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RECENT ADVANCES IN DOPING ANALYSIS (4)

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Occurrence of 19-Nordehydro-Androsterone/Etiocholanolone in Nandrolone Positive Specimens

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Introduction

Nandrolone is the most frequently abused steroid and the one most frequently found in doping control. The metabolism was investigated in 1958 (1), the main metabolites have been confirmed, their structure was elucidated by synthesis (2). These data along with unpublished observations by Schänzer and Donike have been summarized in a recent comprehensive review by Schänzer (3). Routine detection of nandrolone major metabolites, 5α-estran-3α-ol-17-one (norandrosterone), 5β-estran-3α-ol-17-one (noretiocholanolone) and 5α-estran-3β-ol-17-one (norepiandrosterone), is reliable and has never created analytical problems.

However, substantial amounts of unusual metabolites are observed in our laboratory for routine samples containing large amounts of 19-norandrosterone and 19-noretiocholanolone.

Materials and Methods

Nandrolone excretion urines were routine specimens.
19-Norandrosterone (5α-estran-3α-ol-17-one), 19-noretiocholanolone (5β-estran-3α-ol-17-one) and 4-estren-3,17-dione are purchased from Research Plus, Inc., 19-nortestosterone from Sigma.

Assay: Routine screening procedures for conjugated, total and free steroids were applied as follows:

Sample preparation

4 mL of urine are extracted in a glass tube with 4 mL of diethyl ether for steroid free fraction. Ether is separated and evaporated to dryness. Remaining urine is briefly swept with dry nitrogen to remove residual ether and 1 mL of acetate buffer/β-glucuronidaze/ISTD mixture is added and incubated for 3 hours at 52°C.

After hydrolysis the mixture is applied to C18 solid phase extraction column prewashed with

3 mL methanol and 3 mL water. The column is washed with 2 mL 30% acetonitrile in water, steroids eluted with 3 ml methanol. The methanolic eluate is evaporated to dryness.

Derivatization

The dry residues are derivatized with one of the follows:

75 µL of MSTFA/NH4I/Dithioerythritol 1000:2:3, heated for 15 minutes at 70°C

75 µL of MSTFA, heated for 15 minutes at 70°C

50 µL of 4% methoxyamine hydrochloride in pyridine for 30 minutes at 70°C and after pyridine evaporation with 100µL of TSIM at 70°C for 15 minutes

GC/MS parameters

GC/MS HP 5890/5970

column:	HP-1 fused silica, crosslinked methylsilicon, 17m, 0.2mm i.D., 0.11µm film thickness
temperature program:	180°C (0.3 min); 3°C/min - 231°C; 30°C/min - 310°C (1.07 min)

Results and Discussion

Selected ion chromatograms showing urinary nandrolone excretion patterns are presented in Figure 1 for free and in Figure 2 for conjugated fractions. Unusual (-2H) metabolites are detected in both free fraction and at higher concentration in conjugated steroid fraction with a ratio of approximately 6:1. Their enol-TMS EI mass spectra are shown in Figures 3 and 4. These spectra are similar to those of 19-norandrosterone and 19-noretiocholanolone with most ions being 2 mass units less. Based upon comparison of mono-TMS, enol-TMS and MO-TMS derivatives we suggest 19-nor-1,2-en androsterone/ etiocholanolone structures for these compounds. Possible nandrolone metabolite, 19-nor-androstan-3,17-dione, having the same molecular weight of enol-TMS derivative, is not found.

19-Norandrosterone and 19-noretiocholanolone are fully conjugated and appear as expected in conjugated fraction only as does the parent 19-nortestosterone. On the other hand, free fraction contains 4-estren-3,17-dione, apparently a minor metabolite of 19-nortestosterone. The structure of this dione is proved by comparison with a standard, 4-estren-3,17-dione (TMS, enol-TMS and MO-TMS derivatives).

We attempted to incubate negative urine spiked with standards of 19-norandrosterone and 19-noretiocholanolone for several weeks at room temperature with different contaminants such as yeast with visible signs of bacteria growth. However, no clear evidence was obtained that bacterial contamination or any other exogenously catalyzed alteration is the source of the dehydro products; their appearance coincides only with the finding of large amounts of normal metabolites.

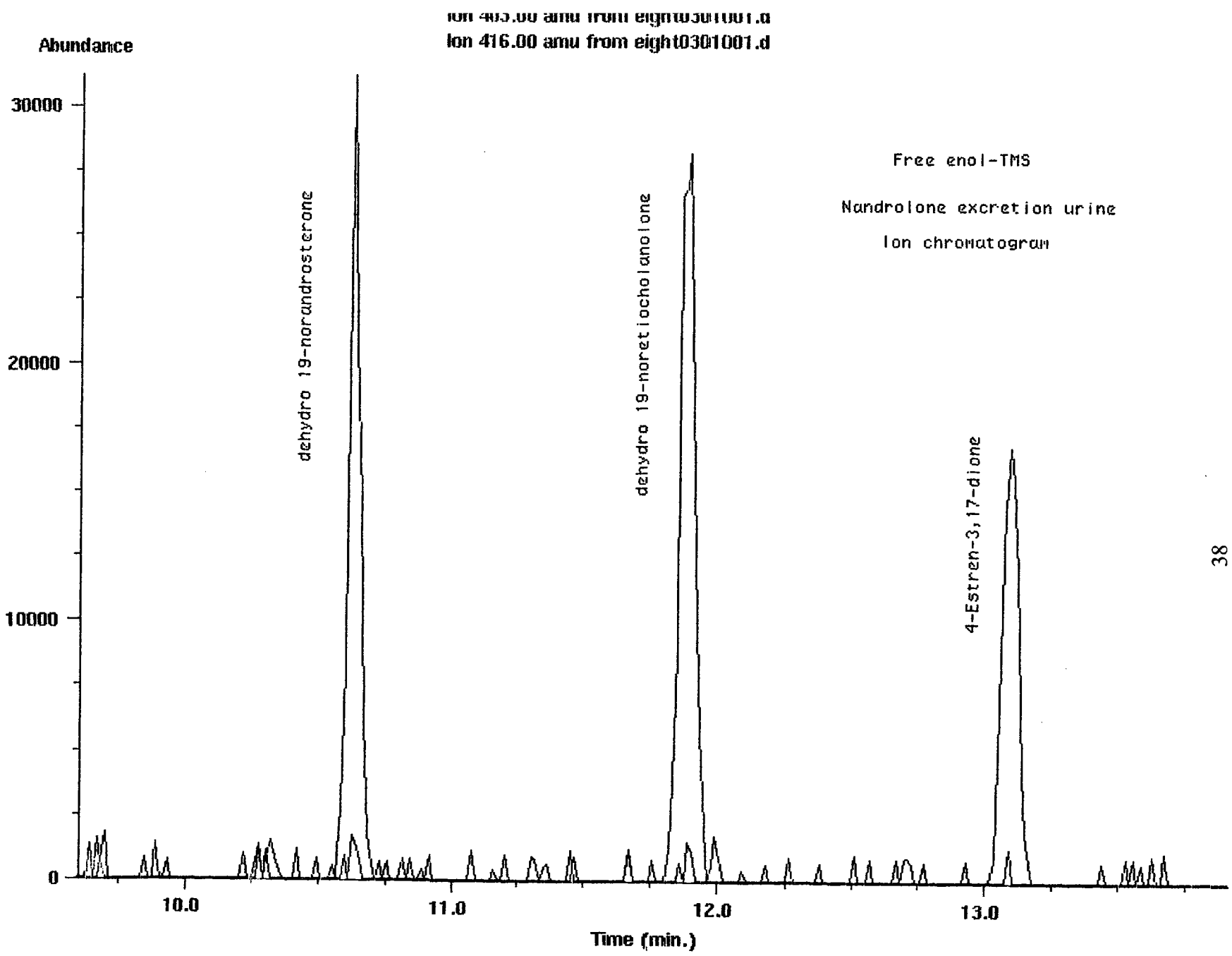
During the seven month study, dehydro nandrolone metabolites were monitored in our laboratory for routine patient's samples along with the normal metabolites, 19-norandrosterone and 19-noretiocholanolone. Of the total sixty nine nandrolone positive specimens during this period, forty four contained various amounts of dehydro products.

References:

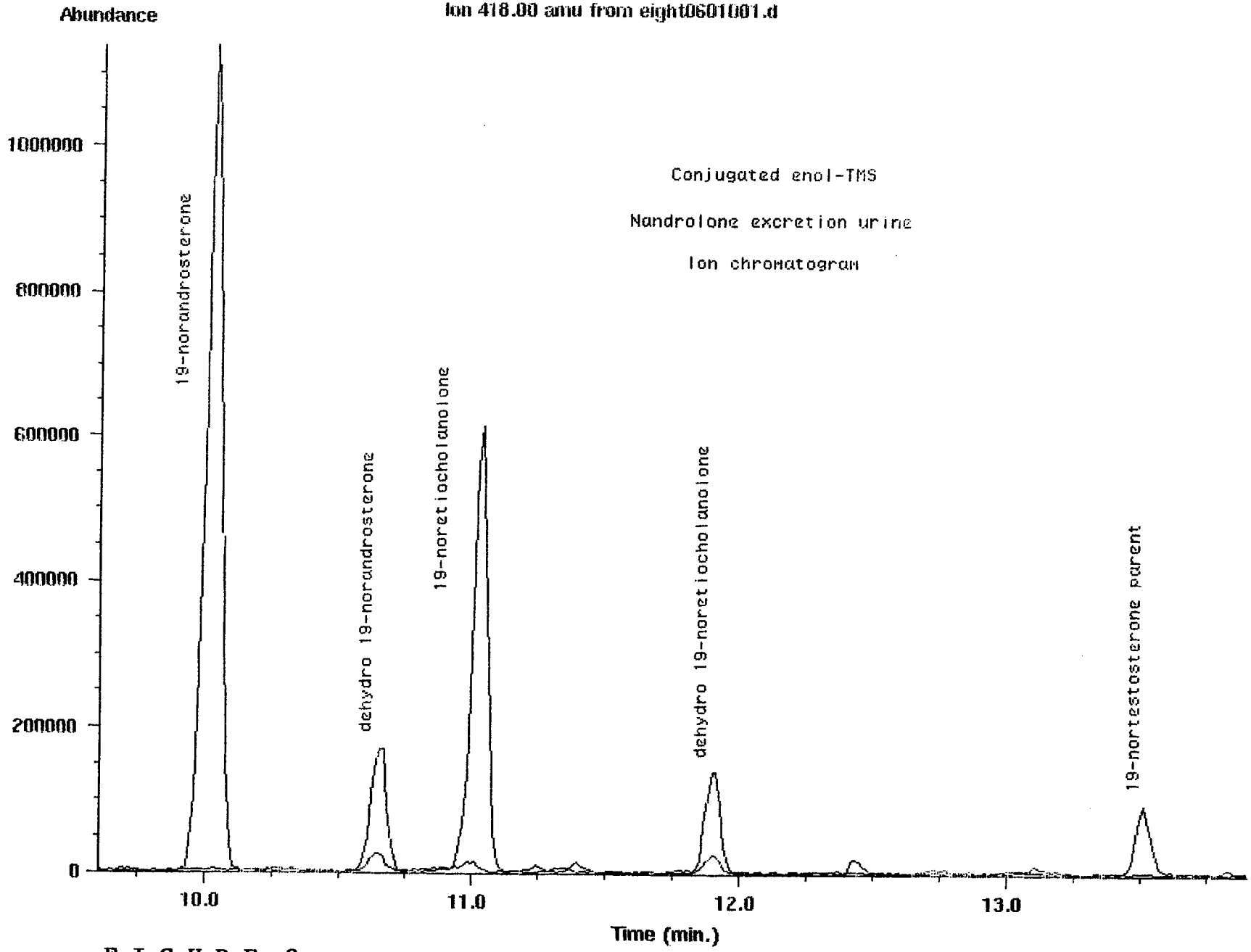
1. Engel LL, Alexander J, Wheeler M. Urinary metabolites of administered 19-nortestosterone. *J Biol Chem*, **231** (1958) 159-165.

2. Kupfer D, Forchielli E, Dorfman RI. 3α-Hydroxy-19-nor-5α-androstan-17-one and 19-nor-5α-androstan-3α,17β-diol. *J Org Chem*, **25** (1960) 1674-1675.

3. Schänzer W. Metabolism of anabolic androgenic steroids. *Clin Chem*, **42** (1996) 1001-1020.


Abundance
Ion 416.00 amu from eight0301001.d
30000
20000
10000
0
dehydro 19-norandrosterone
dehydro 19-noretiocholanolone
4-Estren-3,17-dione
Free enol-TMS
Nandrolone excretion urine
Ion chromatogram
10.0
11.0
12.0
13.0
Time (min.)

F I G U R E 1


Abundance
Ion 418.00 amu from eight0601001.d
1000000
800000
600000
400000
200000
0
19-norandrosterone
dehydro 19-norandrosterone
19-noretiocholanolone
dehydro 19-noretiocholanolone
19-nortestosterone parent
Conjugated enol-TMS
Nandrolone excretion urine
Ion chromatogram
10.0
11.0
12.0
13.0
Time (min.)

F I G U R E 2

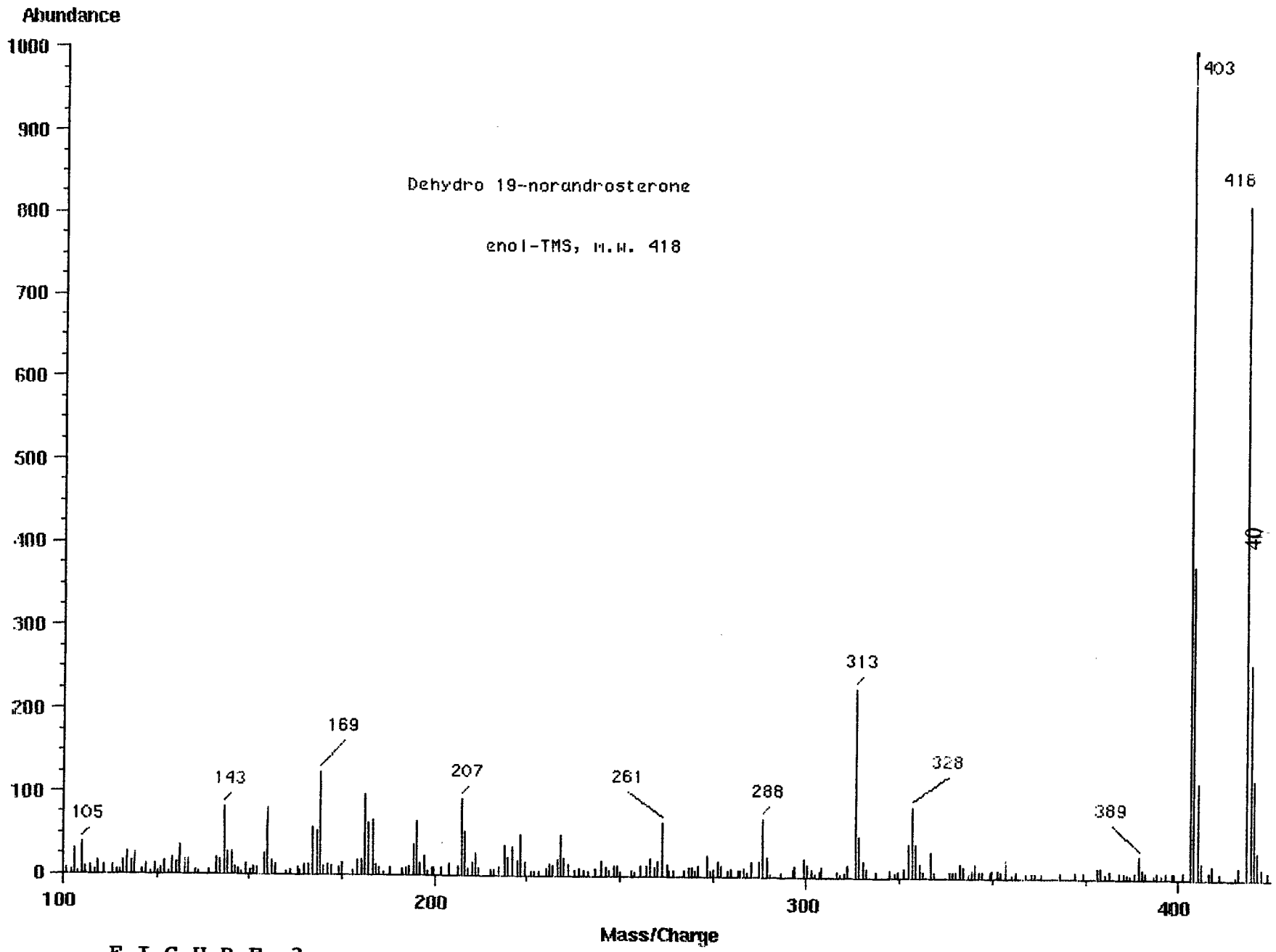
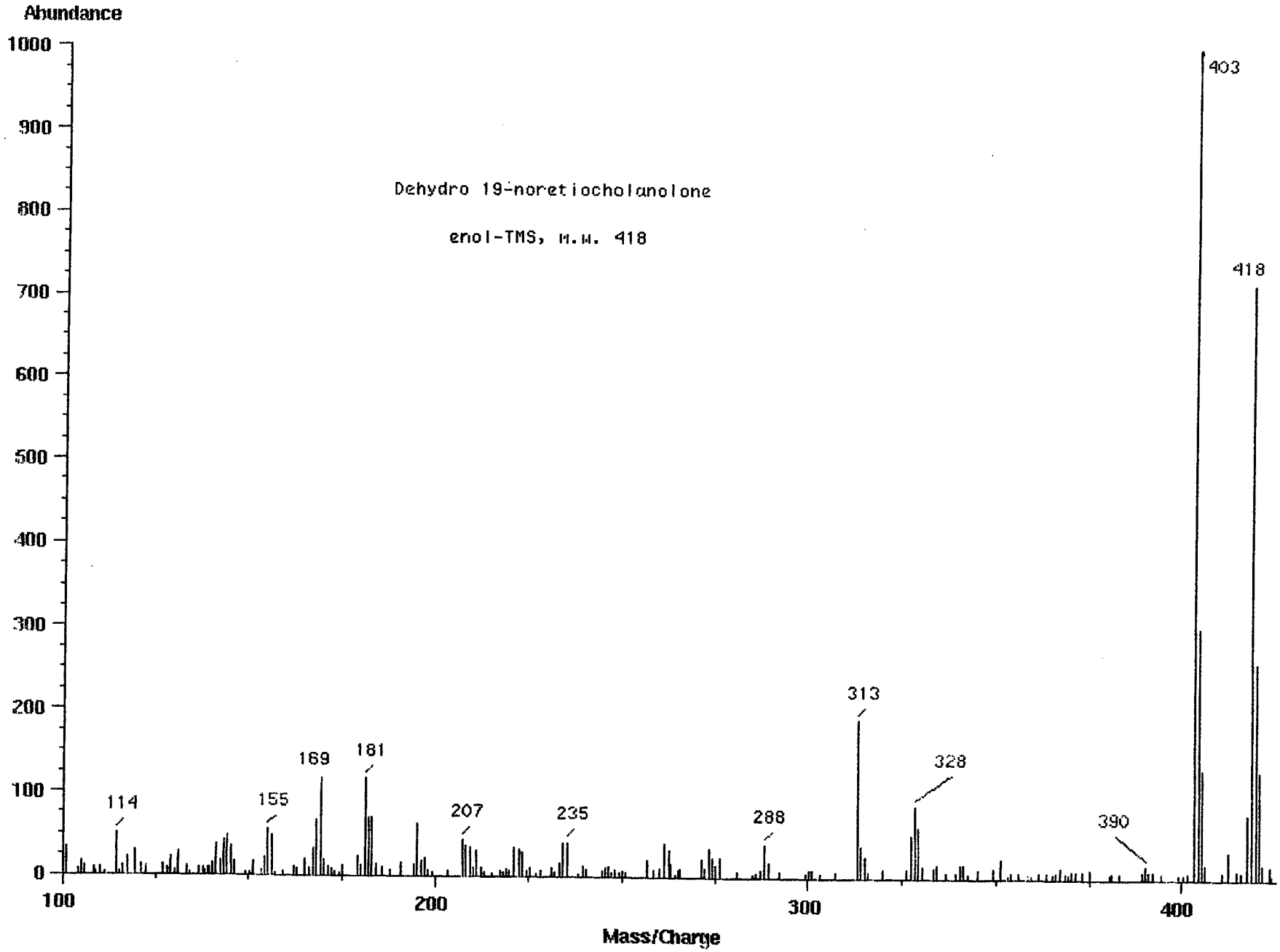

Abundance
1000
900
800
700
600
500
400
300
200
100
0
Dehydro 19-norandrosterone
enol-TMS, M.W. 418
403
416
40
313
169
207
143
261
328
288
105
389
100
200
300
400
Mass/Charge

FIGURE 3


Abundance
1000
900
800
700
600
500
400
300
200
100
0
Dehydro 19-noretiocholanolone
enol-TMS, M.W. 418
114
155
169
181
207
235
288
313
328
390
403
418
100
200
300
400
Mass/Charge

F I G U R E 4