Reprint from

RECENT ADVANCES IN DOPING ANALYSIS (6)

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Sport und Buch Strauß, Köln, 1999

P. VAN EENOO, F.T. DELBEKE, F.H. DE JONG, P. DE BACKER:
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In: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck-Engelke (eds.) Recent advances in doping analysis (6). Sport und Buch Strauß, Köln, (1999) 105-117

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Urinary metabolites of endogenous nandrolone in women: a case study

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1. Introduction

The post-administration detection time for anabolic steroids has recently been extended through analytical improvements, i.e. HRMS[1] or tandem mass spectrometry[2]. Untill now, urinary traces of norandrosterone and noretiocholanolone have been considered evidence of exogenous nandrolone administration. However, endogenous steroids synthetised in small amounts and formerly undetectable in urine now might be retrieved.

For years, the identification of nandrolone residues in urine of meat-producing animals and racehorses has been proof of unallowed nandrolone administration, but recent studies have shown that the urine of stallions and mare follicular fluid contains endogenous traces of nandrolone[5-8]. Furthermore, the endogenous origin of nandrolone in the urine of cows[9-10], pregnant ewes[11] and boars[12-14] was also demonstrated. In horse doping analysis and in the control for anabolic residues in slaughter animals appropriate measures were taken to exclude false positive results.

Similarly, nandrolone has been identified in human ovarian follicular fluid by GC-MS as a possible intermediate in the multistep enzymatic conversion of androgen to estrogen[3]. Norandrosterone was also detected in the urine of women from the 14th week of pregnancy onwards[4].

In the first part of this study, urine samples from pregnant women were analysed for norandrosterone and noretiocholanolone by tandem mass spectrometry.

In July 1997 during a doping test, low amounts of norandrosterone were detected in the urine of a non-pregnant female cyclist. The rules of the International Cyclist Union (UCI) stipulate that prior to conviction of an athlete for doping abuse, a hearing should be organised. Based on the lack of agreement between A- and B- analysis, the strong denial of doping abuse by the athlete and the low amounts of nandrolone detected in the A-sample (estimated concentration ca. 6 ng/ml), the national cyclist federation decided that additional samples needed to be analysed. Therefore during the periods 28/08/97-24/09/97 and 01/11/97-29/11/97, urine samples were collected every other day and analysed for the presence of norandrosterone and noretiocholanolone, the two major metabolites of nandrolone in humans[17]. During the last period, blood samples were also taken on a daily basis and analysed for estradiol (E2), luteinizing hormone (LH) and follicle stimulating hormone (FSH). In the second part of this study, the results of these estimations are described.

2. Material and methods

- The presence of norandrosterone and noretiocholanolone was investigated by means of GC-MS-MS:

Sample preparation:

Urine (5 ml) was extracted with 5 ml n-pentane after enzymatic hydrolysis with S.H.P. (2.5 h at 56°C). The residue was derivatised with 50 µl MSTFA/NH_4I/ethanethiol (380:1:2 vol/wt/vol) for 20 min at 80°C.

Analysis:

Instrument: Finnigan MAT GCQ

Column: 17 m HP-Ultra 1, i.d.=0.2 mm, df= 0.11 µm.

Temperature program: 120°C (1 min) - 40°C/min → 200°C (0.1 min) - 4°C/min → 250°C (0.1 min) - 30°C/min → 300°C (3 min).

1 µl was injected splitless

Instrument parameters: parent ion: m/z=405; collision energy = 1.1; scan window: m/z = 70 - 405

- Human chorionic gonadodotropin (hCG) concentrations were measured using the Abbott IMx-system (Abbott Park, Ill. USA) as previously described[18].

- Plasma LH and FSH were determined using an immuno-enzymometric method from Ortho Clinical Diagnostics (Amersham, U.K.) and plasma estradiol concentrations were measured with a coated tube radioimmunoassay (Diagnostic Products Corporation, LA, CA, USA). Intra-assay variation coefficients were 5.4, 5.8 and 7.2%, respectively.

3. Results and discussion

Recently Mareck-Engelke et al.[16] were able to detetect norandrosterone in urine of women from the 14th week of pregnancy. In the present study however, traces of norandrosterone were detected by GC-MS-MS in urine of women in their 6th week of pregnancy (Fig. 1). However, regarding doping analysis, the detection of low amounts of norandrosterone in pregnant women is not problematic since subsequent hCG determinations could give conclusive evidence of pregnancy.

In the longitudinal study in one female athlete all hCG concentrations were below the recently proposed threshold level of 5 mIU/ml for male athletes[18], thus excluding hCG abuse or pregnancy (Table 1). As illustrated in Fig. 2 and Fig. 3, LH, FSH and E2 profiles were normal. E2 levels reached a maximum on November 11th, one day before maximum FSH and LH concentrations.

Using GC-MS-MS, low amounts of norandrosterone (< 1 ng/ml) were detected in the samples taken on 30/08/97, 07/09/97, 11/09/97, 13/09/97, 15/09/97, 09/11/97 and 11/11/97, whereas noretiocholanolone could only be found in the sample taken on 07/09/97 (Table 1). The daughter spectra of norandrosterone in spiked urine (2 ng/ml) and in the sample taken on 13/09/97 are shown in Fig. 4 and Fig. 5 respectively. The detection of norandrosterone in some urine samples of this athlete, months after the first recordings of these substances in small amounts during a doping test, could be indicative for the endogenous origin of nandrolone in this athlete.

In human ovarian follicular fluid a correlation has been established between the presence of nandrolone and E2 concentrations[15], indicating that nandrolone is an intermediate in the

aromatization process, which leads to the biosynthesis of oestrogens from androgens[19]. This incomplete aromatization could account for the occurrence of norandrosterone in the samples taken on 09/11/97 and 11/11/97 and, if the duration of a normal menstrual cycle is considered, also for the presence of norandrosterone in the samples taken during the period 11/09/97 - 15/09/97. However, as shown in Table 1, norandrosterone was also detected in two out of three post-race samples (30/08/97 and 07/09/97). Although some results are conflicting[20-24], the increase in peripheral E2 concentration after strenuous exercise has been postulated, probably as a result of cortisol stimulated peripheral aromatization.

In 1982, Björkhem and Håkan[25] concluded on a theoretical basis that endogenous nandrolone residues might appear in urine and proposed a threshold level of 20 ng/ml, above which an athlete should be considered as positive. Since then, major analytical improvements have been made and the hypothesis seems to have become reality. Moreover, the presence of small amounts of norandrosterone in samples not coinciding with the ovulation period could be due to the effect of exercise. As a consequence a new statistically supported decision limit will be needed in order to exclude false positive results with the highest possible degree of certainty.

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Date	circumstances	Urinary S.G. (kg/l)	hCG (mIU/ml)	detection
28/08/97	morning	1.022	0.28	
30/08/97	post race	1.030	0.41	NA
01/09/97	morning	1.020	0.11	
03/09/97	post training	1.023	0.07	
05/09/97	morning	1.020	0.11	
07/09/97	post race	1.025	0.28	NA and NE
09/09/97	morning	1.024	0.19	
11/09/97	post training	1.027	0.24	NA
13/09/97	morning	1.018	0.28	NA
15/09/97	morning	1.020	0.15	NA
17/09/97	post training	1.024	0.02	
19/09/97	morning	1.022	0.07	
21/09/97	post race	1.020	0.19	
23/09/97	morning	1.027	0.11	
24/09/97	noon (day of rest)	1.025	0.07	
01/11/97	post training	1.027	0.06	
03/11/97	post training	1.032	0.27	
05/11/97	post training	1.030	0.39	
07/11/97	post training	1.029	0.27	
09/11/97	post training	1.032	0.43	NA
11/11/97	post training	1.030	0.35	NA
13/11/97	post training	1.028	0.27	
15/11/97	post training	1.033	0.22	
17/11/97	post training	1.031	0.14	
19/11/97	post training	1.032	0.51	
21/11/97	post training	1.028	0.27	
23/11/97	post training	1.026	0.47	
25/11/97	post training	1.027	0.51	
27/11/97	post training	1.031	0.47	
29/11/97	post training	1.026	0.62	

Table 1. Summary of the conditions of sampling, the urinary specific gravity (SG), hCG concentration and detection of norandrosterone (NA) and noretiocholanolone (NE).

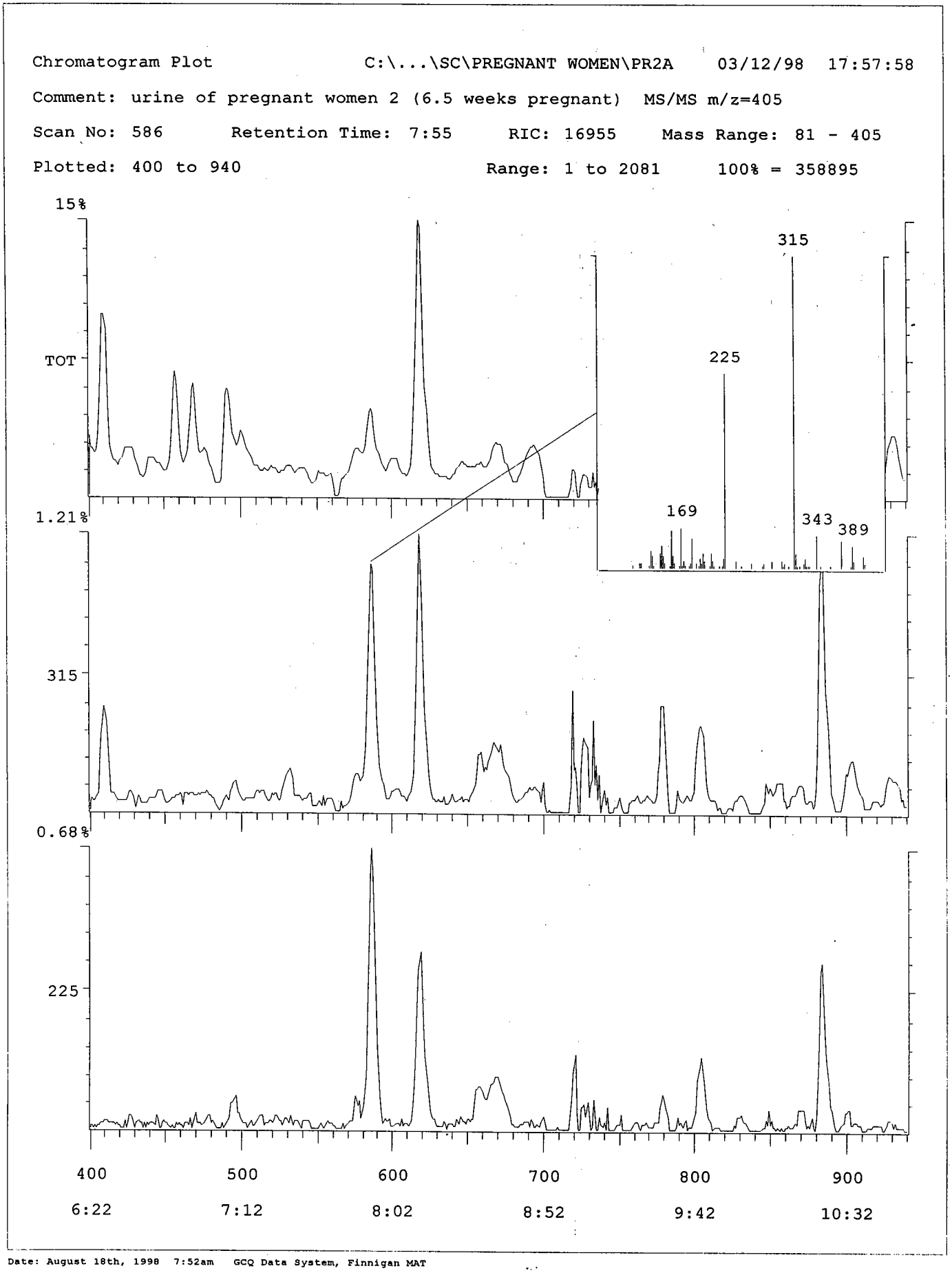

Fig. 1. Ion chromatograms (TIC, m/z=315 and m/z=225) and daughter spectrum of norandrosterone-bis-TMS (R.T.= 7.55 min) in a urine sample of a woman (6.5 weeks pregnant).

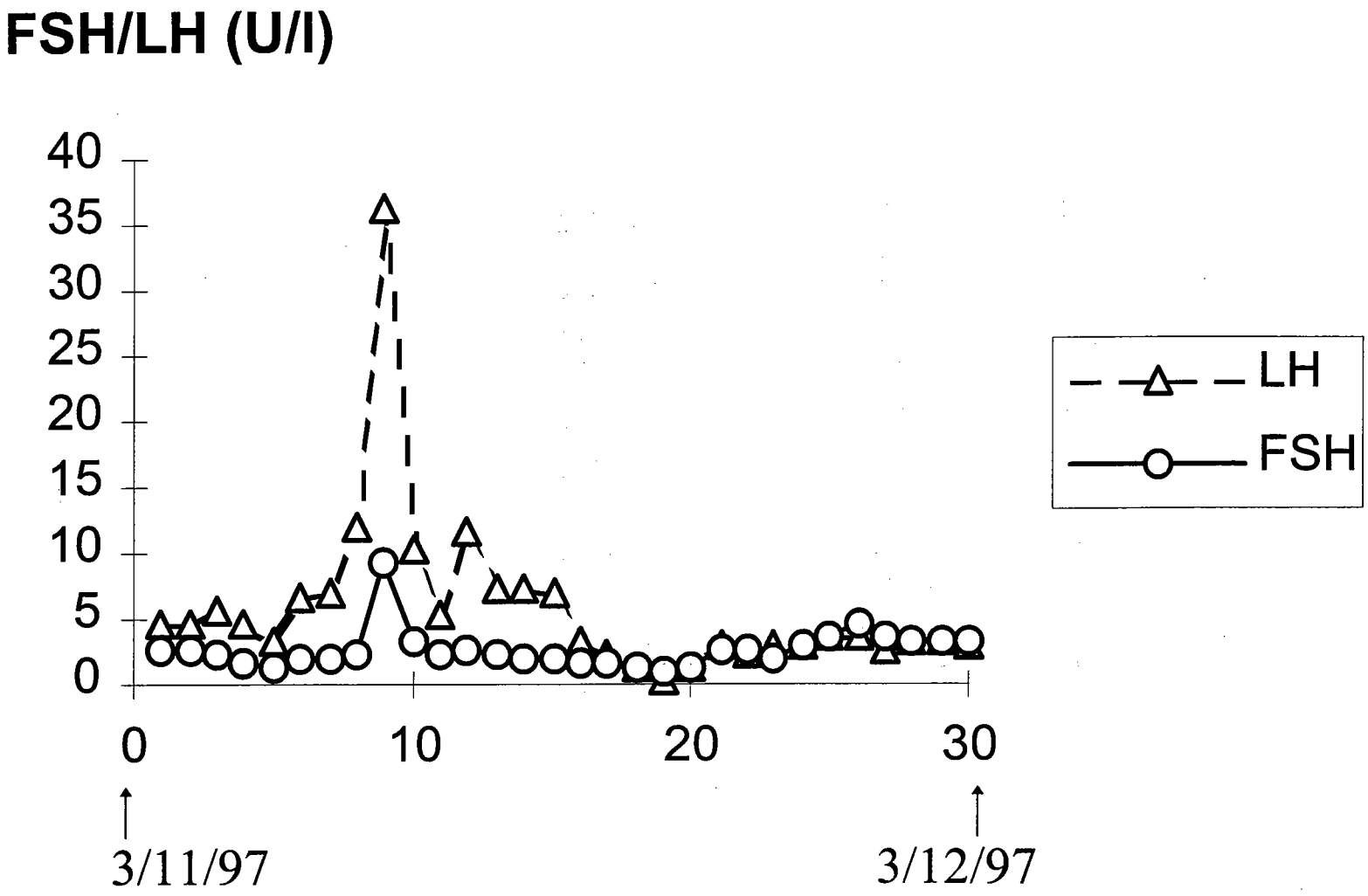

Fig. 2. LH- and FSH- plasma concentrations during the period 01/11/97 -29/11/97.

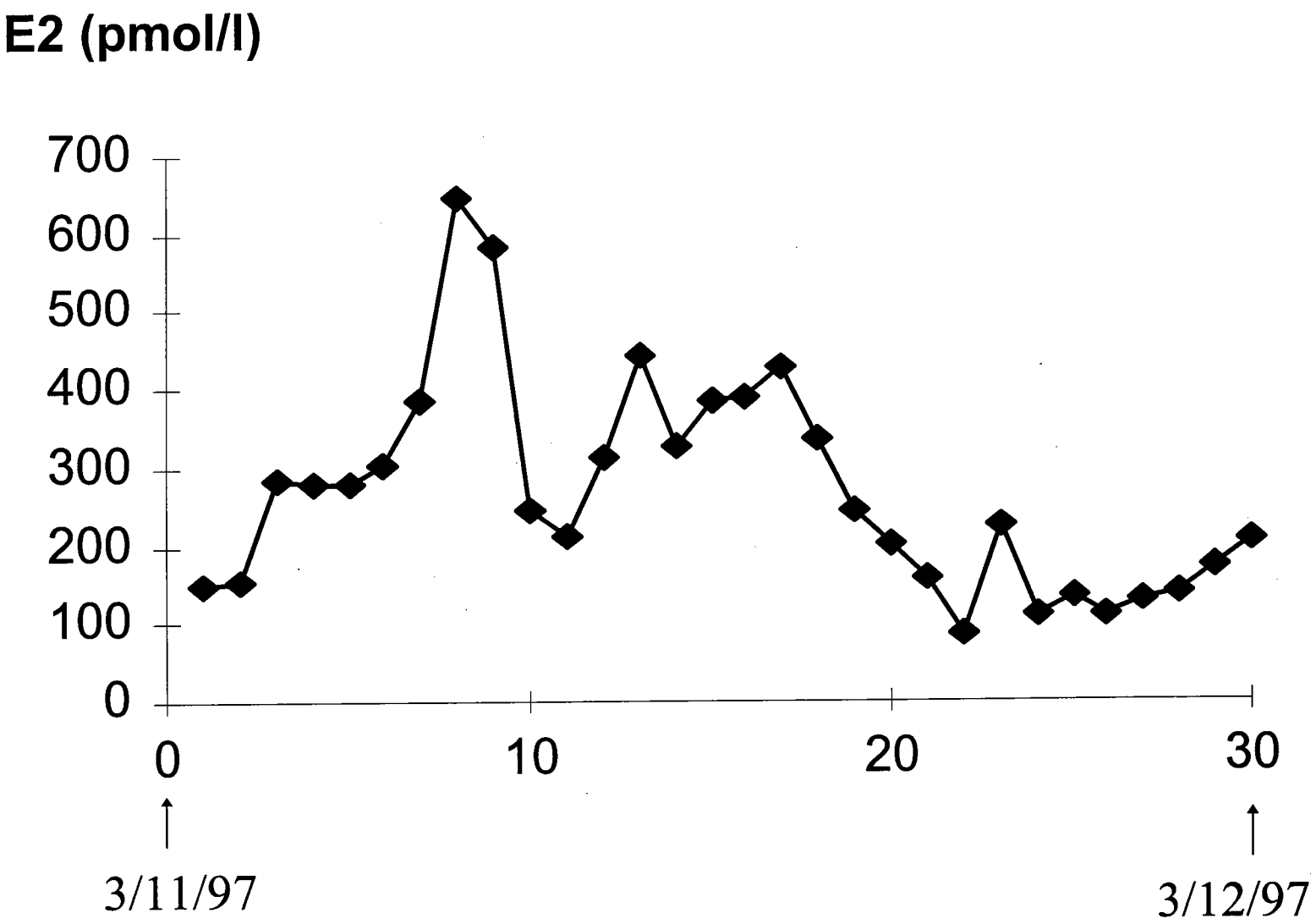

Fig. 3. E2- plasma concentrations during the period 01/11/97 - 29/11/97.

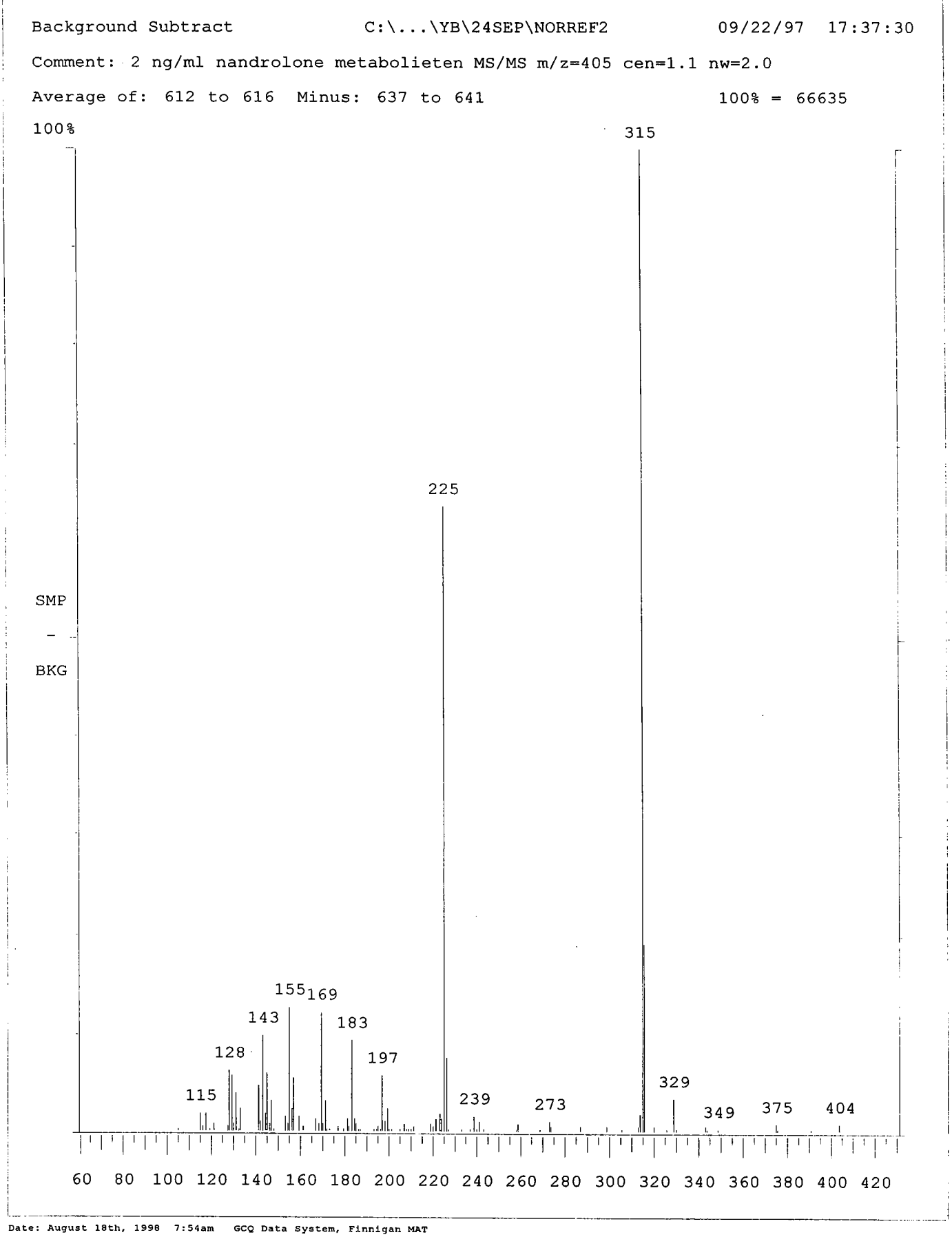

Fig. 4. Daughter spectrum (m/z=405) of norandrosterone-bis-TMS in a spiked urine sample (final concentration = 2 ng/ml)

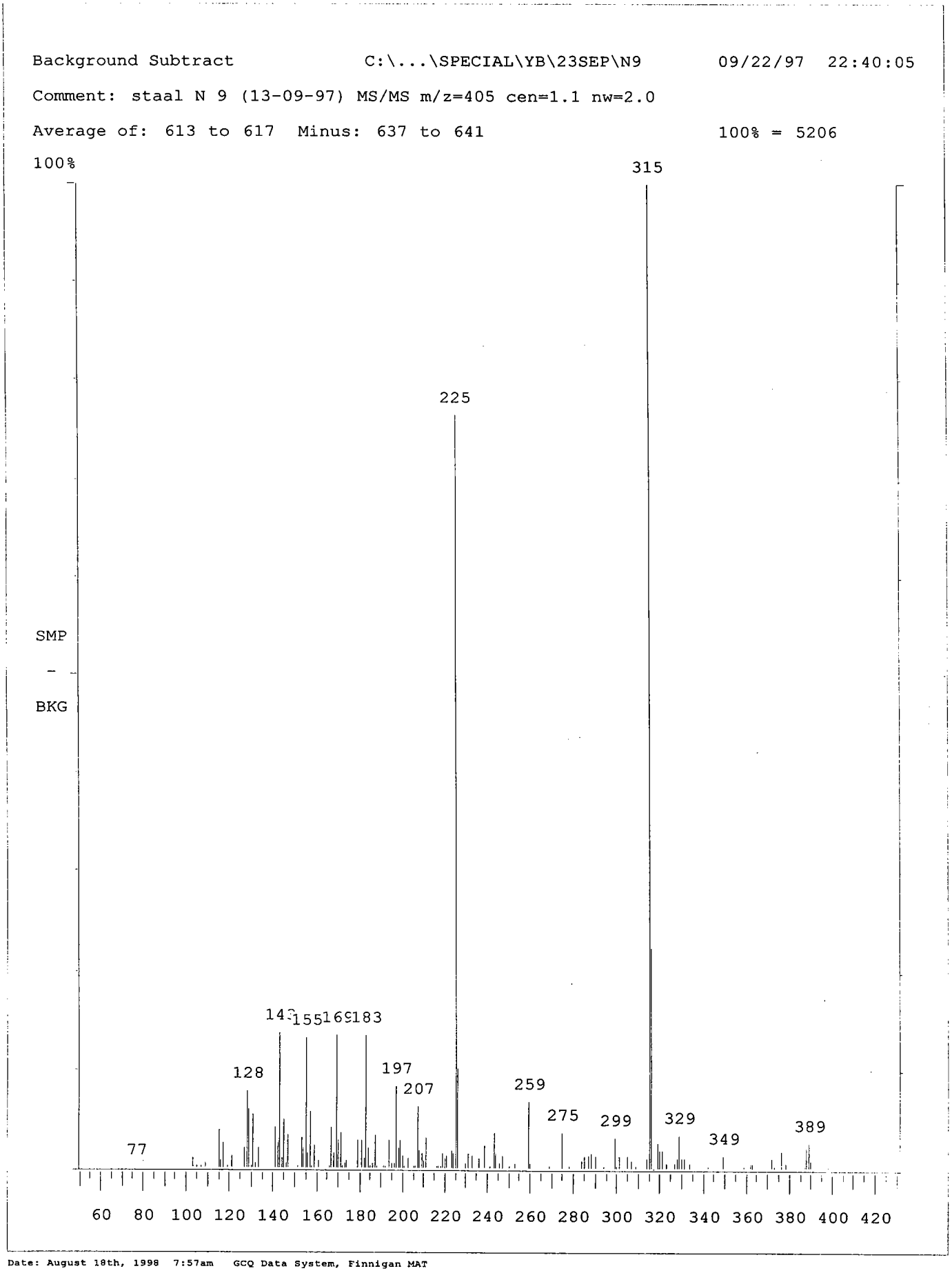

Fig. 5. Daughter spectrum(m/z=405) of the GC-peak in the urine sample taken on 13/09/97.