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CASE REPORT

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Famprofazone as the Source of Methamphetamine and Amphetamine in Urine Specimen Collected During Sport Competition

ABSTRACT: During a sport competition event in Taiwan, one urine specimen was found positive for both methamphetamine (2688 ng/mL) and amphetamine (462 ng/mL). The specimen donor claimed that she had taken Gewolen® (a nonprescription drug manufactured in Taiwan) for treating abdominal pain and the medication was presented. Laboratory investigation confirmed that Gewolen® contains famprofazone, which is known to metabolize to methamphetamine and amphetamine and is included in the prohibited list of the World Anti-Doping Agency. Study on the excretion profiles of three volunteers ingesting 50 mg famprofazone produced the following patterns similar to that observed in the case specimen: (a) the ratio of methamphetamine to amphetamine was approximately 6 to 1; (b) *d*- and *l*-enantiomers of both methamphetamine and amphetamine were present, while the amount of *l*-methamphetamine was 3–4-fold greater than its counterpart. The data suggested that famprofazone (as the ingredient of Gewolen®) was likely the source of the prohibited drugs found in the case specimen.

KEYWORDS: forensic sciences, famprofazone, amphetamine, methamphetamine, athlete, dope testing

Famprofazone, 4-isopropyl-2-methyl-3[*N*-methyl-*N*-(α-methyphenylethyl)-aminomethyl]-1-phenyl-3-pyrazolin-5-one (see Fig. 1 in a later section for the chemical structure), is an analgesic and antipyretic agent available in several European countries as the primary ingredient of Gewolen® (1). It has long been reported to metabolize to methamphetamine and amphetamine (2), which are prohibited in sport competitions as currently regulated by the World Anti-Doping Agency (WADA) (3).

A urine specimen collected during a recent national sport competition in Taiwan tested positive for both methamphetamine and amphetamine. In response to the inquiry conducted by the local Doping Control Commission, the donor of the specimen claimed ingesting a locally manufactured nonprescription Gewolen® tablet before the competitions for treating abdominal pain. While famprofazone has recently been included in the WADA prohibition list, to the best of our knowledge, doping violation related to the ingestion of famprofazone has not yet been reported in the literature. This current report details data derived from the scientific investigation process.

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Materials and Methods

Chemicals and Standards

All reagents were of analytical grade. (*S*)-(–)-*N*-(trifluoroacetyl)-prolyl chloride (*l*-TPC) was purchased from Aldrich (Milwaukee, WI). Trifluoroacetic anhydride (TFAA), cysteine, *t*-butylmethyl ether (TBME), *n*-hexane, ethyl acetate, potassium carbonate, diphenylamine, and isopropanol were purchased from Riedel-de Haën (Wunstorfer Str. 40 Seelze, Germany). Sodium sulfate anhydrous was purchased from Tedia Co. (Fairfield, OH). Ethyl acetate was purchased from Mallinckrodt (St. Louis, MO). *d,l*-Amphetamine, *d,l*-methamphetamine, *d,l*-amphetamine-d_8, and *d,l*-methamphetamine-d_8 were purchased from Cerilliant (Austin, TX). Famprofazone was purchased from Sigma (St. Louis, MO). Gewolen® tablets manufactured by Health Chem. & Pharm. Co. (Taichung, Taiwan) were purchased from a local pharmacy.

Urine Samples

Urine samples included in this study were from two sources: (a) a total of 251 samples (male, $n = 133$; females, $n = 118$) collected from the athletes who were asked to provide at least 75 mL urine for doping control purpose during the 2004 National Junior and High School Games; and (b) samples collected from three healthy male volunteers who orally ingested a single 50 mg dose of famprofazone (two Gewolen® tablets; each tablet contains 25 mg famprofazone, 200 mg *N*-acetyl-*p*-aminophenol, 75 mg isopropylantipyrine, and 25 mg caffeine) (personal communication).

Urine samples from the volunteers were collected at 0 (before drug ingestion), 2, 4, 6, 8, 12, 24, and 48 h postadministration and were stored at -20°C. If more than one void was obtained at each collecting interval, urine samples were pooled as one unit for that specific time point. Specific gravity and pH of each urine sample

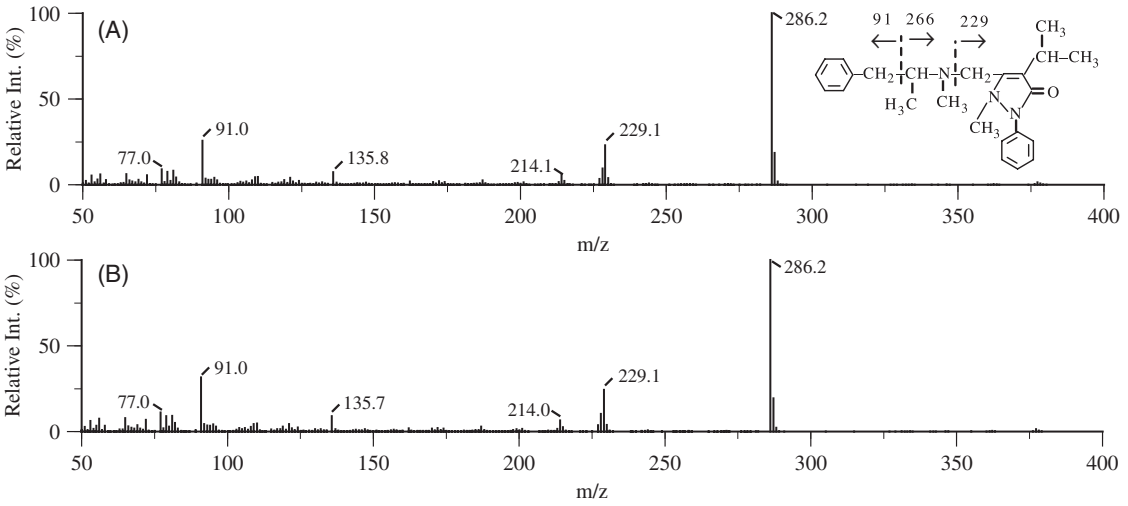

FIG. 1—*Mass spectra of famprofazone reference standard (A) and medication (Gewolen®) (B) prepared in methanol.*

were measured using a refractometer (Atago Co., Tokyo, Japan) and a pH meter (Mettler–Toledo) before sample preparations for chromatographic analysis.

Sample Preparation and Extraction Procedure

Analytical protocols established for detecting a comprehensive list of compounds of interest to sport testing laboratories were used in this study. While procedures commonly adapted by workplace drug-testing laboratories for the analysis of amphetamine and methamphetamine might have been simpler, the procedures described here fit well with the routines established in this laboratory.

Extraction and Derivatization Procedures for Routine Screen and Confirmation

Two 4-mL aliquots were processed for each urine specimen. The first aliquot was prepared by adding 100 mg cysteine and 0.5 mL HC1, followed by incubation at 100°C for 30 min. A volume of 3 mL TBME was then added, followed by shaking and centrifugation. The organic layer was aspirated to waste. The remaining aqueous phase was mixed with 0.6 mL NaOH, 2 g $NaHCO_3$: K_2CO_3 (3:2 w/w), 50 µL phenazine (90 µg/mL; internal standard [IS]), and 3 mL TBME. The mixture was shaken and centrifuged. The organic layer was retained to combine with an extract from the second aliquot.

The second aliquot was mixed with 50 µL phenazine, 2 g $NaHCO_3$: K_2CO_3 mixture, and 3 mL TBME: 2-propanol solution (9:1 v/v). The mixture was shaken and centrifuged at 2000 r.p.m. for 6 min. The organic layer was then combined with that obtained from the first aliquot. The combined extract was evaporated to dryness under nitrogen gas, the resulting residues were incubated with 100 µL TFAA at 70°C for 20 min, and then evaporated to dryness. The resulting products were reconstituted with 500 µL ethyl acetate for gas chromatography–mass spectrometry (GC–MS) analyses (4).

*Separation of the **d**- and **l**-Enantiomers of Amphetamine and Methamphetamine*

d- and *l*-Enantiomers of amphetamine and methamphetamine were separated based on a procedure detailed in a previous report (5). ISs, including 500 µL each of amphetamine-d_8 (10 µg/mL) and methamphetamine-d_8 (10 µg/mL), were added to a 1-mL aliquot of urine. The urine samples were extracted by adding 0.5 mL saturated K_2CO_3 solution, 2 mL *n*-hexane, and 50 µL *l*-TPC (derivatizing agent). The mixture was then shaken for 10 min and centrifuged at 2000 r.p.m. for 5 min. After centrifugation, the top organic layer was transferred to a clean, dry screw-top glass tube and evaporated to dryness under nitrogen gas. The residue was reconstituted with 200 µL *n*-hexane for GC–MS analysis.

Sample Preparation for Quantification by Gas Chromatography–Nitrogen-Phosphorus Detector (GC–NPD)

With high sensitivity and negligible interference from nonnitrogenous compounds, GC–NPD protocols have been well established in sport testing laboratories for simultaneous analysis of a broad list of underivatized drugs (6,7). In this study, amphetamine, and methamphetamine in the samples of interest were quantified using this approach. Specifically, an aliquot of 1 mL urine in a 20-mL glass tube was added, along with 10 µL diphenylamine (IS; 0.4 mg/mL), 100 µL KOH (5 N), 0.6 g NaCl, and 1 mL TBME. The mixture was shaken mechanically for 10 min and centrifuged at 2000 r.p.m. for 8 min. The organic layer was transferred to a glass vial containing 100 mg sodium sulfate and the sample was directly subject to GC–NPD analysis.

Instrumentation and Conditions

GC–NPD—A Hewlett-Packard HP 6890 gas chromatograph (Palo Alto, CA) equipped with a nitrogen–phosphorus detector (GC–NPD) was applied for sample quantification. GC was equipped with an HP-5MS crosslinked 5% diphenyl and 95% dimethylpolysiloxane capillary column (25 m × 0.25 mm × 0.33 µm film thickness). The injector was operated in the split mode (10:1) and the carrier gas was helium at flow rates of 11.0 and 1.1 mL/min. The injection port temperature was set at 250°C. The column was operated with an initial temperature of 100°C (holding time 1 min), followed by increasing 10°C/min to 200°C, and then 20°C/min to 300°C (holding time 4 min). One microliter of sample was injected with an autosampler.

GC–MS—A Hewlett-Packard HP 5890 GC interfaced with a 5972 mass selective detector (MSD) was used in the analyses.

A capillary column (HP-5MS cross-linked 5% diphenyl and 95% dimethylpolysiloxane 25 m × 0.25 mm × 0.33 μm film thickness) was used for GC separation. Helium was used as a carrier gas with a split flow rate of 1.1 mL/min. For routine analysis, the injection port and the interface temperatures were set at 250°C and 300°C, respectively. The initial temperature was 90°C, followed by increasing 15°C/min to 240°C and 10°C/min to 300°C (holding time 15 min). For the *l*- and *d*-enantiomer analysis, samples were injected in the splitless mode, with the injection port and the interface temperatures set at 250°C and 280°C, respectively. The initial temperature was at 150°C and held for 5 min, next programed to 250°C at 20°C/min, and held for 5 min. The electron impact ionization was 70 eV and the mass spectrum was obtained by scanning from *m/z* 50 to 550. The injection volume was 1 μL for all the analyses.

The analysis was carried out in a selected ion monitoring (SIM) mode for initial screen and full scan mode for confirmation. For the routine screen analysis, ions monitored in the SIM mode were *m/z* 140, 118, and 91 for amphetamine-TFA, and *m/z* 154, 118, and 110 for methamphetamine-TFA. For the *l*- and *d*-enantiomer analysis, ions for the analytes were as follows: *m/z* 237, 166, and 91 for amphetamine-TPC; *m/z* 240, 166, and 96 for amphetamine-d_8-TPC; *m/z* 251, 166, and 58 for mathamphetamine-TPC; and *m/z* 258, 166, 65 for methamphetamine-d_8-TPC.

Evaluation of Analytical Parameters

Limit of Quantification (LOQ) and Recovery—The limit of quantification (LOQ) of the assay was determined mathematically by the concentration of amphetamine and methamphetamine that produced an *S/N* ratio of approximately 10 using a single diagnostic ion *m/z* 140 and 154, respectively. The LOQ of 0.1 μg/mL was determined ($n = 6$) for both amphetamine and methamphetamine. To determine the recovery (or extraction efficiency) of the sample preparation procedure, three target concentrations (0.25, 2, and 8 μg/mL) of amphetamine and methamphetamine were used and six replicates for each concentration were analyzed. Two sets (A and B) of blank urine for each concentration were prepared. IS was added to the calibrator and controls before extraction (set A) and after extraction (set B) to the recovery standards. Recovery was calculated by dividing the mean peak-area ratio of set A by the corresponding peak-area ratio of set B and then multiplying by 100%. The mean recoveries of the target concentrations at 0.25, 2, and 8 μg/mL were 119%, 104%, and 101%, respectively, for amphetamine and were 106%, 85%, and 95%, respectively, for methamphetamine.

Linearity, Accuracy, and Precision

Calibration curves were constructed with concentrations ($n = 8$) ranging from 0.1 to 8 μg/mL of amphetamine and methamphetamine. The calibration curve for linear regression analysis of each analyte was constructed by plotting the peak area ratio of the reference standard to that of the IS (diphenylamine) against the known concentrations of the analyte. The linearity of the assay over the 0.1–8 μg/mL range showed a correlation coefficient of $r^2 = 0.997$ and $y = 0.8953x + 0.101$ for amphetamine, and $r^2 = 0.998$ and $y = 0.7682x + 0.0435$ for methamphetamine.

The intra-assay accuracy and precision of amphetamine and methamphetamine were determined from the analyses of six replicates at target concentrations of 0.25, 2, and 8 μg/mL within a single analytical batch. The accuracy of amphetamine for the three target concentrations were 104.4%, 117.0%, and 110.1% with precision (%CV) of 17.7%, 14.5%, and 11.3%, respectively. The accuracy of methamphetamine was 120.9%, 117.6%, and 102.1% with a precision of 17.9%, 15.2%, and 10.1%, respectively. The interassay accuracy and precision (%CV) were determined from six separate runs using the same concentrations as were used in the intra-assay studies. The values of interassay accuracy for the three target concentrations of amphetamine were 93.8%, 94.6%, and 95.3% with a precision of 10.3%, 14.0%, and 9.1%, respectively. The values of interassay accuracy for the three target methamphetamine concentrations were 115.0%, 96.1%, and 90.8% with a precision of 7.4%, 14.6%, and 8.6%, respectively.

Results and Discussion

Urine pH and Specific Gravity

Upon receipt of each batch of urine samples, the pH, and specific gravity for each urine specimen were recorded. The average for pH and specific gravity in 251 urinary samples tested was 6.03 ± 0.47 (mean ± SD; range 4.97–7.37), and 1.019 ± 0.008 (mean ± SD; range 1.002–1.034), respectively. The measurements of pH and specific gravity were required to assess: (a) if urine density is too low (under 1.005), physiologically the doping agent will not concentrate (8); (b) if pH is too high, some basic substances, e.g. amphetamine, may be excreted in very low concentrations and may escape detection (9,10). For the athlete (female) in question, the urinary pH and specific gravity was 6.24 and 1.032, respectively.

Measurements of pH and specific gravity were also performed for the urinary samples obtained from three volunteers (A, B, and C) following oral administration of a single 50 mg dose of famprofazone (Table 1). For these volunteers, the urine pH values (mean ± SD) were 5.8 ± 0.4, 6.0 ± 0.2, and 6.8 ± 0.9, respectively; the values for specific gravity were 1.017 ± 0.007, 1.019 ± 0.005, and 1.019 ± 0.008, respectively. The effect of high pH on reduction of excretion rate of methamphetamine and amphetamine was observed in volunteer C (see Table 1). It was noted that at 4 h (pH 8.1) and 12 h (pH 8.3) postdose, the amounts of excretion were relatively low and accounted for 0.516 and 0.221 μg/mL, respectively, for methamphetamine, and 0.082 and 0.053 μg/mL, respectively, for amphetamine (see Fig. 3). One volunteer's urine sample also showed a reduction for amphetamine and methamphetamine at 4 h but had a normal pH value (pH 6.5). The pH effect on the urinary excretion rate was also reported by Yoo et al. (11).

Analysis of Medication

To ascertain whether the ingredients of the analgesic and antipyretic medication (Gewolen®; MW 377.5) contain famprofazone, a study was carried out to compare the medication and famprofazone reference standard. The drugs were dissolved in methanol and analyzed by GC–MS without derivatization. Fig. 1 shows that mass spectra of both the medication and famprofazone reference standard displayed identical fragmentation patterns with characteristic ions of *m/z* 286 (base ion), 229, and 377 (molecular ion; M^+). These results demonstrated that the medication (Gewolen®) purchased in a local pharmacy by the athlete in question indeed contains famprofazone.

TABLE 1—*Sample pH, specific gravity (Sp. Gr.), volume, amounts of methamphetamine (METH) and amphetamine (AM), and ratio of methamphetamine and amphetamine in urine of the volunteers following 50 mg famprofazone administration.*

Sample (h)	pH	Sp. Gr.*	Volume (mL)	Amount (μg/mL)		Ratio
				METH	AM	
Volunteer A						
0	5.42	1.025	85	0	0	–
2	5.86	1.026	80	1.737	0.282	6.2
4	6.46	1.005	200	0.744	0.124	6.0
6	5.62	1.012	185	1.570	0.282	5.6
8	5.62	1.020	130	2.088	0.390	5.4
12	5.50	1.013	220	1.209	0.243	5.0
24	5.69	1.014	750	0.822	0.162	5.0
48	6.48	1.019	1450	0.349	0.077	4.5
Mean ± SD	5.8 ± 0.4	1.017 ± 0.007				
Volunteer B						
0	5.89	1.025	70	0	0	–
2	6.07	1.025	70	1.226	0.193	6.4
4	6.10	1.016	100	0.830	0.122	6.8
6	5.91	1.015	150	0.859	0.130	6.6
8	6.22	1.016	130	0.661	0.107	6.2
12	5.87	1.012	240	0.488	0.084	5.8
24	6.25	1.021	680	0.453	0.077	5.9
48	6.60	1.018	1000	0.138	0.000	0.0
Mean ± SD	6.00 ± 0.2	1.019 ± 0.005				
Volunteer C						
0	6.60	1.022	75	0	0	–
2	6.45	1.009	275	0.896	0.139	6.4
4	8.10	1.013	180	0.516	0.082	6.3
6	6.18	1.018	110	1.618	0.286	5.7
8	5.96	1.012	110	1.400	0.280	5.0
12	8.30	1.009	380	0.221	0.053	4.2
24	6.19	1.018	370	0.726	0.170	4.3
48	6.25	1.015	780	0.336	0.098	3.4
Mean ± SD	6.80 ± 0.9	1.015 ± 0.005				

*Sp. Gr., specific gravity.

Amphetamine and Methamphetamine in Urinary Samples of the Athlete and the Volunteers

The urinary sample collected from the athlete during the competition was found to contain both amphetamine and methamphetamine during our routine doping analysis. The sample was confirmed with the full scan mode by GC–MS, and its selected ion chromatogram is shown in Fig. 2*A*. The chromatographic retention times for amphetamine and methamphetamine appeared at 5.54 min (RRT = 0.60) and 6.50 min (RRT = 0.63), respectively. The characteristic ions in the mass spectra showed *m/z* 140 (base ion), 118, and 91 for the amphetamine-TFA derivative and *m/z* 154 (base ion), 118, and 110 for the metahamphetamine-TFA derivative (Figs. 2*B* and 2*C*). Identical results for these two metabolites were also found in the urine samples from the famprofazone excretion study (data not shown). In addition to the metabolized methamphetamine and amphetamine in urine following famprofazone administration, other minor amounts of metabolites, such as ephedrine (EPH), phenylpropanolamine (PPA), and cathine were also present in the urine samples of the athlete and the famprofazone excretion study (Fig. 2*A*). As our routine GC–MS analysis was unable to separate PPA and cathine simultaneously, these two diasteroisomers were identified using GC–NPD (4). The famprofazone metabolic products found in the present study were basically in agreement with those reported by others (12,13). Several other metabolites, including *p*-hydroxyamphetamine, *p*-hydroxymethamphetamine, *p*-hydroxydemethylfamprofazone, and 3-hydroxymethylpyrazolone, were also reported (14,15), but were not identified in this study due to unavailability of these reference standards in our laboratory. In addition, pseudoephedrine, also documented to be a metabolite of famprofazone (15,16), was not detected in this study.

Excretion Profiles of Methamphetamine and Amphetamine in Urine of the Volunteers in Reference to the Urine of the Athlete

In the excretion study, each volunteer ($n = 3$) was orally administered 50 mg of famprofazone and urine was collected at different times over a 48-h period. Concentrations for methamphetamine and amphetamine in urine of each collection time were determined by GC–NPD. The excretion profiles of methamphetamine and amphetamine showed identical patterns, although a great interindividual variation was present in terms of time of peak concentrations and concentrations at each time point of urine collection (Fig. 3). The peak of excretion was reached between 2 and 8 h postadministration for both methamphetamine and amphetamine. These results are in agreement with one recent study (13), in which the concentrations of amphetamine and methamphetamine peaked between 3 and 7 h following 50 mg of famprofazone administration. In the present study, both methamphetamine and amphetamine showed dramatic reductions in concentrations after 8 h postadministration (Fig. 3).

The concentrations of methamphetamine and amphetamine within 24 h postdose ranged between 2.088 ± 0.221 and 0.390 ± 0.053 μg/mL, respectively, and their average ratios were between 4.2 and 6.8 (Table 1). The concentrations of methamphetamine and amphetamine in the athlete's urine sample, at the

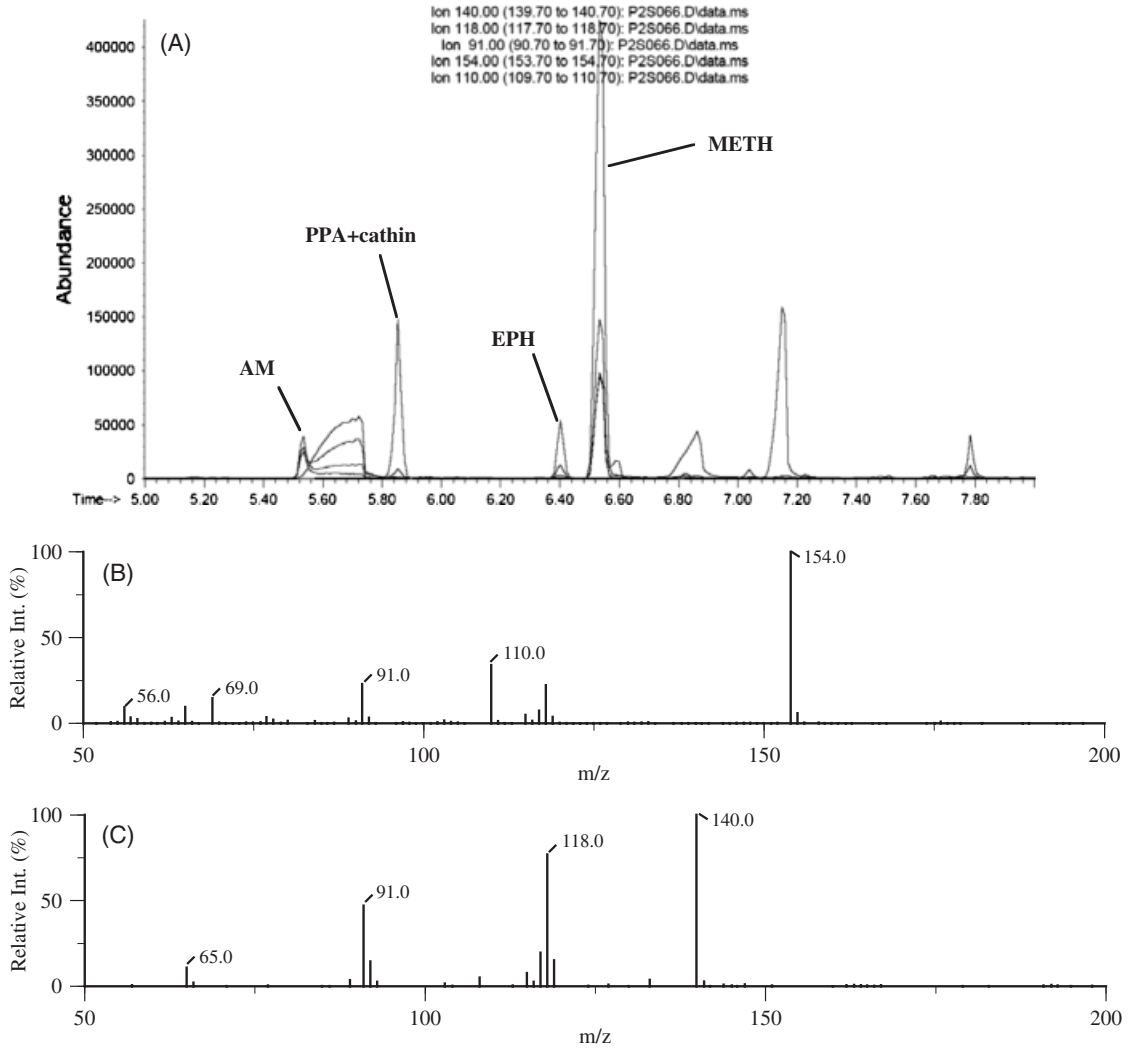

FIG. 2—*Gas chromatography–mass spectrometry (GC–MS) analysis of the athlete's urinary sample. GC chromatogram shows methamphetamine (METH; RT, 6.50 min; RRT, 0.63) and amphetamine (AM; RT, 5.54 min; RRT, 0.60) (A); mass spectra of methamphetamine (B) and amphetamine (C). Note that ephedrine (EPH) and phenylpropanolamine/cathine (PPA/cathine) were also present in the athlete's urine sample.*

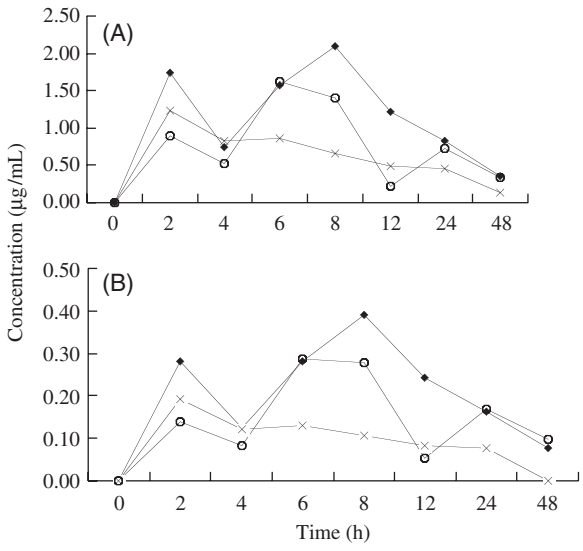

FIG. 3—*Excretion profiles of methamphetamine (A) and amphetamine (B) in urine of the three volunteers over 48 h following famprofazone administration. Symbol identification for the three volunteers: Volunteer A (◆); Volunteer B (×); Volunteer C (○).*

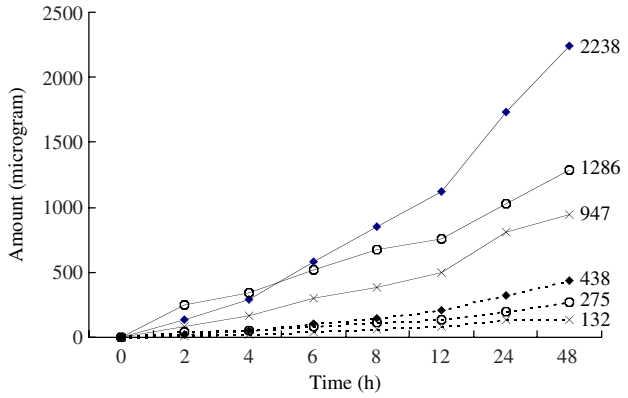

FIG. 4—*Cumulative amounts of methamphetamine (solid line) and amphetamine (dash line) in urine of the three volunteers over 48 h following famprofazone administration. Symbol identification for the three volunteers: Volunteer A (◆); Volunteer B (×); Volunteer C (○).*

time of urine collection for doping control, were found to be 2.688 and 0.462 µg/mL, respectively, and with a ratio of six between these two metabolites. Although the amounts of these two me-

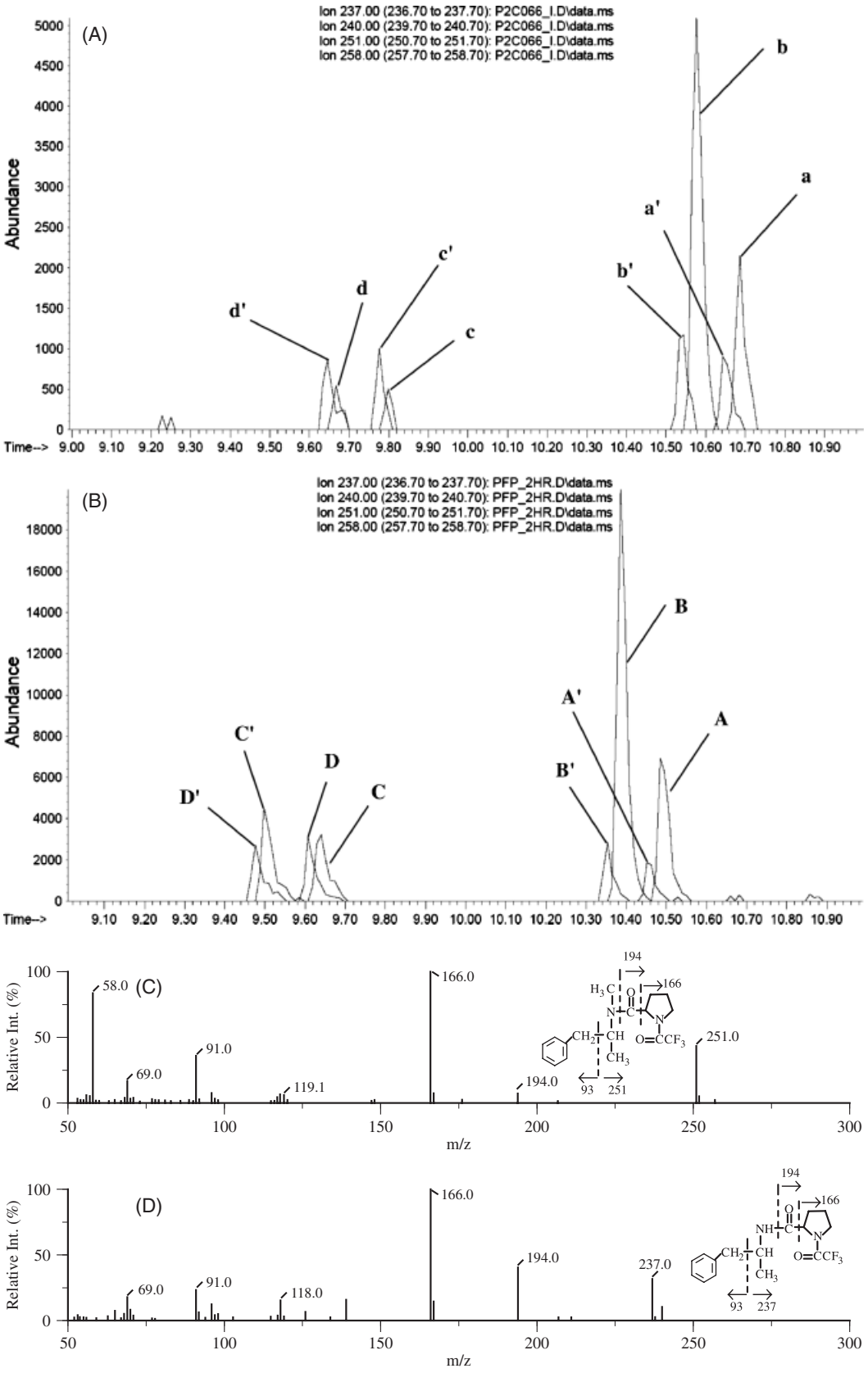

FIG. 5—*Gas chromatography–mass spectrometry (GC–MS) chromatograms and mass spectra of d,l-methamphetamine and d,l-amphetamine enantiomers in urine of the athlete (A) and the volunteer (B). The peaks are identified as follows: d-methamphetamine-TPC (a; A); d-methamphetamine-d_8-TPC (a′; A′); l-methamphetamine-TPC (b; B); l-methamphetamine-d_8-TPC (b′; B′); d-amphetamine-TPC (c; C); d-amphetamine-d_8-TPC (c′; C′); l-amphetamine-TPC (d; D); l-amphetamine-d_8-TPC (d′; D′). Mass spectra of d,l-methamphetamine-TPC (C) and d,l-amphetamine-TPC (D).*

tabolites are higher in the athlete than that in the three volunteers, the results are basically comparable. This discrepancy may be attributed to factors such as individual metabolic differences, dose, and frequency of the drug used, time of urine collection, etc. In this study, we did not know whether single or multiple ingestions were ingested or the exact doses used by this athlete. Significantly higher amounts of methamphetamine over amphetamine have also been reported in recent studies on famprofazone metabolites (13,17). In a single-dose (50 mg) excretion study of famprofazone done by Greenhill et al. (13), the urinary concentrations of methamphetamine and amphetamine reached a peak between 3 and 7 h postdose and the ratio difference could reach *c*. 8 fold at the early stage of excretion.

The cumulative amounts of methamphetamine and amphetamine metabolites excreted in urine were evaluated following famprofazone administration and their profiles are presented in Fig. 4. To obtain the cumulative amounts of methamphetamine and amphetamine, the amounts of metabolites at each time point were first calculated by multiplying the metabolite concentration (in μg/mL) by total volume of urine (in mL) voided during that time period. The cumulative amount of each metabolite in the urine was then evaluated by adding the detected amounts of metabolite to the previous amount obtained. Following 50 mg oral intake of famprofazone, the cumulative amounts (and percents of the dose) over 48 h for these three volunteers were 132 μg (0.3%), 275 μg (0.6%), and 438 μg (0.9%) for amphetamine and 947 μg (1.9%), 1286 μg (2.6%), and 2238 μg (4.5%) for methamphetamine (Fig. 4). These results are comparable with those found in a previous study done by Yoo et al. (11), in which they found that, after 50 mg famprofazone administration, the total amounts of methamphetamine and amphetamine over a 24 h period ranged from 1003 to 1563 μg and from 153 to 284 μg, respectively.

Enantiomer Analysis of Amphetamine and Methamphetamine in Urine of the Athlete and the Volunteers

The urine samples from the athlete and one of the volunteers were used to analyze for metabolic conversion of *d*- and *l*-enantiomers of amphetamine and methamphetamine (as derivatized with *l*-TPC). For the athlete's urine, two pairs of GC chromatographic peaks were present at 10.69 and 10.58 min and at 9.80 and 9.67 min for *d*- and *l*-methamphetamine and *d*- and *l*-amphetamines, respectively (Fig. 5*A*). For the urine of one of the volunteers, the peaks of *d*- and *l*-methamphetamine and *d*- and *l*-amphetamines appeared at retention times of 10.50 and 10.39 min and 9.64 and 9.50 min, respectively (Fig. 5*B*). Both *d*- and *l*-methamphetamine have identical mass spectra and so do *d*- and *l*-amphetamine. The characteristic ions of methamphetamine-TPC and amphetamine-TPC were *m/z* 251, 166, and 58 and *m/z* 237, 166, and 91, respectively (Figs. 5*C* and 5*D*).

Famprofazone exists in both (+)- and (−)-enantiomers and has been documented to be steroselectively metabolized (13,15,16). In a previous study, the proportion of *l*-methamphetamine was found to exceed its enantiomer; initially, the proportion was approximately 70% *l*- and 30% *d*-methamphetamine and increased over time, indicating that *l*-famprofazone was metabolized to *l*-methamphetamine at a faster rate than *d*-famprofazone was metabolized to *d*-methamphetamine (13). In the same study, however, *l*- and *d*-amphetamine amounts were found to be virtually the same in the early excretion samples, with the proportion of *l*-amphetamine increasing as time progressed (13).

In the present study, the urinary sample from the athlete showed that the *d*- and *l*-enantiomers were also present in both methamphetamine and amphetamine metabolites. The proportion between *l*- and *d*-methamphetamine showed a marked difference, with *l*-methamphetamine *c*. 3- to 4- fold greater than its enantiomer based on chromatographic differences in peak areas of these two enantiomers. In contrast, the proportion between *l*- and *d*-amphetamines was *c*. equal at the time when the athlete's urine was collected (Fig. 5*A*). Similar results were also found in the urine of the volunteers (Fig. 5*B*). These results were basically in agreement with those reported by others (13,18). In addition, as most illicit methamphetamine contains only the *d*-enantiomer (13) and the use of a Vicks nasal inhaler contains only *l*-methamphetamine (14,18,19), we can thus rule out the possibility of misuse of only methamphetamine in this particular case.

Although a prohibited substance detected in urine in doping control is legitimate evidence of violation in sport, occasionally athletes may be inadvertently administered medicines that can lead to an adverse test result. In the present study, we confirmed that the medication claimed by the athlete for treating abdominal pain contained famprofazone. In addition, our analytical results provided evidence that supports the athlete's claim that famprofazone was taken before the sport competition based on the following comparable findings in urine of the athlete and famprofazone excretion study: (a) the presence of methamphetamine, amphetamine, and other minor amounts of metabolites; (b) similarity in the concentrations and ratios of methamphetamine and amphetamine; and (c) similarity in the proportions of *l*- and *d*-methamphetamine and their enantiomers.

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