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Study of the behavior of the steroid profile under hydrolysis conditions after 4-androstenedione oral administration by GC/MS

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1-Introduction

Androstenedione(4-androstene-3,17-dione) is secreted endogenously by the adrenal cortex and the gonads, and the small amount is also produced by peripheral conversion. It is an immediate precursor to testosterone in the intrinsic synthetical pathways of androgens [1].

The evaluation of urine steroid profile under normal procedure used for anabolic steroids (1 hour at 55°C) compared to the hydrolysis for about 15 hours at 37°C has been suggested. This change affects several endogenous compounds such as Testosterone, Epitestosterone, Androsterone, Etiocholanolone, 6 α -hydroxyandrostenedione, DHEA, DHT, 5 α diol, 5 β diol, OHA, OHE, Pregnanediol, Pregnanetriol and 16(5 α)-androsten-3 α -ol.

In this study, we expose the results obtained after oral administration of 4-androstenedione and the impact on the steroid profile under different conditions of hydrolysis in terms of concentrations to allow the analysis by GC/IRMS according the TD2004EAAS.

2-Excretion study

A single dose of 100 mg of androstenedione was administrated to a volunteer man. The urine samples were collected prior and after application (10 days) the excretion study is carried out to follow the concentration of the endogenous compounds by GC/MS under different conditions of hydrolysis.

3- Experimental

Sample preparation for GC/MS analysis: After addition of internal standard (17- α -methyltestosterone) (50 μ g/ml) to the two sets of 2 ml urine samples collected after administration of androstenedione, 200 μ l of phosphate buffer (pH 6-7) were added and the urine was hydrolyzed with 40 μ l of β -glucuronidase (isolated from Escherichia coli K 12) in two different ways (1h, 55°C) and (15h, 37°C). The urines was adjusted to pH=11 with

potassium carbonate buffer and extracted with 5 ml of MTBE [2]. The tube was shaken in a mechanic agitator for 10 min. after that, the urinary extract was centrifuged at 2500 rpm for 5 min and frozen to separate the organic layer, then after evaporated to dryness under the nitrogen stream; derivatisation was carried out with a mixture of MSTFA/NH₄I/DTE (1000/1/2) (v:w:w) 30 min at 65°C [2], 3 µl were then injected to GC/MS (Split ratio 1/20).

GC/MS analysis : This analysis was carried out in a HP 5973 series GC/MS system, equipped with a HP 6890 GC, HP 7683 auto-sampler, and HP ChemStation data system. The separation was done using HP-1 column (25m, 0.2mm ID, 0.11 µm). The oven temperature program was:190°C(0min)-2°C/min to 235°C(0min)-15°C/min to 320°C(5 min).

4-Results and discussion

The screening results by GC/MS of the endogenous compounds of samples urines collected after administration of androstenedione show a rapid increase of testosterone and epitestosterone concentrations between 1 to 20 hours under different conditions of hydrolysis (1h, 55°C) and (15h, 37°C). Moreover the T/E ratio remains below 4 before and after 11 hours. The concentration of androsterone is observed with a maximum after 7 hours. 19 hours following administration, the concentration of androsterone still remains beneath 10000 ng/ml. For etiocholanolone, the concentration did not reach the threshold of 10000 ng/ml. The DHEA was not affected by the administration of androstenedione as shown in *figures 1, 2, 3, 4, 5* and *6*.

In the case of testosterone, we can see the increase of its concentration under 37°C hydrolysis by about 19 per cent one night later exceeding the threshold of 200 ng/ml, this is observed in several points as shown in *figure 3* compared to 55°C 1 hour, the first hydrolysis could be an interesting factor to allow the passage to the GC/C/IRMS from the GC/MS analysis; this establishment is not observed in the case of epitestosterone (Increase by about 4% only), therefore pulling T/E ratio up by about 13% as it is shown in *figure 4* and *5*.

The most affected endogenous compounds by 37°C hydrolysis one night were OHA (45.5%) and OHE (69%). In fact, their concentrations exceeded 1000 ng/ml and 400 ng/ml respectively in several points of the excretion study. As for the other compounds, we also notice an increase of concentrations as following : pregnandiol (21%), pregnantriol (35.5%), 5αdiol (35%), 5βdiol (17%) and DHEA (27%) as it is shown in *figures 6, 7, 8, 9, 10, 11* and *12*. On the other hand, DHT decreases by 23% and 16(5α)-androsten-3α-ol by 22% as it is

shown in *figures 13* and *14*. Moreover, the 6α -hydroxyandrostenedione which is affected by about 35% though it is the major androstenedione metabolite (*figure 15*)

Conclusion

As previously indicated in this study, we focused on the behavior of the endogenous profile following two different conditions of hydrolysis (1h, 55°C) and (15h, 37°C) in the screening procedure. We can conclude that better results are registered when a long time hydrolysis is applied to the urine samples in terms of increasing the concentration of the most important endogenous substances detected in urine.

We also had the opportunity to handle different endogenous compounds and examine the variation of steroid profile under the two types of hydrolysis mentioned above in the screening procedure. But it is worth to mention here that some concentrations increased when some others decreased. So far, we do not have an explanation to that phenomenon. However, it can be studied on an other occasion and in a more detailed approach to show the plausible causes of this behavior and try to find some rules or conditions to such types of hydrolysis.

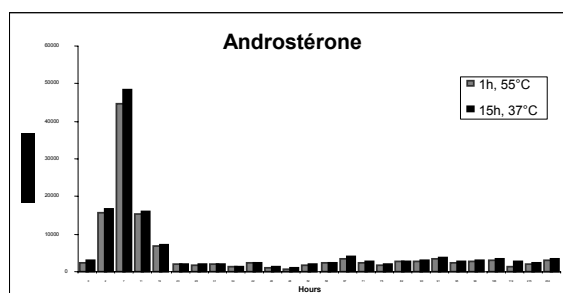


Fig.1

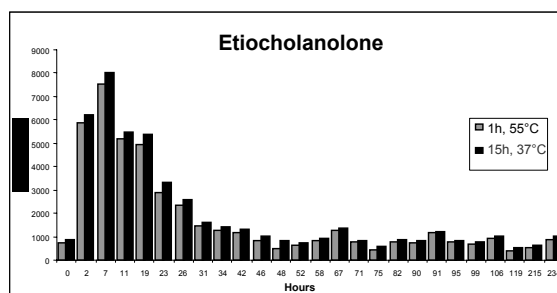


Fig.2

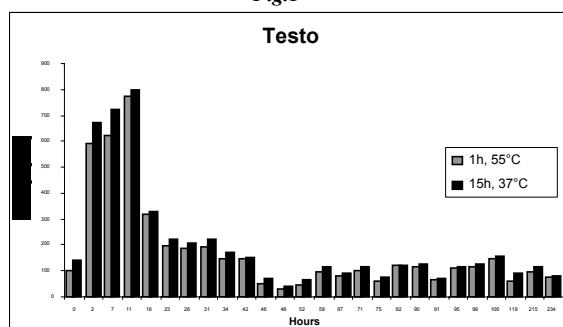


Fig.3

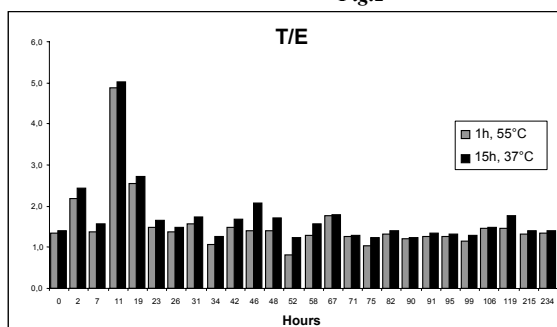


Fig.4

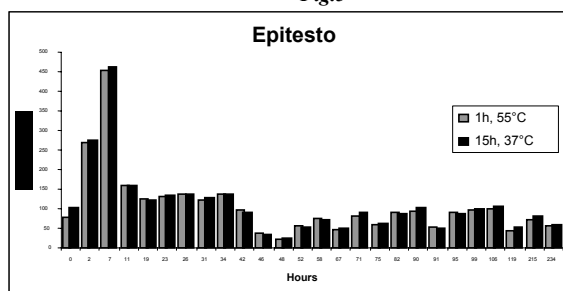


Fig.5

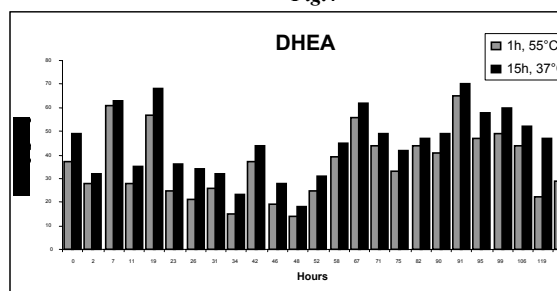


Fig.6

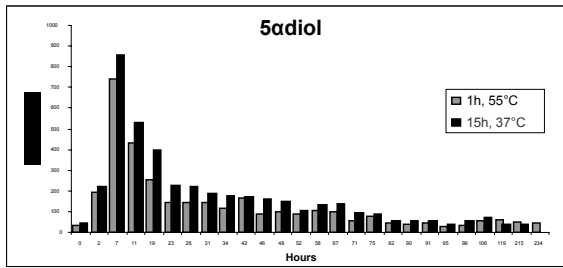


Fig.7

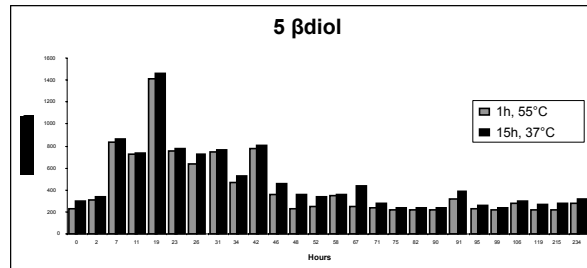


Fig.8

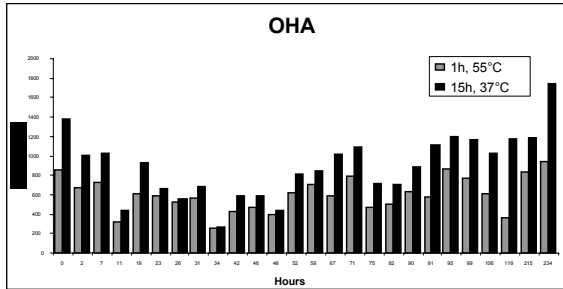


Fig.9

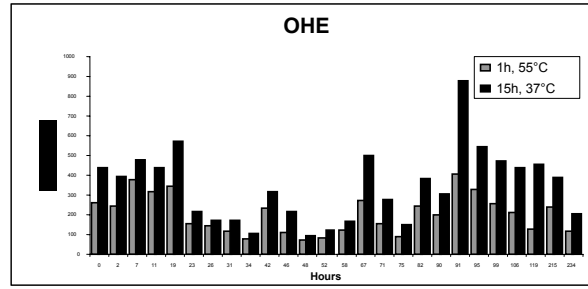


Fig.10

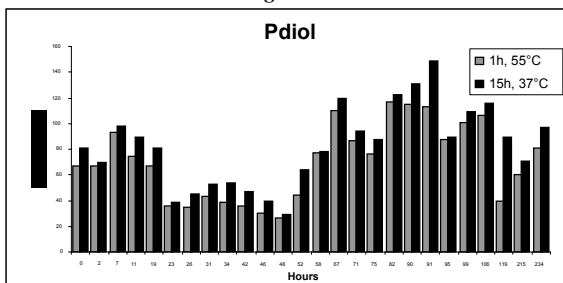


Fig.11

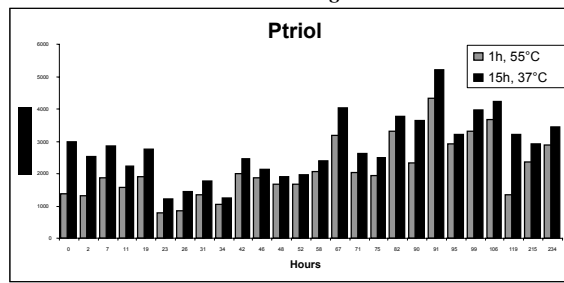


Fig.12

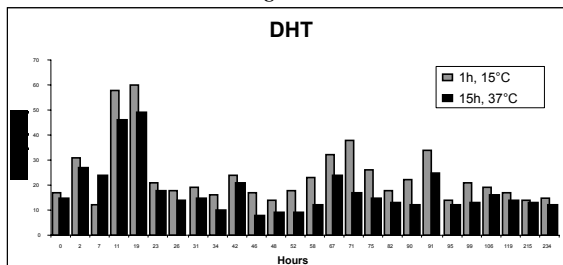


Fig.13

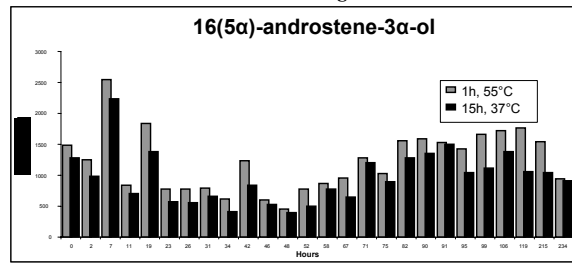


Fig.14

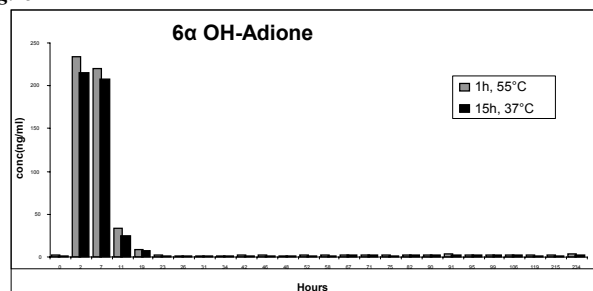


Fig.15

References

- [1] Don H. Catlin, Benjamin Z. Leder, Brian D. Ahrens, Caroline K. Hatton, Joel S. Finkelstein: Effects of androstenedione administration on epitestosterone metabolism in men.
- [2] Uralets VP and Gillette PA. Over-the-Counter Anabolic steroids 4-Androsten-3,17-dione; 4-Androsten-3β,17β-diol and 19-Nor-androsten-3,17-dione: excretion Studies in Men. J Anal Toxicol. 1999; 23(5):357-66.