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Sports drug testing: Analytical aspects of selected cases of suspected, purported, and proven urine manipulation

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ABSTRACT

Manipulation of urine specimens provided by elite athletes for doping control purposes has been reported several times in the past, and in most of these cases urine substitution was eventually proven. Recent findings of suspected and substantiated manipulation have outlined the complexity and diversity of tampering options, sample appearance alterations resulting from non-manipulative influence, and the analytical challenges arising from these scenarios. Using state-of-the-art mass spectrometric and immunological doping control and forensic chemistry methodologies, four unusual findings were observed. One sports drug testing specimen was found to contain an unusually high content of saccharides accompanied by hordenine and Serpine-Z4, while no endogenous steroid (e.g. testosterone, epitestosterone, androsterone and etiocholanolone) was detected. This specimen was identified as non-alcoholic beer filled into the doping control sample container, constituting an undisputed doping offense. A doping control sample of bright green color was received and found to contain residues of methylene blue, which is not considered relevant for doping controls as no masking or manipulative effect is known. In addition, the number of urine samples of raspberry to crimson red coloration received at doping control laboratories has constantly increased during the last years, attributed to the presence of hemoglobin or betanin/isobetanin. Also here, no doping rule violation was given and an impact on routine analytical results was not observed. Finally, a total of 8 sports drug testing samples collected at different competition sites was shown to contain identical urine specimens as indicated by steroid profile analysis and conclusively proven by DNA-STR (short tandem repeat) analysis. Here, the athletes in question were not involved in the urine substitution act but the doping control officer was convicted of sample manipulation.

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1. Introduction

In modern human sports drug testing, urine is by far the most frequently collected doping control specimen complemented by blood or serum samples for selected analytical approaches. Despite stringent regulations concerning the urine sampling procedure, 12 incidences of physical or chemical manipulation of doping control samples were uncovered between 2006 and 2009 [1–4], most of which resulted from urine substitution [5] or (attempted) tampering, e.g. by the addition of proteases [6–9] to interfere with the determination of prohibited peptide hormones such as erythropoiesis-stimulating agents. Consequently, doping control authorities (including the laboratories and national/international anti-doping organizations) have paid particular attention to unusual visual appearances of specimens and atypical analytical results. The latter refers especially to the detection of rare and uncommon substances as well as to conspicuous urinary steroid profile data using population-based statistics as well as individual reference ranges of the tested athletes [10–18], which allowed for the identification of most of the urine substitutions. In the following, four recent cases of suspected, purported or proven urine sample manipulation are presented and the importance of comprehensive analytical approaches to provide evidence for as well as against a doping rule violation by the athlete is discussed.

2. Experimental

Routine doping controls were conducted according to established and accredited methods [19]. Specific follow-up experiments included 1-dimensional gel electrophoresis (1D-GE) with bottom-up identification of Coomassie-stained proteins, ethanol determination, DNA-typing, and liquid chromatography–high resolution/high accuracy (tandem) mass spectrometry (LC–MS/MS) analyses.

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2.1. 1D-gel electrophoresis

1D-GE was performed in agreement with commonly used protocols for doping controls [7,8]. In brief, 4 mL of urine were concentrated employing a centrifugal filter device (10 kDa cut-off) to a final volume of approximately 50 μL. Denaturation of proteins and reduction of disulfide bonds was subsequently achieved by dithiothreitol (DTT) and lithium dodecyl sulphate (LDS) sample buffer, and SDS-PAGE was conducted with 12% Bis–Tris gels. The gels were eventually stained with Coomassie Blue according to the manufacturer's instructions. For protein identification, gel bands were excised, destained, and subjected to trypsin digestion. The generated tryptic peptides were extracted into 50% ACN/1% TFA, dried under reduced pressure, and resolved in 2% acetic acid for LC–MS(/MS) analysis.

2.2. Ethanol determination

The determination of ethanol in suspicious doping control samples was conducted by the Institute of Legal Medicine at the University of Cologne using accredited/validated GC-based methods. Samples were analyzed in duplicate and average values reported.

2.3. DNA typing

Urinary DNA was analyzed at LGC Forensics (Cologne, Germany) using accredited/validated procedures focusing on 12 different short tandem repeat (STR) loci as outlined in Table 1. DNA typing was conducted in duplicate using two separate assays (SEfiler Plus Kit, Applied Biosystems, Darmstadt, Germany, and Nonaplex QS-Kit, Biotype, Dresden, Germany).

2.4. LC–MS/MS analysis

For the determination of low molecular mass analytes, normal flow liquid chromatography was accomplished using an Agilent 1260 HPLC system (Waldbronn, Germany) equipped with a Pyramid C-18 analytical column (50 mm × 2.1 mm, 1.9 μm particle size, Macherey-Nagel, Düren, Germany). Solvent A was 0.1% formic acid and solvent B was acetonitrile. The gradient started at 100% solvent A, increased to 100% solvent B within 8 min, maintained for 0.5 min at 100% B, and re-equilibrated at starting conditions for 3.5 min. The flow-rate was set to 300 μL/min. The LC was interfaced to either to an AB Sciex API4000 QTrap (Toronto, Canada) or an AB Sciex 5600 TripleTOF (quadrupole-time-of-flight, QqTOF) mass analyser operated in positive electrospray ionisation mode at 500 °C. Nitrogen obtained from an N_2-generator (CMC, Eschborn, Germany) was used as collision and auxiliary/sheath gas in both systems, and collision energies employed in product ion scan experiments were optimized for each individual target analyte. The QqTOF mass spectrometer was calibrated (auto-calibration following every 5th injection using the manufacturer's reference compound mixture and calibrant delivery system) in MS and MS/MS mode to ensure mass accuracies <5 ppm.

Peptide and protein analyses were conducted using a Waters (Eschborn, Germany) Acquity nanoUPLC connected via nanospray source to a Thermo (Bremen, Germany) LTQ-Orbitrap mass spectrometer. The nanoUPLC was equipped with a Waters BEH-130 C_{18} (75 μm × 100 mm, 1.7 μm particle size) and Waters Symmetry C_{18} (180 μm × 20 mm, 5 μm particle size) analytical and trapping column, respectively. Solvent A consisted of purified water acidified with 0.1% of formic acid and solvent B was acetonitrile also containing 0.1% of formic acid.

After injection of 1 μL of sample solution, the target analytes were pre-concentrated on the trapping column with 97% of solvent A and a flow rate of 5 μL/min. After 3 min, the flow was diverted to the analytical column at 750 nL/min, and the gradient started with an isocratic step for 1 min with 97% of A. The rate of solvent B increased to 90% in 21 min, followed by a re-equilibration phase at starting conditions for 13 min. The overall runtime was 35 min. The mass spectrometer featured a nanospray source with a coated fused-silica emitter (New Objective, MA, USA) operated in positive ionisation mode. Accurate mass measurement (<3 ppm) was ensured through calibration with the manufacturer's calibration mixture (consisting of caffeine, the tetrapeptide MRFA, and Ultramark), and the gas supply consisted of nitrogen (N_2-generator, same as mentioned above) and helium (purity 5.0). The ionisation voltage was set to 1.5 kV and the temperature of the transfer capillary was adjusted to 150 °C. The resolution was set to 30,000 @ m/z 400 (FWHM), and the collision energy for automated data-dependent MS/MS experiments was 35% (arbitrary units, Xcalibur software version 2.0).

2.5. Data analysis (bottom-up protein identification)

Proteome Discoverer (Version 2.0) including the Swissprot/Uniprot database (2010, Thermo, Bremen, Germany) was used for the evaluation of LC–MS/MS-generated data. The database search was restricted to trypsin digestion but not to any particular species. The identification of proteins was considered successful if at least two peptides were detected and protein sequence coverage of more than 10% was achieved with peptide masses not deviating more than 10 ppm from theory. The mass tolerance for product ions (recorded in low resolution mode) was set to 0.5 Da.

Table 1
Results of DNA-STR typing of doping control urine samples presumably delivered by eight different athletes.

Locus	Urine sample number							
	13969/2009	13970/2009	13971/2009	13972/2009	0154/2010	0155/2010	0156/2010	0157/2010
Amelogenin	X/X	X/X	X/X	X/X	X/X	X/X	X/X	X/X
D18S51	14/14	14/14	14/14	14/14	14/14	14/14	14/14	14/14
D3S1358	16/17	16/17	16/17	16/17	16/17	16/17	16/17	16/17
D2S1338	21/24	21/24	21/24	21/24	21/24	21/24	21/24	21/24
D8S1179	15/15	15/15	15/15	15/15	15/15	15/15	15/15	15/15
D16S539	11/13	11/13	11/13	11/13	11/13	11/13	11/13	11/13
D19S433	12/14	12/14	12/14	12/14	12/14	12/14	12/14	12/14
D21S11	28/32.2	28/32.2	28/32.2	28/32.2	28/32.2	28/32.2	28/32.2	28/32.2
FGA	22.2/25	22.2/25	22.2/25	22.2/25	22.2/25	22.2/25	22.2/25	22.2/25
SE33	21/31.2	21/31.2	21/31.2	21/31.2	21/31.2	21/31.2	21/31.2	21/31.2
TH01	6/9	6/9	6/9	6/9	6/9	6/9	6/9	6/9
vWA	17/17	17/17	17/17	17/17	17/17	17/17	17/17	17/17

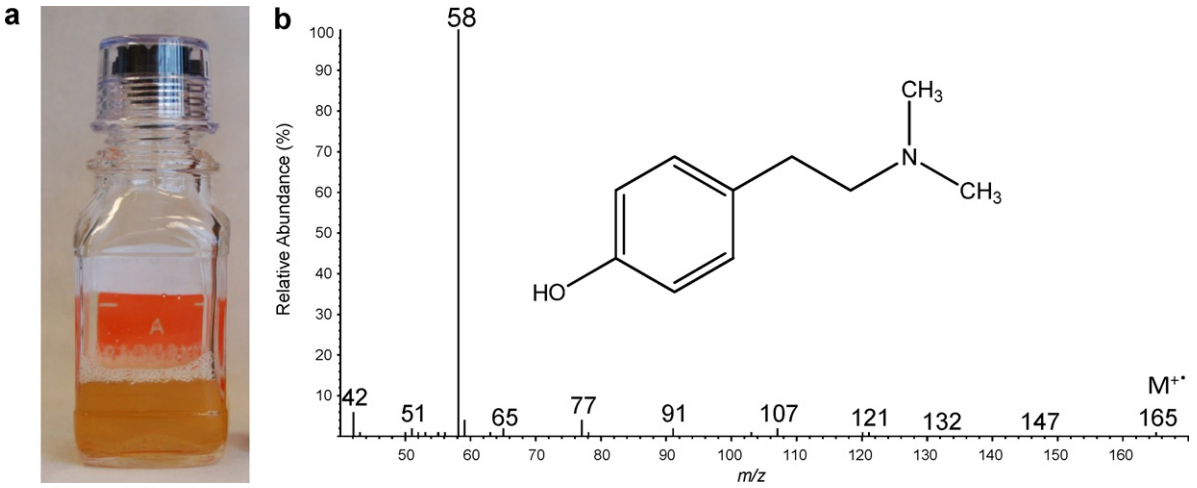

Fig. 1. Doping control sample (a) of physically unremarkable appearance but identified as alcohol-free beer containing amongst other indicators high amounts of hordenine as determined by GC–MS (b).

3. Case vignettes

3.1. Case 1

In 2010, a urine specimen of physically unremarkable appearance (Fig. 1a) was received and forwarded to routine doping control analyses according to the regulations applicable to in-competition testing. Steroid profile analyses as well as GC–MS/NPD measurements however returned highly suspicious (since extremely unusual) results. The steroid screening procedure demonstrated the absence of all commonly observed human urinary steroids such as testosterone, epitestosterone, androsterone and etiocholanolone, which disproved the sample's composition as urinary matrix of human origin. This finding as such represented a violation of anti-doping rules concerning manipulation. In the course of answering the question regarding the specimen's true nature, the determination of comparably high amounts of hordenine (4-(2-dimethylaminoethyl)phenol, Fig. 1b) provided supporting evidence. Hordenine, also referred to as *N*,*N*-dimethyltyramine and structurally elucidated in 1907 [20], is a plant alkaloid being produced, e.g. in germinating barley [21]. Consequently, the consumption of beer brewed from barley has been a major source of hordenine in human urine [22] and is observed frequently in routine doping controls. Further, also olfactometry suggested the admixture or bottling of a brewing product or beverage into the sports drug testing sample, and the specimen was subjected to 1D-GE followed by bottom-up identification of separated and Coomassie-stained proteins. With a total of 8 (proteotypical) peptides contributing to a sequence coverage of 22%, a major component of the endosperm albumin referred to as Serpin-Z4 (composed of 399 amino acids exhibiting an average molecular weight of 43276.4 Da) from barley (*Hordeum vulgare*, UniProt accession #P06293) was identified as the predominant protein in that particular sample. Moreover, the specimen was analyzed concerning its ethanol content revealing a concentration of 120 mg/kg (or 0.015 vol.%), which is in agreement with ethanol residues commonly found in alcohol-free beer ranging between 0.02 and 0.5 vol.%, and the pH of the sample was determined with 3.5.

All these aspects led to the conclusion that a sample manipulation had occurred by substituting the requested urine specimen by an aliquot of alcohol-free beer, which unequivocally represented a doping rule violation according to M2.1 of the WADA prohibited list [23].

3.2. Cases 2 and 3

Between 2009 and 2011, several sports drug testing samples of noticeable coloration, particularly green and raspberry/crimson red, were received at the doping control laboratory in Cologne (Fig. 2a). Despite the peculiar appearance, routine doping controls did not reveal any unusual test result concerning, e.g. anabolic agents, stimulants and masking agents. Nevertheless, since no particular medication [24] that might cause such an eye-catching look (e.g. chlorpromazine, thioridazine and amitriptyline) was declared in the sample documentation sheets, the origin of the colors was investigated.

A common source of reddish discoloration of athletes' urine is hemoglobin resulting, e.g. from intravascular hemolysis (also referred to as march or footstrike hemoglobinuria) as observed after strenuous exertional exercise [25–27]. Moreover, contact sport such as karate was reported to occasionally lead to an increased hemoglobin excretion [28]. In addition to hemoglobin, also myoglobin liberated in the course of severe myopathy can lead to a dark-red dyeing of urine [29,30]. Hence, commercial dipstick tests (Combur 9, Roche Diagnostics, Mannheim, Germany) for hemoglobin were performed on three reddish urine samples, and one returned a positive result. The remaining two specimens were subjected to LC–MS(/MS) analysis (following a 1:10 dilution with deionized water) in order to screen for known as well as potentially unknown substances presumably causing the reddish coloration. As illustrated in Fig. 2b, the two major betacyanins of beetroot referred to as betanin (**1**) and isobetanin (**2**) [31], which represent natural dyes of beetroot, were detected in both samples. The protonated molecule of each stereoisomer was observed at *m*/*z* 551.1509 ($C_{24}H_{27}O_{13}N_2$, error: 0.2 ppm), which generates the product ion at *m*/*z* 389.0982 (representing the aglycon with $C_{18}H_{17}O_8N_2$, error: 0.7 ppm) [32] that subsequently eliminates formic acid (46 Da) to yield the low abundant product ions at *m*/*z* 343.0931 ($C_{17}H_{15}O_6N_2$, error: 1.6 ppm) and 297.0870 ($C_{16}H_{13}O_4N_2$, error: 0.1 ppm) as shown in Fig. 2c. An additional characteristic product ion was found at *m*/*z* 150.0545, which was assigned to an elemental composition of $C_8H_8NO_2$ (error: −2.4 ppm) and suggested to represent the charged residue of 4-ethylidene-1,4-dihydropyridine-2-carboxylic acid. The phenomenon of 'beeturia', the excretion of pink or raspberry-red urine following the ingestion of beetroot, has been observed and elucidated since the 1950s [33–35]. After initially attributing the fact that only 10–14% of humans produce a reddish urine after

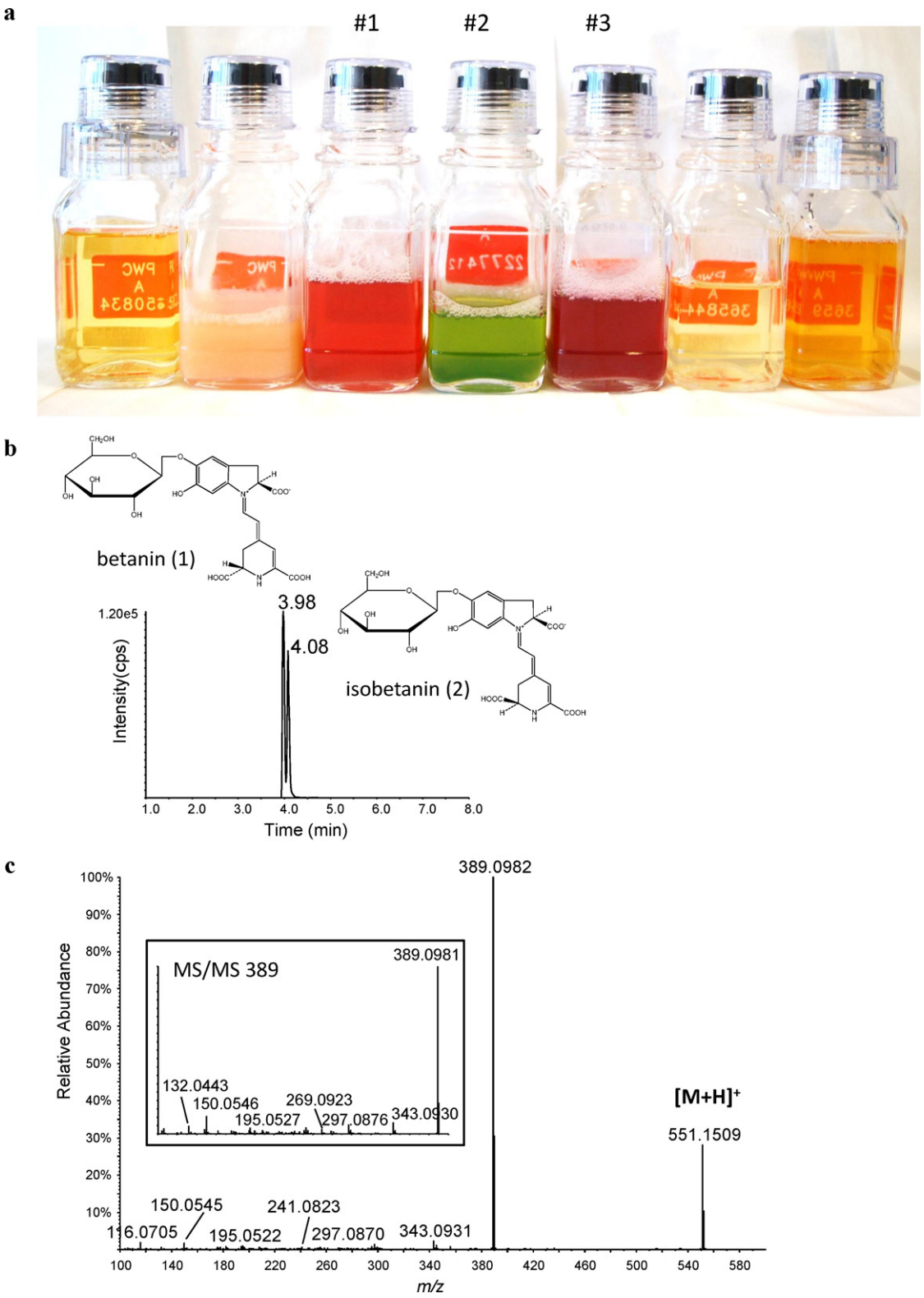

Fig. 2. Doping control samples (a) with eye-catching discoloration. The reddish appearance was attributed to hemoglobin (#1) and betanin/isobetanin (#3) and the green color (#2) to methylene blue. The chromatographic separation of the betacyanins and the ESI product ion mass spectrum of betanin are shown as (b) and (c), respectively.

consumption of beetroot or its juice to genetic and thus metabolic reasons, it was eventually proven that the interindividual variations in urinary excretion of betanin and isobetanin are predominantly (if not exclusively) determined by their gastronintestinal absorption [35–37]. A possible reason for the comparably frequent observation of reddish urine samples in sports drug testing might be the recently increasing interest in dietary nitrate supplementation, which was described to improve exercise performance [38], and the fact that beetroot juice in particular is naturally rich in nitrate. In summary, both scenarios of red urine are not due to sample manipulation and are readily characterized by routine sports drug testing methods.

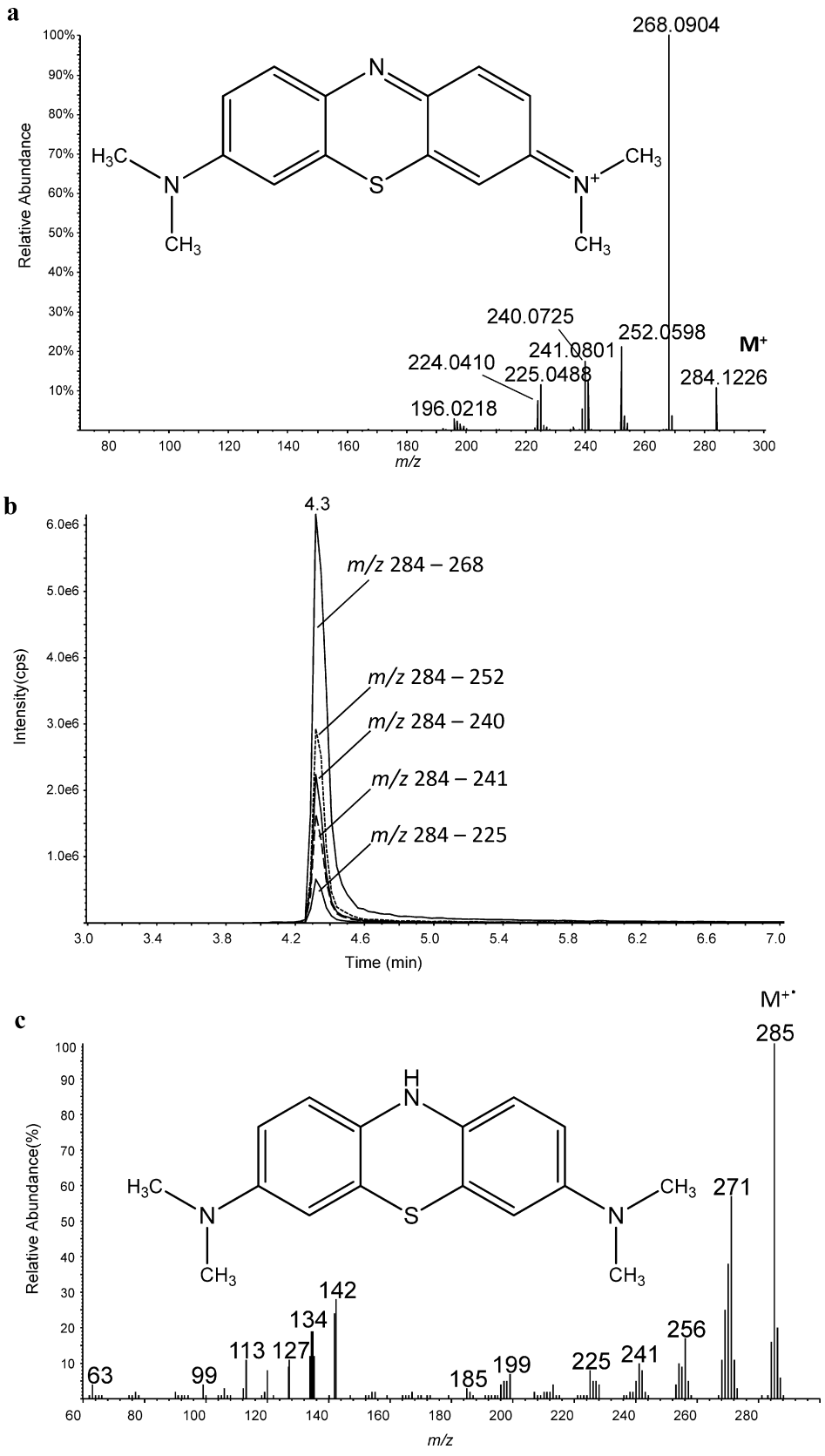

Fig. 3. ESI product ion mass spectrum of the charged molecule M^+ = 284 of methylene blue (a) and extracted ion chromatograms of a greenish urine specimen demonstrating the presence of methylene blue (b). In (c) the EI mass spectrum of methylene blue is shown resulting from a routine doping control analysis employing GC–MS.

Table 2
Routine screening results of the urine specimens 13969/2009–13972/2009 and 0154/2010–0157/2010 with regard to pH, specific gravity and selected steroid profile parameters.

Sample lab code	pH	Density (g/mL)	Steroidprofileparameters			
			T/EpiT	A/E	A/T	Adiol/Bdiol
13969/2009	5.8	1.018	0.440	0.897	337.172	0.489
13970/2009	5.8	1.018	0.418	0.865	341.788	0.514
13971/2009	5.7	1.018	0.426	0.902	346.017	0.501
13972/2009	5.8	1.018	0.421	0.893	344.188	0.497
0154/2010	7.0	1.018	0.501	1.039	272.388	0.642
0155/2010	7.0	1.018	0.531	1.040	254.788	0.638
0156/2010	7.0	1.018	0.610	1.045	240.268	0.639
0157/2010	7.0	1.018	0.586	1.044	245.711	0.650

Compared to the red coloration of urine, green specimens (Fig. 2a) are rarely received in doping control laboratories; however, one sample was obtained in 2008 and submitted to routine test methods. Since all analyses returned inconspicuous data, further measurements were conducted to reveal the origin of the unusual discoloration. In agreement with literature data [24], LC–MS/MS analysis (following a 1:10 dilution of a urine aliquot with deionized water) outlined the presence of methylene blue (Fig. 3a). The positively charged molecule was found at *m*/*z* 284.1227 (error: 3.9 ppm) that eliminates two methane molecules to yield the product ions at *m*/*z* 268.0905 (error: 0.8 ppm) and 252.0599 (error: 3.6 ppm). Moreover, the consecutive losses of N-methylenemethanamine (C_2H_5N, 43 Da) and methane from the precursor ion gave rise to *m*/*z* 241.0725 (error: 3.3 ppm) and *m*/*z* 225.0488 (error: 3.1 ppm), respectively, while the release of the radical composed of C_2H_6N (44 Da) produced the diagnostic ion at *m*/*z* 240.0725 (error: 3.9 ppm). By means of five characteristic ion transitions, the target compound was detected in a doping control specimen as depicted in Fig. 3b. In addition to the dedicated analysis of methylene blue by LC–MS/MS, also routine doping control screening procedures employing GC–MS allow the detection of this substance. In the absence of LC–MS(/MS), the presence of methylene blue was confirmed by the electron ionization mass spectrum (Fig. 3c) of an artifact (attributed to the reduced form of methylene blue) [39] in a doping control specimen in 1990, where the substance was supposedly originating from a drug referred to as "DeWitt's pill", which contained approximately 10 mg of methylene blue per capsule.

The multifaceted drug methylene blue was the first fully synthetic therapeutic agent, which was employed for the treatment of malaria as early as 1891 [40], before its utility in cases of inherited or acute methemoglobinemia as well as ifosamid-induced neurotoxicity was observed. Moreover, its application to virus inactivation in medicinal plasma products [41] or as bacteriostatic genitourinary antiseptic [42] is well established, and recent studies were conducted as to its therapeutic properties in Alzheimer's disease [40]. In addition, methylene blue's potential to act as monoamine oxidase A inhibitor was demonstrated [43]. Despite all of these properties, the use of methylene blue did not constitute a doping rule violation and the sample returned (except for a peculiar appearance) unremarkable doping control analytical data.

3.3. Case 4

Late 2009/early 2010 two sets of 4 in-competition doping control samples were analyzed following routine screening protocols, which include amongst others a comprehensive steroid profile and full scan GC–MS/NPD analysis. Common steroid profiling in sports drug testing considers various urinary concentration ratios, and a selected subset consisting of testosterone/epitestosterone (T/epiT), androsterone/etiocholanolone (A/E), androsterone/testosterone (A/T), and 5α-androstane-3α, 17β-diol/5β-androstane-3α,17β-diol (Adiol/Bdiol) is routinely applied due to their comparably high intra-individual stability [12]. In the present case, each batch of 4 specimens returned conspicuously similar steroid profiles (Table 2) although all samples were, according to the accompanying documents, provided by different individuals. The first set of samples (13969/2009–13972/2009) had a pH of 5.7–5.8, identical densities of 1.018 g/mL, and all steroid profile ratios deviated less than 5% from their average value, indicating a mutual donor for all 4 specimens. In a comparable manner, the second batch yielded data for pH (7.0), density (1.018 g/mL), and steroid profiles that suggested one rather than four individuals as the producer of the doping control samples. Moreover, the similar patterns of steroid profiles measured in both batches led to the assumption that all 8 specimens were provided by one person only. The supposition was verified by DNA-STR typing using 12 loci (Table 1), which unequivocally determined the identical origin of the 8 doping control specimens. Since the only common denominator of all supposedly tested athletes and locations of doping control sampling was the doping control officer (DCO), an investigation was conducted that eventually proved the DCO guilty of having filled all doping control containers with her own urine (her DNA was identical to those of the urine specimens), having forged documents and sent these samples as the athletes' doping control specimens to the laboratory. In consequence, the athletes were not sanctioned for the offense of urine substitution. This case represents another aspect of manipulation that must be considered in sports drug testing: was the athlete *de facto* visited by the DCO.

4. Conclusions

The question whether doping control urine samples are authentic or tampered is of particular importance to sports drug testing authorities. Numerous measures are taken to ensure the integrity of such specimens including visual inspection during sample delivery, use of sealed urine containers, documentation of the entire sampling process, etc. Nevertheless, manipulation has obviously occurred and doping control laboratories in particular are urged to follow-up suspicious results that might indicate attempted or successful tampering. As presented in this overview, urine discoloration is not necessarily an indication for manipulation while sophisticated methods are required to identify urine substitution by non-human liquids (e.g. beverages) or other person's urine.

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References

[1] World Anti-Doping Agency, Laboratory Statistics, 2006, http://www.dshs-koeln.de/y/biochemie/rubriken/07_info/WADA_Statistik_2006.pdf.

[2] World Anti-Doping Agency, Laboratory Statistics, 2007 (26.07.10) http://www.wada-ama.org/rtecontent/document/LABSTATS_2007.PDF.
[3] World Anti-Doping Agency, Laboratory Statistics, 2008 (02.15.08) http://www.wada-ama.org/en/dynamic.ch2?pageCategory.id=594.
[4] World Anti-Doping Agency, Laboratory Statistics, 2009 (26.05.11) http://www.wada-ama.org/Documents/Science_Medicine/Anti-Doping_Laboratories/Lab_Statistics/WADA_2009_LaboratoryStatisticsReport_Final.pdf.
[5] M. Thevis, H. Geyer, U. Mareck, G. Sigmund, J. Henke, L. Henke, W. Schänzer, Detection of manipulation in doping control urine sample collection: a multidisciplinary approach to determine identical urine samples, Anal. Bioanal. Chem. 388 (2007) 1539–1543.
[6] M. Kohler, A. Thomas, H. Geyer, L. Horta, W. Schänzer, M. Thevis, Detection of the protease Bacillolysin in doping-control urine samples, Drug Test. Anal. 1 (2009) 143–145.
[7] M. Thevis, J. Maurer, M. Kohler, H. Geyer, W. Schänzer, Proteases in doping control analysis, Int. J. Sports Med. 28 (2007) 545–549.
[8] A. Thomas, M. Kohler, K. Walpurgis, W. Schänzer, M. Thevis, Proteolysis and autolysis of proteases and the detection of degradation products in doping control, Drug Test. Anal. 1 (2009) 81–86.
[9] S. Lamon, N. Robinson, P.E. Sottas, H. Henry, M. Kamber, P. Mangin, M. Saugy, Possible origins of undetectable EPO in urine samples, Clin. Chim. Acta 385 (2007) 61–66.
[10] M. Donike, S. Rauth, U. Mareck-Engelke, H. Geyer, R. Nitschke, Evaluation of longitudinal studies: the determination of subject-based reference ranges of the testosterone/epitestosterone ratio, in: M. Donike, H. Geyer, A. Gotzmann, U. Mareck-Engelke, S. Rauth (Eds.), Recent Advances in Doping Analysis, Sport & Buch Strauss, Cologne, 1993, pp. 33–39.
[11] M. Donike, M. Ueki, Y. Kuroda, H. Geyer, E. Nolteernsting, S. Rauth, W. Schänzer, U. Schindler, E. Völker, M. Fujisaki, Detection of dihydrotestosterone (DHT) doping: alterations in the steroid profile and reference ranges for DHT and its 5 alpha-metabolites, J. Sports Med. Phys. Fitness 35 (1995) 235–250.
[12] U. Mareck, H. Geyer, G. Opfermann, M. Thevis, W. Schänzer, Factors influencing the steroid profile in doping control analysis, J. Mass Spectrom. 43 (2008) 877–891.
[13] P.E. Sottas, N. Baume, C. Saudan, C. Schweizer, M. Kamber, M. Saugy, Bayesian detection of abnormal values in longitudinal biomarkers with an application to T/E ratio, Biostatistics 8 (2007) 285–296.
[14] P.E. Sottas, C. Saudan, C. Schweizer, N. Baume, P. Mangin, M. Saugy, From population- to subject-based limits of T/E ratio to detect testosterone abuse in elite sports, Forensic Sci. Int. 174 (2008) 166–172.
[15] P.E. Sottas, M. Saugy, C. Saudan, Endogenous steroid profiling in the athlete biological passport, Endocrinol. Metab. Clin. North Am. 39 (2010) 59–73, viii–ix.
[16] P. Van Renterghem, P. Van Eenoo, H. Geyer, W. Schänzer, F.T. Delbeke, Reference ranges for urinary concentrations and ratios of endogenous steroids, which can be used as markers for steroid misuse, in a Caucasian population of athletes, Steroids 75 (2010) 154–163.
[17] P. Van Renterghem, P. Van Eenoo, W. Van Thuyne, H. Geyer, W. Schanzer, F.T. Delbeke, Validation of an extended method for the detection of the misuse of endogenous steroids in sports, including new hydroxylated metabolites, J. Chromatogr. B 876 (2008) 225–235.
[18] P. Van Renterghem, P. Van Eenoo, P.E. Sottas, M. Saugy, F. Delbeke, Subject-based steroid profiling and the determination of novel biomarkers for DHT and DHEA misuse in sports, Drug Test. Anal. 2 (2010) 582–588.
[19] M. Thevis, Mass Spectrometry in Sports Drug Testing – Characterization of Prohibited Substances and Doping Control Analytical Assays, Wiley, Hoboken, 2010.
[20] M.E. Leger, On the constitution of hordenine, Bull. Soc. Chim. Fr. 35 (1907) 868–872.
[21] J.D. Mann, C.E. Steinhart, S.H. Mudd, Alkaloids and plant metabolism – V. The distribution and formation of tyramine methylpherase during germination of barley, J. Biol. Chem. 238 (1963) 676–681.
[22] A.K. Singh, K. Granley, U. Misrha, K. Naeem, T. White, Y. Jiang, Screening and confirmation of drugs in urine: interference of hordenine with the immunoassays and thin layer chromatography methods, Forensic Sci. Int. 54 (1992) 9–22.
[23] World Anti-Doping Agency, The 2011 Prohibited List, 2011 (27.10.10) http://www.wada-ama.org/Documents/World_Anti-Doping_Program/WADP-Prohibited-list/To_be_effective/WADA_Prohibited_List_2011_EN.pdf.
[24] A. Musch, Farbveränderungen des Urins, Med. Monatsschr. Pharm. 28 (2005) 205–208.
[25] R.M. Buckle, Exertional (March) haemoglobinuria: reduction of haemolytic episodes by use of sorbo-rubber insoles in shoes, Lancet 1 (1965) 1136–1138.
[26] R.W. Deitrick, Intravascular haemolysis in the recreational runner, Br. J. Sports Med. 25 (1991) 183–187.
[27] R. Fleischer, Ueber eine neue Form von haemoglobinurie beim Menschen, Berl. Klin. Wochenschr. 47 (1881) 691–694.
[28] J.A. Streeton, Traumatic haemoglobinuria caused by karate exercises, Lancet 2 (1967) 191–192.
[29] S.A. Moeckel-Cole, P.M. Clarkson, Rhabdomyolysis in a collegiate football player, J. Strength Cond. Res. 23 (2009) 1055–1059.
[30] H.B. Schiff, E.T. MacSearraigh, J.C. Kallmeyer, Myoglobinuria, rhabdomyolysis and marathon running, Q. J. Med. 47 (1978) 463–472.
[31] R.A. Harmer, Occurrence, chemistry and application of betanin, Food Chem. 5 (1980) 81–90.
[32] K.M. Herbach, F.C. Stintzing, R. Carle, Identification of heat-induced degradation products from purified betanin, phyllocactin and hylocerenin by high-performance liquid chromatography/electrospray ionization mass spectrometry, Rapid Commun. Mass Spectrom. 19 (2005) 2603–2616.
[33] A.C. Allison, K.G. McWhirter, Two unifactorial characters for which man is polymorphic, Nature 178 (1956) 748–749.
[34] P.H. Saldanha, L.E. Magalhaes, W.A. Horta, Race differences in the ability to excrete beetroot pigment (betanin), Nature 187 (1960) 806.
[35] W.C. Watson, R.G. Luke, J.A. Inall, Beeturia: its incidence and a clue to its mechanism, Br. Med. J. 2 (1963) 971–973.
[36] R.M. Pearcy, S.C. Mitchell, R.L. Smith, Beetroot and red urine, Biochem. Soc. Trans. 20 (1991) 22S.
[37] A.R. Watts, M.S. Lennard, S.L. Mason, G.T. Tucker, H.F. Woods, Beeturia and the biological fate of beetroot pigments, Pharmacogenetics 3 (1993) 302–311.
[38] K.E. Lansley, P.G. Winyard, S.J. Bailey, A. Vanhatalo, D.P. Wilkerson, J.R. Blackwell, M. Gilchrist, N. Benjamin, A.M. Jones, Acute dietary nitrate supplementation improves cycling time trial performance, Med. Sci. Sports Exerc. 43 (2011) 1125–1131.
[39] H.H. Maurer, K. Pfleger, A.A. Weber, Mass Spectral Library of Drugs, Poisons, Pesticides, Pollutants and Their Metabolites, Wiley-VCH, 2007.
[40] R.H. Schirmer, H. Adler, M. Pickhardt, E. Mandelkow, "Lest we forget you – methylene blue…", Neurobiol. Aging, doi:10.1016/j.neurobiolaging.2010.12.012, in press.
[41] S.J. Wagner, Virus inactivation in blood components by photoactive phenothiazine dyes, Transfus. Med. Rev. 16 (2002) 61–66.
[42] J. Clifton, J.B. 2nd, Leikin, Methylene blue, Am. J. Ther. 10 (2003) 289–291.
[43] R.R. Ramsay, C. Dunford, P.K. Gillman, Methylene blue and serotonin toxicity: inhibition of monoamine oxidase A (MAO A) confirms a theoretical prediction, Br. J. Pharmacol. 152 (2007) 946–951.