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W. Schänzer
H. Geyer
A. Gotzmann
U. Mareck
(Editors)

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S. Jain, A. Beotra, T. Kaur:
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S. Jain, A. Beotra and T. Kaur

A Case Study: Detection of 1-Testosterone in Urine by GC-MSD

Dope Control Centre, Sports Authority of India, JLN Stadium, New Delhi, India, 110003

INTRODUCTION

This paper presents the results of incidental detection of a sample tested positive for 1-Testosterone for the first time in Dope Control Centre (DCC), New Delhi. This was just after successful identification of 1-Testosterone in proficiency testing round of World Association of Anti-Doping Scientists (WAADS). This sample showed abnormal steroid profile during screening procedure, when the sample was injected in scan mode, it came out to be suspicious for 1-Testosterone. 1-Testosterone 17-hydroxy- 5a-androst-1-en-3-one is a new steroid in the market.

MATERIAL AND METHODS

25 Urine samples of athletes were received from Athletic Federation of India.

Sample extraction and derivatization

The urine samples were prepared and analyzed according to the standard operating procedures for anabolic steroids.

The sample preparation procedure involved solid liquid extraction using XAD2 resin and methanol, enzymatic hydrolysis with β-Glucouronidase (E. Coli), extraction with diethyl ether, the dry residue was derivatized with 50 µl of MSTFA/TMSI/Dithioerythritol (1000:2:2:v/v/v) and heated for 20 min at 60 C. 2 µl injected in Gas Chromatography Mass Spectrometer Detector (GC-MSD). 17-methyl testosterone was used as an internal standard to calculate the relative retention time (RRT). For confirmation of 1-Testosterone, WAADS sample of 1-Testosterone was considered as validated positive control sample, as DCC was not having certified reference standard for 1-Testosterone (in compliance with clause 5.4.4.2.1 of World Anti Doping Agency (WADA) International Standards for Laboratories).

Instrument Conditions

GC-MS: HP 6890-HP 5973 (Agilent Technologies), **Column**:HP Ultra I, 17m, 0.2 mm i.d., 0.11um film thickness, **Carrier gas**:helium 12psi, **split** 1:9, **Injector Temperature**:280 ^{o}C, **Temperature program**: 180^{o}C -3 ^{o}C/min to 229^{o}C, 229^{o}C –40^{o}C/min. to 300 ^{o}C, hold for 4 min. , **Ionization mode**:EI, 70 eV, **Column head pressure**: 110 kPa

RESULTS & DISCUSSION

Out of 25 samples one sample (female athlete) showed abnormal values as mentioned below. When the sample was injected in scan mode, it came out to be suspicious for 1-Testosterone. It was found that 1-Testosterone and its metabolites were interfering with steroid profile. (Table-1: Steroid Profile). Steroid profile of this sample showed higher values of androsterone, testosterone, epitestosterone and DHEA. The peak of 1-Androsterone was interfering with etiocholanolone because it started at the tail of etiocholanolone. It was also interfering with 5α-Androstan-3α, 17β-diol and 5β-Androstan-3α, 17β-diol peaks. Peak of 1-Testosterone was interfering with peak of epiandrosterone whereas peak of 1-androsterone eluted at the same time of Dehydroepiandrosterone (DHEA).

1-Testosterone: Total ion chromatograph of suspicious sample is shown below in fig. –1. Which shows various peaks. The peak of 1-Testosterone (parent) was detected at RRT 0.816.Peaks detected at RRT 0.748, 0.771 and 0.792 were probably 1- Androsterone, 1-Androstenediol, 1-Androstenedione respectively as best three major metabolites in high abundance(only assumptions) because no reference substances were available. Based on the mass spectrum of peak 6, 8 and 3 that were similar to mass spectrum of peak 2, 4 and 7 respectively. It is assumed that mass spectrum of peak 6, 8 and 3 may be 1-Etiocholonalone (Peak-6), some isomer of 1-Androstenediol (Peak-8) and 1-Epitestosterone (Peak-3). Zang Yinong et al. has also reported the presence of some exogenous metabolites of 1-Testosterone including 1-Testosterone,Androst-1-ene-3, 17-dione and another unidentified metabolites. In the present case, 7 metabolites (assumptions) were observed. Mass spectra of 1-Testosterone and its three metabolites in this sample were comparable with mass spectra of 1-Testosterone and its metabolites in validated urine sample received from WAADS. Hence this sample was reported with adverse analytical finding showing positive for 1-Testosterone.

CONCLUSION

On the basis of the above findings it was concluded that the sample was reported as adverse analytical finding for 1-Testosterone. No request was received for B-sample analysis.

REFERENCES

1. Counsel etal, Anabolic Agents, Derivatives of 5 alpha- androst-1-ene, J. Org. Chem., 27(1962), 248-251.
2. Zhang Yinong, Liu Xin, Wu Moutian, Wang Jingzhu, Zang Huyue: Analytical Data of 1-Testosterone and the preliminary Results of Excretion Study with 1- Testosterone in: W. Schanzer, H. Geyer, A. Gotzmann, U. Mareck-Engelke (eds.) Recent advances in doping analysis Primary (12). Sport und Buch Strauβ, Koln (2004) 81-90

Table-1: Steroid profile of the sample suspicious for 1-Testosterone

MultiVu - [C:\MSDCHEM\1\DATA\M52207A4\027.D\report.txt]

File Edit Search Window

Quantitation Report (QT Reviewed)

Data File : C:\MSDCHEM\1\DATA\M52207A4\027.D Vial: 22
Acq On : 23 Jul 2004 4:17 Operator: S JAIN
Sample : 6043 Inst : MSD-5
Misc : Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: STEROID.E
Quant Time: Jul 23 04:20:05 2004 Quant Results File: ASSIM.RES

Quant Method : C:\MSDCHEM\1\METHODS\ASSIM.M (Chemstation Integrator)
Title : Steroid
Last Update : Mon Jul 26 18:51:23 2004
Response via : Initial Calibration
DataAcq Meth : ASSIM

Internal Standards	R.T.	QIon	Response	Conc	Units	Dev(Min)
1) ISTD	15.95	446	11453797	500.00	ng/ml	0.07
Target Compounds						Qvalue
2) Androsterone	11.56	434	250648661m	40627.89	ng/ml	
3) Etiocholanolone	11.79	434	59964105m	9854.89	ng/ml	
4) Testosterone	14.15	432	3047089m	818.45	ng/ml	
5) Epitestosterone	13.30	432	3923987m	425.65	ng/ml	
6) 11-OH-Andro.	14.50	522	7827320m	911.31	ng/ml	
7) 11-OH-Etio	14.74	522	872459m	144.18	ng/ml	
8) DHT	13.53	434	131157m	1.77	ng/ml	
9) 5a-3a-diol	11.95	241	1101506m	251.95	ng/ml	
10) 5b-3a-diol	12.00	241	687712m	703.89	ng/ml	
11) 5a, 3b-diol	13.16	421	152217	59.08	ng/ml	100
12) Epiandrosterone	12.98	434	0	N.D.		
13) DHEA	12.64	432	774399m	1115.80	ng/ml	
14) Androstenedion e	13.81	430	382815m	5.13	ng/ml	
15) 6-OH-androstenedione	17.18	518	617410m	219.54	ng/ml	

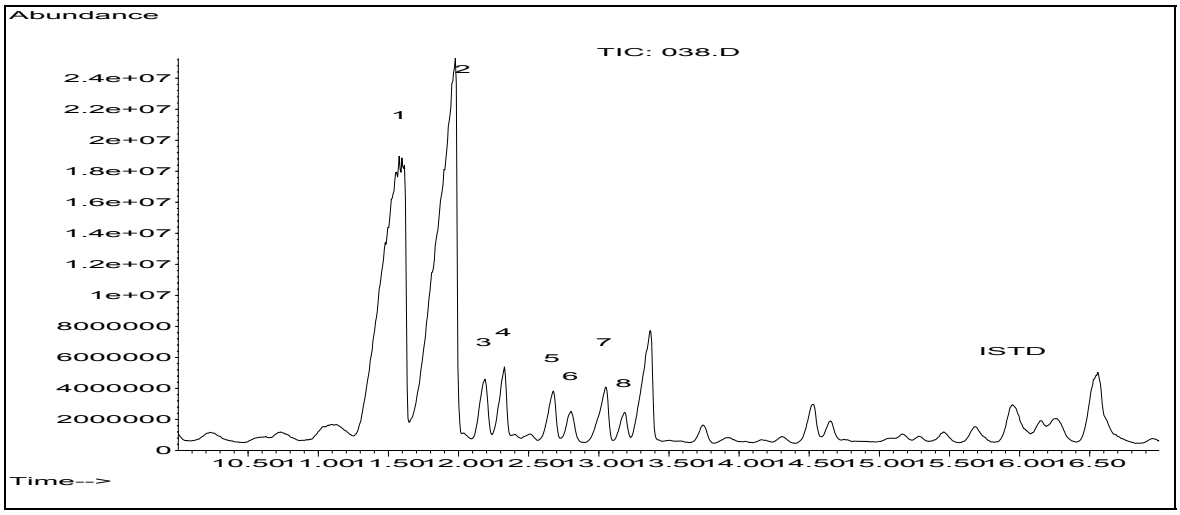

Fig. –1: Peak-1 (Androsterone), 2(1-Androsterone), 3(1-Epitestosterone?), 4(1-Androstenediol), 5(1-Androstenedione), 6(Unknown), 7(1-Testosterone -Parent), 8(unknown) and ISTD)

Mass spectrums of various peaks are shown below: (fig- 2 to fig. 5).

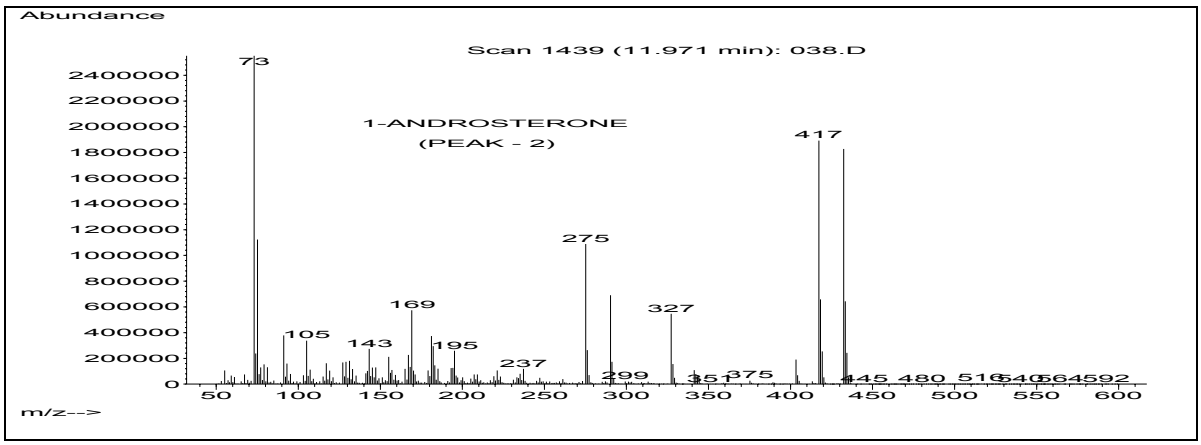

Fig. 2: Mass Spectrum of 1-Androsterone-Bis-TMS

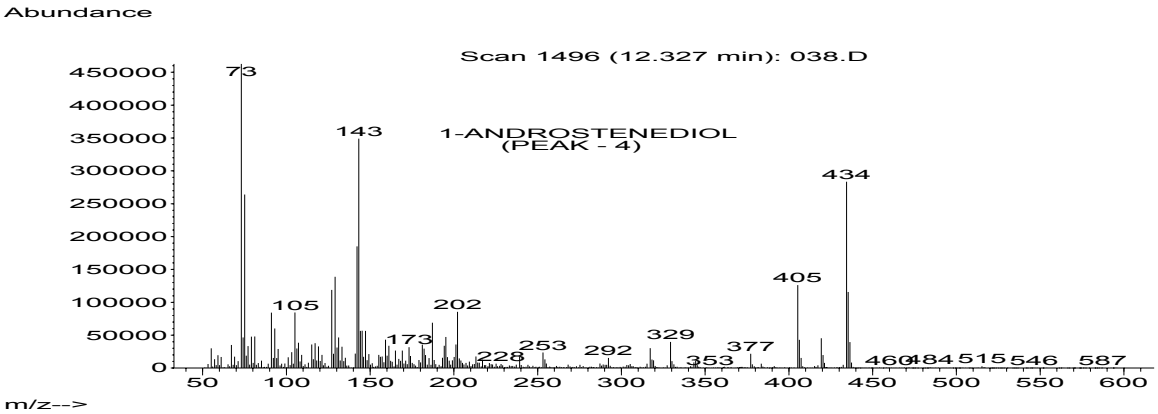

Fig. 3: Mass Spectrum of 1-Androstenediol-Bis-TMS

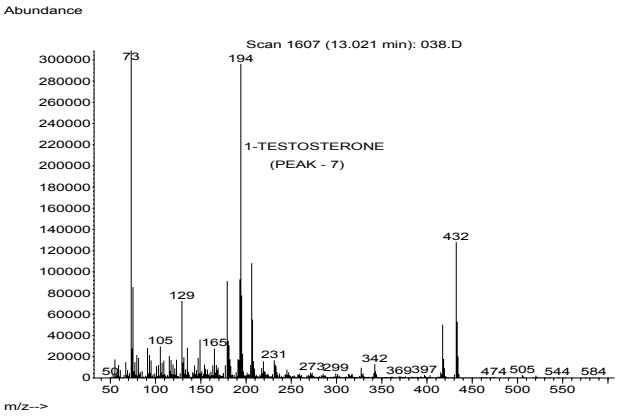

Fig. 4:Mass Spectrum of 1-Testosterone-Bis-TMS (Parent)

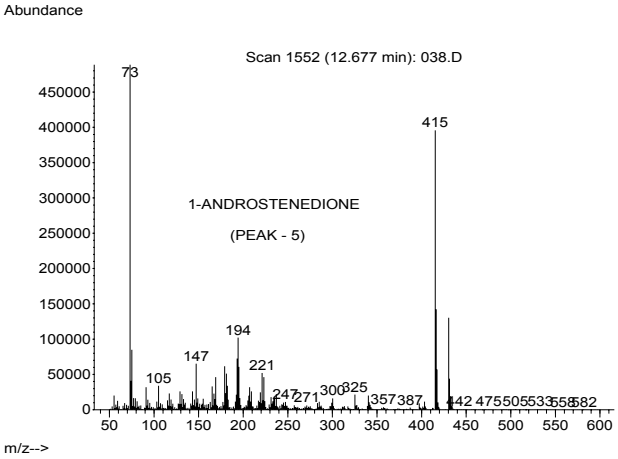

Fig. 5: Mass Spectrum of 1-Androstenedione- Bis-TMS