as 567 ng/ml. In the serum sample methamphetamine was determined at a concentration of 12.8 ng/ml and no amphetamine was detected. Using the enantioselective method, *l*-methamphetamine exceeded *d*-methamphetamine in the urine sample with 69% *l*-methamphetamine (Fig. 2) and amphetamine enantiomers were determined with 54% *l*-amphetamine.

Following standard extraction procedures propyphenazone and acetaminophen were identified in the serum sample by GC/MS. Analysis of the urine sample revealed positive results for the unchanged parent drug famprofazone. Selected ion chromatograms indicating the presence of famprofazone and its metabolite are shown in Fig. 3. Famprofazone and propyphenazone metabolites were identified by comparison of their mass spectra with reference libary spectra (Fig. 4) [9]. Ephedrine and norephedrine were identified in the urine sample in addition to amphetamine, methamphetamine and acetaminophen.

Table 1 Results of toxicological analysis of urine and serum

Urine	Serum
• Immunochemical amphetamine test positive (CEDIA)	• Immunochemical amphetamine test positive (CEDIA)
• Methamphetamine 2831 ng/ml (69% *l*-methamphetamine)	• Methamphetamine 12.8 ng/ml (59% *l*-methamphetamine)
• Amphetamine 567 ng/ml (54% *l*-amphetamine)	• Acetaminophen
• Ephedrin, norephedrine	• Propyphenazone
• Acetaminophen	
• Propyphenazone with metabolites	
• 3-hydroxymethylpropyphenazone	
• Unchanged famprofazone	

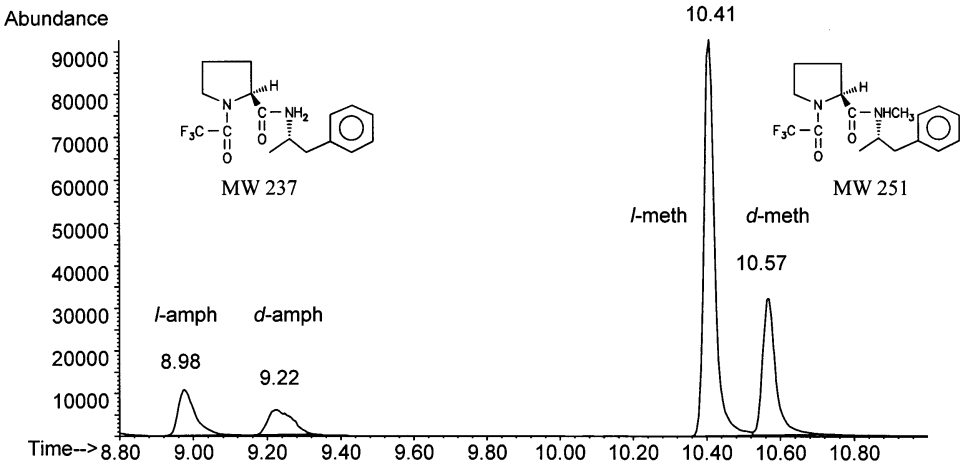

Fig. 2 Selected ion chromatograms with characteristic mass fragments of S-trifluoroacetyl-prolyl-*R,S*-amphetamine and *S*-trifluoroacetyl-prolyl-*R,S*-methamphetamine (structures of *d*-isomer TFP derivatives)

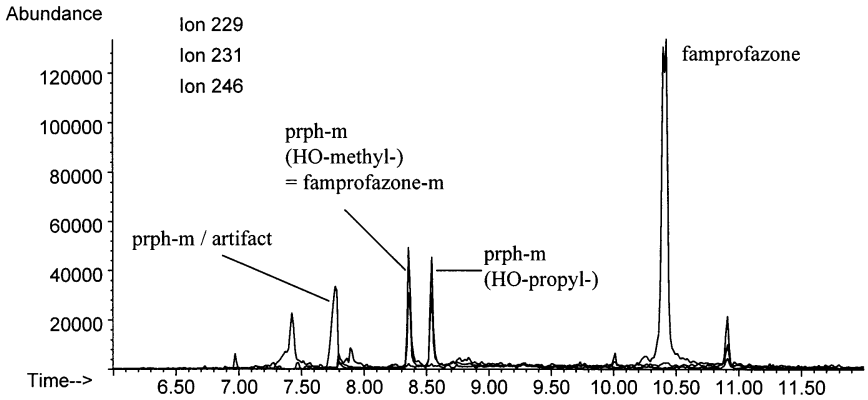

Fig. 3 Selected ion chromatograms indicating the presence of famprofazone and propyphenazone metabolites (prph-m) in the acetylated extracts of a urine sample. As a common metabolite of propyphenazone and famprofazone 3-hydroxymethylpyrazolone was identified

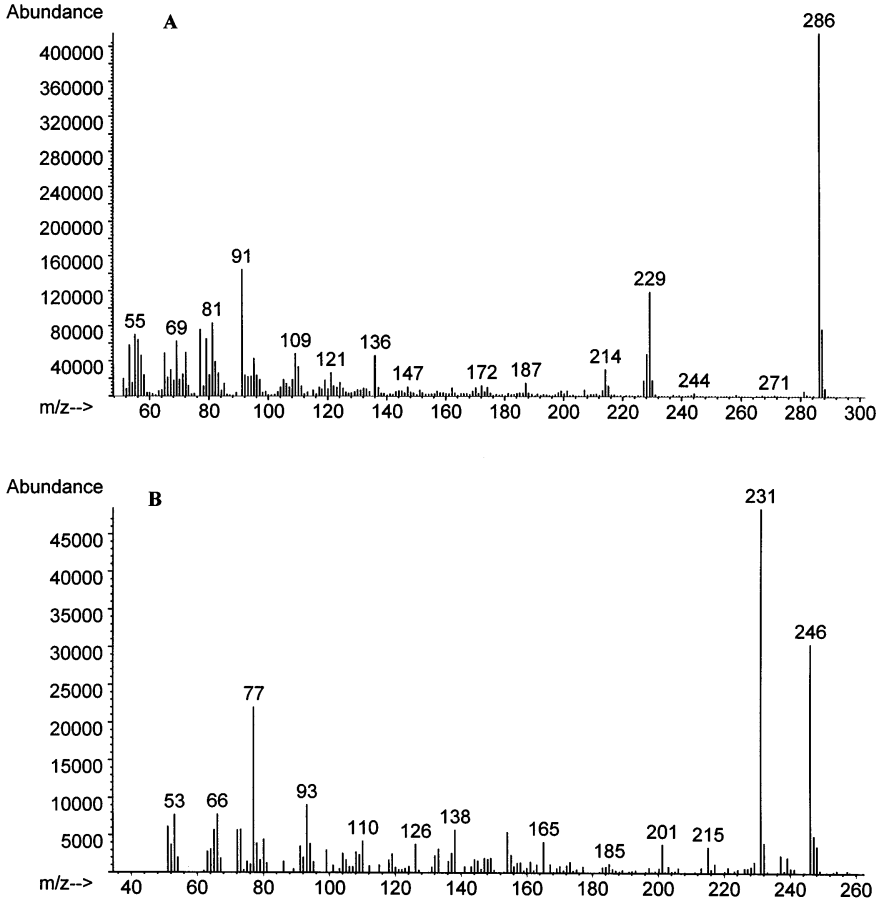

Fig. 4 Mass spectra of famprofazone (M^+ = 377) (**A**) and 3-hydroxymethylpyrazolone (**B**)

Discussion

Following oral administration of famprofazone, methamphetamine and the conjugate of 3-hydroxymethylpyrazolone have been detected in the urine of mice [6] and man [7]. 3-Formylpyrazolone, unchanged famprofazone, hydroxylated famprofazone, and 3-carboxylpyrazolone were not detected. Oh et al. [8] quantified methamphetamine and famprofazone in plasma as well as in urine after oral administration of famprofazone to volunteers. Following administration of 100 mg famprofazone, methamphetamine appeared in the plasma within 1 h of ingestion and the concentration was maintained at 24–44 ng/ml over 2–12 h. In urine samples intact famprofazone was not detected after a dose of 100 mg, whereas the major metabolite *N*-hydroxy-*N*-methylaminomethylpyrazolone formed by the Cope elimination was observed. After a single dose of 50 mg famprofazone Yoo et al. [12] found 44–105 μg methamphetamine in urine collected over 24 h. Cody [3] investigated the enantiomeric composition of amphetamine and methamphetamine metabolically derived from famprofazone by administration of 50 mg of famprofazone to a volunteer followed by collection of urine over the next 6 days. Peak concentrations of amphetamine and methamphetamine were seen in urine samples collected 14 h post-dose with 420 and 1996 ng/ml, respectively. The percentage of famprofazone metabolized to methamphetamine was 14.6%. The amount of *l*-methamphetamine exceeded *d*-methamphetamine in each sample beginning with 67% *l*-methamphetamine in the first sample, increasing to 100% in the last few samples. The percentage of amphetamine enantiomers was much closer beginning at approximately 50% *l*-amphetamine but rose only to approximately 55% in the later samples. This is in accordance with our findings on the enantiomeric composition of amphetamine and methamphetamine. Since *d*-methamphetamine represents the vast majority of illicit methamphetamine and metabolism of famprofazone results in both *d*- and *l*-methamphetamine, the author proposed that any sample which contains only d-methamphetamine could not have come from the use of famprofazone and that it leads to the suspicion of an illegal drug abuse. Identification of the 3-hydroxymethylpyrazolone metabolite was discussed to be a definitive proof of famprofazone administration.

Shin et al. [11] identified for the first time unchanged famprofazone as well as a new metabolite, hydroxydesmethylfamprofazone, in urine samples. Famprofazone was detected up to 6 h and the metabolite up to 32 h in human urine following administration of two tablets of Gewodin. The sum of the two compounds excreted in urine was approximately 1.5% of the dose.

Recently, Shin [10] studied the stereoselective metabolism of famprofazone. Following administration of racemic famprofazone the *l*-forms of methamphetamine, amphetamine, p-hydroxyamphetamine and p-hydroxymethamphetamine were excreted in greater amounts in urine samples than their enantiomers, as well as the *l*-enantiomers of ephedrine, pseudoephedrine, norephedrine and norpseudoephedrine. Famprofazone was metabolized by product and substrate stereoselective N-dealkylation, β-hydroxylation, and p-hydroxylation.

In the case presented here, methamphetamine and amphetamine were detected following ingestion of famprofazone in similar concentrations as described before and *l*-enantiomers also exceeded the *d*-enantiomers. Additionally, 3-hydroxymethylpyrazolone was identified in the urine sample. This metabolite of famprofazone was previously held to be definitive proof of ingestion of Gewodin [3]. However, the author did not take into account the other ingredients of Gewodin, especially propyphenazone. 3-Hydroxymethylpyrazolone is a common metabolite of propyphenazone and famprofazone. Therefore identification of this metabolite cannot be discussed as evidence of administration of Gewodin. A definitive proof is only given by identification of the unchanged parent drug.

In the case presented here the suspected man was exonerated from the suspicion of illegal drug abuse. However, it should clearly be stated that misinterpretation of positive immunoassay and even GC/MS results is possible, since the parent compound is detectable for a shorter time period than the metabolites amphetamine and methamphetamine.

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