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The oral administration of DHEA : The efficiency of steroid profiling.

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Introduction

Dehydroepiandrosterone (DHEA) and its sulphate form (DHEA-S) are weak androgens mainly produced by the adrenal glands. Both can be converted in peripheral tissues to androstenedione, testosterone and 5α-dihydrotestosterone (5α-DHT) and both are aromatized to estrogens [1]. It is estimated that 30 to 50 % of total androgens in men and 75 % in pre-menopausal women are synthesised in peripheral intracrine tissues from DHEA and DHEA-S. The gonads would produce the balance [2-4]. Considered as dietary supplements, androgen precursors such as DHEA are available over the counter in the USA although in many other countries, its distribution and importation are illegal being regulated substances. Athletes may use these products mainly to increase their levels of testosterone and 5α-DHT. DHEA is related to testosterone and as such its use was not permitted in Olympic Sports. Since it may be purchased *legally* in the USA, in January 1997, the medical commission of the International Olympic Committee (IOC) listed by name DHEA in the class of prohibited anabolic agents.

In the last two years, only few research groups have studied effects of the oral administration of DHEA in human on the steroid profile. Three studies described no increase of the T/E ratio to levels above 6:1 when 50 mg of DHEA was given [5-7]. On the other hand, Bowers observed an important increase of the T/E ratio, from 2.4 to 8.1, also following a 50 mg dose [8]. Two probes were proposed to detect the administration of DHEA. According to Dehennin *et al*, a urinary concentration of DHEA glucuronide exceeding a level of 300 ng/mL, corrected to a specific gravity of 1.020, would indicate its administration [5] while, Ueki *et al* suggested to use the ratio of the concentration of DHEA over terahydrocortisol (THC) and tetrahydrocortisone (THE) [9].

The main objectives of our study are to evaluate the effects of oral administration of DHEA supplements on the common steroid profile, to locate and identify characteristic $C_{19}O_3$ metabolites and ultimately, to propose an approach for the detection of DHEA abuses.

Experimental

A 200 mg dose of DHEA from " Ultimate Nutrition ® " was given to 3 males of 23, 25 and 33 years old and to 1 female of 32 years old. Urine samples were collected over 48 hours. The usual procedure was used to isolate the free and glucuronide fractions [10]. For the hydrolysis of the sulphate fraction, we used 1 ml of THF and 2 μl of 4M H_2SO_4 as a solvolysis mixture. The solution was heated at 50°C for 1 hour. All samples were derivatized as TMS-enol TMS-ether and analysed by GC/MS.

Results and Discussion

Different changes in the steroid profile were observed following the oral administration of a 200 mg dose of DHEA. We noticed first, that the variation of the T/E values depended on the initial or basal ratio of the male subject involved. For our subject A, which had a normal T/E value of 0.25, only a slight increase of the T/E ratio was observed after the single dose. For the second male subject, the initial ratio of 1.8 increased to 3.5 14 hours following the administration. In both cases, the T/E ratios did not break the 6:1 limit. From an initial value of 2.5, the T/E ratio of our third male volunteer rose to 7.5 in about 15 hours and got back to normal 20 hours after the dose. This increase is related to high levels of testosterone glucuronide. For our three male volunteers, we can conclude that the magnitude of the increase depends on the initial T/E values. A subject having a naturally elevated ratio will have a more important increase then someone with a ratio under 0.5. This statement is not applicable for females. From an initial value of 1.3, the T/E ratios of our only female subject increased to 12 in 10 hours and got back to normal within 20 hours.

The variations of the androsterone (A) and etiocholanolone (Et) concentrations and of the A/Et ratio were similar to those reported previously by Kazlauskas [7]. Following the administration of DHEA, the A/Et ratio increased and then felt below the initial value during the

first 48 hours. The decrease of the A/Et ratio was caused by a second peak in etiocholanolone glucuronide excretion that occurred between 10 and 20 hours following the administration while the levels of androsterone remained normal. In a punctual viewpoint, the ratio A/Et is not informative enough to be used as a probe. On the other hand, the urinary concentration of androsterone and etiocholanolone glucuronides are quite interesting. Elevated levels exceeding those observed in the male athletes' population were maintained during 24 hours for androsterone and more than 48 hours for etiocholanolone. Therefore, the first alteration noticed during the GC/MS screening of a urine sample, is the high levels of androsterone and etiocholanolone, metabolites of the androgens. This should be the signal prompting investigation.

Many reactions are involved in the metabolism of steroids such as DHEA. Reduction, oxidation, hydroxylation and conjugation are the main ones. In the past few years, we paid more attention to hydroxylated metabolites of natural steroids such as DHEA, androstenedione and androstenediol. We observed that natural precursors of testosterone have important effects on the profile of the $C_{19}O_3$ steroids. In the case of the DHEA, its administration generated an important increase of the excretion of C-7 hydroxylated metabolites: 7α and 7β-OH-DHEA. These two products are naturally present in the urine. They would be formed in several tissues from dog, rat, mouse and human [11-14]. Because both metabolites possess 3β-hydroxy-groups, they are mainly excreted as sulfoconjugated steroids. The administration of DHEA caused major variations in the $C_{19}O_3$ sulphate steroid profile.

We observed that five major $C_{19}O_3$ steroids were naturally present in the sulphate fraction of a male urine: the 16α-OH-DHEA (m/z 520, 505, 415 and 147), the 16α-OH-androsterone (m/z 522, 507, 417 and 147), the 16α-OH-etiocholanolone (m/z 522, 507, 417 and 147), the 7β-OH-DHEA (m/z 520, 430, 415, 325 and 169) and the 7α-OH-DHEA (m/z 520, 430, 415, 325 and 169). The structure of all these products was confirmed by the comparison of the TMS-enol TMS-ether and TMS-ether mass spectra and with the retention time of authentic standards. The mass spectra of 7β-OH-DHEA and 16α-OH-androsterone are presented at figures 1A and 1B. In a "normal" $C_{19}O_3$ sulphate steroid profile, 16α-OH-DHEA and 16α-OH-androsterone are the most abundant steroids and the area ratio between the ion at m/z 430 of 7β-OH-DHEA and the ion at m/z 507 of the 16α-OH-androsterone seemed to be lower than 1 in the subjects we studied

so far (Figure 2A). After a 200 mg dose of DHEA, important qualitative variations in the $C_{19}O_3$ steroid profile were observed (Figure 2B):

1. Increased levels of 7α- and 7β-OH-DHEA
2. Suppression of the C-16α hydroxylated steroids
3. Presence of 4ξ-OH-DHEA(proposed structure) (m/z 520, 430, <u>391</u>, 301, 325, 340)

During more than three hours after the administration of DHEA, the ion at m/z 430 of 7β-OH-DHEA was nearly ten times more abundant than the ion at m/z 507 of 16α-OH-androsterone. The time-period during which the ratio 7β-OH-DHEA to 16α-OH-androsterone seemed altered varied between 12 to 23 hours for our male subjects. This ratio was not measured for our female volunteer. Therefore, we think that the 7β-OH-DHEA/16α-OH-androsterone ratio is an interesting probe for the detection of DHEA oral administration. This conclusion being drawn after qualitative analysis of the profiles, further studies are needed to definitely estimate the efficiency of this ratio.

Conclusion

Since the T/E ratio is not always modified by the administration of DHEA, it cannot be used as a universal probe. The increase of the ratio seems to be directly related to its initial value. The first signal of DHEA use is given during the GC/MS analysis by the measurement of high urinary concentrations of androsterone and etiocholanolone glucuronides. When these exceed the concentration usually observed in the male or female athletes' populations, it would be interesting to look at the $C_{19}O_3$ sulphate steroid profile for the presence of DHEA characteristic metabolites (7α and 7β-OH-DHEA), signs of suppression of the C-16α hydroxylated steroids and an elevate 7β-OH-DHEA/16α-OH-androsterone ratio if further studies confirm its usefulness.

An important point that we must keep in mind is that stress can affect substantially the excretion of DHEA. Spiteller and his team evaluated that 10 to 20 % of the population would

have high excretion levels of DHEA [15, 16]. The effects of stress on the $C_{19}O_3$-steroid profile still haven't been evaluated.
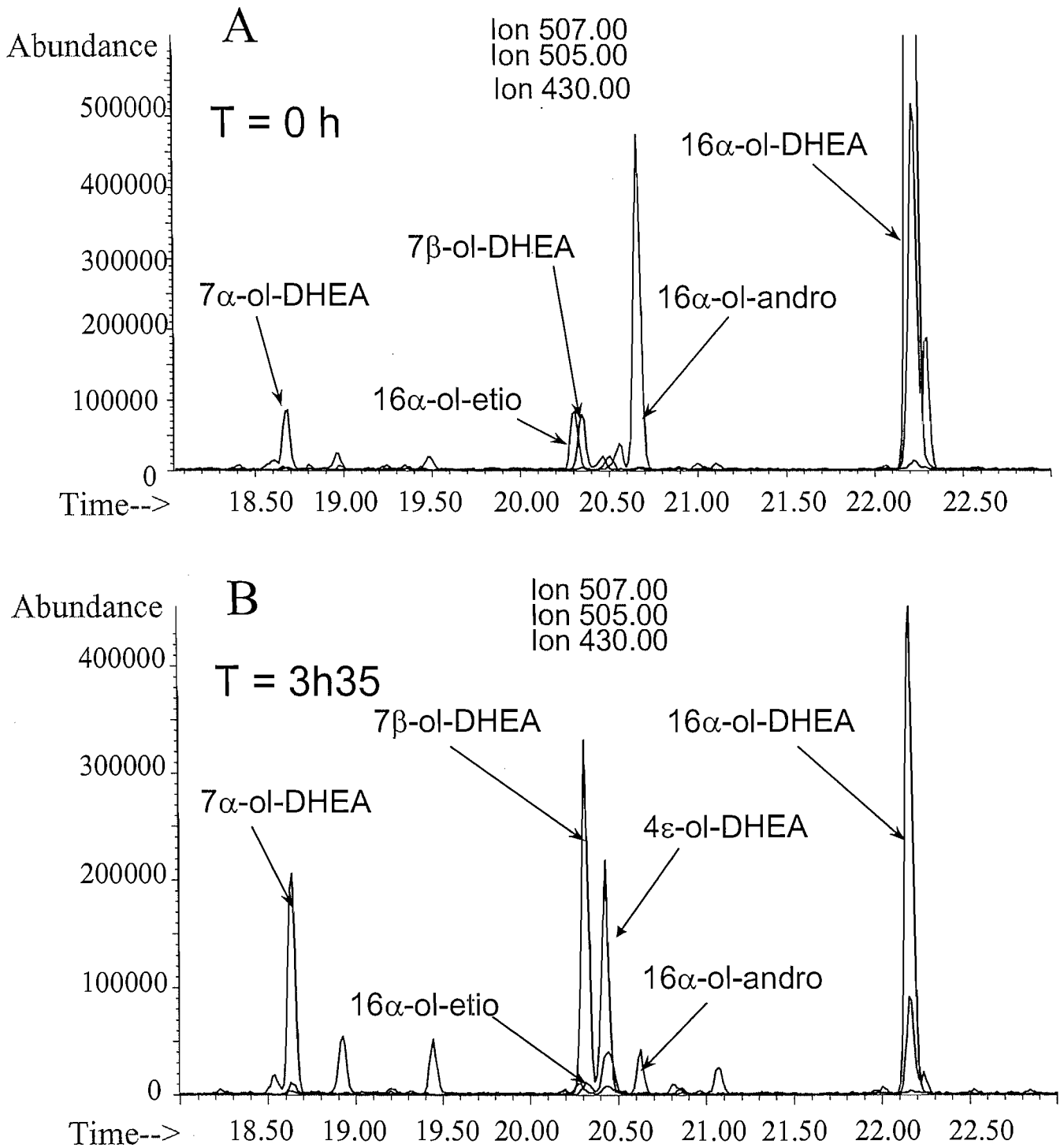
Acknowledgement

References

[1] Longcope, C.. 1996. Dehydroepiandrosterone metabolism. J. Endrocinol., vol. 150, pp S125-S127

[2] Labrie, F.. 1991. Intracrinology. Moll. Cell Endrocrinol., vol. 78, pp C113-C118

[3] Labrie, F., A. Dupont, A. Bélanger. 1985. Complete androgen blockade for the treatment of prostate cancer
in : deVite VT, S. Hellman, SA Rosenberg (eds). Important Advances in Oncology. JB Lippincott, Philadelphia, pp.193-217

[4] Bolander, FF.. 1996. Introduction in : Molecular Endocrinology Academic Press, San Diego, London, pp 1-23

[5] Dehennin, L., M. Ferry, P. Lafarge, G. Pérès, JP Lafarge. 1998. Oral administration of dehydroepiandrosterone to healthy men: Alteration of the urinary androgen profile and consequences for the detection of abuse in sport by GC/MS. Steroids. Vol. 63. pp. 80-87

[6] Bosy, TZ, KA Moore, A. Poklis. 1998. The effect of oral DHEA on the urine T/E ratio in human male volunteers. J. Anal. Toxicol.. vol. 22, pp. 455-459

[7] Kazlauskas, R.. Effects of dehydroepiandrosterone on urinary steroids. In Schänzer W, ed. Recent Advances in Doping Analysis (5). Proceedings of the Manfred Donike 15th Cologne workshp on dope analysis, 1997. Cologne Sport und Buch Strauß, 1998. pp. 83-90

[8] Bowers, LD.. 1999. Oral dehydroepiandrosterone supplementation can increase the T/E ratio. Clinical Chemistry. Vol. 45. pp 295-297

[9] Ueki, M., M. Okano and A. Ikekita. Nagano strategy against natural hormone doping-Testosterone, DHT, Androstenedione and DHEA. In Schänzer W, ed. Recent Advances in Doping Analysis (6). Proceedings of the Manfred Donike 16th Cologne workshp on dope analysis, 1998. Cologne Sport und Buch Strauß, 1999. pp. 145-146

[10] Ayotte, C., D. Goudreault and A. Charlebois. 1996. Testing for natural and synthetic anabolic agents in human urine. J. Chromatogr., vol. 687, pp 3-25

[11] Morfin, R., I. Leav, JC Orr, D. Picard and P. Ofner. 1980. C_{19}-steroid metabolism by canine prostate, epididymes and perianal glands. Application of the twin-ion technique of GC/MS to establish 7α-hydroxylation. Eur. J. Biochem., vol. 109, pp 119-127

[12] Isaacs, JT, IR McDermott, DS Coffey. 1979. Characterization of the two new enzymatic activities of the rat ventral prostate : 5α-androstane-3β,17β-diol 6α-hydroxylase and 5α-androstane-3β,17β-diol 7α-hydroxylase. Steroids. Vol. 33, pp 675-692

[13] Morfin, R. and G. Courchay. 1994. Pregnenolone and dehydroepiandrosterone as precursors of native 7-hydroxylated metabolites which increase the immune response in mice. J. Steroid Biochem. Molec. Biol.,
vol. 50, pp 91-100

[14] Morfin, R., S. DiStefano, JF Charles and HH Floch. 1977. Precursors for 6ξ amd 7α-hydroxylation of 5α-androstane-3β,17β-diol by normal and hyperplactic prostate. Biochimie, vol. 59, pp 637-644

[15] Ludwig, H., M. Spiteller, HJ Egger and G. Spiteller. 1977. Correlation of emotional stress and physical exertions with urinary metabolite profile. Isr. J. of Chem., vol. 16, pp 7-11

[16] Pfeifer, P. and G. Spiteller. 1981. Steroid profiles on healthy individuals. J. Chromatogr., vol. 223, p. 21-32
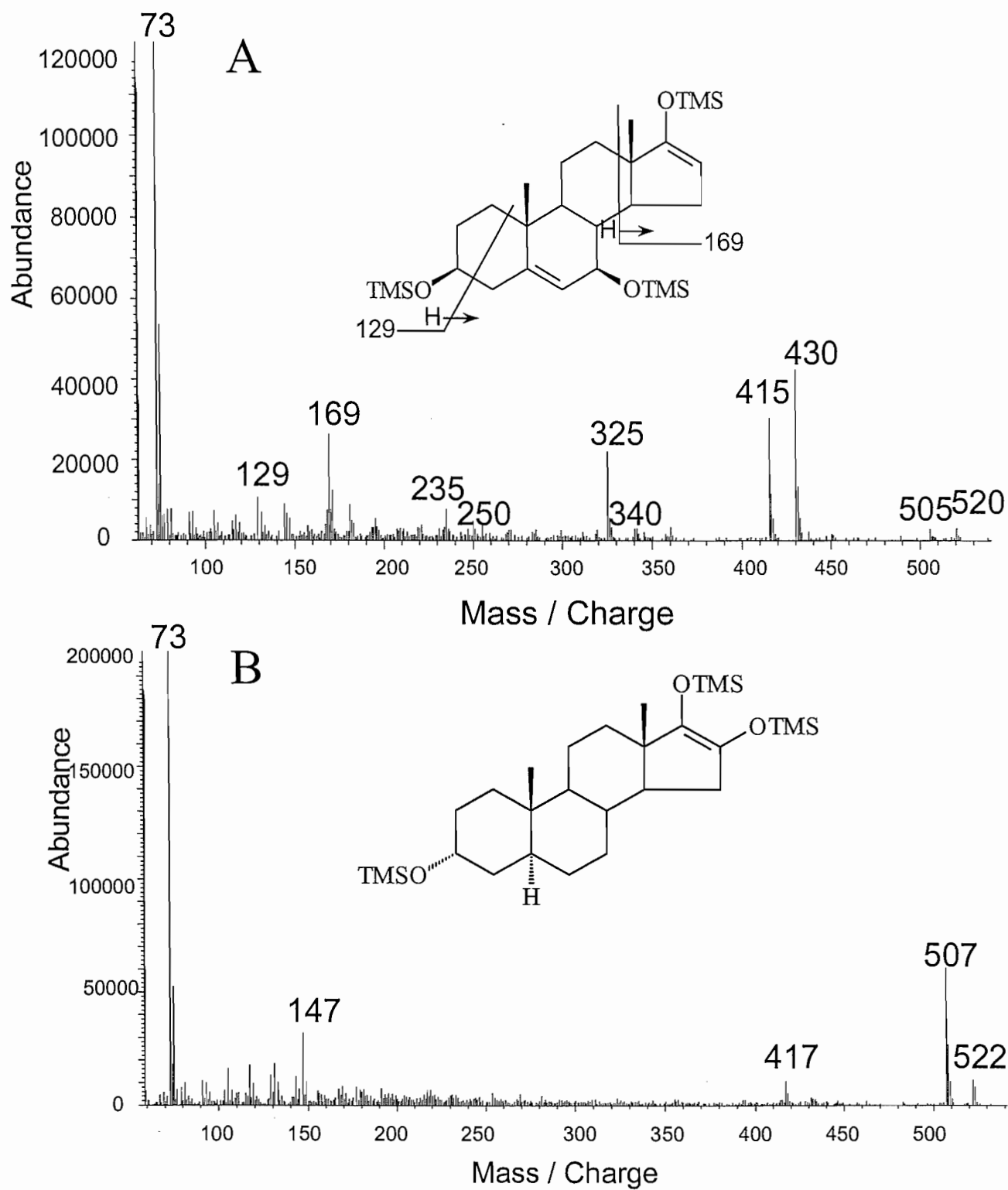
Figure 1: Mass spectra of the TMS-enol TMS-ether derivatives of 7β-OH-DHEA (**A**) and of 16α-OH-androsterone (**B**).

A

Abundance

T = 0 h

Ion 507.00
Ion 505.00
Ion 430.00

500000

400000

300000

16α-ol-DHEA

7β-ol-DHEA

200000

7α-ol-DHEA

16α-ol-andro

100000

16α-ol-etio

0

Time-->   18.50 19.00 19.50 20.00 20.50 21.00 21.50 22.00 22.50

B

Abundance

T = 3h35

Ion 507.00
Ion 505.00
Ion 430.00

400000

7β-ol-DHEA

16α-ol-DHEA

300000

7α-ol-DHEA

4ε-ol-DHEA

200000

16α-ol-andro

100000

16α-ol-etio

0

Time-->   18.50 19.00 19.50 20.00 20.50 21.00 21.50 22.00 22.50

Figure 2: Profiles of the $C_{19}O_3$ sulfate steroids before (A) and 3h35 after (B) the oral administration of DHEA to a male subject (#2).