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Contribution of ion trap mass spectrometry in the determination of fragment origin for steroids

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Abstract

In doping control analysis the used technology is chromatography coupled with a mass spectrometer for screening analyses and for the confirmation purpose, by choosing the characteristic fragments for the target compounds. In order to improve the efficiency of anti-doping programs, laboratories have defined new analytical strategies like tandem mass spectrometry (MS-MS) as a good tool for improving sensitivity. Moreover, the MS/MS technique is used to study the behavior of the precursor ion and also to highlight the origin of some fragments conventionally known such as fragments issued from 17-oxo steroids.

Today, the ion trap technology is available, and it is used as a good confirmation tool. It provides a full mass spectrum that is induced, not by the molecular mass but by lower masses which lead to the characteristic fragments of the compound. The purpose of this study is to emphasize the MS/MS technique for lower masses of the 17-oxo steroids, 19-norandrosterone (19-NA), 19- norethiocholanolone (19-NE) and their deuterates, as well as to demonstrate that some fragments have different origins.

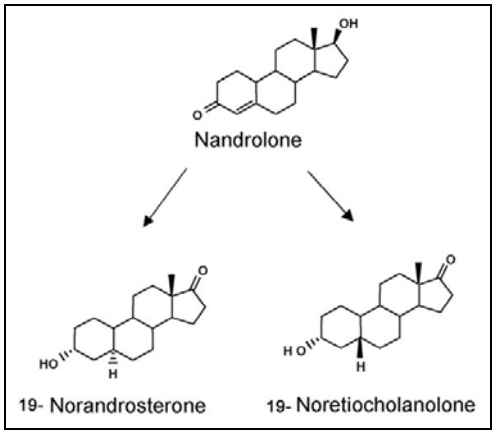

Fig.1. Metabolites pathway

Introduction

It is well known that silylated 17-oxo steroids produce a characteristic fragment m/z 169 indicated by $C_9H_{17}OSi$ as an elemental composition with bond cleavages in-between carbons 11-12, 13-14 and/or 14-15 with charge retention on the 17-OTMS part. However, the fragmentation of precursor ions of nandrolone metabolites, using tandem mass spectrometry,

indicates the presence of a second origin of the fragment ion formation m/z 169. In fact, the dissociation of ion m/z 225, resulting from the loss of 2*(HOTMS) from a precursor ion 405 m/z of the two nandrolone metabolites, indicates the presence of a fragment ion at m/z=169. These results suggest that ions m/z= 169 could be derived by the cleavage of the A-ring. The purpose of this work was to demonstrate a second origin of formation of such ions with m/z 169. Tandem mass spectrometry MS^n data revealed that an elementary equivalent ion of m/z 169 could derive from the cleavage of the A-ring. Moreover, the use of deuterated keto steroids, such as D_4 19-NA and D_4 19-NE, shows the presence of fragment 169 m/z. This investigation illustrates a non conventional formation of ion fragment 169 m/z using MS/MS technique.

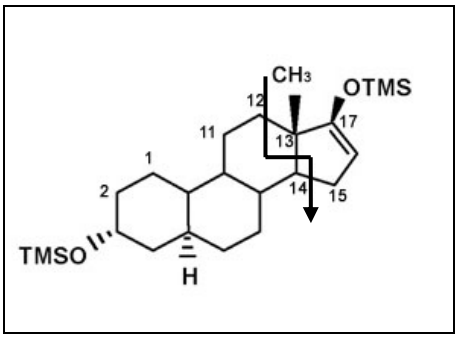

Fig.2. 19-Norandrosterone bis TMS

Materials and Methods

Materials

Norandrosterone (3α–hydroxy-5α-estran-17-one), noretiocholanolone (3α–hydroxy-5b-estran-17-one), 2,2,4,4 $^2H_4$ 19-NA (D_4 19-NA) and 2,2,4,4 $^2H_4$ 19-NE (D_4 19-NE) were obtained from the National Measurement Institute (NMI, Australia).

N-Methyl-N-trimethylsilyltrifluoacetamide (MSTFA), ammonium iodide and dithioerythritol were purchased from Sigma.

Sample preparation

All steroids samples are derivatised with MSTFA which is prepared at a final concentration of 50 ng/ml of MSTFA/NH_4I/Dithioerythrithol ((1000:1:2), v/w/w) and heated at 65˚C for 30 mn. Following the derivatisation, samples were injected into the GC/MS.

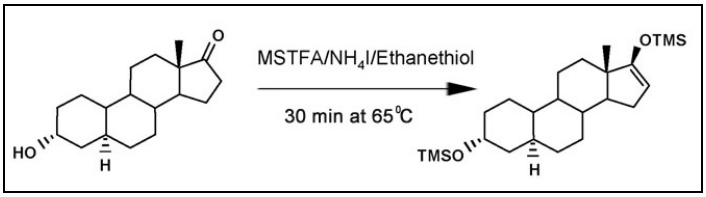

Fig. 3. Derivatisation with MSTFA/NH_4I/Dithioerythritol

Gas Chromatography/mass spectrometry

Analyses of synthetic samples, by GC/MS/MS, were performed on a VARIAN GC/ITD instrument consisting of a VARIAN 3800 GC coupled to a VARIAN SATURN 2000. Two microlitres of samples were injected in split mode 1/10 in the injector VARIAN 8200 and kept at 280˚C. A constant flow mode was used.

Injection parameters	Volume 2 µl, temperature 280 ˚C
Column	HP Ultra of methylsilicone 1; 25m ; 0.2 mm ID ; 0.11µm film thickness
Carrier gas	Helium, spilt 1/10, constant pressure 21.2 psi
Oven program	180˚C -- 15˚C / mn -- 270˚C -- 50˚C/mn -- 300˚C, final time 10 mn
Ionisation	70eV, EI
CID type	MS/MS/MS, non resonant
Ion trap temperature	200˚C
Interface temperature	300˚C

Results and discussion

On the one hand, it was demonstrated that when we use the multiple fragmentation method MS^n, the ion 225 m/z, generated by MS^3 after the successive dissociations of the major ion 405 m/z of the 19-NA and 19-NE, lead us to the appearance of the fragment with an m/z of 169. On the other hand, the use of deuterated homologous molecules (2,2,4,4-$^2H_4$ 19-NA and 2,2,4,4-$^2H_4$ 19-NE) gives us the same result.

In short, we see that the ion at m/z 169 can be obtained through a different fragmentation scenario. However, the study of the fragmentation of the 229 m/z ion, after isolation and dissociation, leads to the production of the ion fragment 169 m/z. This result suggests possible bond-cleavages between carbon 1-10 and carbon 4-5.

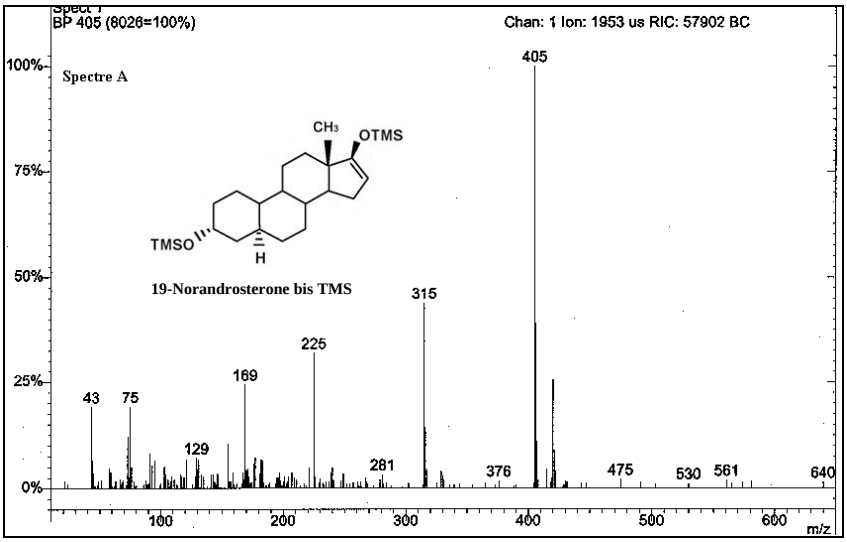

Fig.4. EI Spectrum of 19-Norandrosterone bis TMS

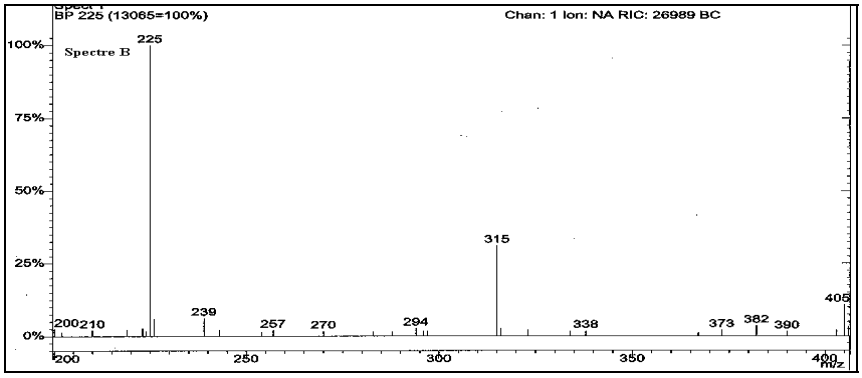

Fig.5. MS/MS spectrum of 19-Norandrosterone PI 405

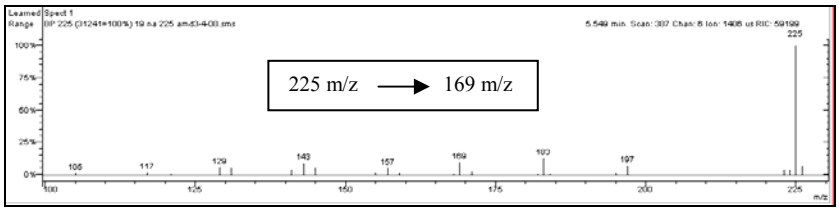

Fig.6. MS/MS Spectrum of 19-NA PI 225

References

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