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Oxidation of testosterone by permanganate and its implication in sports drug testing

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Running head: Oxidation of urinary testosterone by permanganate

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Abstract

Manipulations of urine sample by urine substitution, urine dilution, and urine adulteration with highly oxidative chemicals to escape detection in doping control analysis have been reported in the past. Adulteration of urine with oxidising chemicals such as potassium permanganate, cerium ammonium nitrate, pyridinium chlorochromate etc can lead to considerable changes in the endogenous steroidal profile parameters and thus mask the abnormality in steroidal profile following steroid abuse. In this study we have identified the formation of two stable oxidation products on reaction of potassium permanganate with testosterone, an important endogenous urinary steroid. Isolation and characterisation of these oxidation products were performed using chromatography and spectroscopy and the products were elucidated as 4α,5α-dihydroxytestosterone and 4*β*,5*β*-dihydroxytestosterone. Formation of these two molecules in human urine after adulteration with potassium permanganate has been demonstrated by liquid chromatography-mass spectrometry (LC-MS) analysis. The products 4α,5α-dihydroxytestosterone and 4*β*,5*β*-dihydroxytestosterone have not been previously reported in urine and hence has the potential to be included in routine drug testing program for monitoring possible testosterone abuse and permanganate adulteration of urine.

Keywords: sports drug testing, doping control, testosterone, dihydroxytestosterone, urine manipulation, permanganate

1. Introduction

Manipulations of urine sample by urine substitution, urine dilution, and urine adulteration with highly oxidative chemicals to escape detection in doping control analysis have been reported several times in the past[1-4]. Even though the World Anti-Doping Agency (WADA) has imposed stringent regulations on sample collection process, recent reports of suspected and substantiated manipulation outline the complexity and diversity of tampering options[1]. Access to unethical health professionals and other advisors has greatly increased the sophistication of both doping and masking strategies[5]. A battery of adulterants ranging from common household chemicals like chlorine bleach, liquid drain cleaner and chemicals e.g. potassium permanganate and ammonium cerium nitrate which are readily available from scientific laboratories, chemist shops, supermarkets and internet sites to commercial adulterants such as Urine luck (pyridinium chlorochromate), Stealth (peroxidase), Klear (potassium nitrite), Whizzies (sodium nitrite), and Instant Clean (gluteraldehyde) are being used to render false negative results during urinanalysis[6-8]. These adulterants act by either interfering with immunoassay procedures or by converting the target drugs to other compounds[8].

Due to the vital importance of the steroid profile in the fight against doping in sports, we investigated the influence of various oxidising chemicals on the steroid profile of human urine as part of an anti-doping research program (ADRP). We have reported that some popular oxidizing adulterants can selectively and significantly reduce the absolute concentrations of certain endogenous steroids in human urine and yet maintain steroid ratios within the acceptable population-based ranges[9,10]. The findings support the view that urine manipulation by oxidising chemicals can be an effective strategy to mask steroid abuse by reducing the elevated steroid levels back to the "normal" ranges. Among the various oxidants tested, potassium permanganate, potassium nitrite and ammonium cerium nitrate were found to exhibit selective reactivity against testosterone and epitestosterone. A significant decrease in the recoveries of testosterone and epitestosterone from urine was observed after the addition of these oxidising agents. It is known that urine adulteration with oxidising agents conceals the presence of the drugs and can also lead to formation of reaction products that are specific to the oxidising agent and drug[11-14]. In the present study we report isolation and characterisation of new oxidation products formed from the reaction of potassium permanganate with testosterone applying normal phase chromatography, nuclear magnetic resonance (NMR) and mass spectrometry (MS). A qualitative confirmation of the formation

of these products in human urine following permanganate exposure using liquid chromatography-mass spectrometry (LC-MS) studies has been demonstrated.

2. Experimental

2.1 General

Testosterone standard was obtained from the National Measurement Institute (North Ryde, NSW, Australia). Potassium permanganate, *β*-glucuronidase from *Helix pomatia* (Type H-3) was purchased from Sigma Aldrich (Sydney, NSW, Australia). Surine (synthetic urine devoid of human urinary steroids) was purchased from Ceriliant (Austin, TX, USA). Potassium bicarbonate, potassium carbonate, sodium acetate and glacial acetic acid were obtained from Univar (Ingleburn, NSW, Australia). All other reagents were of analytical grade and obtained from Lab Scan (Seacliff, SA, Australia).

2.2 Collection of urine

Blank urine samples were obtained from healthy donors (n=10) and pooled to obtain a representative biological urine matrix. All urine samples were stored at −20 °C before being used for the study. Ethics approval for this study was obtained from the UTS Human Research Ethics Committee (HREC), Ethics Approval No. UTS HREC 2010-268A.

2.3 Thin layer chromatography (TLC) and column chromatography

TLC was performed on normal phase silica gel 60 F254 precoated TLC plates with fluorescent indicator from Merck (Kilsyth, Victoria, Australia). The developing solvents were a mixture of n-hexane and ethyl acetate (4:1 v/v). The TLC was visualized under UV light and with the aid of methanolic sulphuric acid treatment and heating. Column chromatography was performed on in-house prepared glass columns packed with silica gel 60 (230-400 mesh), Merck (Kilsyth, Victoria, Australia). The eluting solvents used were mixtures of n-hexane and ethyl acetate at varying ratios.

2.4 Isolation of oxidation products from Surine

The oxidation reaction of permanganate (1M) was performed in surine (10 x1 mL) spiked with testosterone at 2 mg/mL. The reaction mixture was left at room temperature for 1 h and monitored by TLC for formation of oxidation products. A liquid–liquid extraction was subsequently performed with diethyl ether. The organic layer was recovered after extraction,

combined and dried down using a gentle stream of nitrogen gas at 30 °C. The concentrated residue (~7.0 mg) was subjected to column chromatography on silica gel (80 mg). The column was eluted with 20% ethyl acetate in n-hexane (500 mL), 30% ethyl acetate in n-hexane (500 mL), and finally 40% ethyl acetate in n-hexane (800 mL). Fractions were collected at every 20 mL and were monitored by TLC. Repeated column chromatography on pooled fractions resulted in the isolation of two oxidation products P1 (Rf 0.32) and P2 (Rf 0.34) which were later identified as 4*α*,5*α*-dihydroxytestosterone and 4*β*,5*β*-dihydroxytestosterone.

2.5 Detection of oxidation products in Urine

To qualitatively analyse the formation of reaction products in human urine, the oxidation reaction was repeated in pooled human blank urine. A standard urinary steroid screening procedure was adapted to analyse the formation of P1 and P2 in urine[15,16]. A range of concentrations of potassium permanganate was reacted with 1 mL of male and female urine for a time span of 2 h at room temperature. After the reaction, hydrolysis was carried out with *β*-glucuronidase from *H. Pomatia*. The hydrolysate was mixed with 1 mL of carbonate buffer (pH 9). Diethyl ether (5 mL) was added and the tube capped and extracted for ten minutes on a Ratek roller mixer (Boronia, VIC, Australia). The diethyl ether layer was then transferred into a screw cap glass tube and evaporated to dryness under nitrogen at 35 °C. The residue was dissolved in methanol then transferred to a 1.5-mL auto-sampler vial for LC-MS analysis.

2.6 NMR

Nuclear magnetic resonance (NMR) spectroscopy analyses were performed on an Agilent Technologies 500 MHz /54mm bore premium shielded NMR spectrometer coupled with a 7510 AS auto sampler. P1 and P2 were dissolved in deuterated methanol and chloroform respectively and spectra were recorded at room temperature. ^{1}H, ^{13}C, COSY, HSQC, HMBC and NOESY experiments were performed.

2.7 LC-MS

LC-MS analysis was performed on an Agilent 6490 Triple Quadrupole mass spectrometer with an ESI source (positive ion mode), interfaced with an Agilent 1290 LC system. Chromatographic separation was achieved on an Agilent Zorbax eclipse XDB-C18 column (2.1 mm x 50 mm, 1.8 µm), set at 40 ºC, by gradient elution of 0.1% formic acid in deionised

water (solvent A) and 95% acetonitrile in water (solvent B). A linear gradient was run at a flow rate of 0.5 mL/min. With initial solvent B composition at 2%, it was increased to 5% at 6 min, 30% at 12 min, 70% at 17 min and 95% at 19 min; held at 95% to 21 min, and decreased to 2% at 21.1 min and maintained at 2% for 4 min. Samples prepared were injected in 1 µL volume to obtain full scan, product ion scan and multiple reaction monitoring (MRM) spectra. A fragmentor voltage of 380V and collision energy of 25 eV (for product ion scan and MRM experiments) were applied. The scanning mass range was set at m/z 100-1000 (scan time = 500 ms). The sheath gas temperature and flow were set to 250 ^{o}C and 11 L/min, respectively. The capillary and nozzle voltages were 3000 V and 1500 V, respectively.

2.8 HRQToFMS

High resolution quadrupole Time-of- Flight mass spectrometry (HRQToFMS) experiments were carried out on an Agilent 6510 Accurate Mass QToF Mass Spectrometer, equipped with an electrospray ionization source (ESI) source operated in positive ion mode using the following operation parameters: Capillary voltage: 3500 V; nebulizer pressure: 40 psi; drying gas: 12.0 L/min; gas temperature: 350 °C; fragmentor voltage: 200 V; skimmer voltage: 60 V. HRQToFMS accurate mass spectra were recorded across the range from m/z 100 to 1000 at a scan rate of 1 scan/s. Mass resolving power was on average FWHM 23,500. The chromatographic conditions and column used were same as described above. The mass axis was calibrated using the mixture provided by the manufacturer over the m/z 50-3200 range. A second orthogonal sprayer with a reference solution was used as a continuous calibration using the following reference masses: m/z 121.0509 and 922.0098.

3. Results and discussion

The oxidation reaction of testosterone with potassium permanganate was performed in surine. The reaction mixture was chromatographed on silica gel to afford two oxidation products P1 and P2. The identification of these oxidation products was carried out based on spectroscopic evidence as detailed below. Even though these molecules have been proposed before[17], this is the first time to report a comprehensive determination of their structures.

P1 was obtained as a white amorphous powder. Its molecular formula was assigned as $C_{19}H_{30}O_4$ on the basis of HRTOFMS data ($[M+Na]^+$ at m/z 345.2047, calc. value 345.2041). Permanganate is a common *syn*-dihydroxylating agent reported to yield *cis* glycols (4*α*,5*α*-

and 4*β*,5*β*-diols) with D-4 steroids[18] and an increase in 34 Da in molecular weight was speculative of a dihydroxylated product. The position and configuration of the introduced hydroxyl groups were determined mainly based on changes in NMR spectra compared with the ones of the starting material and data in literature[19]. The ^{1}H NMR and ^{13}C NMR assignments of P1 are listed in Table 1. A singlet representing one proton was observed at *δ* 4.23 ppm (H-4) which was characteristic of a proton attached to an oxygen bearing carbon, which was further confirmed by HSQC spectrum. The downfield signal of *δ* 210.88 ppm could be assigned as a carbonyl carbon (C-3). The loss of conjugation for the carbonyl carbon (C-3) was evident from this downfield chemical shift (*δ* 210.88 ppm) compared to *δ* 199 ppm for testosterone. The carbon signals at *δ* 77.83 and 80.36 ppm could be assigned to the carbons bearing hydroxyl group at C-4 and C-5, which was further confirmed by the HMBC correlations between the signals of C-2 protons and C-4, as well as between the signals of C-19 methyl protons and C-5. The C-5 hydroxyl group exhibited γ-gauche interaction with C-7, C-1 and C-9 as evidenced from ^{13}C- NMR spectra[20]. As a result of γ-gauche substituent effect and subsequent shielding of carbon atoms due to the hydroxyl group in the axial position, upfield shifts were observed for these carbons. The presence of two steroidal methyl groups was confirmed by the methyl proton signals at *δ* 0.75 ppm (s) and *δ* 1.27 ppm (s) and the corresponding carbon signals at *δ* 10.28 and 14.6 respectively in the HSQC spectrum. COSY and HMBC correlations are shown in Fig 1 and NOESY correlations in Fig 2.

The absolute configuration of the hydroxyl groups was determined based on the NOESY spectrum. As reported previously, the hydroxylation across the olefinic double bond occurs via *syn* addition, which allows stable addition to the same side of the ring resulting in the formation of 4*α*,5*α*- and 4*β*,5*β*-diols [17,18,21,22]. In the 5*β*- configuration, a *cis* junction exists between rings A and B which results in a strong bending of the steroid nucleus along the line through atoms C (6) and C (10) and apparently in a non-steroid conformation in which ring A is tilted away from planar axis, resulting in a curved structural shape. Whereas in the 5*α*-configuration, a *trans* junction exists between rings A and B and the molecule exists in a more planar geometry[21, 23]. The NOESY spectrum of P1 (Fig 3) showed key correlation of angular methyl group at C-19 to H-2, H-4 and H-6[24]. In addition, the presence of cross peaks between H-2, H-4 and H-6 in the NOESY spectra established that rings A and B are planar. Thus P1 was elucidated as 4*α*,5*α*-dihydroxytestosterone (4*α*,5,17β-trihydroxy-5*α*-androstan-3-one).

P2, the less polar compound was obtained as a white powder, its molecular formula was deduced as $C_{19}H_{30}O_4$ according to the HRToFMS results which showed an adduct ion at m/z 345.2034 ($[M+Na]^+$,calc. value 345.2041). The ^1H and ^{13}C NMR spectral data of P2 were found to be similar to those of P1 as listed in Table 1. The NMR spectrum exhibited proton signals at δ 0.78 ppm and δ 1.06 ppm correlating to carbon signals at δ 11.14 ppm and δ 16.50 ppm in the HSQC spectrum attributable to two angular methyl groups. The observed chemical shift of the H-4 proton (s, δ 4.51 ppm) indicated its attachment to the oxygen bearing carbon[25]. The presence of hydroxyl bearing carbons and the keto carbonyl group was revealed by ^{13}C NMR signals at δ 74.14 ppm (C-4), δ 80.86 ppm (C-5) and δ 210.79 ppm (C-3). The axial hydroxyl group at C-5 position exerted γ-gauche effect on C-7 and C-9 as evidenced from ^{13}C NMR spectra. HMBC spectrum revealed the key correlations being, H-19 → C-5 and C-1; H-4→C-10, C-2 and C-6; H-1 → C-5 and C-3; H-17 → C-15. NOESY spectrum (Fig 4) showed that the signal of hydrogen at C-4 (H-4) was correlated to H-9 and H-7, indicative of a *cis* fused A/B ring system. Based on the aforementioned data P2 was identified as 4*β,*5*β*-dihydroxytestosterone (4β,5,17β-trihydroxy 5β-androstan-3-one), a diastereoisomer of P1.

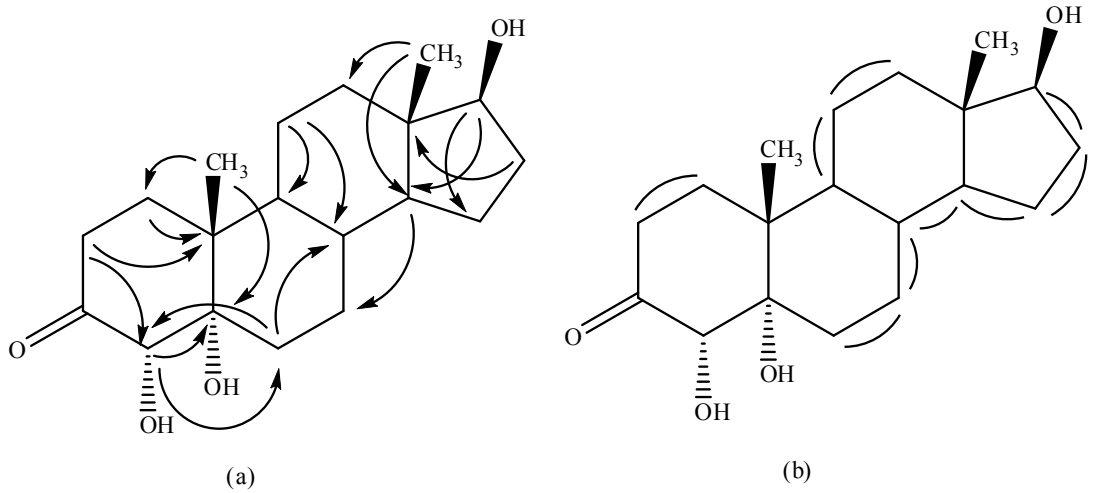

Figure 1. Key HMBC (a) and ^1H-^1H COSY(b) correlations of compound P1

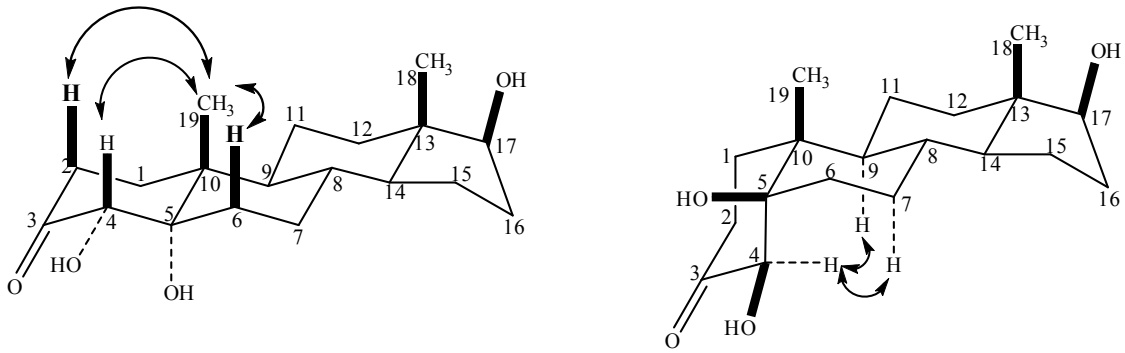

Figure 2. Key NOESY correlations of compound P1 and P2

	4*α*,5*α*-Dihydroxytestosterone (P1)		4*β*,5*β*-Dihydroxytestosterone (P2)	
	δ_C ppm	δ_H ppm	δ_C ppm	δ_H ppm
1	33.02	1.87, 1.74	31.67	1.89, 2.03
2	35.36	2.32, 2.58	34.91	2.38, 2.43
3	210.88		210.79	
4	77.83	4.23	74.14	4.51
5	80.36		80.86	
6	28.09	1.70, 1.83	30.90	1.60, 1.90
7	25.60	1.23, 1.51	27.96	1.10, 1.72
8	34.54	1.51	34.91	1.62
9	45.13	1.00	43.98	1.50
10	40.13		41.15	
11	20.40	1.42, 1.45	21.09	1.49, 1.58
12	36.69	1.07, 1.84	36.67	1.13, 1.90
13	42.74		43.01	
14	50.77	1.00	51.20	1.04
15	22.91	1.28, 1.60	23.35	1.32, 1.64
16	29.31	1.49, 1.98	30.59	1.47, 2.09
17	81.08	3.56	81.71	3.65
18	10.28	0.75	11.14	0.78
19	14.60	1.27	16.50	1.06

Table: 1 ^{1}H and ^{13}C NMR spectral assignment of 4*α*,5*α*-dihydroxytestosterone (P1) and 4*β*,5*β*-dihydroxytestosterone (P2)

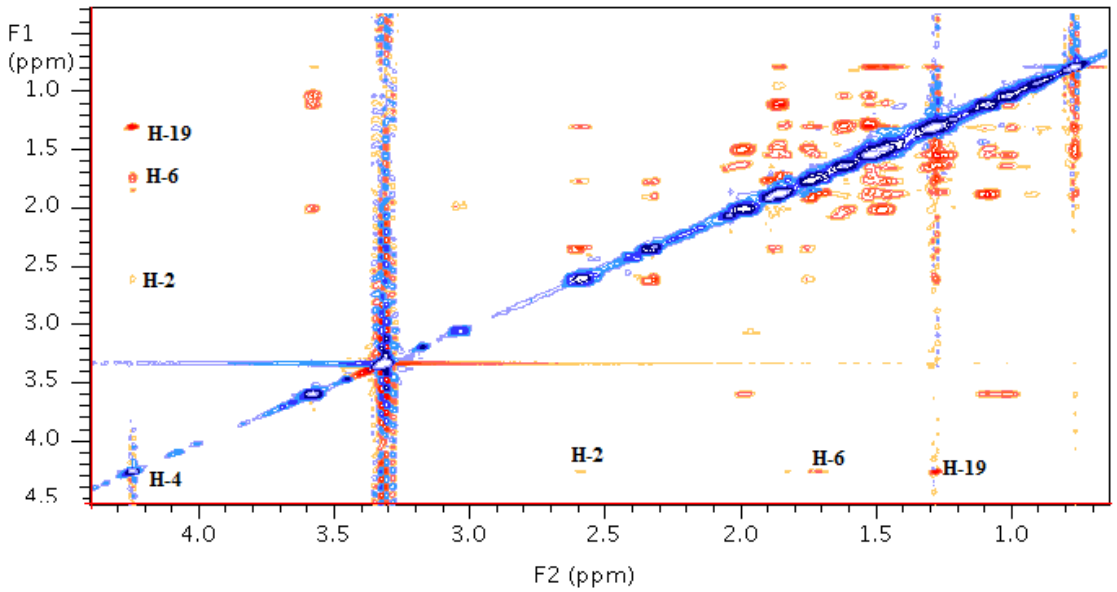

Figure 3. NOESY spectrum of 4*α*,5*α*-dihydroxytestosterone (P1)

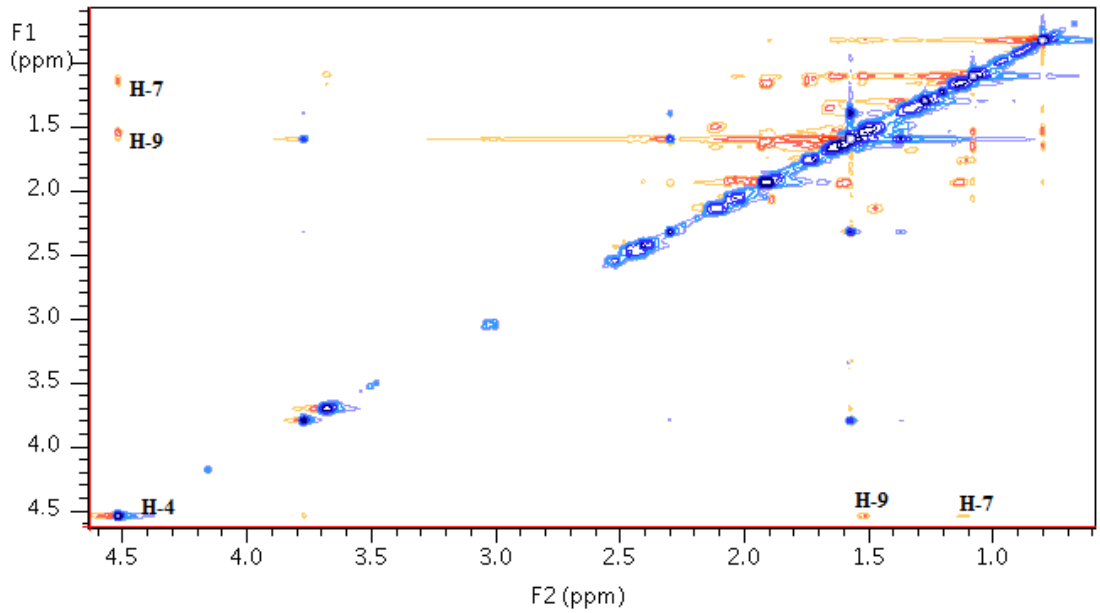

Figure 4. NOESY Spectrum of 4*β*,5*β*-dihydroxytestosterone (P2)

To demonstrate if the oxidation products P1 and P2 can be formed in real urine samples, pooled human urine samples from healthy individuals were adulterated with permanganate. Three concentrations of permanganate were investigated at 2, 5, 10 and 20 mM under both physiological and acidic pH conditions. These concentrations have been reported to deplete urinary testosterone concentration at physiological and acidic pH[9]. Following the oxidation reaction, the urine samples were treated with glucuronidase to hydrolyze steroid glucuronide conjugates and analysed by LC-MS in MRM mode. Three transitions were selected for both P1 and P2, i.e. m/z 323→305, m/z 323→287, m/z 323→269 to qualitatively determine their formation in urine (Fig 5). These transitions were based on product ions observed during collision-induced dissociation (CID) of the protonated molecules of P1 and P2. It was found that both P1 and P2 were detectable in urine samples adulterated with permanganate at concentrations 5, 10 and 20 mM. Urine pH did not appear to have any effect on the formation of P1 and P2. Figure 5 shows the representative total ion current (TIC) chromatogram of urine steroid extract following treatment of permanganate at 20 mM under physiological pH, illustrating the formation of both P1 and P2. The reaction mixture was further monitored over time and the products were detectable until 7 days after adulteration. Longer time windows were not monitored.

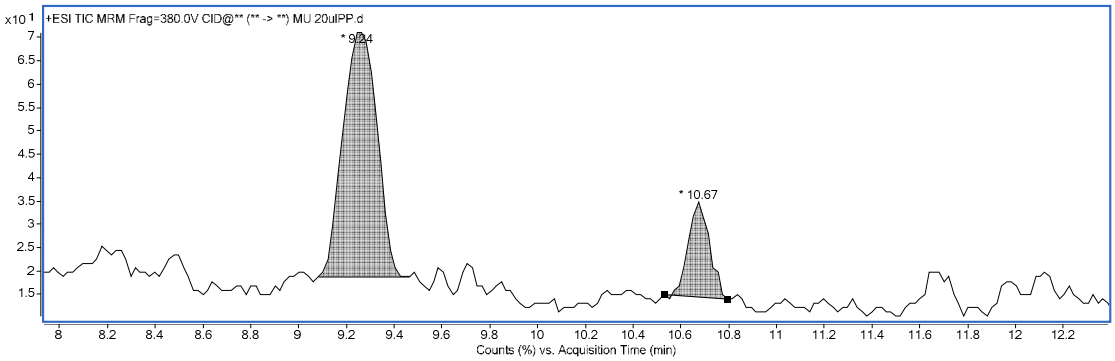

Figure 5. TIC chromatogram of hydrolysed urine extract following urine adulteration with 20 mM potassium permanganate under physiological urine pH illustrating the formation of P1 (Rt 10.67 min) and P2 (Rt 9.24 min)

There are no previous reports on these molecules in urine and dihydroxylation at olefinic double bond position is not a known route of metabolism for testosterone in human. Considering these factors and the long window of detection of these molecules as observed in LC-MS studies, 4*α*,5*α*-dihydroxytestosterone and 4*β*,5*β*-dihydroxytestosterone have the potential to be included as marker analytes in drug testing analysis to monitor adulteration with permanganate. Although permanganate is known to confer strong colouration to aqueous

solutions, which may raise suspicion of laboratory testing personnel on the validity of the testing samples, our observation was that this strong colouration (purple colour) was only noticeable when the permanganate concentration was exceeding 10 mM and this purple colour faded significantly on standing. In a routine drug testing setting, one would expect colour difference among urine specimens (from light yellow to dark brown) due to the natural variation of urine in the human population. This variation can be attributed to diet, as well as physiological and pathologic conditions[26, 27]. Urine adulteration by permanganate would not be readily recognisable just by observing urine colour, especially when permanganate concentration is not too high and when there is a delay from sample collection to sample analysis. Permanganate would be considered as an effective urine adulterant given its known and demonstrated oxidising capability. It has been previously reported that adulteration of urine with permanganate leads to absolute loss of both testosterone and epitestosterone in urine but insignificant change for the ratio of the two species[9]. This means that testosterone abuse could potentially go undetected if a drug cheat ingests both testosterone and the masking epitestosterone and then manipulates the collected urine samples with permanganate following the current WADA drug testing protocol[28]. Inclusion of the two oxidation products identified from this study as additional drug analytes in the routine test methods may be an effective approach to detect possible testosterone abuse and urine adulteration with permanganate.

4. Conclusion

The present study shows that potassium permanganate is capable of depleting testosterone in human urine, and at the same time leading to formation of two distinctive reaction products, namely, 4*α*,5*α*-dihydroxytestosterone and 4*β*,5*β*-dihydroxytestosterone. The formation of these molecules in real urine under both physiological and acidic conditions was observed. These oxidant-specific products have the potential to serve as unique markers for drug testing laboratories to develop methods that can detect steroid abuse and/or chemical manipulation involving permanganate by athletes. This will in effect widen the sensitivity of drug screening programmes and benefit the global effort in fighting for doping in sports.

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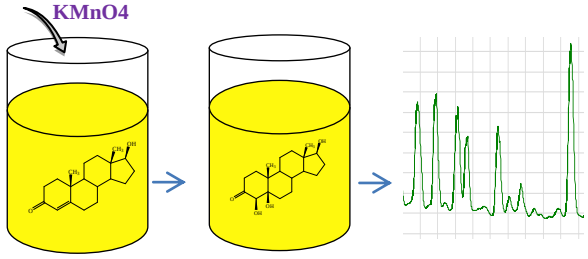
References

1. M. Thevis, H. Geyer, G. Sigmund and W. Schänzer, *Journal of Pharmaceutical and Biomedical Analysis*, 2012, **57**, 26-32.
2. M. Thevis, H. Geyer, U. Mareck, G. Sigmund, J. Henke, L. Henke and W. Schänzer, *Anal Bioanal Chem*, 2007, **388**, 1539-1543.
3. M. Thevis, M. Kohler and W. Schänzer, *Drug Discovery Today*, 2008, **13**, 59-66.
4. P.-E. Sottas, N. Robinson, O. Rabin and M. Saugy, *Clinical Chemistry*, 2011, **57**, 969-976.
5. T. Werner and C. Hatton, *J Chem Educ*, 2010, **88**, 34-40.
6. S. Mikkelsen and K. Ash, *Clin Chem*, 1988, **34**, 2333-2336.
7. B. D. Paul, *Journal of Analytical Toxicology*, 2004, **28**, 599-608.
8. A. H. B. Wu, B. Bristol, K. Sexton, G. Cassella-McLane, V. Holtman and D. W. Hill, *Clinical Chemistry*, 1999, **45**, 1051-1057.
9. U. Kuzhiumparambil and S. Fu, *Steroids*, 2013, **78**, 288-296.
10. U. Kuzhiumparambil and S. Fu, *Analytical Methods*, 2013, **5**, 4402-4408.
11. S. Luong and S. Fu, *Drug Testing and Analysis*, 2014, **6**, 277-287.
12. A. Q. N. Pham, T. Kelly and S. Fu, *Analytical Methods*, 2013, **5**, 3948-3955.
13. U. Mareck, H. Geyer, G. Opfermann, M. Thevis and W. Schänzer, *Journal of Mass Spectrometry*, 2008, **43**, 877-891.
14. S. Luong, A. T. Ung, J. Kalman and S. Fu, *Rapid Communications in Mass Spectrometry*, 2014, **28**, 1609-1620.
15. P. Van Renterghem, P. Van Eenoo, H. Geyer, W. Schänzer and F. T. Delbeke, *Steroids*, 2010, **75**, 154-163.
16. R. Ahmadkhaniha, A. Shafiee, N. Rastkari, M. R. Khoshayand and F. Kobarfard, *Journal of Chromatography B*, 2010, **878**, 845-852.
17. T. Kubota, K. Yoshida and F. Watanabe, *Chemical and Pharmaceutical Bulletin*, 1966, **14**, 1426-1430.
18. J. A. R. Salvador, S. M. Silvestre and V. M. Moreira, *Current Organic Chemistry*, 2008, **12**, 492-522.
19. K. Hayamizu and O. Kamo, *Magnetic Resonance in Chemistry*, 1990, **28**, 250-256.
20. H. Eggert, C. L. VanAntwerp, N. S. Bhacca and C. Djerassi, *The Journal of Organic Chemistry*, 1976, **41**, 71-78.
21. J. T. Edward and J. M. Ferland, *Canadian Journal of Chemistry*, 1966, **44**, 1317-1322.
22. S. Hrycko and P. Morand, *Journal of the Chemical Society, Perkin Transactions 1*, 1990, 2899-2904.
23. A. W. Roszak and P. W. Codding, *Acta Crystallographica Section C*, 1990, **46**, 1700-1704.
24. H. L. Holland, W. Xu and D. W. Hughes, *Journal of the Chemical Society, Chemical Communications*, 1989, 1760-1761.
25. S. Deng, S.-N. Chen, P. Yao, D. Nikolic, R. B. van Breemen, J. L. Bolton, H. H. S. Fong, N. R. Farnsworth and G. F. Pauli, *Journal of Natural Products*, 2006, **69**, 536-541.
26. J.T. Cody and S. Valtier, *Journal of Analytical Toxicology.* 2001**, 25**, 466-470.
27. A.H.B.Wu, *Journal of Clinical Ligand Assay.* 2003, **26**, 11-18.
28. World Anti-doping Agency, http://www.wada-ama.org, accessed 17th July 2014.



Adulteration of urine with permanganate can lead to depletion of testosterone and formation of new reaction products (4α,5α- and 4*β*,5*β*-dihydroxytestosterone)