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# Doping control analysis: the 6th World Championships of Athletics, Athens, Greece

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**The first official report on the organization, analytical methodologies, and the results of the doping control analysis performed by the International Olympic Committee (IOC)-accredited Doping Control Laboratory of Athens, Greece, during the 6th World Championships of Athletics, held in Athens on 1–10 August 1997, is presented. The significance of the various analytical parameters of the doping control is shown. The results include 20 positive cases, of which two were detected in screening procedures and confirmed exclusively by high resolution mass spectrometry. The screening results and analytical data on the excretion of a new black-market doping agent, phenylpiracetam or carfedon, which was unknown in the literature, are also presented.** 

*Keywords:* Doping control; Phenylpiracetam; Carfedon; High-resolution mass spectrometry; Stanozolol

## 1. Introduction

Doping control analysis of athletes' biological fluids (mainly urine) is performed during competitions or during training periods, in order to constrain the abuse of drugs for enhancing athletic performance. The doping analysis is organized by the Medical Commission of the International Olympic Committee (IOC) and performed by the 25 IOC-accredited doping control laboratories worldwide. The IOC Medical Code [1] contains all the definitions, the rules and the list of prohibited compounds.

Doping control is a specialized and complex task, comprising the following activities:

- sample collection in specific, sealed containers, where the collected urine sample is divided between two separate bottles (A and B),
- screening analysis [2] in an IOC-accredited laboratory of the A-bottle urine, using mainly GC–MS,
- confirmatory analysis of the samples regarded as suspect from the screening analysis and announcement of the positive and negative results, and
- analysis of the B-bottle urine of the corresponding positive samples, if requested [1].

Doping control during major competitions imposes a significantly increased daily analytical capacity on the laboratory in charge. The requirements of the International Amateur Athletic Federation (IAAF) that were set for the IOC-accredited Doping Control Laboratory of Athens, Greece, were as follows: analysis of 50 samples per day, reporting the negative results within 24 h after sample collection, reporting the pos-

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Table 1
Instrumentation list of the Doping Control Laboratory of Athens

| Technique | Instrument | Chromatographic columns | Analytical procedures[a] |
|---|---|---|---|
| GC–NPD | HP5890–NPD[b] (2) | HP Ultra 1[e(i)] | Screening procedure I<br>Quantitation of ephedrines |
| GC–MS (quadrupole analyzer) | HP5890–HP5971 MSD[b] | HP Ultra 2[f] | Screening procedures II, V |
| | HP5890–HP5970 MSD[b] | HP Ultra 2[f] | Screening procedure IVα |
| | HP5890–HP5970 MSD[b] | HP Ultra 1[e(ii)] | Screening procedure IVβ<br>Confirmation procedure IVβ |
| | HP6890–HP5973 MSD[b] | HP Ultra 1[e(ii)] | Screening procedure IVβ<br>Confirmation procedure IVβ |
| | HP6890–HP5973 MSD[b] | HP Ultra 2[f] | Confirmation procedures I, II and IVα<br>Quantitation of ephedrines |
| GC–HRMS (electrostatic-magnetic analyzer) | HP6890[b]–Autospec[c] | HP Ultra1[e(ii)] | Screening procedure IVβ and confirmation |
| HPLC–DAD (UV–Vis) | HP1090[b] | Lichrospher 100 RP18 (5 μm) | Clean-up procedures, caffeine quantitation |
| IRMA | γ-counter[d] | – | Screening, confirmation procedure VI |

[a]For details, see Tables 2 and 3 and text. [b]Manufacturer: Hewlett-Packard. [c]Manufacturer: Micromass. [d]Manufacturer: Packard Crystal. [e]Capillary crosslinked methylsilicone gum, (i) 12.5 m × 0.2 mm × 0.33 μm, (ii) 17 m × 0.200 mm × 0.11 μm. [f]Capillary crosslinked 5% phenyl-methylsilicone gum 12 m × 0.200 mm × 0.33 μm.

itive samples within 48 h and the B analysis of the positive samples to be conducted during the competition period. Over the years, detailed accounts of the doping activities of several major international competitions have been published [3–5].

During the 6th World Championships of Athletics, 198 countries participated with a total of 1882 athletes. Four hundred and one samples were analyzed in 10 days (40 samples per day) and resulted in 20 positive cases; seven B samples were analyzed for eight prohibited substance violations. The main methodological and analytical aspects are outlined and the analytical results obtained are discussed below. The use of high-resolution mass spectrometry (HRMS) for the detection of certain anabolic steroid traces (parents and metabolite compounds) was considered to be a milestone with regard to the sensitivity of analysis, as will be discussed later, and led to the exclusive detection of two positive samples. Finally, analytical data on the excretion of a new black-market doping agent, phenylpiracetam or carfedon, which was unknown in the literature, are presented.

## 2. Analytical procedures

The laboratory staff consisted of 22 scientists and technicians working in two shifts that provided support to the laboratory on a 24-h basis. The morning staff's tasks were the analysis of the samples collected during the qualification rounds that took place in the morning, evaluation of analytical results and, if necessary, the confirmation of suspect positive cases. All B-sample analyses were scheduled and also carried out during the morning period. The night shift worked mainly on the analysis of the samples collected during the evening events.

Solvents used in the extraction procedures were of analytical grade. Water used in the HPLC was doubly distilled. All HPLC solvents were of HPLC grade. Other reagents were of analytical grade quality. β-Glucuronidase from *Escherichia coli* (Boehringer Mannheim) and β-glucuronidase arylsulfatase from *Helix pomatia* (Sigma Chemicals) were used for enzymatic hydrolysis. Derivatization reagents were purchased from Macherey-Nagel. The working internal standard solution of deuterated steroids used for the evaluation of the testosterone–epitestosterone ratio (in suspect positive cases), along with 4α-OH-stanozolol and the quality control urine used in HRMS analysis were kindly provided by the Cologne, Germany, Doping Control Laboratory. Epiandrosterone glucuronide (Epiandr-G) was purchased from Steraloids Inc., USA.

The main analytical instrumentation used is listed in Table 1. Maintenance of the instruments before the games comprised cleaning of all the electron ionization (EI) sources and replacement of the chromatographic columns with new ones. Maintenance during the games involved additional cleaning of two EI sour-

ces and replacement of two columns. The HRMS inner electron impact source was in operating condition after the end of the games. Baking of the HRMS analyzer tube was performed only once (for 2 h) during the games.

The analytical procedures applied are described in Tables 2 and 3. Procedures I (screening of stimulants, narcotics, analgesics), II (screening of stimulants, narcotics, analgesics, local anesthetics, cannabinoids, β-blockers, $\beta_2$-agonists), IV (screening of anabolic steroids, $\beta_2$-agonists, pemoline, amineptine, cocaine, probenecid, as free and combined fractions) were applied to all samples, and procedure VI (human chorionic gonadotropin screening) to all samples from males. Procedure V (screening of diuretics, like acetazolamide, etacrynic acid, furosemide, hydrochlorothiazide, etc.) was carried out on urine samples that had a low specific gravity and/or abnormally alkaline pH, as well as all positive samples. Luteinizing hormone (LH) measurement was also performed on suspected abnormal steroid profile samples.

All analytical batches included several quality control samples. In order to evaluate reproducibility of retention times, an internal standard and a sample containing various substances were used, either spiked or from an excretion study, which were screened in the specific procedure. As a sensitivity control, a sample spiked with several characteristic analytes was applied, at the concentration of the detection limit or the threshold limit for threshold substances, depending on the procedure [1]. The enzymatic hydrolysis of combined steroids fractions was checked using Epiandr-G.

The confirmation of positive samples involved reanalysis of the suspect sample according to a suitably modified screening analytical procedure, along with a positive control or a urine sample from an excretion study, a blank urine, and a reagent blank sample. The

Table 2
Examples of classes of prohibited screened substances, and standard solutions used in the six screening procedures applied during the 6th World Championship of Athletics

| Screening procedure | Classes of substances screened | Control samples |
|---|---|---|
| I | Volatile nitrogen-containing compounds | Control urine sample for retention times and detection limit, containing amphetamine, caffeine, cocaine, nor- and pseudo-ephedrines, methylphenidate, pipradol and strychnine |
| II | Heavy volatile nitrogen-containing compounds | Control urine samples for retention times and detection limit, containing ethamivan, etilephrine, fenoterol, methadone, morphine, pethidine, pentazocine, pholedrine, sydnocarb and terbutaline |
| IVα | Free fraction of anabolic steroids | Sensitivity control urine sample containing 3′-OH-stanozolol (0.01 ppm) |
| IVβ (GC–MSD) | Conjugated and free fraction of anabolic steroids, β-agonists, cocaine, pemoline, probenecid | Control urine sample for retention times (from an excretion study) containing clostebol, androstanolone, fluoxymesterone, mesterolone, methenolone, nandrolone, norethandrolone, oxymesterone, oxymetholone and probenecid |
| | Endogenous steroid profile | Steroid profile calibration curve (see text)<br>Sensitivity control urine sample containing 19-norandrosterone, 19-noretiocholanolone and 3′-OH-stanozolol |
| IVβ (GC–HRMS) | Conjugated and free fraction of certain anabolic steroids | Control urine sample for retention times and detection limit, containing clenbuterol, 17α- and 17β-methyl-5α-androstane-3α,17β-diol, epimethenediol, 19-norandrosterone, 19-noretiocholanolone, 18-nor-17,17-dimethyl-5β-androst-1,13-dien-3α-ol, 3′-OH-stanozolol, 4β-OH-stanozolol |
| V | Diuretics | Control urine sample for retention times and detection limit, containing acetazolamide, benzoflumethiazide, bumetamide, canrenone, chlortalidone, clopamide, dichlorphenamide, etacrynic acid, furosemide, hydrochlorothiazide, indapamide and probenecid at a concentration of 1 ppm |
| VI | Human chorionic gonadotropin (hCG) | Six hCG standards calibrated against 1st IRP/3rd IS 75/537<br>Positive control |
| | Human luteinizing hormone (LH) | Six LH standards calibrated against 1st IRP 68/40<br>Positive control |

Table 3
Sample preparation and analytical parameters for chromatographic screening procedures I–V used in the 6th World Championships of Athletics

| | Screening procedures | | | | |
|---|---|---|---|---|---|
| | I | II | IVα | IVβ | V |
| Urine (ml) | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| ISTD[a] (μg/ml) | Diphenylamine (2.5) | Codeine (1.0) | 4α-OH-Stanozolol (0.1) | Methyltestosterone (0.08)<br>Epiandr-G (0.5) | Mefruside (2.0) |
| Solid-phase extraction | – | – | | – | XAD-2 (elution with $CH_3OH$) |
| Hydrolysis | – | 100 mg Cysteine, 0.50 ml HCl, 6 *M*, 100°C, 30 min | – | *E. coli* β-glucuronidase, pH 7, 1.5 h, 50°C | – |
| Extract. solvent (ml) | Diethyl ether (2.0) | Diethyl ether (2.5), isopropanol (0.5) | Diethyl ether (5.0) | Diethyl ether (5.0) | – |
| Buffering pH solution | KOH 6 *M* | $H_3BO_3$ 2.7 M–KOH 10 M<br>400 mg $Na_2CO_3$, pH 9.6 | $NaHCO_3$–$K_2CO_3$ 2:1 (w/w)<br>pH 9.6 | $K_2CO_3$ 30% (w/v)<br>pH 9.6 | |
| Anhydrous salt (g) | $Na_2SO_4$ (3) | $Na_2SO_4$ (3) | $Na_2SO_4$ (3) | $Na_2SO_4$ (3) | |
| Derivatization | – | MSTFA[b] (100 μl)–MBTFA[c] (30 μl) 80°C, 10 min, 80°C, 5 min | MSTFA:TMS–Imidazole 100:2 50 μl, 80°C, 30 min | MSTFA:$NH_4I$:DTE[d] 1000:2:4 100 μl, 60°C, 30 min | $CH_3I$ (20 μl), $K_2CO_3$ (100 mg), $CH_3CN$ (200 μl), 60°C, 5 h |
| Analysis | GC–NPD[e] | GC–MS | GC–MS | a, GC–MS; b, GC–HRMS | GC–MS |
| Injection mode | Splitless | Split 1:10 | Split 1:10 | Split 1:10 | Split 1:10 |
| Volume (μl) | 2 | 2 | 2 | a, 2; b, 1 | 2 |
| Carrier gas, flow | $N_2$, 1 ml/min | He, 0.7 ml/min | He, 0.7 ml/min | He; a, 0.8; b, 1.2 ml/min | He, 0.7 ml/min |
| Injector/detector temperature (°C) | 200/300 | 250/300 | 250/300 | 250/a,310; b, 280 | 250/300 |
| Temperature program | 80°C, 2 min, 25°C/min, 250°C, 5 min, 30°C/min, 310°C, 1 min | 100°C, 20°C/min, 290°C, 5 min | 180°C, 20°C/min, 300°C, 4 min | a: 180°C, 3°C/min, 235°C, 30°C/min, 310°C, 3.15 min<br>b: 150°C, 0.5 min, 12.5°C/min, 310°C, 2.5 min | 180°C, 22°C/min, 300°C, 5 min |
| Acquisition mode | – | Full scan mode | SIM | a, SIM; b, SIR | SIM |

[a]Internal standard. [b]*N*-Methyl-*N*-trimethylsilyltrifluoroacetamide. [c]*N*-Methyl-bis(trifluoroacetamide). [d]Dithioerythritol. [e]Nitrogen–phosphorus detector.

samples for the confirmation analyses were injected in the following order: reagent blank, suspect sample, blank urine sample, and positive control or urine sample from an excretion study.

In Procedure IVβ the steroid profile of the athlete was also monitored by quantitative analysis of the main endogenous steroids, such as testosterone (T), epitestosterone (E), androsterone, etiocholanolone, dihydrotestosterone (DHT), dehydroepiandrosterone (DHEA), 5α-androstane-3α,17β-diol (5α-diol) and 5β-androstane-3α,17β-diol (5β-diol), as their trimethylsilyl (TMS) derivatives, based on a 3-point calibration curve prepared daily. The parameters of the urinary steroid profile, especially the steroid ratios, are stable and can be influenced by the application of endogenous steroids, anabolic androgenic steroids, diuretics, ethanol, bacterial activities, etc., leading to characteristic patterns. The criteria for the steroid profiles indicating the possible application of exogenous testosterone, DHT, 5α-androstanedione, and DHEA, are mainly based upon disturbed endogenous steroid ratios such as that for T/E [6].

## 3. Results and discussion

The laboratory received 401 urine samples (56% from males and 44% from females) during the nine competition days. A mean of 45 samples was received per day. Batches of aliquots were delivered for screening and processing two or three times a day.

Of the 401 samples received at the laboratory, 64 were from athletes who declared that they had taken no drugs before the sporting events. Of the other 337 cases, 252 of them had used multi-medication, of which 18% declared they had consumed analgesics, 25% anti-inflammatory agents, 48% vitamins and electrolytes, 9% hormones (estrogen, birth control pills, melatonin, etc.) and 3% anti-asthmatic drugs (salbutamol, terbutaline). Of the 10 cases of β-agonists declared, nine were detected and reported as positive cases.

Most of the samples (85%) showed pH values between 5.0 and 6.0 with 10% between 6.0 and 7.0. Seven samples showed values $8 \geq pH > 7$ and only one sample had a $pH \leq 4$. As far as specific gravity (SG) is concerned, 87% of the samples had values between 1.010 and 1.030 and 9% had SG $\leq 1.010$. Consequently, these samples were screened for diuretics, with negative results, and a 10-ml aliquot was analyzed in screening procedure IVβ. Measurements of pH and SG were conducted both at the collection site using dip-sticks and instrumentally during sample reception at the laboratory. Measurements on-site would prevent delivery of diluted urine samples caused by high water intake. The majority of the samples presented a standard negative difference, ranging from 0.002 to 0.008 in absolute values, between the SG values measured at the collection site and those measured in the laboratory, while a standard positive difference of 0.5 in pH values is observed for more than 50% of the samples. Such differences are to be expected, bearing in mind the low accuracy of the measuring method used on-site. Nevertheless, the distribution of SG and pH values does not change.

Table 4
Positive doping control cases reported to the Medical Commission of the 6th World Championships of Athletics

| Doping class[a] | Number | Substance reported | Remarks |
|---|---|---|---|
| Stimulants | 1 | Ephedrine | Real: Female |
| | 1 | Ephedrine | Control: Female |
| | 2 | Ephedrines | Real: Male |
| | 1 | Strychnine | Control: Female |
| β-Agonists | 8 | Salbutamol | Real: 3 Males, 5 Females |
| | 1 | Terbutaline | Real: Female |
| Anabolic steroids | 1 | Methyltestosterone metabolites[b] | Control: Male |
| | 1 | Oxandrolone | Control: Male |
| | 2 | Stanozolol | Real: GC–HRMS, Females |
| | 2 | T/E[c] | Real: Male |
| Peptide hormones | 1 | hCG | Real: Male |

[a]According to the classification of the IOC International Olympic Charter against Doping in Sport [1].
[b]17α-Methyl-5α-androstan-3α,17β-diol and 17α-methyl-5β-androstan-3α,17β-diol.
[c]$T/E > 6$.

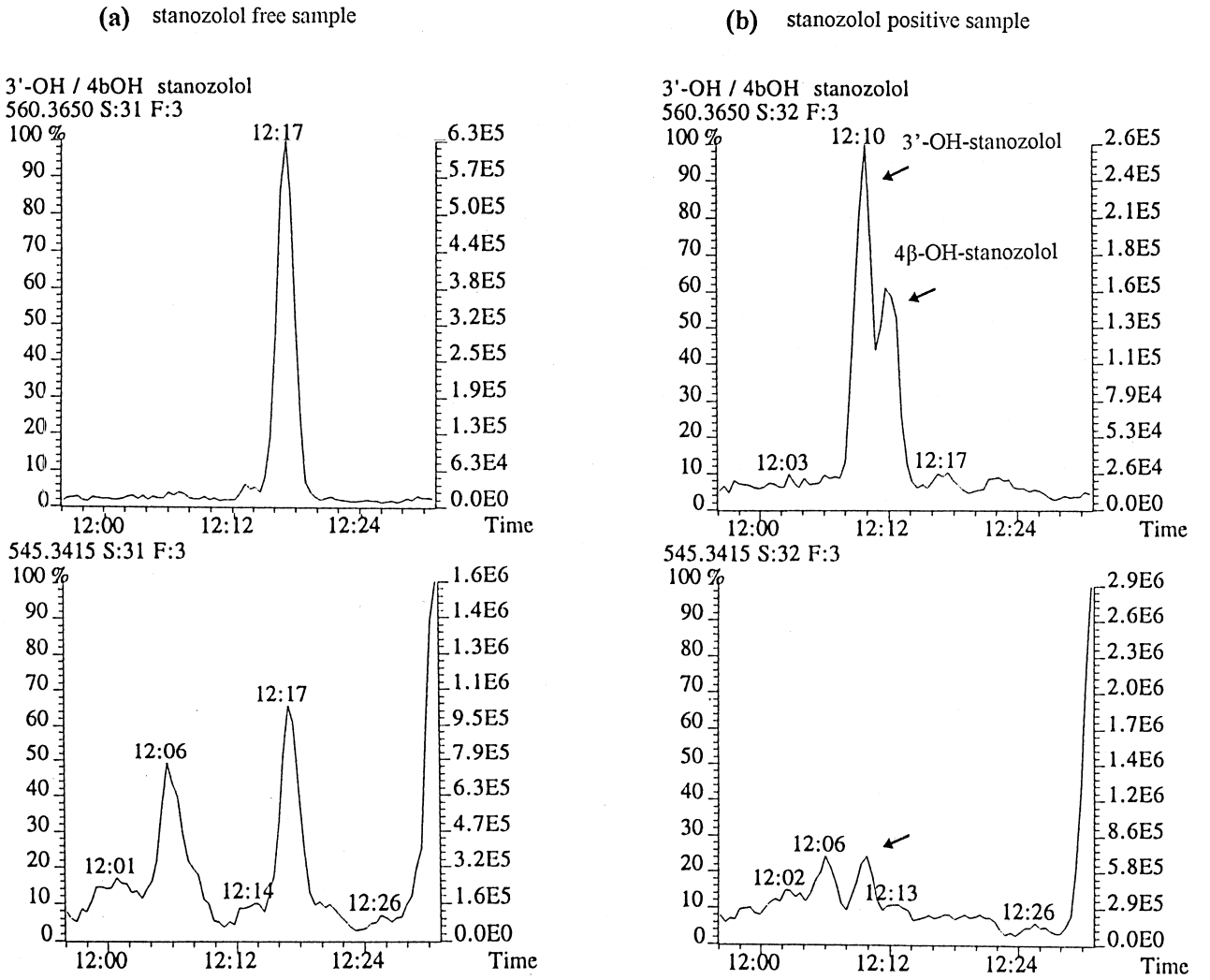


Fig. 1. Comparison of the ion chromatograms ( *m/z* 560.3650, 545.3415) of two samples analyzed in sequence according to screening procedure IVβ (GC–HRMS). a: Stanozolol-free sample; b: one of the stanozolol-positive samples.

Although caffeine is a common stimulant found in several commercial beverages, for doping it is considered a banned threshold substance. In 43% of the samples analyzed, low caffeine concentrations ($\geq$0.5 μg/ml) were observed, while in 48% of the samples caffeine concentrations ranged between 1 and 4 μg/ml. Only five samples had a caffeine concentration $\geq$10 μg/ml, but none exceeded the limit of 12 μg/ml. Quantitation of caffeine was carried out after analysis according to screening procedure I.

Testosterone is an endogenous anabolic steroid and is also a banned substance. The IOC-MC rule considers positive any sample with a T/E ratio in urine higher than 6.0. The general overview of the T/E ratios obtained for 401 samples analyzed by procedure IVβ showed that 91% of the samples (89% of the male and 93% of the female samples) presented $0 \leq T/E \leq 3$. Thirty-five samples had $4 \leq T/E \leq 6$ and three samples, two male and one female, had values $> 6$ in the initial analysis. When the triplicate analysis was repeated, the two male samples were reported as positive cases, giving T/E = 7.6 and 7.7.

### 3.1. Positives Cases

When a urine sample was confirmed as containing banned substances or metabolites, a comprehensive written analytical report was created. Twenty positive

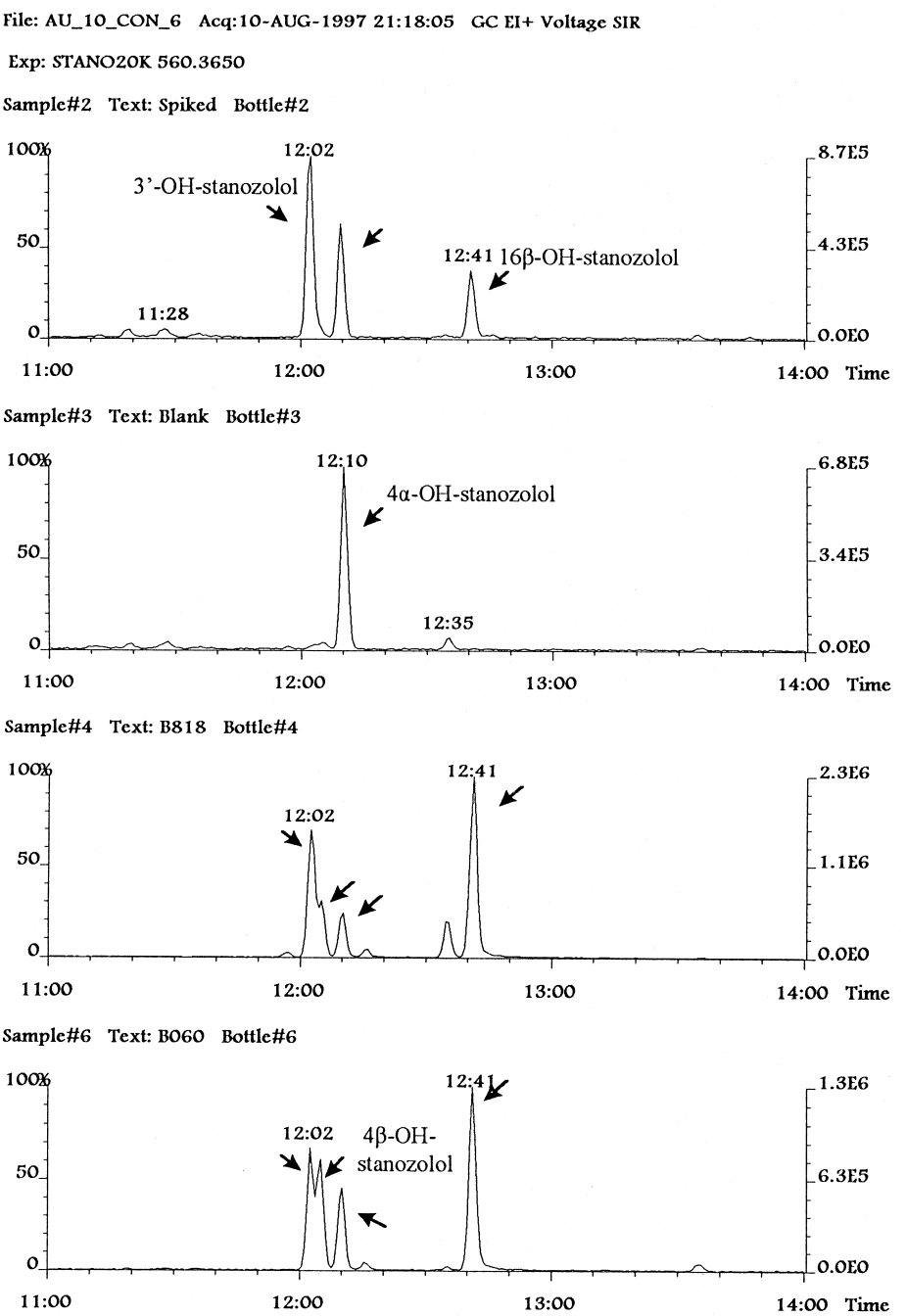


Fig. 2. Comparison of the ion chromatogram ( *m/z* 560.3650) of stanozolol metabolites and internal standard, 4α-OH-stanozolol, in the two stanozolol-positive cases, the negative control sample and the positive control sample analyzed during the confirmatory analysis of B samples (GC–HRMS). #2, positive control sample; #3, negative control sample; #4, first B sample; #6, second B sample.

cases were reported, four of them being control samples introduced by the IAAF Medical Commission to verify the proper functioning of the laboratory. Confirmatory analysis (analysis of B samples) was requested for seven cases, and the initial results were re-established. The banned substances detected and reported to the Medical Commission of the Games are listed in Table 4.

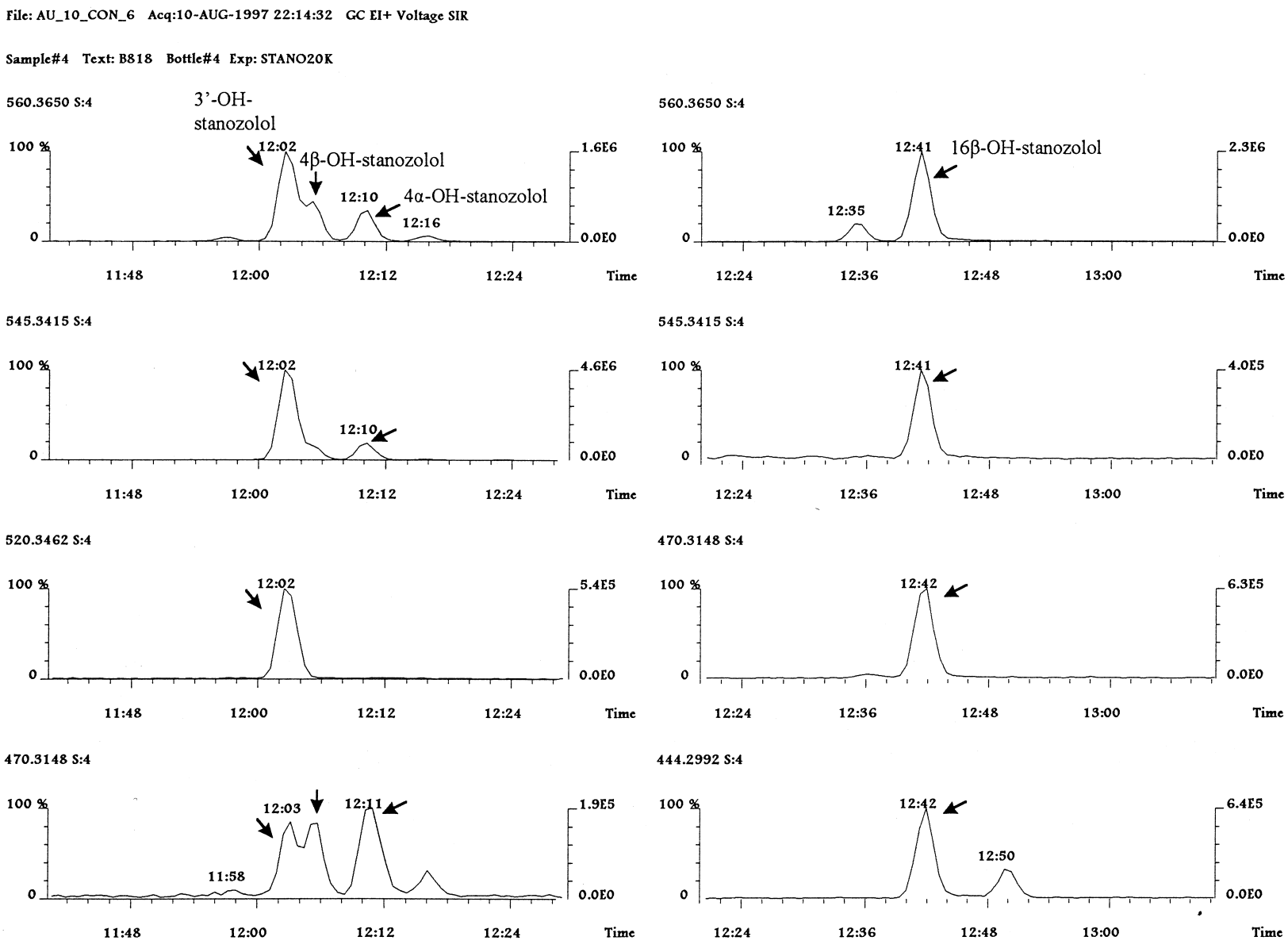


Fig. 3. Ion chromatograms ( $m/z$ 560.3650, 545.3415, 520.3462, 470.3148, 444.2992) of stanozolol metabolites and internal standard, 4α-OH-stanozolol, in one of the stanozolol-positive cases analyzed during the confirmatory analysis of B samples (GC–HRMS).

Table 5
SIR mass spectral data of one of the stanozolol-positive cases (B sample) reported in the 6th IAAF World Championships of Athletics and the stanozolol-positive sample used in the confirmatory analysis of B samples, according to the IOC guidelines [1] and the European Union guidelines for mass spectrometry (No. L118/64, 14 May 1993)

| *m/z* | Relative abundances | | | | | |
|---|---|---|---|---|---|---|
| | B sample | | | Positive control | | |
| | 3′-OH-Stanozolol | 4β-OH-Stanozolol | 16β-OH-Stanozolol | 3′-OH-Stanozolol | 4β-OH-Stanozolol | 16β-OH-Stanozolol |
| 560.3650 | 37.4% | 100% | 100% | 37.5% | 100% | 100% |
| 545.3415 | 100% | 97.5% | 16.7% | 100% | 93.4% | 13.6% |
| 520.3462 | 11.7% | | | 10.9% | | |
| 470.3148 | 3.3% | 21.6% | 27.9% | 3.4% | 20.4% | 27.4% |
| 444.2992 | | | 28.0% | | | 26.1% |

On the second day of the games, the presence of ephedrines was suspected after GC–NPD and GC–MS screening analysis and confirmed after GC–MS analysis of the sample freshly prepared according to the properly modified procedure I (internal standard codeine, derivatization according to procedure II) following the specifications set by the IOC and described previously. The MSD was acquiring data in scan mode ($m/z$ = 50–400). Quantitation of ephedrine was also performed using a 10 ppm ephedrine control urine. The following ions were of diagnostic structural importance: methylephedrine-OTMS (relative to the internal standard having retention time (RRT) = 0.345, $M_r$ = 251): 72, 163 (M-TMS-$CH_3$), 236 (M-$CH_3$), norephedrine-NTFA, bis-*N*,*O*-TMS (RRT = 0.417, $M_r$ = 391): 179, 212, 376 (M-$CH_3$), norpseudoephedrine NTFA, bis-*N*,*O*-TMS (RRT = 0.430, $M_r$ = 391): 179, 212, 376 (M-$CH_3$), ephedrine-NTFA, OTMS (RRT = 0.439, $M_r$ = 333): 179, 154, 318 (M-$CH_3$) and pseudoephedrine-NTFA, OTMS (RRT = 0.455, $M_r$ = 333): 179, 154, 318 (M-$CH_3$).

The use of human chorionic gonadotropin (hCG) results in a natural increase of testosterone in the body [7]. Since it is endogenous testosterone that is being leveled up, doping cannot be detected by determination of the T/E ratio. Therefore, hCG measurement was conducted on all male samples as mentioned above. The majority of the samples (95%) had hCG values ≤0.8 mIU/ml, 11 samples presented a value between 1 and 5 and, on the second day of the games, one had a value of 59 mIU/ml and reported positive. After 8 days, a urine sample was collected from the same athlete and subjected to analysis. The hCG was found to be 1.35 mIU/ml.

The abuse of exogenous testosterone, indicated by a T/E ratio higher than six in two male samples collected on the second and the third day of the games, was confirmed by quantitative analysis (GC–MS) of the corresponding samples in triplicate, prepared according to the adequately modified procedure IVβ (deuterated internal standard solution, discarding free fraction, double extraction with pentane) [8]. The free fraction was analyzed for free testosterone and the mass spectra of testosterone and epitestosterone compared to spectra obtained from standard solutions of the compounds.

The presence of oxandrolone and its metabolite, epioxandrolone, was suspected after GC–MS and GC–HRMS screening analysis of a urine sample, prepared according to procedures IVα and IVβ, and introduced by IOC as a control into the routine of the fourth day of the games. The confirmatory analysis was performed by GC–MS using a sample freshly prepared according to a slightly modified procedure IVα (double extraction with dichloromethane at the pH of urine) following the specifications set by IOC. The MSD acquired data in the scan mode. Oxandrolone-monoTMS and epioxandrolone-monoTMS have the molecular ion $M^+$ = 378, and RRT = 0.783 and 0.727, respectively.

The presence of salbutamol and terbutaline was suspected after GC–MS analysis of the corresponding samples according to procedure IVβ and confirmed after GC–MS analysis of the same sample, freshly prepared according to a slightly modified procedure IVβ (70 µl of derivatization reagent, for 60 min at 80°C, with analysis on an Ultra-2 column) following the specifications set by the IOC. The MSD acquired data in the selective ion monitoring (SIM) mode, the following ions being of diagnostic importance for each of the above β-agonists: salbutamol-trisTMS (RRT = 0.648): 455 ($M^+$), 440 ($M^+-CH_3$), 369

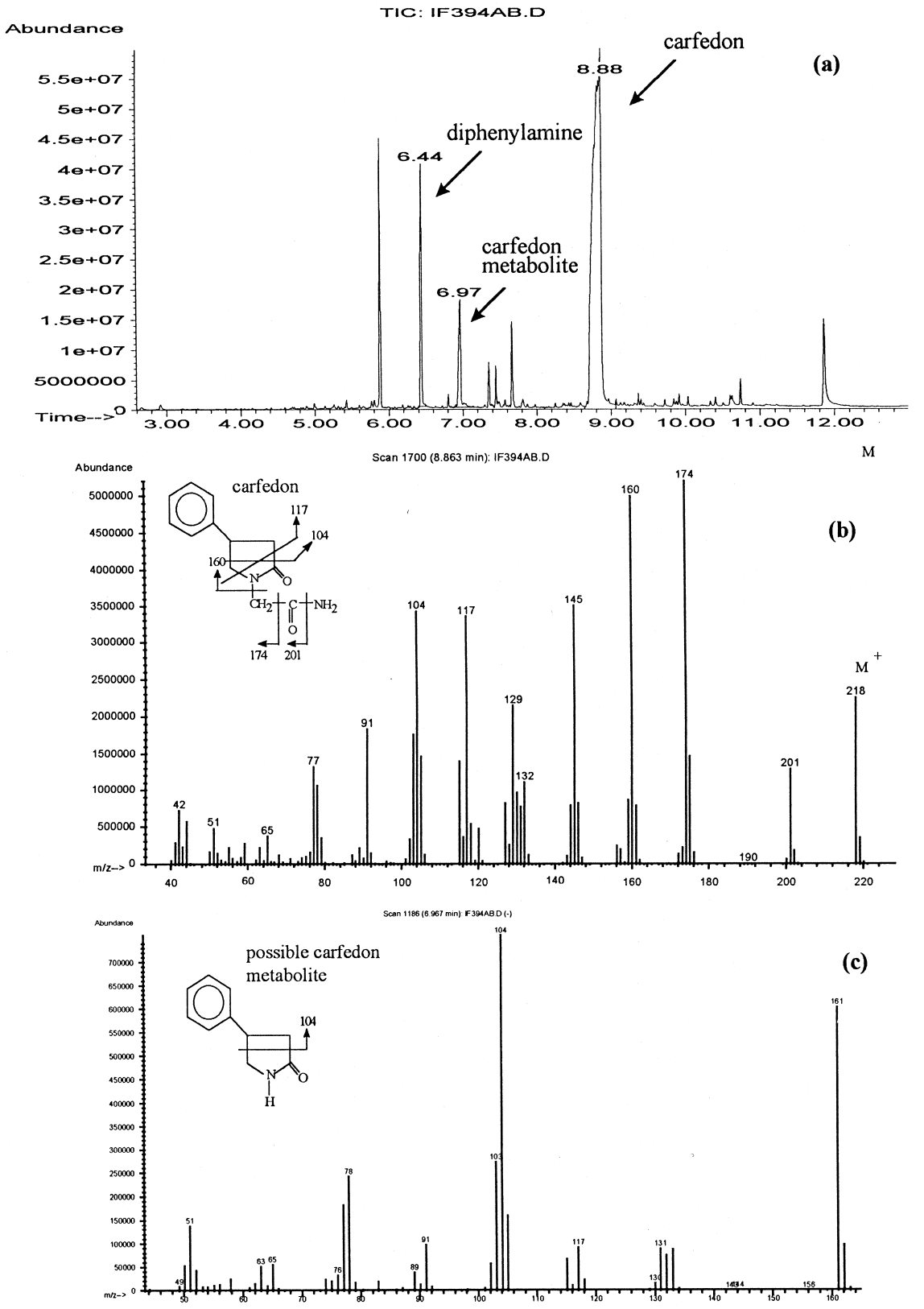
TIC: IF394AB.D
Abundance
carfedon
8.88
(a)
diphenylamine
6.44
carfedon metabolite
6.97
Time-->
Scan 1700 (8.863 min): IF394AB.D
carfedon
(b)
M+
Scan 1186 (6.967 min): IF394AB.D (-)
possible carfedon metabolite
(c)
m/z-->

Fig. 4. Total ion current chromatogram of a carfedon-positive urine sample (a), and mass spectra of the parent compound (b) and a possible carfedon metabolite (c). The sample was prepared according to procedure I and analyzed according to procedure II. Internal standard, diphenylamine.
←

($M^+-CH_2NHC(CH_3)_3$), 86 ($CH_2NHC(CH_3)_3$) and terbutaline-trisTMS (RRT = 0.606): 426 ($M^+-CH_3$), 370 ($M^+-NHC(CH_3)_3$), 356 ($M^+-CH_2NHC(CH_3)_3$), 86 ($CH_2NHC(CH_3)_3$).

The presence of strychnine was suspected after GC–NPD analysis of a sample introduced by the IOC, as a control, into the routine of the sixth day of the games. This was prepared according to procedure I and confirmed after GC–MS analysis of the same sample, freshly prepared following the specifications set by the IOC. The MSD acquired data in scan mode ($m/z$ = 40–400). Strychnine has the molecular ion $M^+$ = 334 and RRT = 1.95.

The presence of methyltestosterone metabolites, 17α-methyl-5α-adrostane-3α,17β-diol and 17α-methyl-5β-adrïstane-3α,17β-diol, was suspected after GC–HRMS and GC–MS analysis of a urine sample, introduced by the IOC as a control into the routine of the eighth day of the games, prepared according to procedure IVβ and confirmed after GC–MS analysis of the same sample freshly prepared according to a slightly modified procedure IVβ (double extraction with pentane, internal standard nor-androsterone) following the specifications set by the IOC. The MSD acquired data in the SIM mode and the following ions are of diagnostic importance for each methyltestosterone metabolite: 5α-diol-bisTMS (RRT = 1.375): 450 ($M^+$), 435 ($M^+-CH_3$), 360 ($M^+-$HOTMS), 255, 143 and 130 5β-diol-bisTMS (RRT = 1.392): 450 ($M^+$), 435 ($M^+-CH_3$), 360 ($M^+-$HOTMS), 255, 143 and 130.

After GC–HRMS screening, the presence of stanozolol metabolites was suspected in two female samples, collected on the seventh and eighth day of the games. They were prepared according to procedure IVβ and confirmed after GC–HRMS analysis of the same sample, freshly prepared according to a slightly modified procedure IVβ (internal standard 4α-OH-stanozolol, double extraction). Additionally, a method according to Schanzer et al. [9] involving an immunoaffinity chromatography purification step was applied, because of the low concentration of the stanozolol metabolites. Three metabolites of stanozolol were detected in both samples: 3′-OH-stanozolol, 4β-OH-stanozolol and 16β-OH-stanozolol at the following concentrations (ng/ml): first sample 0.8, 0.4 and 7, second sample 0.4, 0.4 and 4, respectively. The HRMS acquired data in the selective ion recording (SIR) mode. The following ions are of structural diagnostic importance: 3′-OH-stanozolol-trisTMS (RRT = 0.989), 4β-OH-stanozolol-trisTMS (RRT = 0.994), 16β-OH-stanozolol-trisTMS (RRT = 1.042): 560.3650 ($M^+$), 545.3415 ($M^+-CH_3$), 520.3462 ($M^+-CH_2CH_2CH_2$), 470.3148 ($M^+-$OTMS), 455.2914 [$M^+-$(OTMS, $CH_3$)]), 444.2992 ($M^+-CH_2CHOTMS$). In Fig. 1, chromatograms from a stanozolol-positive sample and a stanozolol-free sample are shown. In Fig. 2, the ion 560.3650 chromatogram for stanozolol metabolites in the two positive cases, in the urine sample spiked with stanozolol, and in the negative control sample used for the confirmatory analysis of B samples are presented. In Fig. 3 the ion chromatograms of all diagnostic ions of stanozolol metabolites and internal standard, 4α-OH-stanozolol, used in the SIR confirmatory analysis of one B sample are shown. The respective spectral data are summarized in Table 5.

Further indication of the presence of stanozolol metabolites was provided by the detection of their N-TFA, bis-OTMS derivatives after additional derivatization with MBTFA: 3′-OH-stanozolol-NTFA, bis-OTMS (RRT = 0.999): 569.2842 ($M^+-CH_3$), 4β-OH-stanozolol-NTFA, bis-OTMS (RRT = 0.958): 569.2842 ($M^+-CH_3$), 16β-OH-stanozolol-NTFA, bis-OTMS (RRT = 1.018): 584.3077 ($M^+$).

In addition, 16 cases of carfedon (phenylpiracetam), an amphetamine-class stimulant 'street' drug, whose use was being monitored during the games, were reported upon request of the IAAF. Samples with carfedon were reported negative but treated as positives (confirmation procedure: sample preparation according to procedure I, analysis according to procedure II). In most carfedon cases, apart from the parent compound (RRT = 1.38), one additional peak was consistently present in the NPD and total ion count (TIC) chromatogram (procedures I and II) with relative retention time 1.08, whose mass spectra could correspond to a possible metabolite, which has lost the acetylamino group of the *N*-carfedon ring. In Figs. 4 and 5, a TIC and the mass spectra of carfedon and carfedon-possible metabolite, free and derivatized, are shown. Exact mass measurement of the pos-

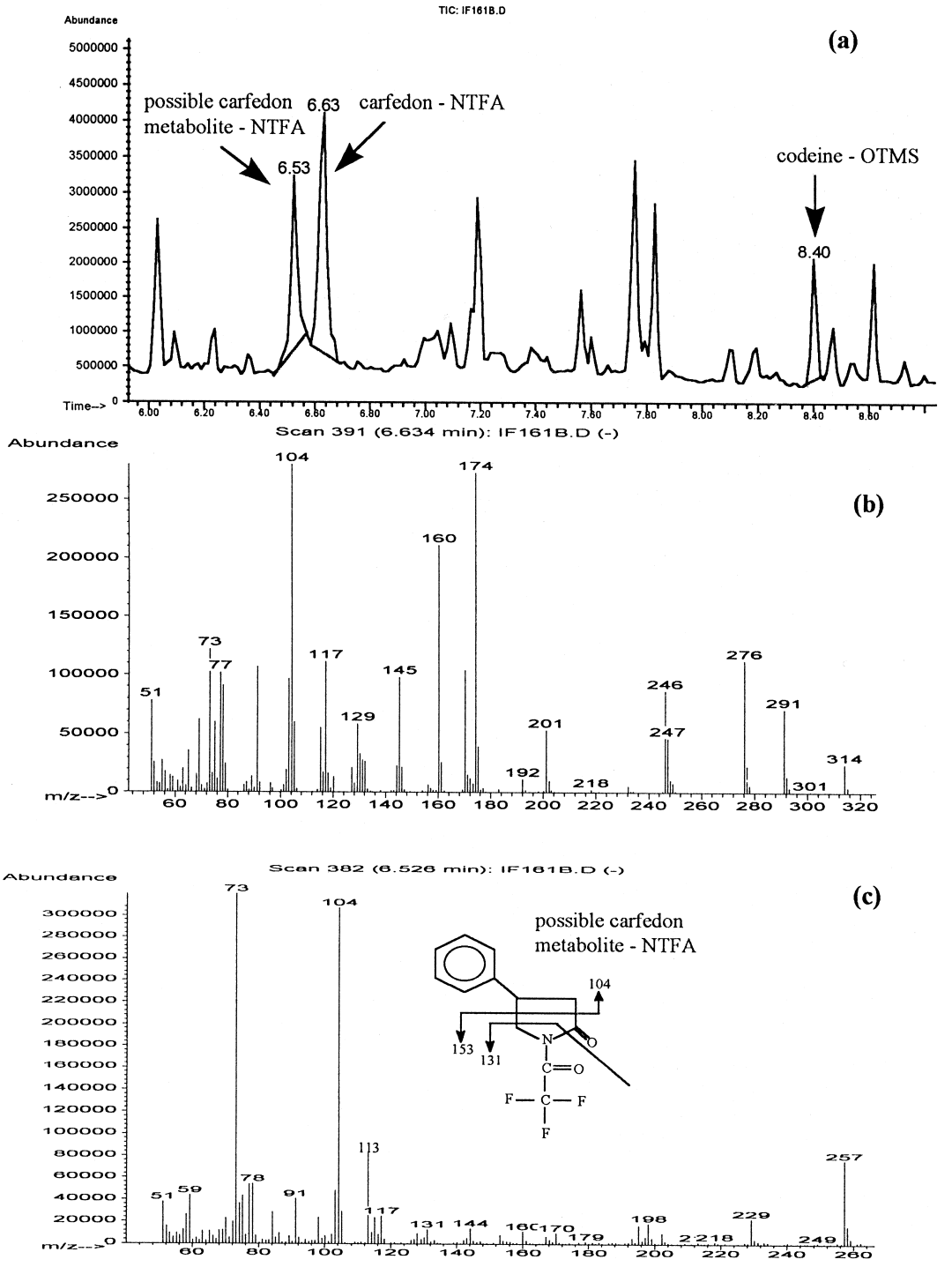


Fig. 5. Total ion current chromatogram of a carfedon-positive urine sample (a), and mass spectra of the parent compound (b) and a possible carfedon metabolite (c), as N-TFA derivatives. The sample was prepared and analyzed according to procedure II. Internal standard, codeine.

sible carfedon metabolite was carried out using GC–HRMS. The numbers of C, N, H and O atoms of carfedon were given as maxima. Only one possible molecule was indicated having 10 C, 11 H, 1 N and 1 O atoms, which coincides with the proposed structure of carfedon's derivative (Fig. 5). The calculated accurate mass of the molecular ion was $M^+ = 161.084281$ compared to the real mass 161.084064 ($ppm = \Delta m/m = -1.3$, $mDa = -0.2$) and the accurate mass of the main fragment was $m/z = 104.061963$, compared to 104.062600 ($ppm = 6.1$, $mDa = 0.6$). Carfedon is excreted free in urine, while the metabolite is excreted mainly free, but also conjugated in minor amounts.

## 4. Conclusions

During the 6th World Championships of Athletics, Athens, Greece, 1997, 401 samples were analyzed in 10 days for stimulants, narcotics, anabolic steroids and hCG and resulted in 20 positive cases. Successful screening of the most important of the anabolic steroid metabolites at a concentration level below 1 ppb proved the superiority of HRMS over the benchtop quadrupole MS. Finally, the current doping trend of athletes who try to escape giving positive tests by seeking new substances such as carfedon was revealed.

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