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Testosterone with Different Delta Value

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Introduction

During the study on detection of doping with 1-testosterone, it was noticed that the delta values of 1-testosterone preparations were from -15‰ to 20‰⁽¹⁾ and 1-testosterone was occasionally mixed with testosterone as by-product during 1-testosterone synthesis. The testosterone as impurity in 1-testosterone was separated by HPLC then identified by GC/MS and NMR. The delta value of -16‰ for this separated testosterone was confirmed by GC/C/MS with our normal routine procedure.

Experiment

1. Instrumentation:

HPLC for separation: Waters 2797 Bioseparation Module with Waters 2996 DAD

GC/MSD for identification: Agilent 5973 N MSD coupled with 6890 GC

GC/C/MS for delta values: Finnigan Delta Plus coupled with 6890 GC

NMR for ¹H and ¹³C spectra: Jeol GNM-ECA-600

2. Chemicals and Reagents:

1-Testosterone was synthesized in Pharmaceutical Faculty, Fudan University. All other chemicals and reagents were commercial available with analytical chemistry degree.

Results

Fig. 1 was the chromatogram with DAD detector. Testosterone (retention time: 11.15 min) was well separated from 1-testosterone (retention time: 11.78 min). The fraction of testosterone was collected by automated fraction collector controlled by the time.

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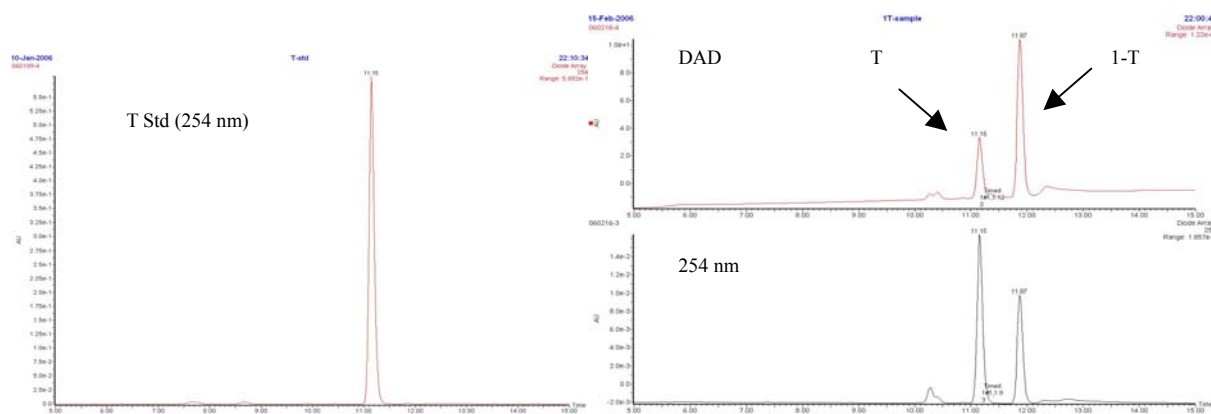


Fig. 1 HPLC chromatogram of T and 1-T

The testosterone collected from HPLC separation was injected into GC/MSD in EI mode without any derivatization. The mass spectrum and the search result with NIST database⁽²⁾ was showed in Fig. 2. This mass spectrum was matched with the quality of 99.

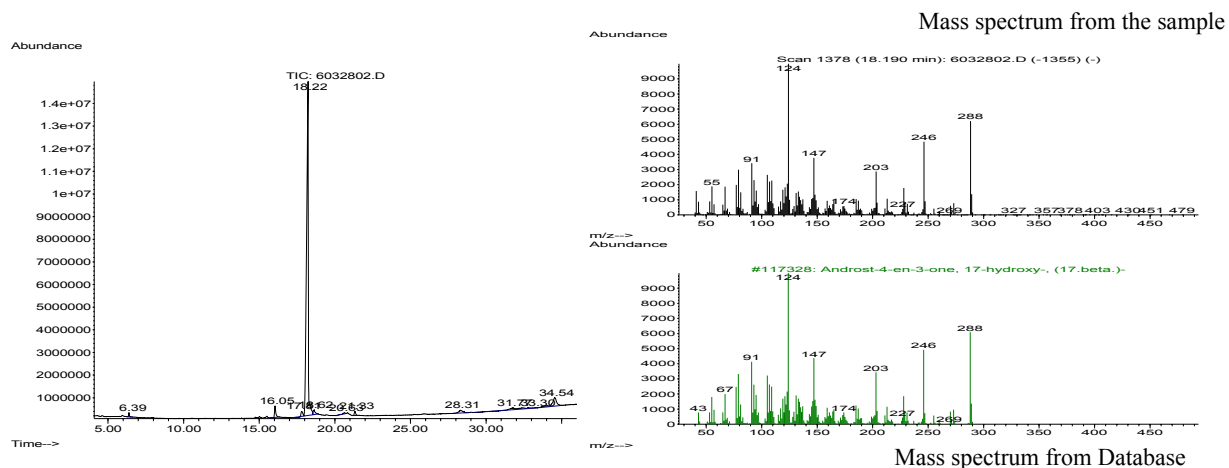


Fig. 2 TIC and Mass Spectrum

The testosterone collected from HPLC separation was analyzed with NMR. Also the NMR spectra of ¹H and ¹³C were identical as the data of testosterone reported in the literature⁽³⁾. The NMR spectra were showed in Fig. 3. Further DEPT, H-H-COSY, HMBC and HMQC spectra confirmed the structure of testosterone too.

The delta values of normal testosterone and of testosterone from 1-testosterone mixture were measured with GC/C/MS using normal routine procedure without any derivatization. The normal testosterone showed us the delta value around -32‰ but that from 1-testosterone mixture around -16‰. The GC/C/MS were showed in Fig. 4. The delta values were in the blue color.

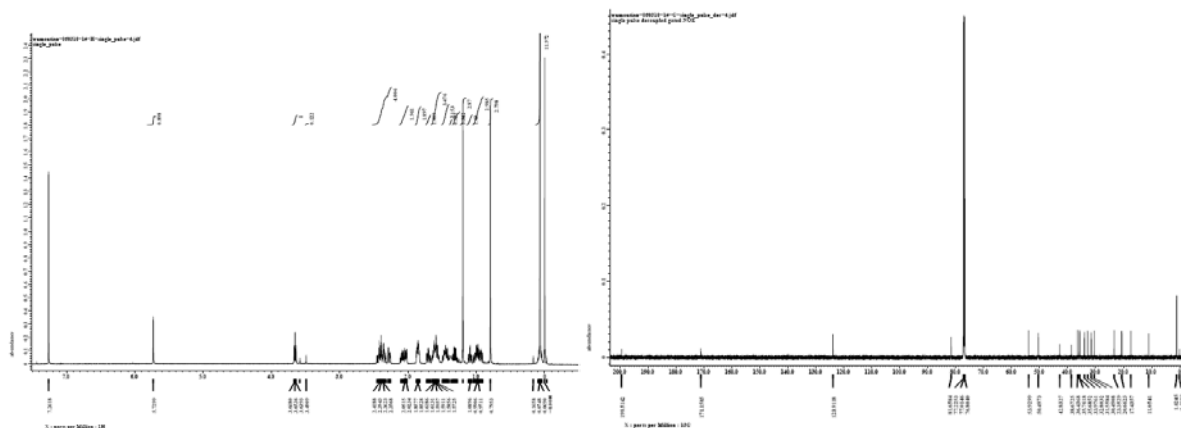


Fig. 3 ¹H (left) and ¹³C (right) NMR spectra

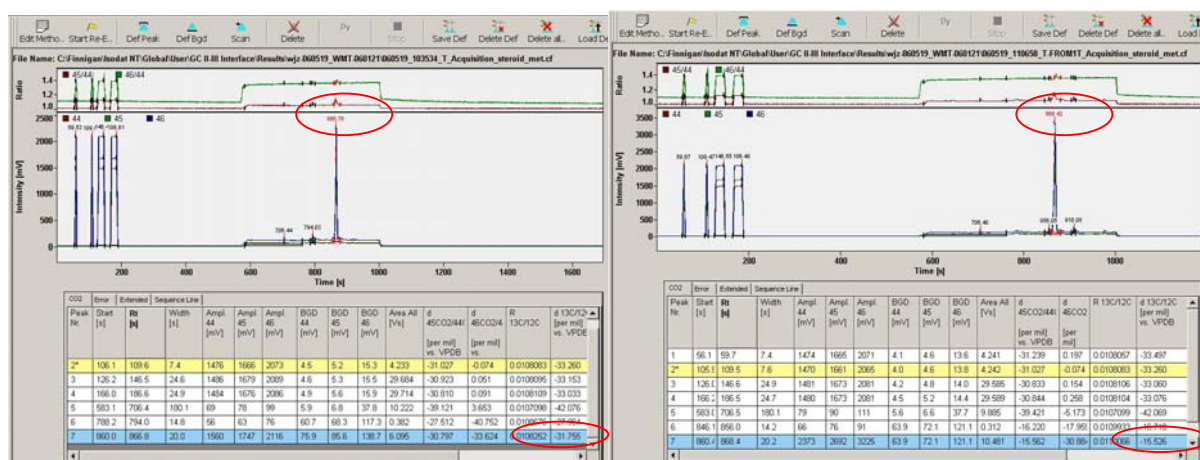


Fig. 4 GC/C/MS and the delta Values

Discussion

1) As we presented in Cologne Workshop years ago⁽¹⁾, the different delta values of testosterone were due to the different starting materials for the testosterone synthesis. Normally testosterone was synthesized from diosgenin, one of steroidal saponin, while 1-testosterone from tigogenin obtained from hemp. These two different kinds of plants determine the different delta values of the synthesized products. As the testosterone and 1-testosterone showed only the difference in position of one double bond, the testosterone as the by-product separated from 1-testosterone, of course, has the same delta value as 1-testosterone.

2) Though both delta values of the normal testosterone and that from 1-testosterone were out of the normal population reference range, the mixture of these both testosterones could show totally manipulated delta values with different proportion of these. Fig. 5 showed the GC/C/MS with the delta value of -25% in blue color for the manipulated testosterone.

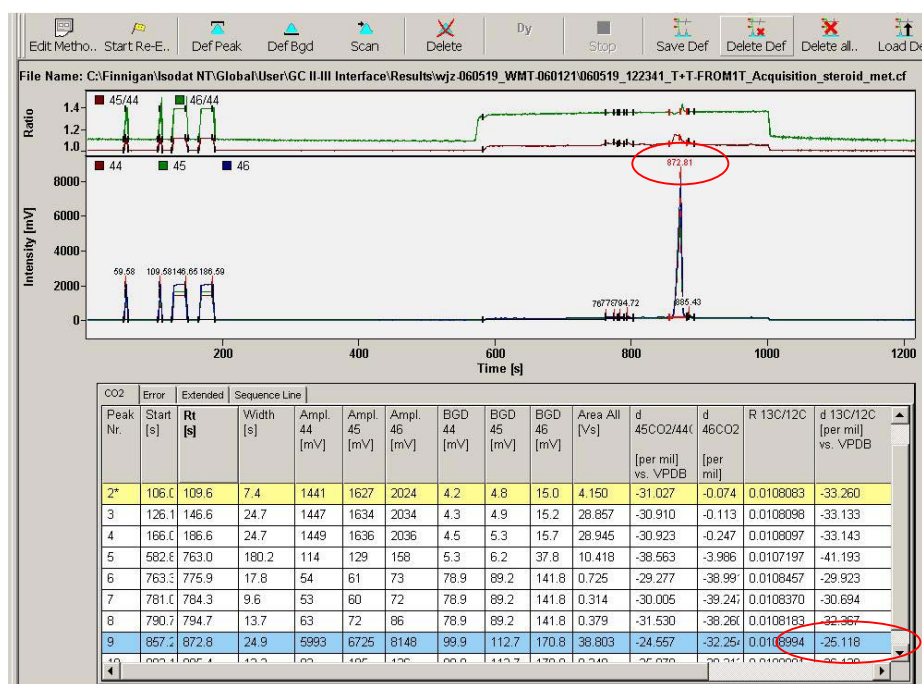


Fig. 5 GC/C/MS and delta Value of Mixed Testosterone

3) This finding presented in this paper confirms us that the targeted substance can not be confirmed as endogenous even though its delta values are very similar with the internal bio-markers. To make the conclusion for confirming no exogenous origin should be very careful and need further evidence. This finding does not mean any peradventure for concluding the administration of some precursors or some preparations of endogenous steroid with the criteria in WADA TD2004EAAS⁽⁴⁾.

Reference

- 1) Wang Jingzhu et al. δ values of different steroids, Recent advances in doping analysis (12) (2005) p.241
- 2) The NIST mass spectral program for the NIST/EPA/NIH mass spectral library, version 2.0 d build Apr. 26, 2005
- 3) E. Pretsch et al. Structure determination of organic compounds, Springer-Verlag (2000)
- 4) WADA Technical Document 2004TDEAAS

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