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Androstenedione metabolism: end of the story ...

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Introduction

In the past years, different groups have investigated the metabolism of androstenedione (4-androstene-3,17-dione), a biosynthetic precursor of testosterone banned by the IOC but available for oral self-administration in some countries and by the Internet, in order to set up criteria for detecting its administration during routine controls (1, 2). They observed some changes in the steroid profiles, such as the increased concentration of androsterone, etiocholanolone as well as dihydrotestosterone. Also reported were the presence of urinary androstenedione, the alteration of the T/E values by modification of the excretion of testosterone and epitestosterone, and the detection of several characteristic metabolites such as the isomeric hydroxylated androstenediones.

In 1999, our group reported the identification of 6α-hydroxyandrostenedione in urine samples collected in the hours following the administration (3). We also proposed structures for characteristic "trioxygenated" metabolites such as 6ß-hydroxyandrosterone, 6ß-hydroxyetiocholanolone and 6ß-hydroxyepiandrosterone (Fig. 1). We have now completed the characterization of these three 6ß-hydroxylated metabolites. The detailed synthesis will be published elsewhere.

In this paper, we describe the detection, characterization and quantification of these metabolites in the glucuro- and sulfoconjugated fractions of urine samples collected after the oral administration of a single dose of androstenedione (100 mg).

Experimental

One capsule of "Androstenedione complex" (100 mg/ capsule of androstenedione) (3) was administrated to two male volunteers (23 and 30 years old) after verification of the content by GC/MS analysis. Informed consent was obtained for the administration of one single dose of the commercial capsule. Urine samples were collected 24 hours before and up to 48 hours after the administration. The steroids fractions (free, glucuronide and sulfate) were analysed separately (3,4). 6ß-hydroxyepietiocholanolone authentic standard was synthesized in our laboratory and used as the internal standard for the quantification. The authentic standard of 6α-hydroxyandrostenedione was purchased from Steraloids Inc. (Wilton, NH)

Results

Characterization of the synthesized standards

The ion chromatograms from the GC/MS analysis (scan mode) (Fig. 2) of a mixture of the three reference standards show the baseline resolution between the 6ß-hydroxyandrosterone and 6ß-hydroxyetiocholanolone and the different retention times of 6ß-hydroxyepiandrosterone and 6ß-hydroxyandrosterone on a HP-1 column (12.5m, length, methylsilicone phase). The mass spectra of the TMS-ether, TMS-enol derivatives show characteristic fragmentation of O-TMS groups at m/z 522 ($M^{+\cdot}$), 507 (M-15), 417 (M-105) and 327 (M-105-90) (Fig. 3). The ions attributed to the presence of a 6ß-OTMS group have also been observed at m/z 295 and 377 (Fig. 4). In the seventies, Harvey and Vorous have reported and explained the formation of similar ions arising from the fragmentation of TMS-derivatives of 6ß-OH-cholestane (5). The ion at m/z 377 is an important fragment of the mass spectrum of the 6ß-hydroxyetiocholanolone isomer, whereas it is very low in the spectrum of the authentic standard of 3ß,6ß-dihydroxy-5ß-androstan-17-one standard (not shown) and that could be attributed to the presence of the 5ß-H and the 3α-hydroxyl group.

Characterization of the androstenedione urinary metabolites

None of these 3ε,6ß-dihydroxy-5ε-androstan-17-one metabolites were detected in the free fraction. The ion chromatograms obtained from the analysis of the glucuronide (Fig. 5) and sulfate fraction of the urine samples collected following the administration of androstenedione are presented along with the same urine samples spiked with 6ß-hydroxyandrosterone, 6ß-

hydroxyetiocholanolone and 6ß-hydroxyepiandrosterone synthesized standards (Fig. 6). That confirms the chemical structure of three isomers of 3ε,6ß-dihydroxy-5ε-androstan-17-one (Fig.1) proposed as unique metabolites of androstenedione. The 6ß-hydroxyandrosterone and 6ß-hydroxyetiocholanolone were present in the glucuro- and sulfoconjugated fractions while the 6ß-hydroxyepiandrosterone was as expected, mainly sulfoconjugated. The mass spectra of the compounds found in the urine samples were identical to those of the authentic synthesized standards (not shown).

Quantification of androstenedione urinary metabolites

The concentration of the three isomeric 3ε,6ß-dihydroxy-5ε-androstan-17-one metabolites and of 6α-hydroxyandrostenedione was estimated in the glucuro- and sulfoconjugated fractions of urine samples collected after the administration of androstenedione. The results obtained are summarised in Table 1, which also provides an approximate period of detection as well as the range of concentrations measured. It is worth mentioning that the isomeric 6ß-hydroxyandrosterone, 6ß-hydroxyetiocholanolone and 6ß-hydroxyepiandrosterone metabolites are excreted in larger amounts and for a longer period of time in the sulfate fraction. 6ß-hydroxyepiandrosterone is the only metabolite that can be detected for more than one day after the administration of a single dose (100 mg) of androstenedione.

Conclusion

The urinary metabolites 6ß-hydroxyandrosterone, 6ß-hydroxyetiocholanolone and 6ß-hydroxyepiandrosterone are specific to androstenedione oral administration. Being excreted for a longer period of time than 6α-hydroxyandrostenedione, it is important to look for these metabolites when an abnormal steroid profile (altered T/E values, abnormally high levels of androsterone, etiocholanolone) is obtained.

References

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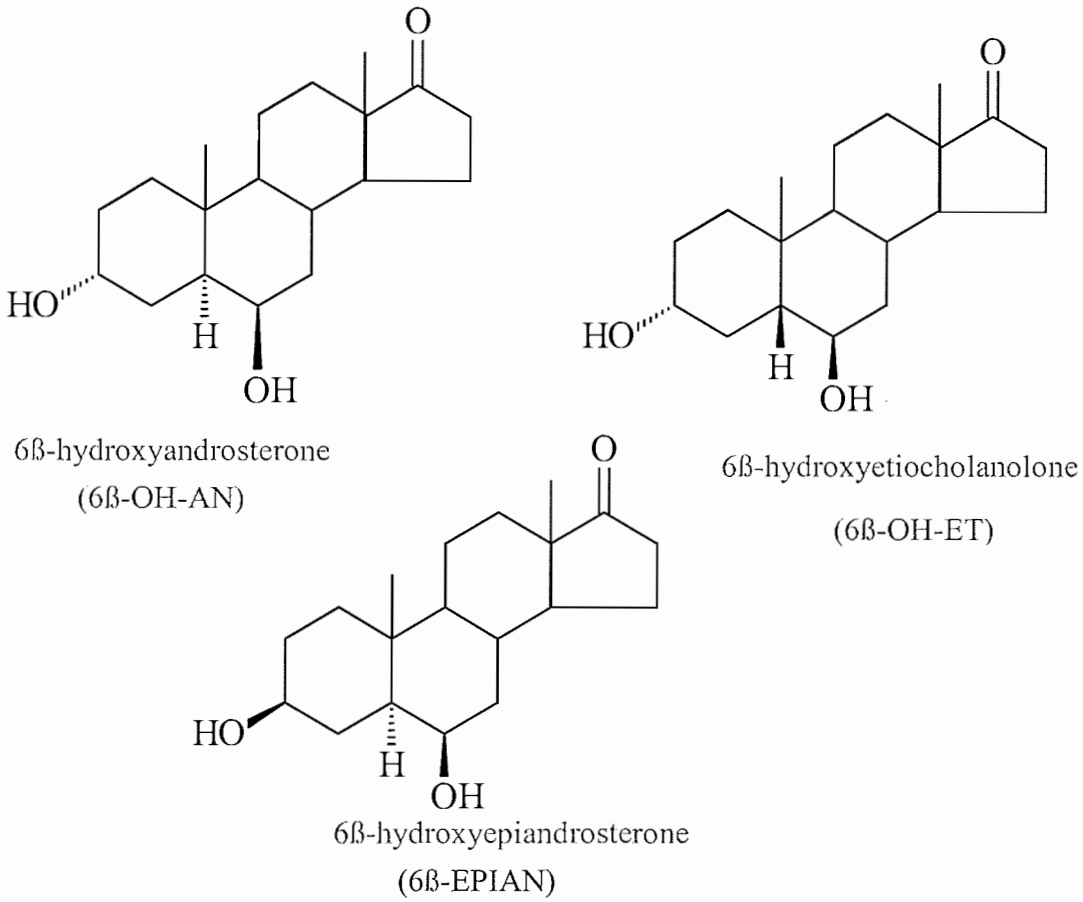

Figure 1: Proposed structures of 3ε,6ß-dihydroxy-5ε-androstan-17-one metabolites

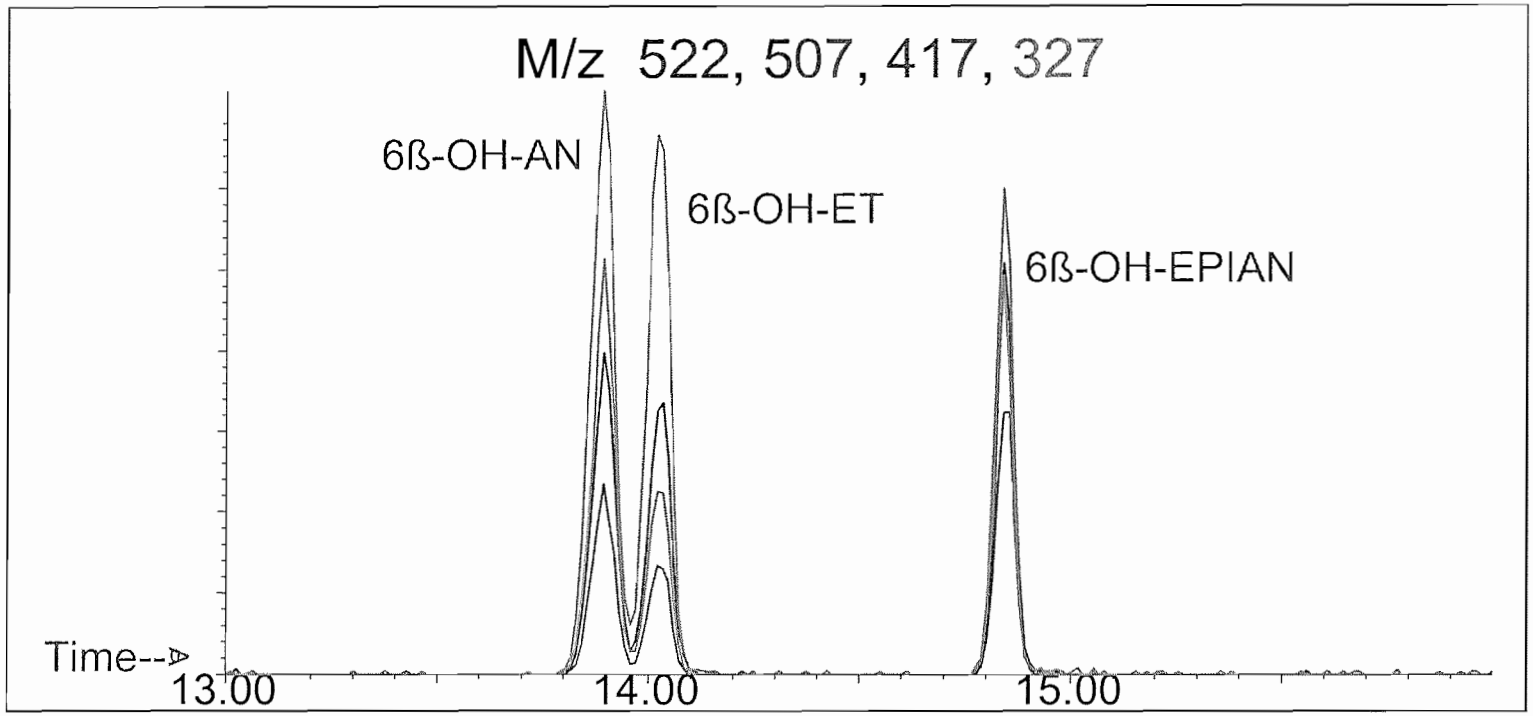

Figure 2: Ion chromatograms from GC/MS analysis (full scan mode) of 6ß,3ε-dihydroxy-5ε-androstan-17-one reference standards as their TMS-enol TMS-ether derivatives (analysed on HP-1 column (12.5m))

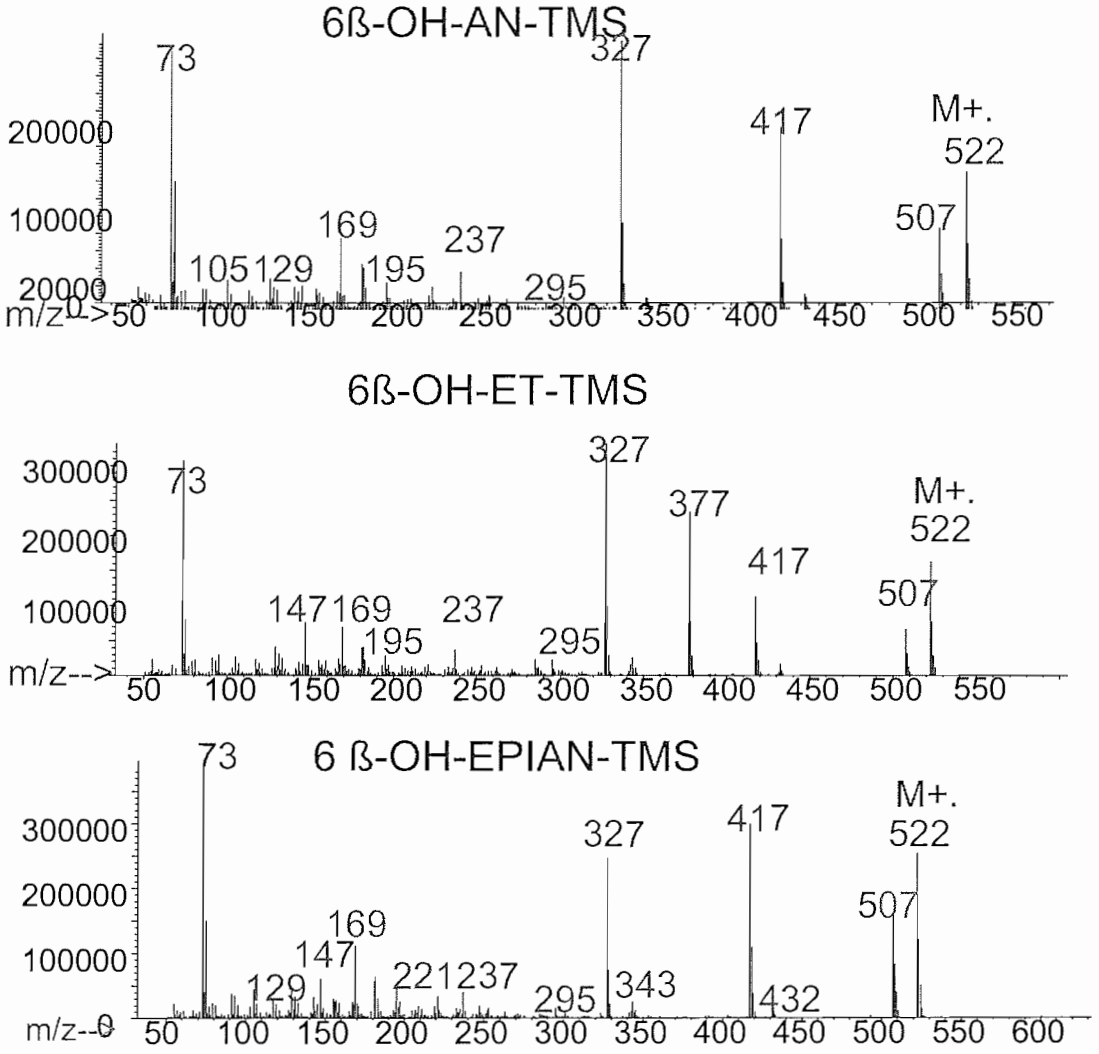

Figure 3: Mass spectrum of reference standards of 6ß-OH metabolites as their TMS-enol TMS-ether derivatives

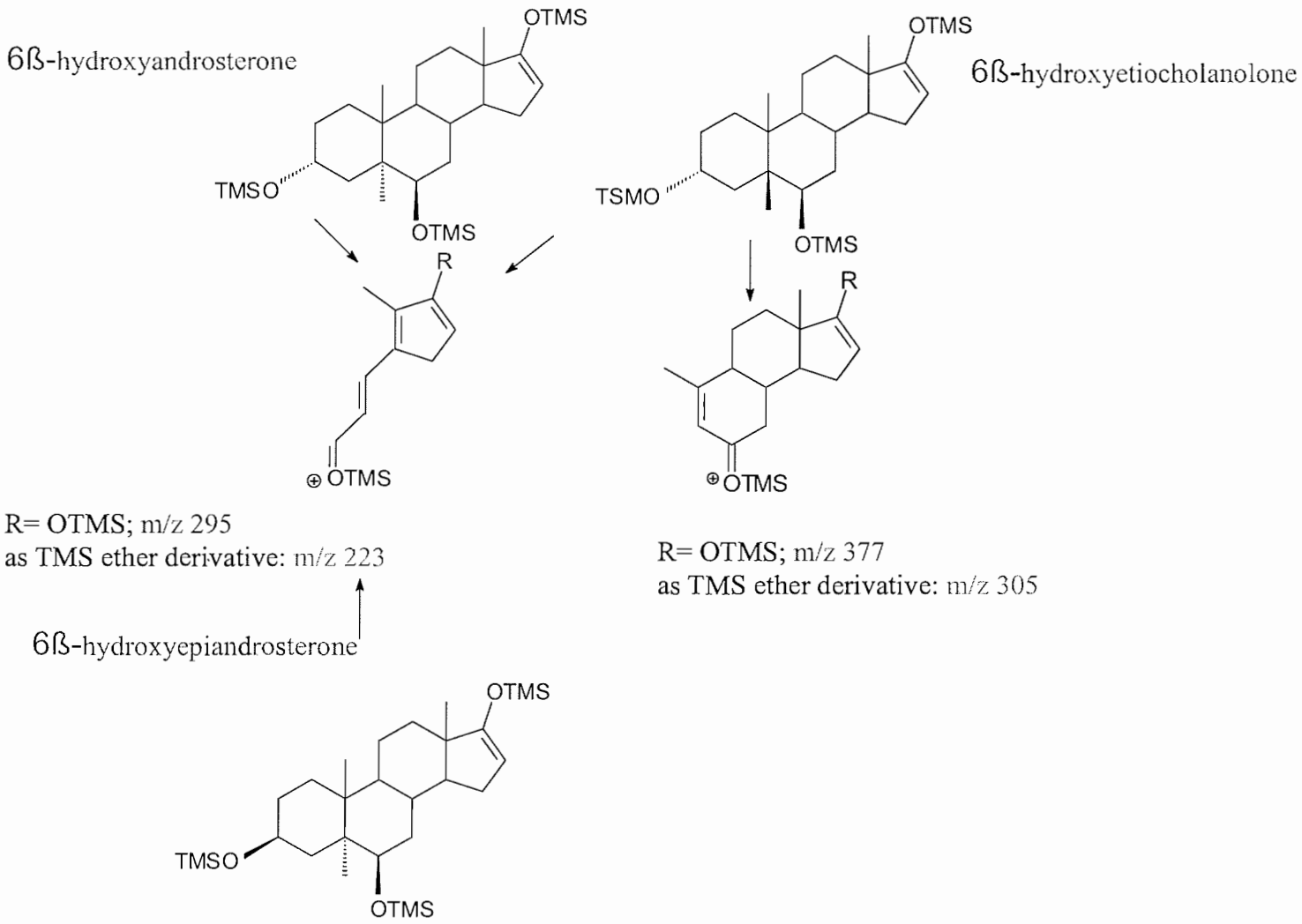

Figure 4: Proposed characteristic fragments of 6ß-OH metabolites (TMS-derivatives)

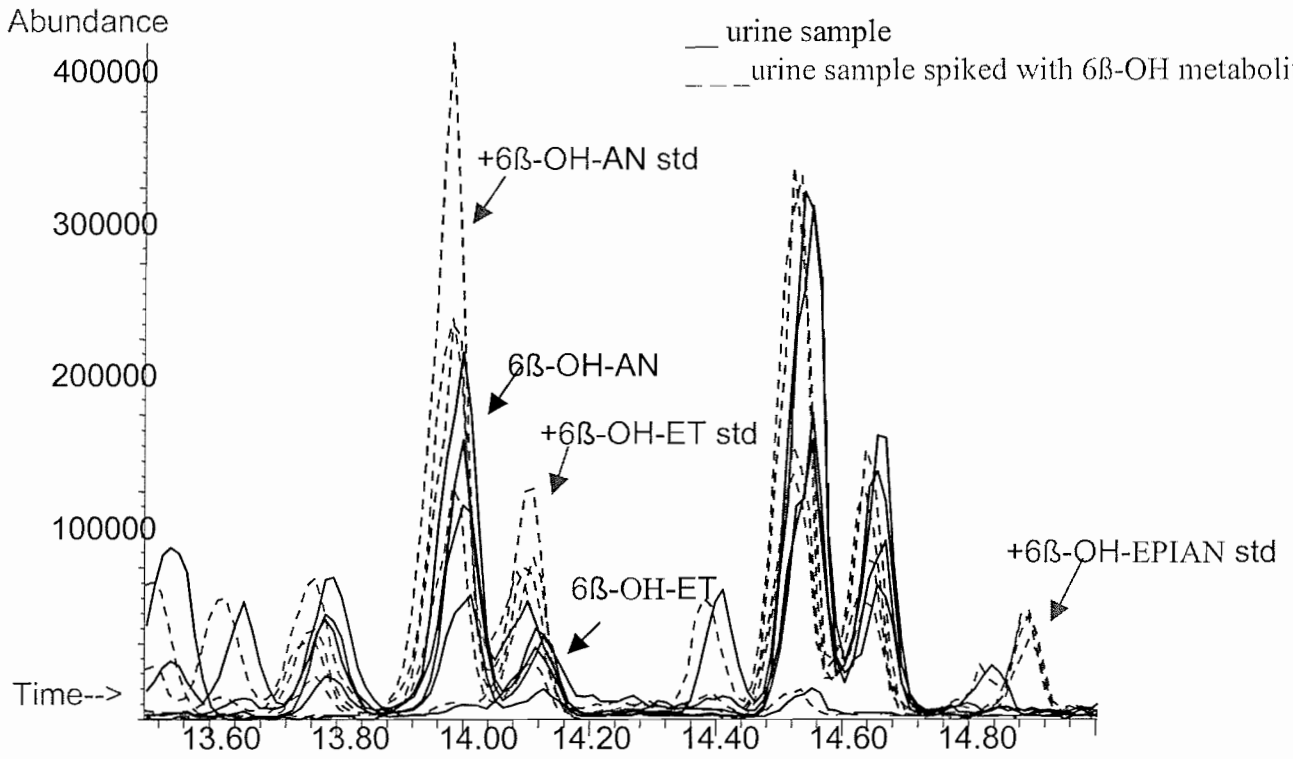

Figure 5: Ion chromatograms (GC/MS, scan mode) of androstenedione reference urine (100 mg, 4.7h) (glucuronide fraction)

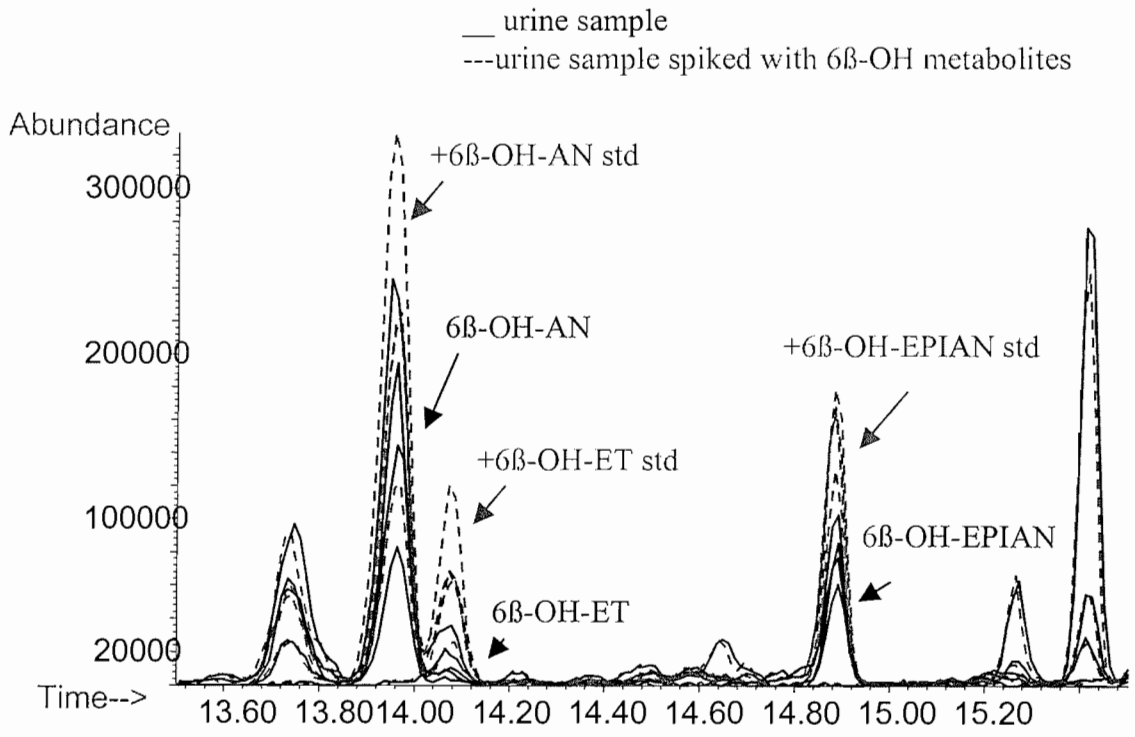

Figure 6: Ion chromatograms (GC/MS, scan mode) of androstenedione reference urine (100 mg, 4.7h) (sulfate fraction)

Table 1: Quantitation of androstenedione metabolites			Range of concentration (ng/mL)
Glucuronide fraction	Subject and dose	Time of detection (h) (gc/ms, sim mode)	
6α-hydroxyandrostenedione	#1- 100 mg	12.5	4-42
	#2- 100 mg	7	2-51
6ß-hydroxyandrosterone	#1- 100 mg	8	6-12
	#2- 100 mg	7	16-55
6ß-hydroxyetiocholanolone	#1- 100 mg	?	Unable to quantity
	#2- 100 mg	?	Unable to quantity

Sulfate fraction	Subject and dose	Time of detection (h) (gc/ms, sim mode)	Range of concentration (ng/mL)
6ß-hydroxyandrosterone	#1- 100 mg	**12.5**	**28-210**
	#2- 100 mg	**10**	**19-285**
6ß-hydroxyetiocholanolone	#1- 100 mg	17	5-58
	#2- 100 mg	7	9-23
6ß-hydroxyepiandrosterone	#1- 100 mg	**12.5**	**18-145**
	#2- 100 mg	**33**	**17-140**

Note: Androstenedione 100 mg: *Androstenedione Complex* from Price's Power International

Concentrations were normalized to a specific gravity of 1,020