Reprint from

# RECENT ADVANCES IN DOPING ANALYSIS (6)

W. Schänzer
H. Geyer
A. Gotzmann
U. Mareck-Engelke
(Editors)

Sport und Buch Strauß, Köln, 1999

---

XIN LIU, MOUTIAN WU, YINONG ZHANG, CHANGJIU ZHANG:
Application of TSQ-7000 in Doping Control Analysis
In: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck-Engelke (eds.) Recent advances in doping analysis (6). Sport und Buch Strauß, Köln, (1999) 257-267

Xin Liu, Moutian Wu *, Yinong Zhang and Changjiu Zhang

# Application of TSQ-7000 in Doping Control Analysis

China Doping Control Center, Beijing, China

## Abstract

This paper is focused on the application of TSQ-7000 in doping control analysis. The feasibility of using TSQ-7000 to analyze the urine sample with low concentrations is investigated. With Immunoanffinity Chromatograph for samples purification TSQ-7000 detected the 5 substances with the level of about 2 ng/ml successfully. The reproducibility of the method and its related parameters are discussed. In daughter mode the mass spectra obtained from TSQ-7000 could supply reasonable fragments for identification and for the study on the mechanism of fragmentation pathway.

## 1. INTRODUCTION

The TSQ-7000 is a triple-stage quadrupole mass spectrometer. It is reported that its longer quadrupoles improve the resolution, a non-linear octapole collision cell provides efficient collision-induced dissociation (CID) and an advanced 15 kV conversion dynode detector enhances the sensitivity.

The purpose of our study is focused on the application of TSQ-7000 in doping control analysis, especially in analyzing the urine samples with low concentrations of the 5 substances with the MS/MS features.

## 2. EXPERIMENTTAL

### 2.1 The Instrument Parameters

A gas chromatograph model 5890 series II plus (Hewlett-Packard) was connected to the TSQ-

*: corresponding author

7000 (Finnigan). The separation was carried out with a HP 1 column (17m, 0.2 mm I.D., 0.11 μm filmthickness). The injector operated in split mode (1:18 split ratio) and the interface were both maintained at 280°C. The temperature program was: initial 180°C, rate 1: 3.3°C/min to 231°C, rate 2: 30°C/min to 310°C and maintained for 2min at 310°C. Helium was used as a carrier gas at flow rate 0.8ml/min (at 180°C). The pressure was kept constant automatically during the running. The transferline was set at 280°C. For GC-MS/MS the first MS worked in EI mode, (electron energy 70 eV). Manifold was set at 70°C, ion source at 180 °C, conversion dynode at 13 kV, scan rate of the mass spectra at 500 a.m.u./s, dwell time in RIM mode at 100 ms/per ion. Emission current was 400 μA, electron multiplier voltage in the range of 1100~1300. PFTBA was used as the calibrator for tuning. Collision gas was argon gas at 1.8~2 mTorr. Collision energy depended on substances in the range of -5 ~ -30 V. The correct factor (MSMSC) was 0.7~1.0.

2.3 Extraction and Derivatization

as Procedure IV for total fraction.

## 3. RESULTS AND DISCUSSION

3.1 Analyzing the Urine Sample with Low Concentrations

A standards mixture solution containing clenbuterol, 19-norandrsterone, 17α-methyl-5β-androst-1-ene-3α,17β-diol(Epimetendiol), 17α-methyl-5α-androstan-3α,17β-diol (5α-THMT) and 3'-OH-stanozolol in the level of 2 ng/μl was used to check the retention times and the relative abundance. A urine sample was spiked with the 5 substances in the level of 1 ng/ml urine. Some different blank urines were used to investigate the interference of biomatrix. The TSQ-7000 was operated in MS/MS daughter mode with Q3 (the second MS) selected reaction monitoring. The MS detection parameters are listed in the following table 1.

Tab.1 The Detection Parameters

| Substance Name | Parent Ion | Daughter Ions Monitored | Energy | Retention |
|---|---|---|---|---|
| Clenbuterol | 335 | 227 262 300 335 | -15 | 4.60 |
| 19-Norandrosterone | 405 | 169 225 315 405 | -17 | 9.40 |
| Epimetenediol | 358 | 196 253 301 358 | -20 | 9.70 |
| 5α-THMT | 435 | 173 255 345 435 | -17 | 12.60 |
| 3'-OH-Stanozolol | 545 | 147 387 455 545 | -30 | 18.40 |

The ion chromatograms of the 5 standards (upper) and the spiked urines (2 ng/mL, lower) were showed in Fig.1, respectively. Their S/N values were larger than 5 even with the level of 2 ng/mL urine. The blank urine showed no interference in the retention time range of the first 4 substances. Though the signal of 3′-OH-stanozolol could be seen in the ions chromatogram some blank urines showed also a signal with the retention time due to the interfering of the biomatrix (see the chromatograms in Fig.2). Though the tandem MS/MS could greatly eliminate the interference of biomatrix, a further purification procedure for 3′-OH-stanozolol was needed in this case. With ICA procedure using a commercial gel the signal of 3′-OH-stanozolol in the level of 2 ng/ml urine appeared in the TIC chromatogram while no signal in the same rentention time range appeared in the chromatogram of the blank urine (see Fig.3).

For reference the daughter mass spectra obtained in our experiment conditions with Q3 scan are listed in Fig.4. Compared with other daughter mass spectra these daughter mass spectra from TSQ-7000 supply more reasonable fragments that may be very helpful for identification.

3.2 Reproducibility and Related Parameters

Standards mixtures with the 5 substances in a series of concentrations of 0.2 ng/μl, 1.0 ng/μl and 10.0 ng/μl were prepared for the test of reproducibility of relative abundance in daughter mass spectra. After 8 injections in a day with a same tune table the relative abundances were obtained through normalization without background subtraction. The statistical results are listed in Table 2.

The CV% values of relative abundance of all daughter ions monitored were less than 9%, Most of them were less than 5%. With the concentration increased the CV% values decreased. It is believed that when the biomatrix is introduced the CV% values grow. It is believed also that when the ion volume gets dirty the reproducibility of the relative abundance becomes worse.

As we know that in the tune file of GC-MSD (e.g. HP 5972) one value of a parameter is set for optimization of all three tune ions m/z 69, 219 and 502. But tuning in TSQ-7000 is operated on a kinetic tune table. It means that different values can be chosen for different tune ions for a same parameter. This technique supplies more possibilities to achieve different specific aims. In this case the tune table plays an important role in the reproducibility of relative abundance of mass spectra. Although the auto tune was always used for tuning, the mass spectra could be changed due to different tune values and other parameters. The mass spectra of 17α-methyl-5α-androstan-3α,17β-diol obtained on Nov. 1997 (showed in Fig.5) and on Sep. 1997 (see Fig.4) respectively showed some different ratio and fragments. When the tune values were kept constant the same mass spectra could be obtained even after a lapse of

several months. The chromatogram and the daughter mass spectrum of 19-Norandrosterone were obtained on 25 May, 1998 from our routine work in Fig.6. This mass spectrum is nearly identical with the mass spectrum in Fig.4 obtained from pure standard.

Tab. 2 Reproducibility of Relative Abundance of MS/MS in Daughter Mode

| Clenbuterol | *X* | | | *S* | | CV% | |
|---|---|---|---|---|---|---|---|
| Parent 335 | m/z 227 | m/z 262 | m/z 300 | m/z 262 | m/z 300 | m/z 262 | m/z 300 |
| 0.2 ng/μl | 100 | 32.75 | 71.30 | 0.499 | 1.012 | 1.37 | 1.42 |
| 1.0 ng/μl | 100 | 30.62 | 71.96 | 0.686 | 0.838 | 2.24 | 1.16 |
| 10.0 ng/μl | 100 | 32.91 | 74.55 | 0.087 | 0.583 | 0.26 | 0.78 |
| Norandrosterone | *X* | | | *S* | | CV% | |
| Parent 405 | m/z 169 | m/z 225 | m/z 315 | m/z 169 | m/z 315 | m/z 169 | m/z 315 |
| 0.2 ng/μl | 61.70 | 100 | 21.16 | 1.046 | 0.799 | 1.70 | 3.78 |
| 1.0 ng/μl | 58.64 | 100 | 21.19 | 1.573 | 1.344 | 2.68 | 6.30 |
| 10.0 ng/μl | 59.93 | 100 | 23.97 | 0.426 | 0.193 | 0.71 | 0.80 |
| Epimetenediol | *X* | | | *S* | | CV% | |
| Parent 358 | m/z 253 | m/z 301 | m/z 343 | m/z 253 | m/z 343 | m/z 253 | m/z 343 |
| 0.2 ng/μl | 6.88 | 100 | 6.22 | 0.345 | 0.340 | 5.01 | 5.47 |
| 1.0 ng/μl | 6.20 | 100 | 5.97 | 0.112 | 0.219 | 1.81 | 3.67 |
| 10.0 ng/μl | 7.41 | 100 | 7.19 | 0.092 | 0.117 | 1.24 | 1.63 |
| 5α-THMT | *X* | | | *S* | | CV% | |
| Parent 435 | m/z 173 | m/z 225 | m/z 345 | m/z 173 | m/z 225 | m/z 173 | m/z 225 |
| 0.2 ng/μl | 14.46 | 89.69 | 100 | 0.402 | 1.119 | 2.78 | 1.25 |
| 1.0 ng/μl | 11.45 | 82.85 | 100 | 0.811 | 4.002 | 7.08 | 4.83 |
| 10.0 ng/μl | 12.56 | 82.61 | 100 | 0.454 | 1.714 | 3.61 | 2.07 |
| 3′-OH-Stanozolol | *X* | | | *S* | | CV% | |
| Parent 545 | m/z 147 | m/z 387 | m/z 455 | m/z 387 | m/z 455 | m/z 387 | m/z 455 |
| 0.2 ng/μl | 100 | 8.42 | 33.07 | 0.753 | 1.906 | 8.94 | 5.76 |
| 1.0 ng/μl | 100 | 7.09 | 34.97 | 0.239 | 1.076 | 3.37 | 3.08 |
| 10.0 ng/μl | 100 | 5.70 | 34.78 | 0.152 | 0.768 | 2.67 | 2.21 |

3.3 Other Applications in Doping Control Analysis

The determination of T/E ratio is sometimes problematic due to the interference of the biomatrix with the ion m/z 432. We have had a case of elevated T/E ratio, but we could not quantitate the T/E ratio precisely with the routine procedure because of the interference (see Fig.7). After reextraction the T/E ratio was determined with MSD. On the other hand, MS/MS

was used to quantitate the T/E ratio of the urine sample without any purification. The ion chromatogram in daughter mode (parent ion m/z 432 and monitored ion m/z 209) and a daughter mass spectrum

Of epitestosterone in Fig.8 shows a clean background. The T/E ratio values obtained from MSD with purification and from MS/MS without purification were quite same.

Testosterone and epi-testosterone are episomers. There are only some slight differences in their EI mass spectra of TMS derivatives. But the daughter mass spectra of the molecular ion m/z 432 are quite different (see Fig.9). With the increase of collision energy the differences between the two mass spectra of testosterone and epi-testosterone TMS derivatives became bigger. Sometimes the ratio of peak areas in ion chromatogram is different from that of the concentrations of testosterone- and epi-testosterone-TMS derivatives. It may be caused by the different mechanism of their fragmentation.

MS/MS can be used to study the structures and the fragmentation pathway. We have tried to use MS/MS to study the mechanism of fragmentation pathway of 5α- and 5β-isomers, 17α- and 17β-isomers.

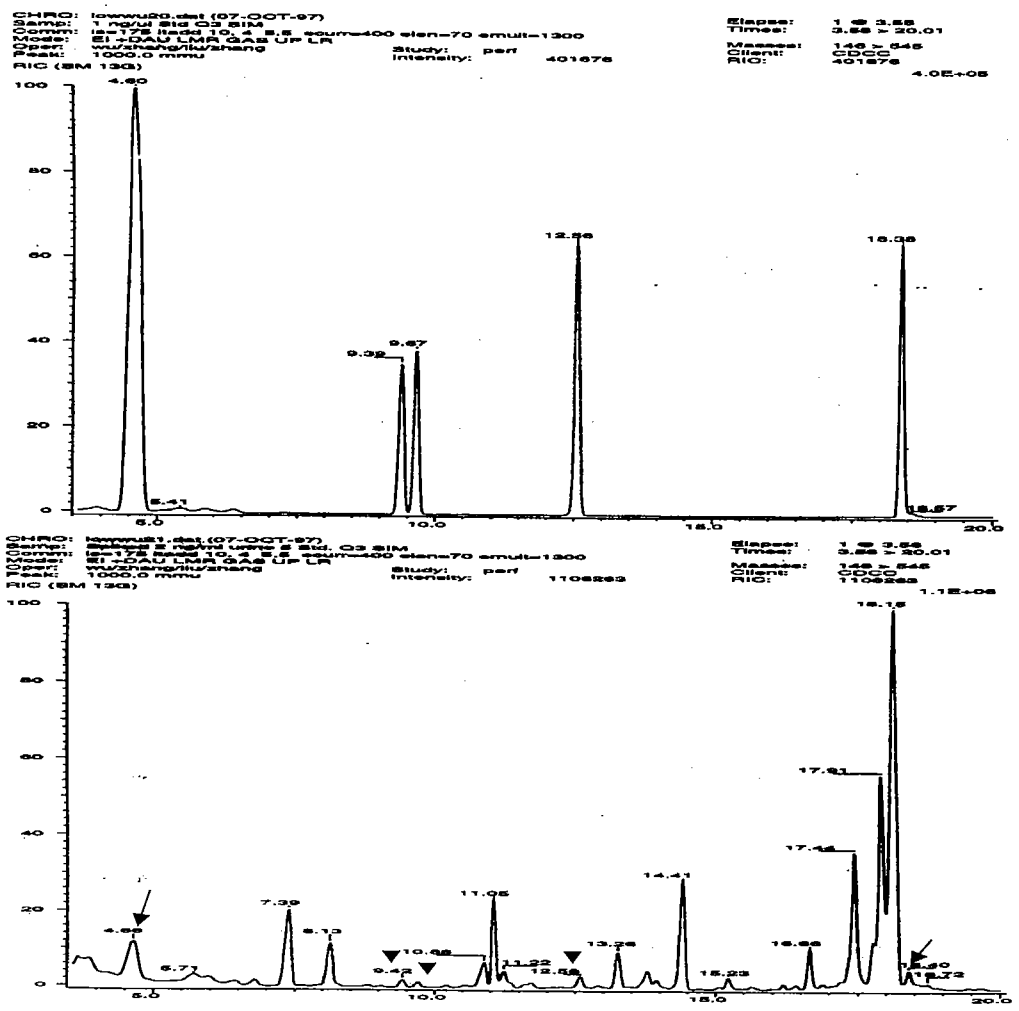

Fig.1 Ions Chromatograms of 5 Standards (upper) and Spiked Urine with the 5 Standards(lower)

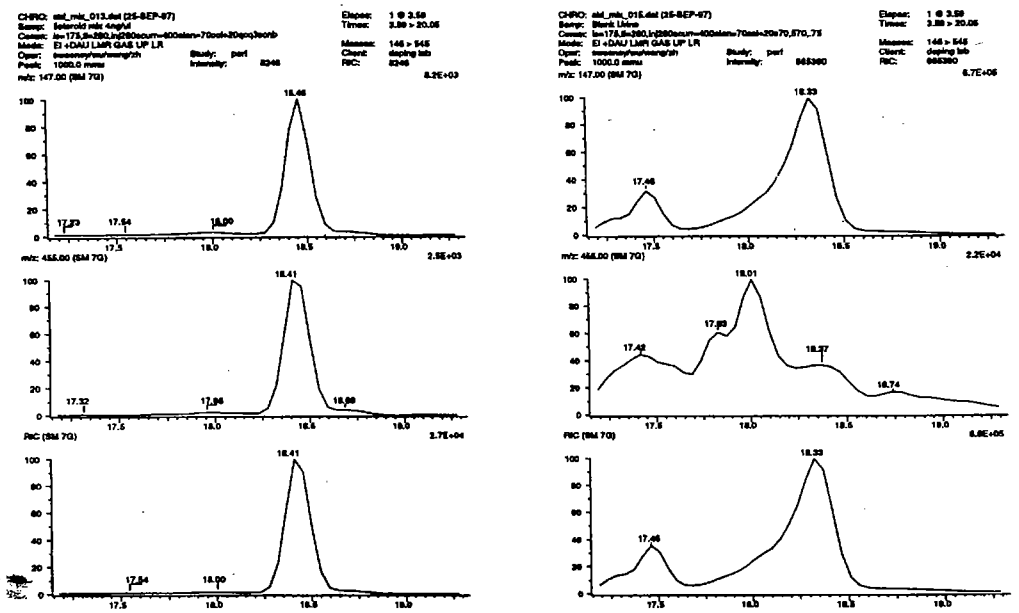

Fig.2 The Chromatograms of the Standard of 3'-OH-Stanozolol and a Blank Urine

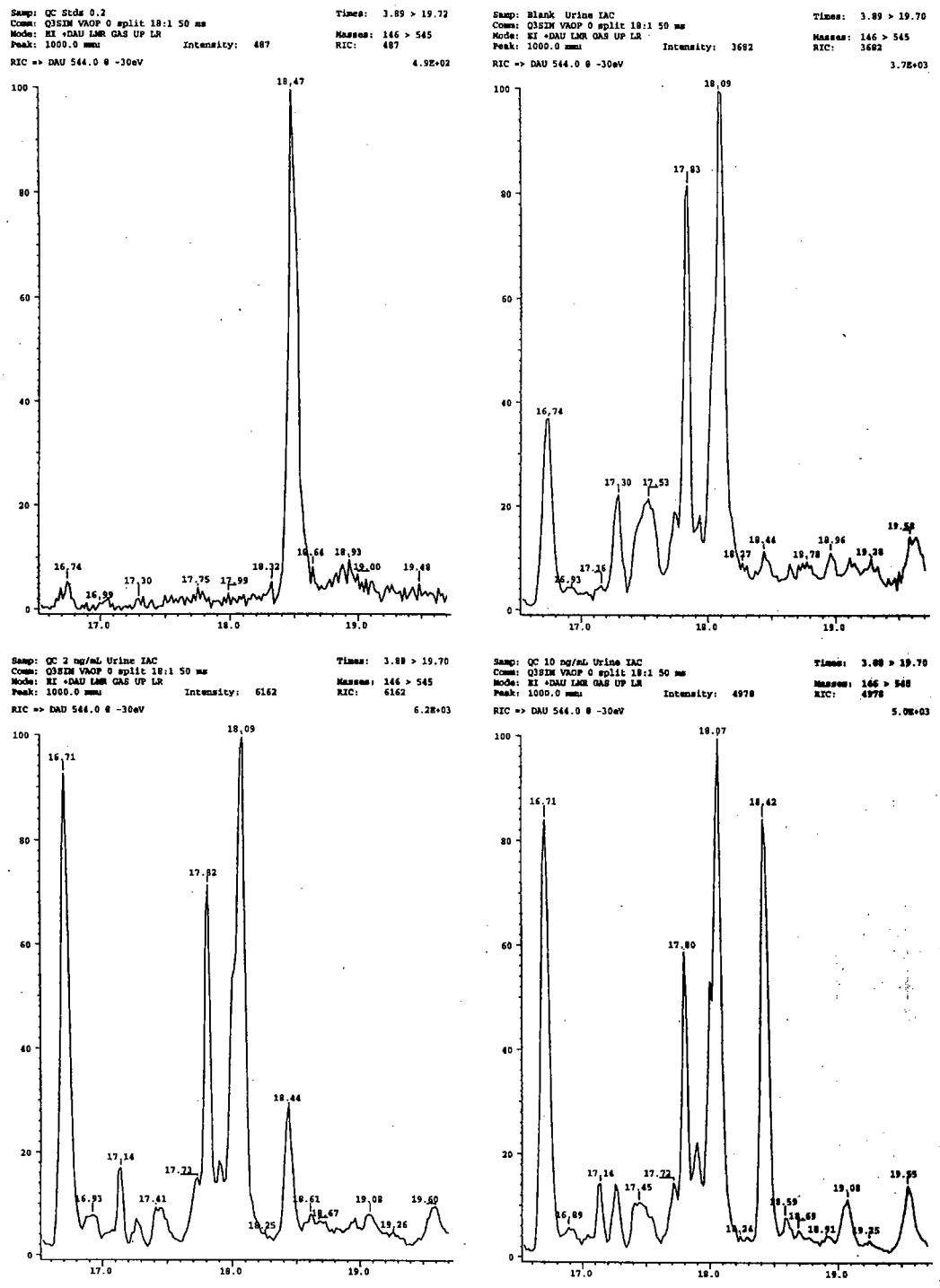


Fig.3 The Ion Chromatograms of 3'-Stanozolol, Blank Urine and Spiked Urine with IAC

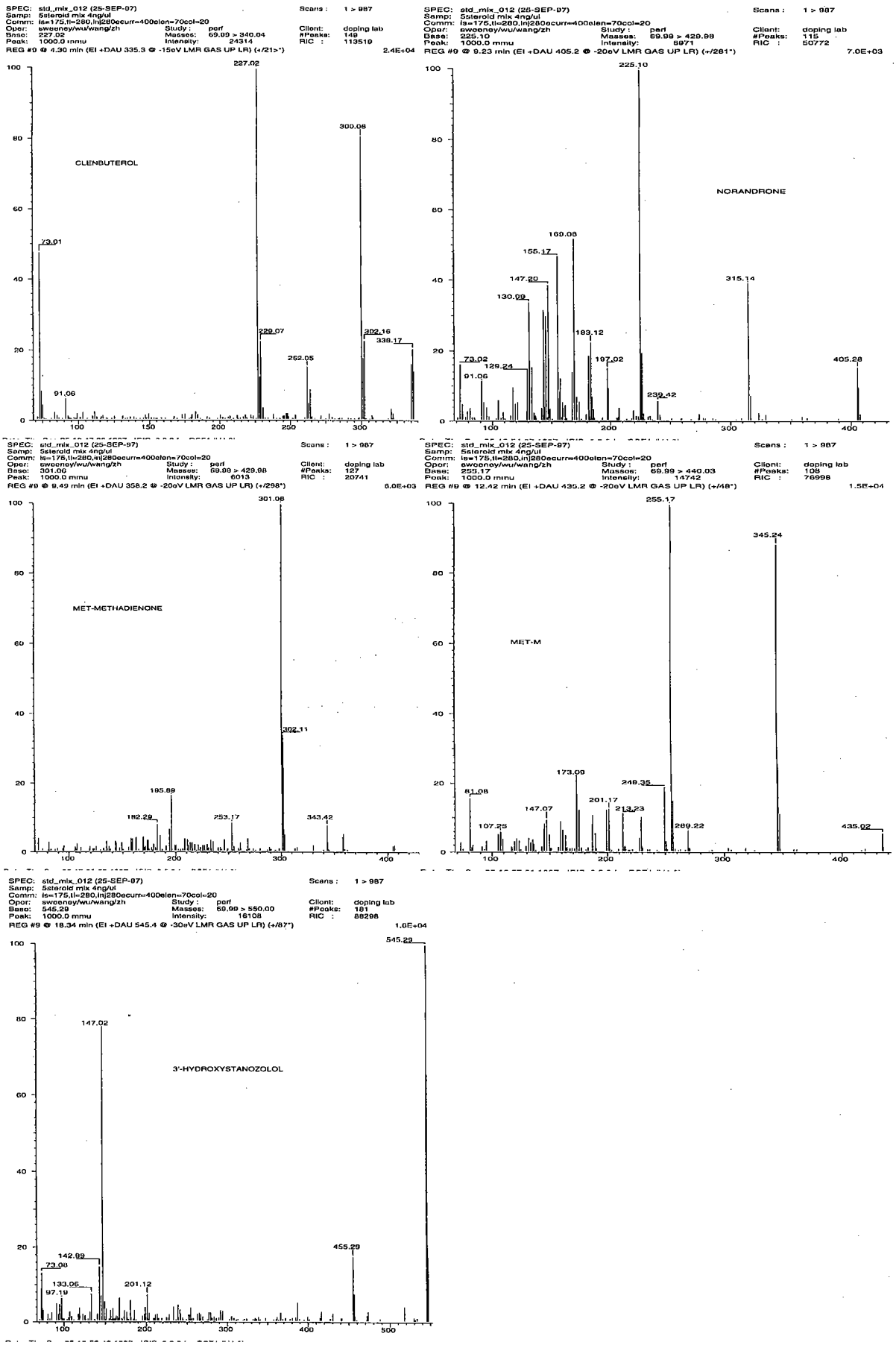


Fig.4 The Daughter Mass Spectra of the 5 Standards

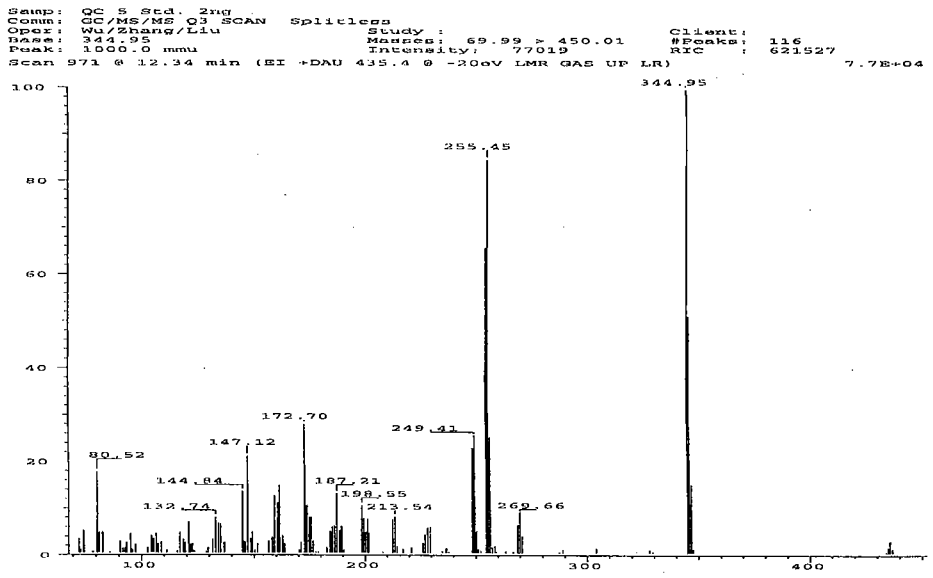


Fig.5 The Daughter Mass Spectrum Changed by Tune Table

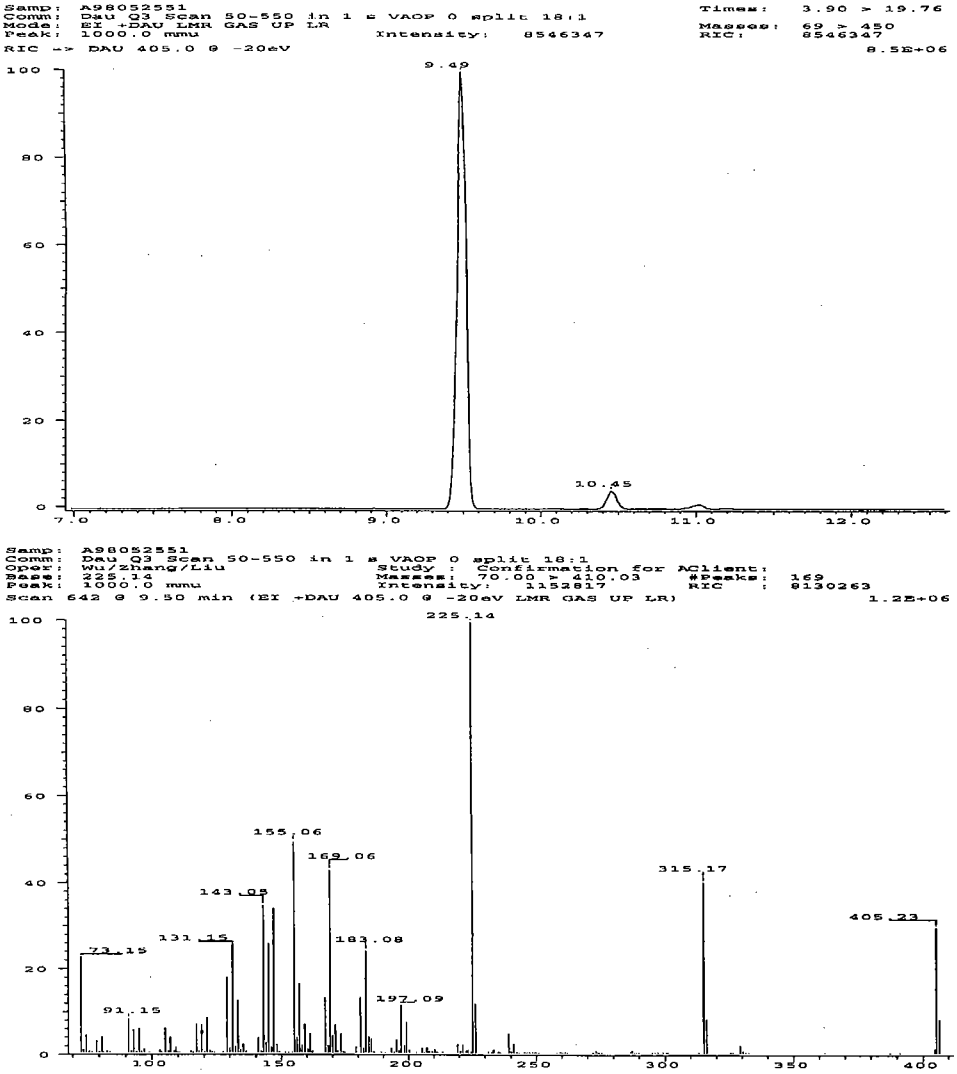


Fig.6 The Chromatogram and Daughter Mass Spectrum of a Nandrolone Positive Urine

```
File        : C:\HPCHEM\1\DATA\980529\E29-A01A.D
Operator    : zhang liu wu
Acquired    : 29 May 98  12:44      using AcqMethod 97PERF
Instrument :    GC/MS Ins
Sample Name: 98052512
Misc Info   :
Vial Number: 3
```

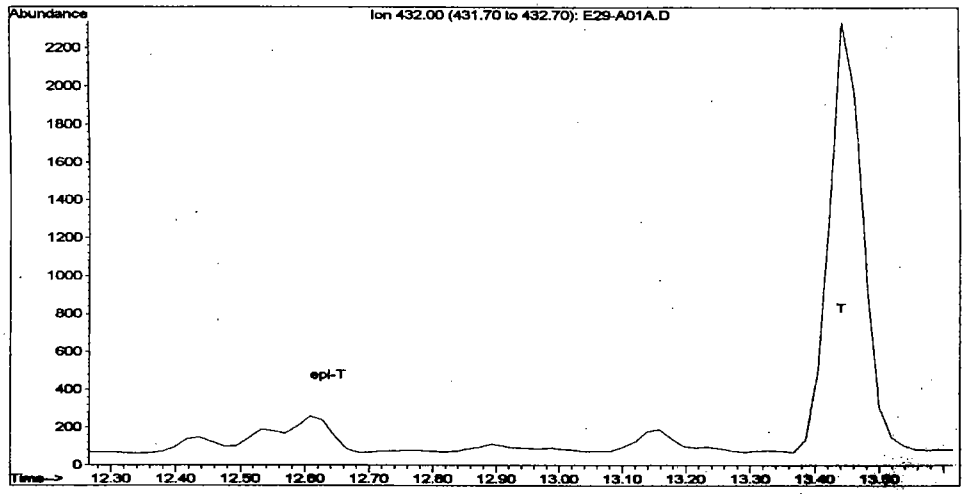


Fig.7 The Interference from Biomatrix for Epitestosterone

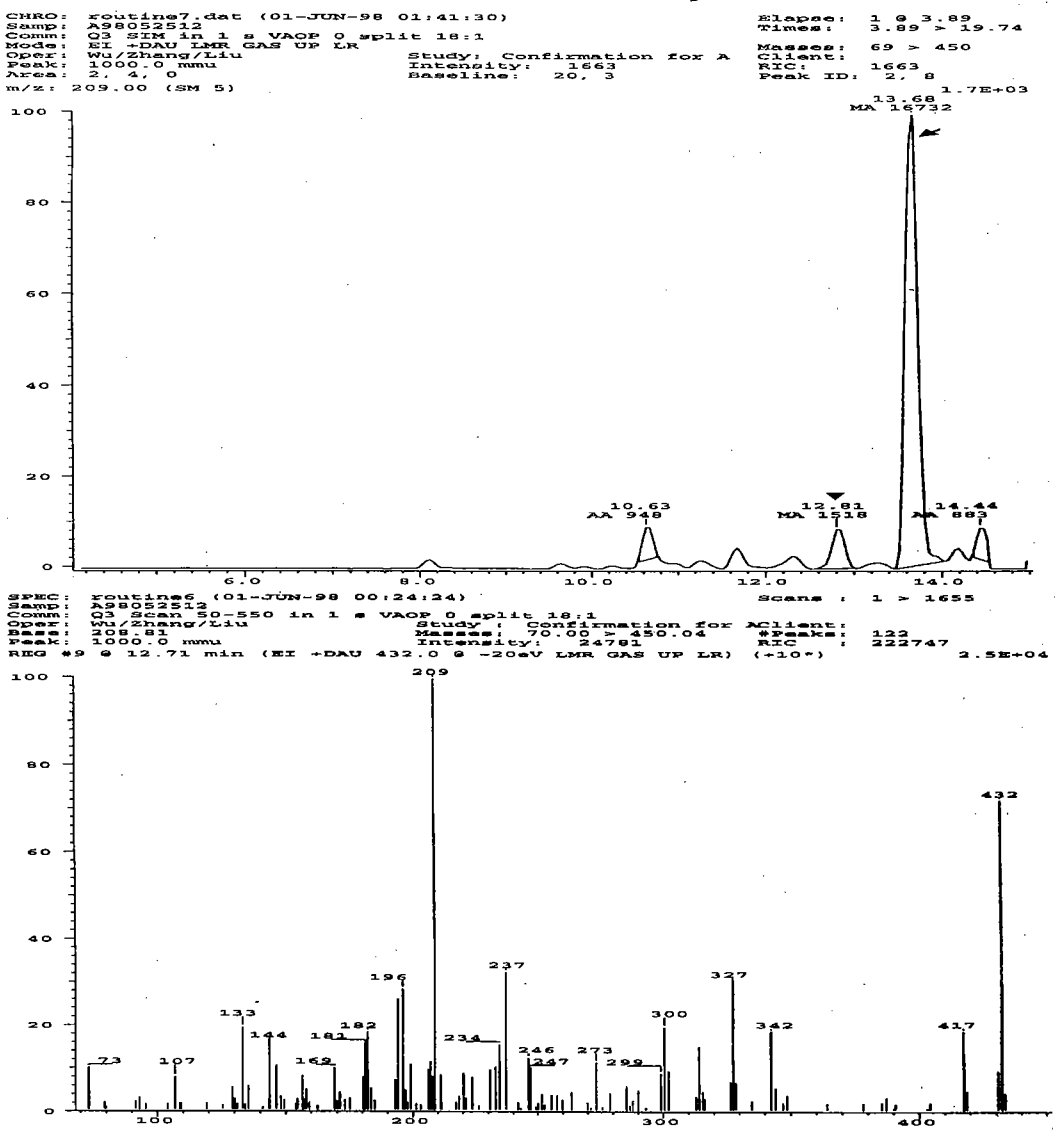


Fig.8 The Ion Chromatogram and the Daghter Mass Spectrum of Epi testosterone

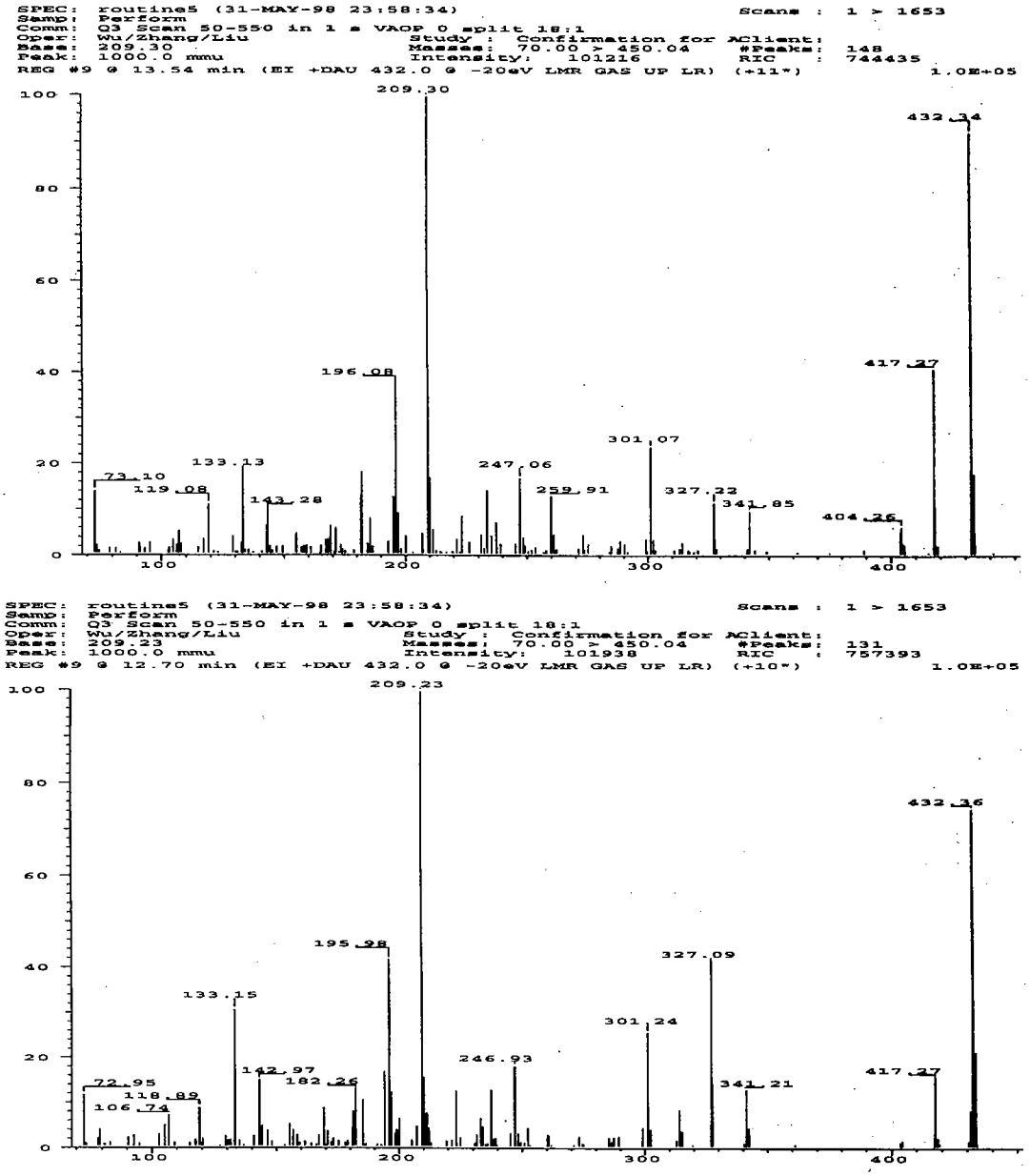


Fig.9 The Daughter Mass Spectra of Testosterone-(upper) and Epitestosterone (lower)-TMS