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RECENT ADVANCES IN DOPING ANALYSIS (7)

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Criteria for the detection of *Androstenedione* oral administration

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Introduction

4-Androstene-3,17-dione can be *legally* purchased in the USA for oral self-administration. Although its importation is illegal in many countries, it was found to be easily available particularly by the Internet. It grew in popularity after an American baseball star disclosed his use of androstenedione and creatine in his race to home runs record. Androstenedione is advertised as being a *legal* steroid and a precursor of testosterone that was developed for athletes by the former East German scientists. As a testosterone related substance, androstenedione has always been a prohibited substance. Its availability for oral self-administration prompted the IOC and the International Sport Federations to list by name androstenedione as an anabolic agent in 1998 (1). Androstenedione is known to be a biosynthetic precursor of testosterone and many scientific publications described in the last thirty years its in vivo and in vitro metabolism (2).

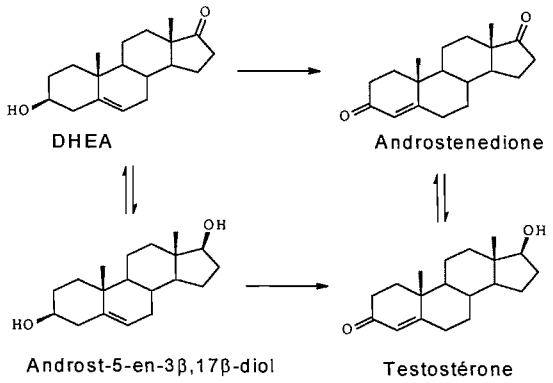

Figure 1: Testosterone and its commercially available precursors.

The in vivo metabolites reported were androsterone, 6β-hydroxyandrostenedione, 16α- and 16β-hydroxyandrostenedione, testosterone, androstandione and in vitro, 5α-androstandione,

5α-androstan-3α,17β-diol, androsterone, epiandrosterone, testosterone, epitestosterone, androstenedione. The conversion to estrogens was also studied. In March 1998, three groups reported during the Cologne Workshop the results of their investigations on 4-androsten-3,17-dione oral administration (3). They described increased excretion of androsterone and etiocholanolone, dihydrotestosterone, androstenedione and alteration of the T/E values that went up or down following the increased excretion of testosterone or epitestosterone in some male subjects. Uralets et al. also reported the presence of an hydroxyandrostenedione metabolite.

This paper presents some criteria to detect the oral administration of androstenedione by GC/MSD.

Experimental

"Androstenedione complex" (100 mg/capsule of androstenedione) was purchased with an authorisation from Health Canada (8572.090.98) from Price's Power International (International Nutrition and Export, Newport News, Virginia 23608, USA). One capsule was administered to three males (23, 24 and 33 year old) and one female (43 years old) volunteers after verification of the content by GC/MS analysis. Urine samples were collected 24 hours before and up to 48 hours after the administration. The urine samples were prepared and analysed according to anabolic agents procedure. The glucuronide and sulfate fractions were analysed separately (4). Authentic standards were purchased from Steraloids Inc. (Wilton, NH 03086).

Results

Alteration of the steroid profiles

The T/E values and the concentration of androsterone, etiocholanolone, testosterone and epitestosterone were determined and were compared to the "reference ranges" of the male and female athletes' populations that are presented in table 1. The results obtained are presented in figures 2 to 5. The urinary T/E values and testosterone concentration increased rapidly following the administration of androstenedione for two males and in the female volunteers but remained unchanged for a fourth male volunteer. Some of the T/E values measured exceeded the threshold of 6:1 and the urinary concentration of testosterone (corrected for a specific gravity of 1.020) was also elevated when compared to the norm of the reference populations in two of the volunteers.

Table 1 : Normal ranges of testosterone, epitestosterone, androsterone, etiocholanolone[1], androsterone/etiocholanolone (A/Etio) and T/E values	
Male athletes	Female athletes
Cologne, 1996 (97.5%) n=5000 (5) Testosterone: 137.4 ng/mL Epitestosterone: 112 ng/mL T/E: 5.19 Androsterone 6703 ng/mL Etiocholanolone: 5294 ng/mL A/Etio lower : 0.55 higher: 2.87	Cologne, 1996 (97.5%) n=1700 Testosterone: 57.3 ng/mL Epitestosterone: 42.2 ng/mL T/E: 6.31 Androsterone: 6439 ng/mL Etiocholanolone: 6107 ng/mL A/Etio lower : 0.42 higher : 2.15
Montréal, 1999 (97.5%) Testosterone :106 ng/mL (n = 9500) Epitestosterone : 94 ng/mL T/E: 4.2 (n = 11000) Androsterone: 6689 ng/mL (n = 9500) Etiocholanolone : 4716 ng/mL A/Etio lower: 0.59 higher: 3.39	Montréal, 1999 (97.5%) Testosterone : 26.4 ng/mL (n = 3740) Epitestosterone: 31.3 ng/mL (n = 4000) T/E: 3.2 (n= 4667) Androsterone: 5170 ng/mL (n = 4200) Etiocholanolone: 4938 ng/mL A/Etio lower: 0.39 higher: 2.32

[1] Concentrations corrected to specific gravity of 1.020

The only parameter of the steroid profile that was altered for all the volunteers was the increased concentration of androsterone and/or etiocholanolone.

Characteristic metabolites

Several metabolites found in the glucuro- and/or sulfoconjugated fractions, appear in the urine samples provided after a single oral dose of androstenedione. The structures were proposed after studying the mass spectrum of the TMS-ether and TMS-enol, TMS-ether derivatives and the reference standards were synthesised chemically or using enzymatic reactions or purchased. All the metabolites identified will be reported elsewhere. The mass spectra of two diagnostic metabolites are presented in figures 6 and 7. The first one, 6α-hydroxyandrostenedione (6α-hydroxy-4-androsten-3,17-dione) is found in the glucuroconjugated fraction during 9 to 11 hours following the administration and the authentic standard is commercially available. Since last

year, we have incorporated the ions at m/z 518 and 319 in the screening method for anabolic agents and found it to be absent from males and females samples and it was not formed in samples that showed signs of bacterial degradation. Three isomers, 6β-hydroxyandrosterone (m/z 522, 517, 417, 327, 169), 6β-hydroxyetiocholanolone (m/z 522, 517, 417, 377, 327, 169) and 6β-hydroxyepiandrosterone (figure 7) that possess characteristic mass spectra were isolated for the first two in the glucuro- and sulfoconjugated fractions, the latter being exclusively sulfoconjugated. The structures were proposed after comparison of the spectrum of available or synthesised standards of 6α- and 6β-hydroxyetiocholanolone and 6α-hydroxyepiandrosterone. The synthesis of the other isomers is currently pursued. The 6β-hydroxyandrosterone and etiocholanolone are found up to 13 hours and 6β-hydroxyepiandrosterone is persistent and present up to 24 hours after a single oral dose.

Conclusion

The oral administration of androstenedione can result in an increased T/E value and testosterone excretion, but not systematically. The urinary concentration of androsterone and/or etiocholanolone is increased to abnormal levels and the presence of the characteristic metabolites 6α-hydroxyandrostenedione, 6β-hydroxyandrosterone, 6β-hydroxyetiocholanolone, 6β-hydroxyepiandrosterone is also diagnostic. The self-administration of androstenedione may involve much higher dosages and reports are made that the athletes use up to 500 mg per day. In a specimen analysed in the laboratory and for which the athlete had declared the use of androstenedione on the doping control form, we have found very high amounts of androsterone, etiocholanolone, testosterone and epitestosterone (134,000 ng/mL, 60,000 ng/mL, 1200 ng/mL and 207 ng/mL respectively). The characteristic metabolites, 6α-hydroxyandrostenedione, 6β-hydroxyandrosterone, 6β-hydroxyetiocholanolone, 6β-hydroxyepiandrosterone were also found along with free and glucuroconjugated androstenedione and androstandiones that were found in low amount following a single dose.

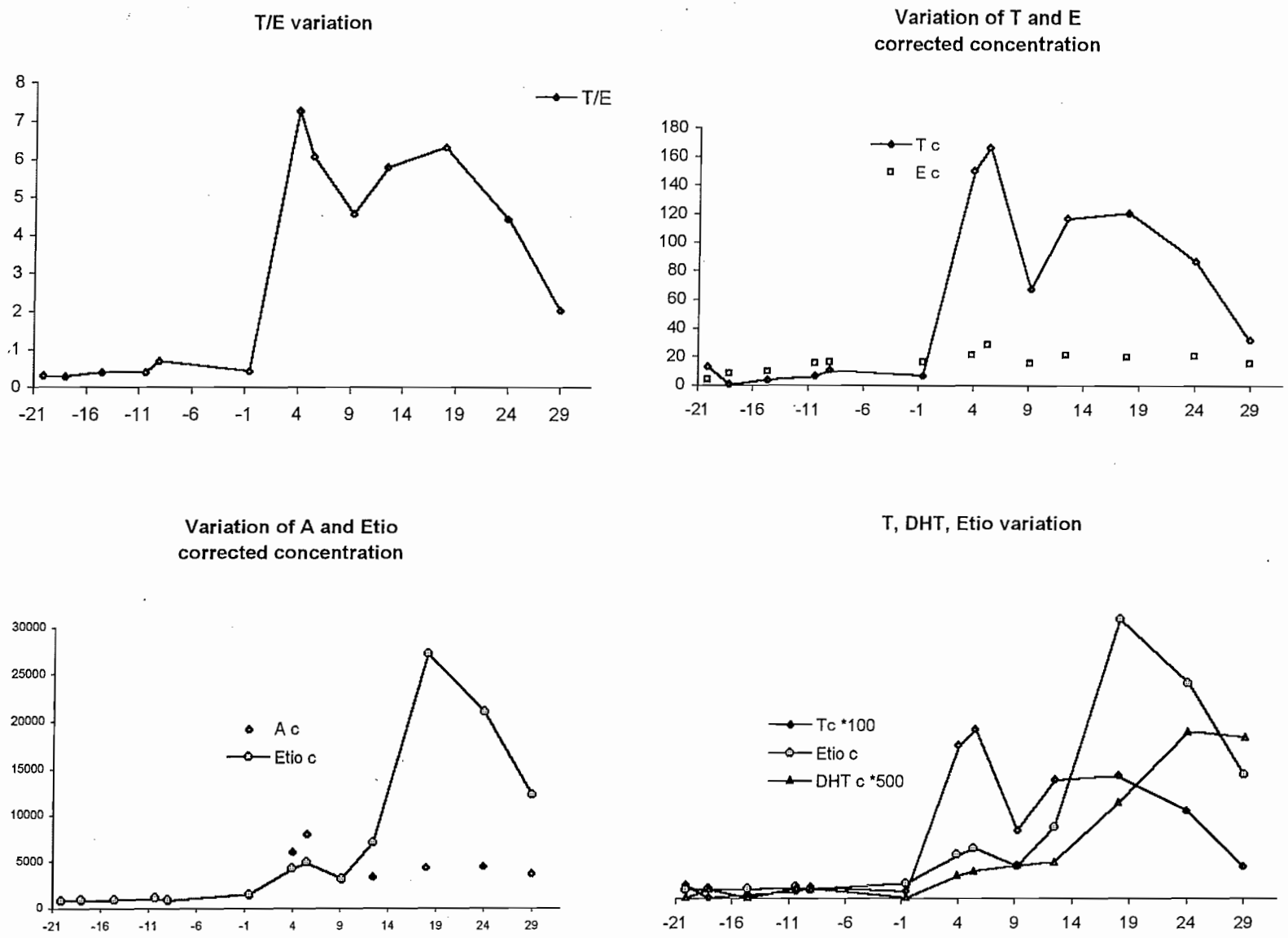

Figure 2 : Alteration of the steroid profile following the administration of androstenedione (100 mg) – female volunteer

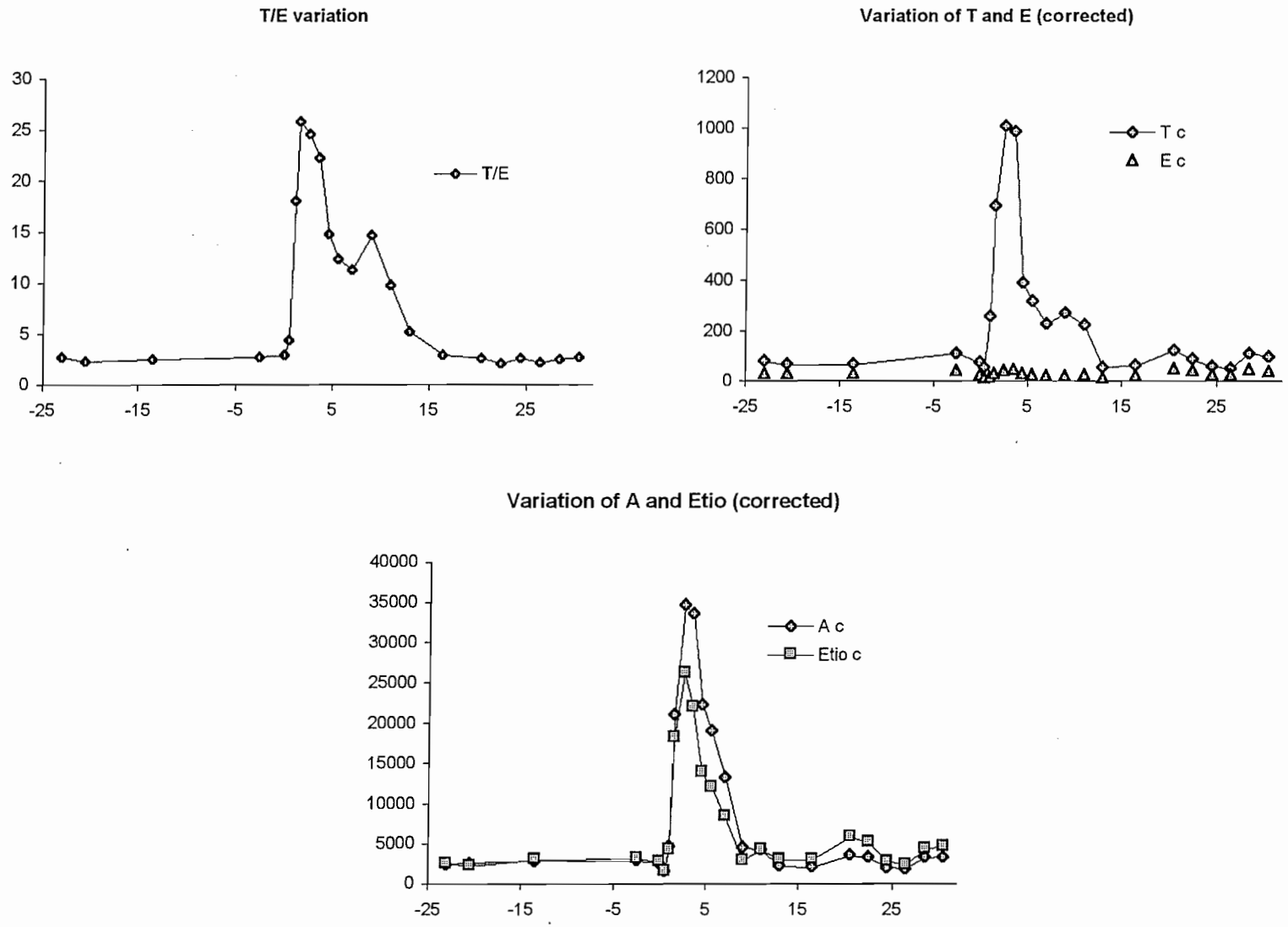

Figure 3 : Alteration of the steroid profile following the administration of androstenedione (50 mg, authentic standard) – Male volunteer 1

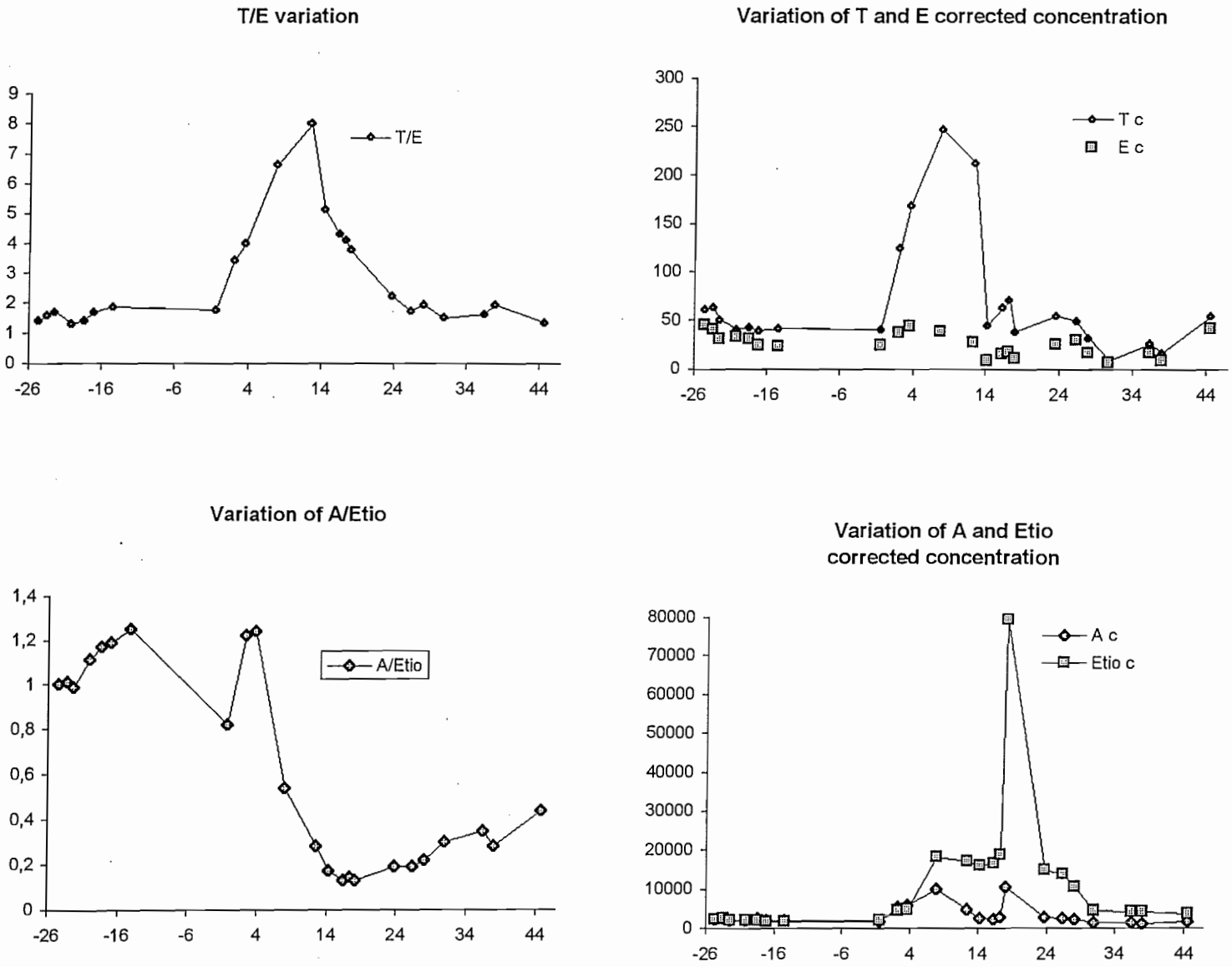

Figure 4 : Alteration of the steroid profile following the administration of androstenedione (100 mg) – Male volunteer 2

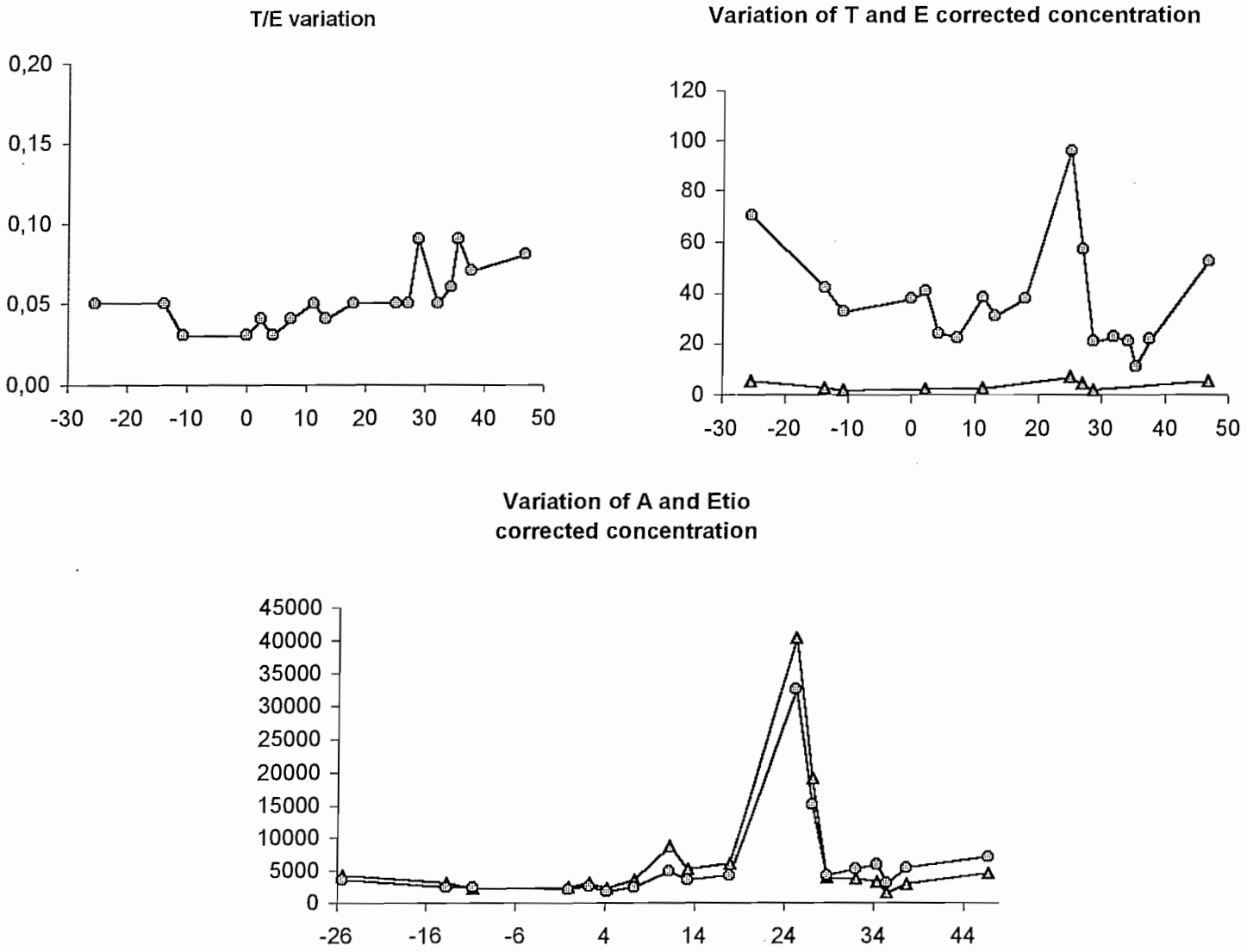

Figure 5 : Alteration of the steroid profile following the administration of androstenedione (100 mg) – Male volunteer 3

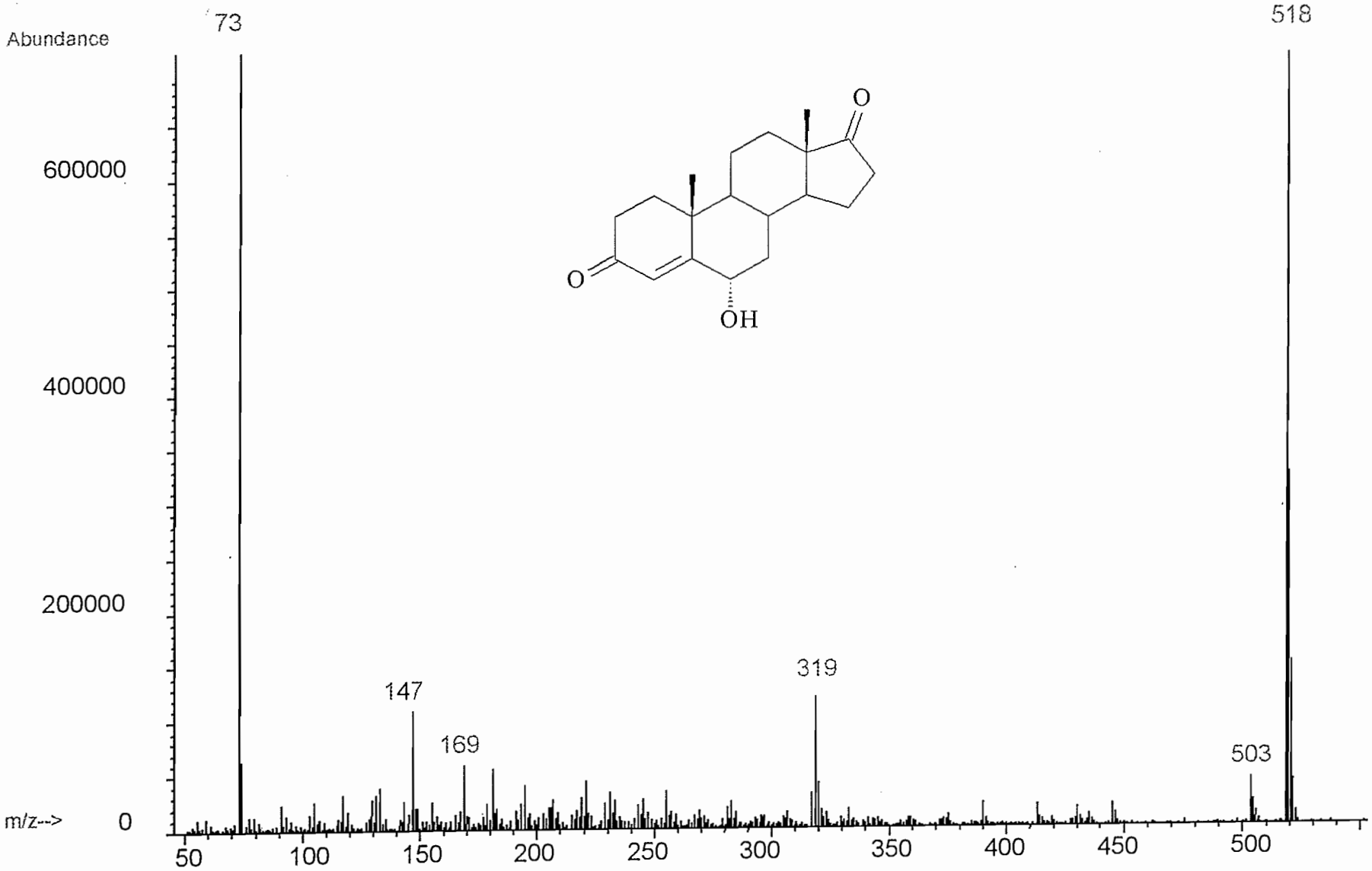

Figure 6 : Mass spectrum of the TMS-derivative of **6α-hydroxyandrostenedione** (glucuroconjugated, authentic standard)

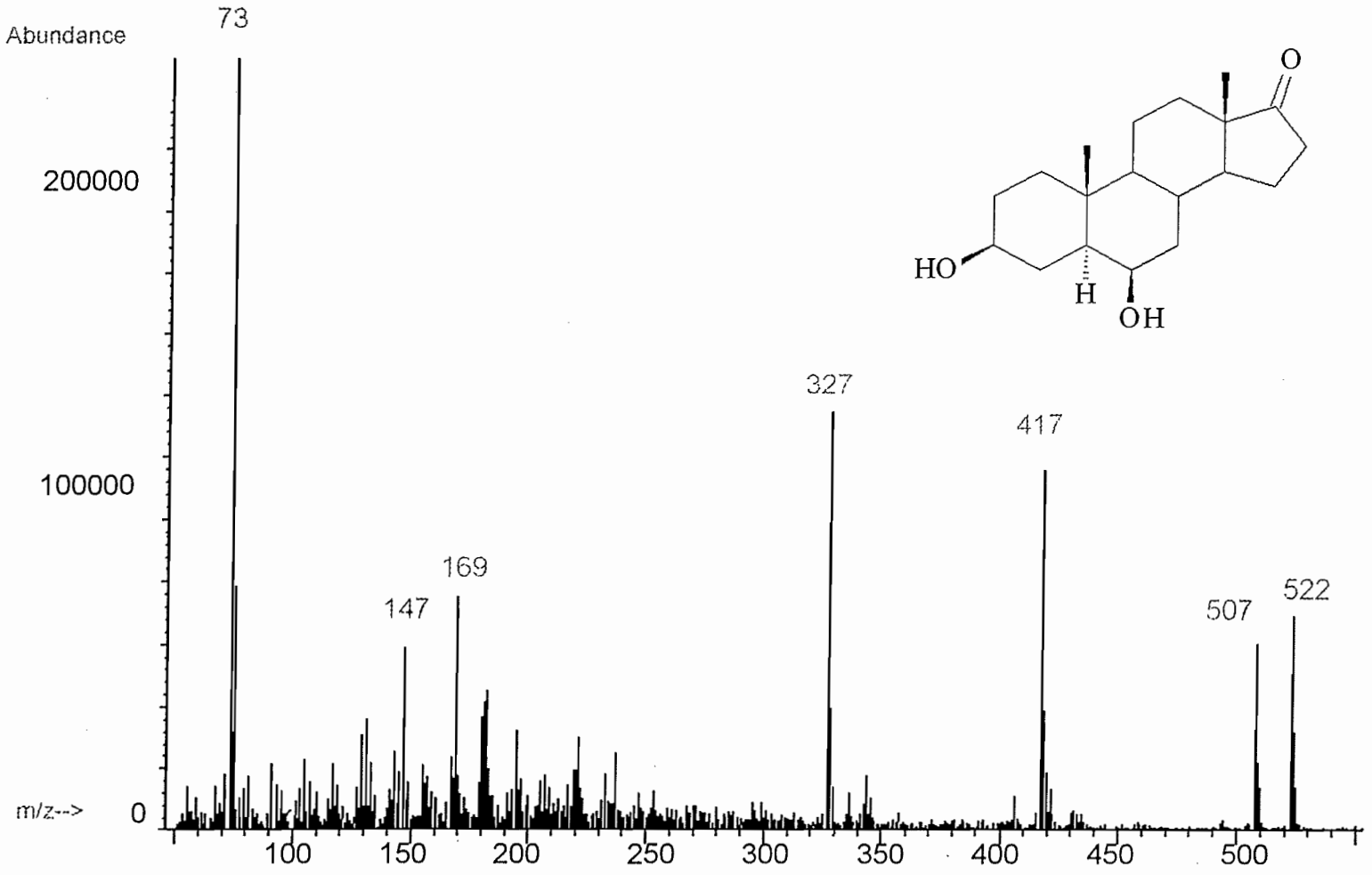

Figure 7: Mass spectrum of the TMS-derivative of **6β-hydroxyepiandrosterone** (sulfoconjugated, proposed structure - synthesis)

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