The Relevance of the Urinary Concentration of Ephedrines in Anti-Doping Analysis: Determination of Pseudoephedrine, Cathine, and Ephedrine After Administration of Over-the-Counter Medicaments

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Abstract: This article describes a method for the detection and quantitation of cathine, pseudoephedrine, ephedrine, and methylephedrine in urine, using their deuterated analogues as internal standards and derivatization to form the corresponding trimethylsilyl derivatives after a simple liquid–liquid extraction. The study was designed to evaluate whether the urinary cutoff values set by the World Anti-Doping Agency for the banned ephedrines (cathine >5 μg/mL, ephedrine and methylephedrine >10 μg/mL) can be exceeded after the normal self-administration of common over-the-counter medicaments containing nonbanned ephedrines. The present method, after validation, has been applied on real urine samples obtained from 9 healthy volunteers taking different doses of over-the-counter preparations containing ephedrines. Results obtained from excretion studies show high interindividual differences in the urinary concentrations of both pseudoephedrine and cathine, not dependent on body weight or sex nor, in some instances, on the administered dose. The same typical therapeutic dose of pseudoephedrine (60 mg) produced a urinary concentration of more than 5 μg/mL for cathine and of more than 100 μg/mL for pseudoephedrine in 2 of 9 subjects only. When a dose of 120 mg was administered, cathine concentration exceeded 5 μg/mL in 4 of 7 subject, and also concentrations of pseudoephedrine above 100 μg/mL. After administration of 5 × 120 mg of pseudoephedrine (120 mg administered every 7 days for 5 weeks) to one of the subjects exceeding the urinary threshold values, the urinary concentration of cathine and pseudoephedrine exceeded 5 μg/mL (peak concentration 14.8 μg/mL) and 100 μg/mL (peak concentration 275 μg/mL), respectively. When the same subject took 180 mg of pseudoephedrine, the urinary concentration values were below 5 μg/mL for ephedrine and 100 μg/mL for pseudoephedrine. In the case of ephedrine administration in a sustained-release formulation containing 12 mg of ephedrine, 2 of 3 subjects exceeded the urinary cutoff value of 10 μg/mL. The high interindividual variability is still significant even if the urinary concentration values are adjusted by specific gravity and/or creatinine. These results confirm a high interindividual variability in the urinary concentration of ephedrines after the administration of the same therapeutic dose of a preparation.

Key Words: doping analysis, ephedrines, drug monitoring, mass spectrometry, urine analysis, pharmacokinetics

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INTRODUCTION

Stimulants were the first substances to be prohibited in sport doping. They were already included in the first list of prohibited substances released by the Medical Commission of the International Olympic Committee in the late 1960s to early 1970s, when the first official anti-doping programs and tests were activated on the occasion of multisport international events.

Ephedrines, a class of sympatomimetic amines comprising ephedrine, phenylpropanolamine (norephedrine), pseudoephedrine, cathine (norpseudoephedrine), and methylephedrine, are included in the class of stimulants of the World Anti-Doping Agency (WADA) Prohibited List because their administration can have a positive effect on the performance of sports because of their activity on the respiratory and cardiovascular system. Apart from cathine and methylephedrine, all the above-mentioned drugs can be found in over-the-counter products in most countries. Ephedrines are banned "in competition," with a threshold value for their respective urinary concentration, fixed to discriminate between therapeutic (allowed) administration and performance-enhancing (prohibited) use. More specifically, ephedrine, methylephedrine, and norpseudoephedrine (cathine) are presently included in the WADA list, but with a cutoff value for the urinary concentration, respectively, of 10 μg/mL for the first two and of 5 μg/mL for cathine[1]; in 2004, pseudoephedrine and norephedrine, previously also forbidden (with a cutoff value of 25 μg/mL for both of them), were removed from the anti-doping list. As a result, their presence in urine, even at high concentration values, no longer represents a doping offense. Since then, it has become problematic to evaluate correctly the relevance, in terms of violation of the anti-doping rules, of a urinary concentration of cathine exceeding the threshold

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value: Because cathine is the main metabolite of pseudoephedrine, the concentration of cathine (a banned substance) could, in principle, exceed the threshold value also due to the administration of a sufficiently high dose of pseudoephedrine (a nonbanned substance).

The status of pseudoephedrine is presently under evaluation; its reintroduction into the list, with a higher threshold value (100 μg/mL instead of 25 μg/mL), has been proposed and is under discussion.

The use of these drugs, especially pseudoephedrine, is widespread among athletes, not only because of its decongestant action but also for its stimulant activity. We noted an increase in the number of samples presenting with an elevated concentration (100 μg/mL and higher) of pseudoephedrine both in particular periods of the year, probably due to the typical seasonal diseases (allergic rhinitis, influenza), and in correspondence of specific sport competitions.

During the 2006 Winter Olympic Games, 3 positive cases were reported for cathine (exceeding the cutoff values) At the same time, the presence of cathine was also detected in several other samples at a concentration not exceeding 5 μg/mL (and therefore not reported as adverse analytical findings), all of them with very high concentrations of pseudoephedrine (> 300 μg/mL). The same trend was also observed in the following months, with an increasing number of cases with high concentrations of pseudoephedrine (above 50 μg/mL), with cathine either exceeding or less than the cutoff.

There are few studies on the interindividual variability of ephedrines/metabolites excretion rates.[2–6] This is an important issue because these substances and/or their metabolites (cathine) are prohibited only when their concentration is above a cutoff.

The present preliminary study was designed to verify whether a "population" threshold could discriminate between the administration of pseudoephedrine for therapeutic uses and the administration of pseudoephedrine (alone and in association with cathine) with the aim of improving sport performance. Preliminarily, a method for the accurate quantitative determination of ephedrines in urine was set up and fully validated, by an implementation of the method described by Forsdahl and Gmeiner,[7] using as internal standards ephedrine-D_3 for ephedrine, pseudoephedrine, and methylephedrine, and norephedrine-D_3 for cathine and norephedrine. The method involved a simple liquid–liquid extraction of the sample and the addition of anhydrous sodium sulfate to the organic phase to remove any residual water. The method was then applied to observe the variability of concentrations of ephedrines/metabolites found in the urine samples of subjects taking these medications in therapeutic doses for self-medication (for the treatment of allergic rhinitis or against influenza symptoms) and, if so, at what administered dose the threshold value was exceeded.

MATERIALS AND METHODS

Chemicals and Reagents

Ephedrine-D_3, norephedrine-D_3 (used as internal standards), and norpseudoephedrine were obtained from LGC Promochem (Milano, Italy); ephedrine, pseudoephedrine, methylephedrine, phenylpropanolamine (norephedrine), *N*-methyl-*N*-trimethylsilyl trifluoroacetamide (MSTFA), tert-butyl-methyl ether, sodium hydroxide, and trimethylchlorosilane (TMCS) were supplied by Sigma–Aldrich (Milano, Italy); Actifed and Reactine were purchased from Pfizer Consumer Health Care (Latina, Italy); and Fienamina was supplied by Recordati (Milano, Italy). Reagents for creatinine determination according to the Jaffe method were purchased from Giesse Diagnostics (Rome, Italy).

Excretion Studies

All the subjects gave informed consent before participation in the study and were submitted to medical evaluation. Excretion studies on pseudoephedrine were performed in 9 subjects: 4 males (age: 23–41 years, weight: 76–95 kg) and 5 females (age: 24–41 years; weight: 42–55 kg) taking either Actifed (pseudoephedrine 60 mg, triprolidine 2.5 mg), a galenic preparation containing 60 mg of pseudoephedrine, or Reactine (pseudoephedrine 120 mg, cetirizine 5 mg, in a sustained-release formulation). Ephedrine excretion studies were performed in 3 subjects, who took a single dose of Fienamine (ephedrine 12 mg, chlorfenamine 10 mg, sustained release).

All the subjects were followed after the administration of a single dose of pseudoephedrine (60 mg each). Seven subjects also took a double dose (120 mg) of pseudoephedrine; 1 subject took also 180 mg of pseudoephedrine in a single dose and 2 doses of 120 mg each with a delay of 12 hours; the same subject also took 2 doses of pseudoephedrine (120 mg) 5 times. One subject took 3 doses of 60 mg with a delay of 12 hours between each other. In each study, a control urine sample was collected immediately before drug administration, followed by all urine produced in the first 12 hours. Subsequently, at least the first and last urine samples of the second day were collected. Urine samples were collected in pharmaceutical reservoirs and the pH values were measured with pH indicator strips. The urine samples were stored at −20°C until analysis.

Correction for Specific Gravity

Specific gravity was measured with an RE50 refractometer from Mettler Toledo (Milano, Italy). Concentration values were then adjusted for a specific gravity of 1.020 using the following formula:

$$\text{Adjusted concentration} = \text{Measured concentration}\ [(1.020 - 1)/(\text{specific gravity of the sample} - 1)]$$

Creatinine Determination

Creatinine was measured by a 100 scan UV spectrophotometer (Varian, Torino, Italy) at 492 nm, after reaction with picric acid in alkaline medium (NaOH 4%), following the manufacturer's instructions.

Calibration Curves

Methanolic standard stock solutions of the substances of interest were prepared at a concentration of 1 mg/mL and of 0.1 mg/mL for internal standards, by diluting the reference solutions in methanol, and stored at −20°C.

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Calibration curves were prepared by addition of the appropriate amount of ephedrine, cathine, methylephedrine, pseudoephedrine, and norephedrine to 1 mL of blank urine to obtain the following concentrations: 0.5, 1, 2.5, 5, 10, and 15 μg/mL for cathine, ephedrine, methylephedrine, and norephedrine, and 1, 2.5, 5, 10, 15, 25, 50, and 100 μg/mL for pseudoephedrine.

Sample Preparation

To 1 mL of sample 25 μL of ephedrine-D_3 and norephedrine-D_3 (100 μg/mL) was added and the mixture was alkalinized by the addition of 2 drops of sodium hydroxide 1M. To this was added 200 mg of sodium chloride, and the mixture was extracted with 2 mL of tert-butyl-methyl ether. The organic layer was separated, and 200 mg of anhydrous sodium sulfate was added, vortexed, transferred in another vial, dried under a gentle stream of nitrogen at room temperature, and derivatized with 50 μL of MSTFA/TMCS (1%) at 70°C for 30 minutes. One microliter of the derivatized extract was injected directly into the gas chromatography–mass spectrometry (GC/MS).

GC/MS Conditions

The GC/MS system was an Agilent HP6890 gas chromatographer coupled to a 5973 mass spectrometric detector. Chromatographic conditions were the following. Supelco custom-made 5% phenyl-methylsilicone capillary column (17 m × 0.2 mm inner diameter, 0.33-μm film thickness); the oven temperature was held at 130°C for 1 minute, increased to 200°C at 8°C/min, increased to 280°C at 40°C/min (held 2 minutes); the injection port was set at 270°C in split mode (split ratio 20/1); and helium was used as carrier gas at a constant pressure of 20 psi.

The mass detector operated in electron ionization at 70 eV in scan mode (scan range from 47 to 400). Ions used for quantitation were 131 for ephedrine and pseudoephedrine, 117 for norpseudoephedrine and norephedrine, 236 for methylephedrine, 134 for ephedrine-D_3, and 120 for norephedrine-D_3.

Method Validation

The method for qualitative and quantitative analysis of ephedrines has been validated taking into consideration the following parameters: limit of detection (LOD) and limit of quantification, specificity, linearity, intra- and interassay accuracy, and repeatability (precision).

The LOD was defined as the lowest concentration of the analyte with an identifiable peak with signal to noise ratio of >3 for at least 3 ions.

The limit of quantification was defined as the lowest concentration of the analyte with an identifiable peak that could be quantified with an acceptable % Coefficient of Variation (CV%) (<10), with a signal to noise ratio always >10 for at least 3 diagnostic ions.

Specificity was studied analyzing 10 blank samples and studying the presence of interfering peaks at the retention times of the analytes. Also samples containing amphetamine-like substances [amphetamine, methamphetamine, methylenedioxyamphetamine, methylenedioxymethamphetamine, methylenedioxyethylamphetamine, ethylamphetamine, fenfluramine, phendimetrazine, and phenmetrazine] and common nonsteroidal anti-inflammatory drugs (ibuprofen, diclofenac, acetyl salicylic acid, tramadol) were studied to evaluate interferences.

Linearity was determined by the preparation of calibration curves ranging from 0.5 to 15 μg/mL for cathine, ephedrine, methylephedrine, and norephedrine, and from 1 to 100 μg/mL for pseudoephedrine, considering the area ratio between the analytes and their correspondent deuterated internal standard (ephedrine-D_3 for ephedrine, pseudoephedrine, and methylephedrine, and norephedrine-D_3 for cathine and norephedrine).

Intra-assay precision (intended as CV%) and accuracy (mean relative error) were determined using 10 spiked samples at 2 concentrations (5 and 10 μg/mL for ephedrine, methylephedrine, and cathine, and 10 and 100 μg/mL for pseudoephedrine). Interassay precision and accuracy were determined by analyzing 3 samples at the above concentrations on 5 different days using 3 different instruments. The stability of the derivatized compounds was tested analyzing by quantifying the same extracts on 3 different days.

RESULTS

The validated method demonstrated its suitability for the accurate quantitative determination of ephedrines in urine. Liquid–liquid extraction allowed a very rapid pretreatment of the sample. The addition of anhydrous sodium sulfate permitted the complete removal of any water residue, thus ensuring complete derivatization. For quantification, the use of the selected ions with lower abundances (*m/z* 131 for ephedrine and pseudoephedrine, 117 for cathine, and 236 for methylephedrine) with respect to the base peaks (*m/z* 130, 116, and 72, respectively) produced better results in terms of correlation coefficient, precision, and accuracy. Validation results are summarized in Table 1 and are satisfactory for the scope of a quantitative analysis.

TABLE 1. Validation Results: Correlation Coefficient, LOD/LOQ, Repeatability, (CV%) and Accuracy

Substance	R^2	LOD/LOQ (μg/mL)	CV% (10 μg/mL)	CV% (5 μg/mL)	% Error (10 μg/mL)	% Error (5 μg/mL)
Ephedrine	1.000	0.25/0.5	0.47	1.5	0	1.1
Methylephedrine	0.999	0.25/0.5	2.2	4.5	0.5	4.8
Cathine	1.000	0.25/0.5	1.2	1.1	1.3	0.8
Pseudoephedrine	0.999	0.25/0.5	0.54	3.1 (100 μg/mL)	0.11	4.7 (100 μg/mL)
Norephedrine	0.999	0.25/0.5	2.5	4.5	3.5	4.8

LOQ, limit of quantification.

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No interfering peaks were detected at the expected retention times of the analytes of interest. The method allowed the separation of the optical isomers (ephedrine/pseudoephedrine and cathine/norephedrine) with well-resolved peaks.

The method was then applied to real samples obtained from excretion studies. Although the method was linear for pseudoephedrine up to 100 μg/mL, samples showing pseudoephedrine concentrations higher than 50 μg/mL were diluted 1/10 before pretreatment to avoid peak overload.

Results obtained from the excretion studies show high interindividual differences in the urinary concentrations for both pseudoephedrine and cathine, which are still significant even if the data are adjusted for the specific gravity and/or the creatinine concentration of the urine (Figs. 1, 2); the variability does not seem to depend on the body weight, nor on the sex or, in some instances, on the administered dose. The total amount of urine produced varied greatly between the subjects, as recorded by self-reports, regardless the value of the specific gravity of the urine.

Experiments performed after the urinary excretion of pseudoephedrine and cathine after the administration of different doses of pseudoephedrine gave the following results.

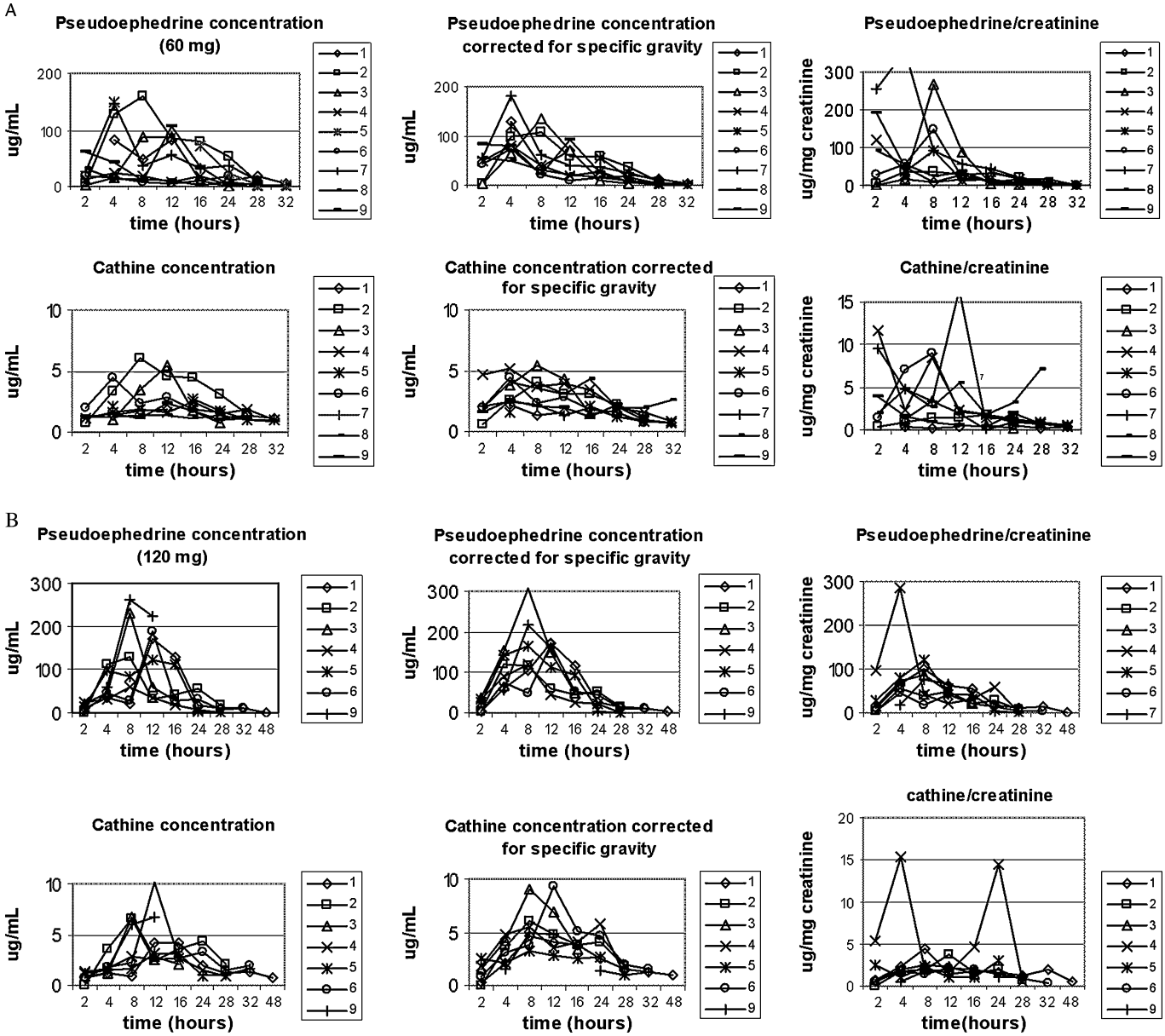

FIGURE 1. A, Cathine and pseudoephedrine concentrations after oral administration of 60 mg of pseudoephedrine to 9 subjects. Subject 1: ♀, age 40 years, weight 52 kg; subject 2: ♂, age 26 years, weight 77 kg; subject 3: ♀, age 24 years, weight 53 years; subject 4: ♂, age 40 years, weight 82 kg; subject 5: ♂, age 41 years, weight 79 kg; subject 6: ♂, age 25 years, weight 95 kg; subject 7: ♀, age 22 years, weight 52 kg; subject 8: ♀, age 28 years, weight 52 kg; subject 9: ♀, age 38 years, weight 42 kg. B, Cathine and pseudoephedrine concentrations after oral administration of 120 mg of pseudoephedrine to 7 subjects.

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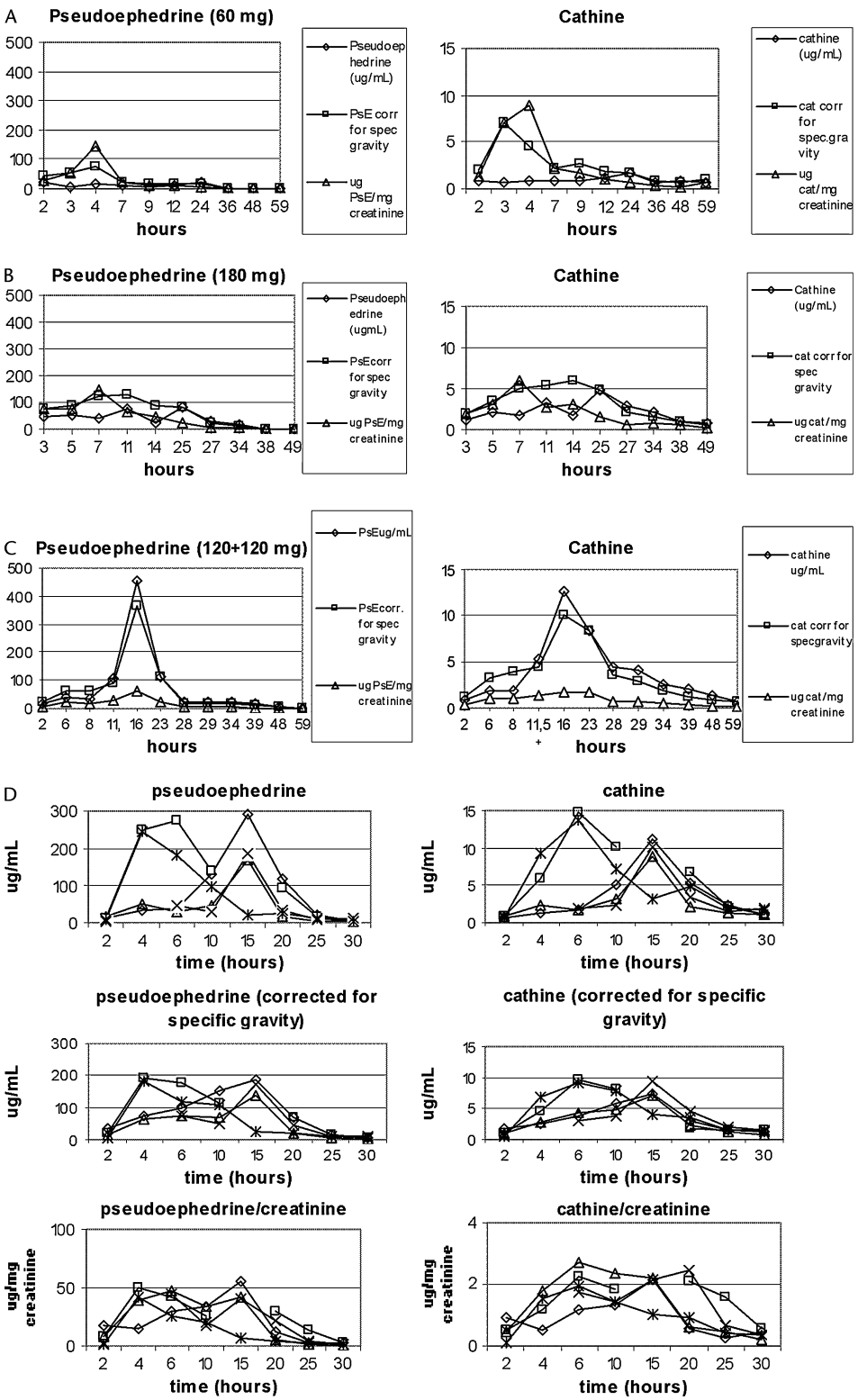

FIGURE 2. Subject 6. A, Cathine and pseudoephedrine concentrations after administration of 60 mg of pseudoephedrine, B, Cathine and pseudoephedrine concentrations after administration of 180 mg of pseudoephedrine in a single dose. C, Cathine and pseudoephedrine concentrations after 2 administrations of 120 mg of pseudoephedrine with a delay of 11 hours. D, Cathine and pseudoephedrine concentrations after 5 administrations of 120 mg of pseudoephedrine.

The administration of the same therapeutic dose (60 mg) of pseudoephedrine produced 2 results over 5 μg/mL for cathine (WADA cutoff value) in 2 different subjects, and 2 cases of pseudoephedrine exceeding 100 μg/mL, one of which with a low concentration of cathine (Fig. 1).

When a dose of 120 mg was administered, 4 of 7 subjects exceeded 5 μg/mL, with concentrations of pseudoephedrine above 100 μg/mL (Fig. 1).

After administering 3 doses of 60 mg (for a total of 180 mg), each dose given every 12 hours (subject 9: female,

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weight 42 kg), the cutoff value was exceeded only after the second administration. It is worth noting that when the same amount of pseudoephedrine was given as a single dose to a different subject (subject 6: male, weight 95 kg), the concentrations of both cathine and pseudoephedrine always remained below 5 μg/mL and 100 μg/mL, respectively.

Nonetheless, we also observed a high intraindividual variability of the urinary concentration of both pseudoephedrine and cathine found after the administration to the same subject (subject 6: male, age 25 years, weight 95 kg): in the case of the administration of 60 and 180 mg of pseudoephedrine, the subject did not exceed the cutoff values for both substances. When 2 doses of 120 mg were taken with a time interval of 11 hours (a typical therapeutic approach in the case of rhinitis), the cutoff value for cathine was exceeded immediately after the second administration, with concentrations reaching 12.6 and 456 μg/mL for pseudoephedrine. Then, the subject took 120 mg of pseudoephedrine every fifth day, and in all cases, the cutoff for cathine and the value of 100 μg/mL for pseudoephedrine were exceeded in at least 1 urine sample for each administration, with concentrations reaching the maximum values of 14.8 μg/mL (cathine) and 275 μg/mL (pseudoephedrine). Higher concentrations of analytes were associated with lower pH values (values between 5.5 and 6). Quite reasonably, the correction of concentrations for specific gravity and for creatinine produced more reproducible results for this subject, but only in the case of administration of the same 120-mg dose (Fig. 2). When the same subject took a sustained-release dose of 120 mg of pseudoephedrine, he never exceeded the cutoff for cathine, but concentrations of pseudoephedrine were higher than 100 μg/mL in 3 urine samples.

The peak of excretion for all subjects was between 8 and 14 hours after administration (10.9 ± 2.9 hours), depending on the urine pH (the peak concentration was observed in the most acidic urine comprised between 8 and 14 hours); the peak was always associated to a pH value of 5–5.5, whereas other values ranged between 6 and 8. Considering the concentrations of both substances at this peak time, the mean values obtained for 120 mg administration were 5.8 μg/mL (SD = 1.9 μg/mL) for cathine, with values ranging from 1.7 to 14.8 μg/mL, and 160 μg/mL (SD = 69.4 μg/mL) for pseudoephedrine, ranging from 34 to 291 μg/mL. If one considers only the cases in which a single dose of 60 mg was administered, the mean values were 2.8 μg/mL for cathine (SD = 1.74 μg/mL) and 63.6 μg/mL for pseudoephedrine (SD = 49 μg/mL).

Mean percent amount of cathine with respect to pseudoephedrine was 4.8% (SD = 1.9) at the peak of excretion, and 6.5% (SD = 4.5) considering all the determinations, excluding samples with concentrations of pseudoephedrine lower than 10 μg/mL. In all cases, cathine showed longer excretion times compared with pseudoephedrine.

Finally, we considered the excretion profile of ephedrine after the administration of Fienamine, a sustained-release form of ephedrine (the only pharmaceutical product containing ephedrine marketed in Italy). Although the number of subjects studied could not permit statistical evaluation, we did observe a remarkable interindividual variation. With a single oral dose, 2 subjects exceeded the WADA cutoff of 10 μg/mL in 3 urine samples, 1 subject in the first 7 hours after administration and the other after 12 hours, whereas a third did not have concentrations higher than the cutoff (Fig. 3). No significant differences were observed if the concentration values were corrected for specific gravity or creatinine.

DISCUSSION

The results presented here show a large interindividual variability in the value of the urinary concentration of both pseudoephedrine and cathine after the administration of therapeutic doses of pseudoephedrine. More specifically, our results show that the threshold of 5 μg/mL of cathine can be exceeded, even if only for a period of a few hours, in some individuals after the administration of therapeutic doses of pseudoephedrine. At the same time, the threshold is not necessarily exceeded in other individuals after the administration of higher doses of pseudoephedrine. The peak urinary concentration of pseudoephedrine is also variable and seems to

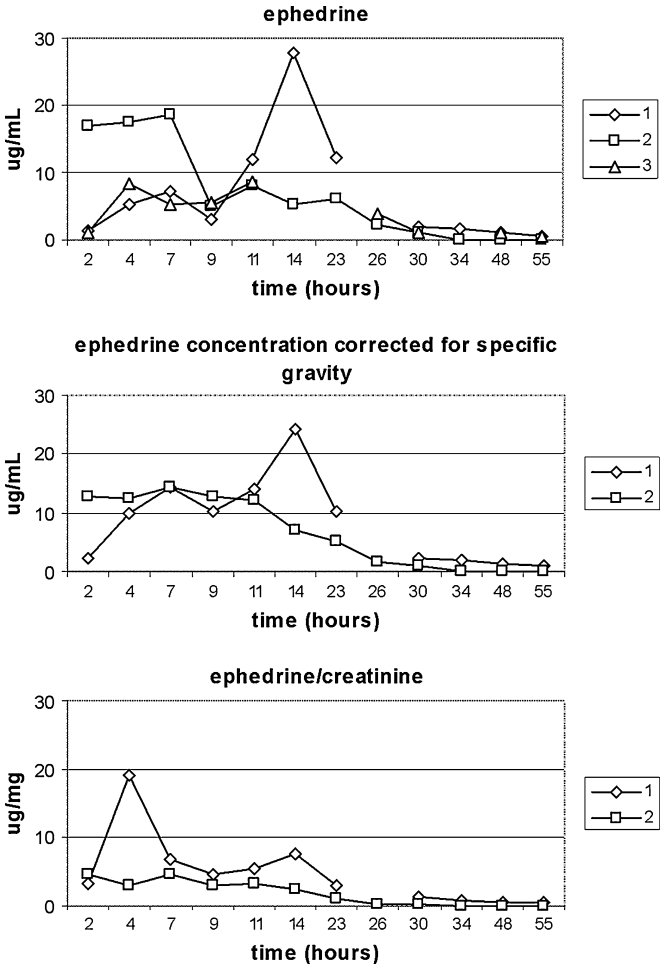

FIGURE 3. Concentrations of ephedrine in 3 subjects after administration of 12 mg of ephedrine. Subject 1: ♀, age 40 years, weight 52 kg; subject 2: ♂, age 25 years, weight 95 kg; subject 3: ♀, age 38 years, weight 50 kg.

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depend more on the individual pharmacokinetics than on the administered dose.

In the case of ephedrine administration at a reduced therapeutic dose in a sustained-release formulation, the WADA threshold (10 μg/mL) was exceeded in 2 out of 3 subjects.

A limitation of this study was the heterogeneity of the doses used and of the time intervals of sample collection; this was because of the fact that this was an observational study on subjects who took these substances for self-medication. On the other hand, this reflects the typical sample delivered to the doping control laboratories. This did not allow us to collect the all volumes of urine produced or the total amount of pseudoephedrine eliminated with respect to each dose. However, as reported by Chester et al,[3] 81% of the assumed dose of pseudoephedrine is excreted unchanged and an additional 6% in the cathine form; other minor metabolites are produced in very minor amounts and can be ignored.

The variability of the results does not seem to be because of differences in the dilution of urine samples. Although the specific gravity of the urine is considered in anti-doping analysis only for the quantitative determination of anabolic steroids that can also be produced endogenously, we have applied the adjustment of the values of the measured urinary concentrations for the specific gravity and also for the creatinine concentration, for the substances considered in the present study. Because the low values of specific gravity of many samples in this study, the correction would cause an increase in concentration values that would exceed cutoff in some cases, whereas in others, correction for specific gravity would lower concentrations below the cutoff. The same trend was observed in the case of correction for creatinine. Values of specific gravity and creatinine concentration showed a correlation value of 0.75.

Based on the experimental evidence obtained in the present work, it seems that it is not easy to define a "population" threshold value for the urinary concentration of pseudoephedrine to discriminate its therapeutic administration from its use as a doping substance. A urinary concentration above the threshold for cathine can be measured, even if only for a few hours, also after the administration of a single therapeutic dose (60–180 mg) of pseudoephedrine.

Consequently, should a population-based threshold value be fixed for pseudoephedrine, athletes should be clearly warned that even the administration of a therapeutic dose of pseudoephedrine can give rise, although for a time interval limited to a few hours after the administration, to urinary concentrations of cathine higher than 5 μg/mL and, also, of pseudoephedrine higher than 100 μg/mL. The expression of the enzymes responsible for the metabolism of ephedrine (eg, cytochrome P isozymes)[8] should in principle also be considered in the case of an adverse analytical finding for cathine in the presence of an elevated concentration of pseudoephedrine; also the pH value of the urine seems to play a role in the concentration of ephedrines, which are excreted preferably in acidic urines. Furthermore, analyzing the excretion of different doses of pseudoephedrine in the same subject, it seems that there is an inductive effect with excretion of higher amounts of substances after various administration. The subject did not exceed the cutoff for cathine after administration of a 180-mg dose but always exceeded 5 μg/mL of cathine and 100 μg/mL of pseudoephedrine after serial administration of 120 mg of pseudoephedrine, although with at least a 5-day interval between each administration. Any positive case for cathine (more than 5 μg/mL) in the presence of pseudoephedrine should activate further studies to discriminate a potential cathine coadministration.

A slight normalization of results could be obtained by a correction for specific gravity or by creatinine but it would not aid discrimination.

It is known that ephedrine-based medications have a stimulant effect and are therefore often abused by athletes, even if used in therapeutic doses. Because pseudoephedrine was removed from the list, the detection of this drug in high concentrations (even higher than the previous threshold of 25 μg/mL) is a common observation.

Additional experiments are currently in progress, aimed at verifying the complementary information that could be obtained by the analysis of saliva.

REFERENCES

1. The World Anti-Doping Code. *The 2008 Prohibited List International Standard*. Montreal, Canada: World Anti-Doping Agency; 2008. Also available at www.wada-ama.org/rtecontent/document/2008_List_En.pdf. Accessed October 10, 2008.
2. Tseng YL, Shieh MH, Kuo FH. Metabolites of ephedrines in human urine after administration of a single therapeutic dose. *Forensic Sci Int*. 2006;157: 149–155.
3. Chester N, Mottram DR, Reilly T, et al. Elimination of ephedrines in urine following multiple dosing: The consequences for athletes, in relation to doping control. *Br J Clin Pharmacol*. 2004;57:62–67.
4. Tseng YL, Hsu HR, Kuo FH, et al. Ephedrines in over-the-counter cold medicines and urine specimens collected during sport competitions. *J Anal Toxicol*. 2003;27:359–365.
5. Lai CM, Stoll RG, Look ZM, et al. Urinary excretion of chlorpheniramine and pseudoephedrine in humans. *J Pharm Sci*. 1979;68:1243–1246.
6. Haller CA, Jacob P III, Benowitz NL. Pharmacology of ephedra alkaloids and caffeine after single-dose dietary supplement use. *Clin Pharmacol Ther*. 2002;71:421–432.
7. Forsdahl G, Gmeiner G. Investigation of the silylation of ephedrines using *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide. *J Chromatogr B*. 2004;811: 201–208.
8. De la Torre R, Farré M, Navarro M, et al. Clinical pharmacokinetics of amphetamine and relates substances. *Clin Pharmacokinet*. 2004;43: 157–185.

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