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Detection of Testosterone Esters in Human Plasma by GC/MS and GC/MS/MS

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1. Introduction

Urine analysis of exogenous synthetic anabolic steroids has been well studied and today, many robust and reliable methods are available for the detection of their consumption. Furthermore with the introduction of the out-of-competition testing programs, the detection of their consumption is not a major problem. Some difficulties remain when the substance abused is a normal endogenous compound. This is the case of testosterone where the positivity of a urine sample, according to the International Olympic Committee (IOC), is based on population studies of the testosterone to epitestosterone ratio (T/E). However, as different physiological states have been found to be characterized by an unusually high T/E ratio *(Oftebro 1992)*, other markers of testosterone misuse like T/LH *(Kicman et al. 1990)* or T/17OHP *(Carlström et al. 1992)* had been investigated to elucidate this cases. Nevertheless, unequivocal confirmation of testosterone misuse can only be achieved by the detection of the unchanged drug administered.

Testosterone is administered as different 17ß-hydroxy esters. Nearly complete hydrolysis of these testosterone esters by esterases give rise to the active testosterone. Nevertheless, minute amounts of unchanged esters remain in the body. The detection of testosterone esters in blood would therefore provide an unambiguous demonstration of exogenous administration of testosterone.

The aim of this work has been to investigate the detectability of testosterone esters in plasma. Extraction and derivatization procedures for nine different testosterone esters with side chains from 2 to 11 carbon atoms have been investigated by GC/MS and GC/MS/MS analysis in order to set-up adequate conditions for their detection and identification. Plasma samples collected after intramuscular or oral administration of different testosterone esters were also analyzed.

2. Materials and methods

2.1. Reference steroids

Testosterone (T), epitestosterone (E), methyltestosterone (MT), testosterone acetate (TA), testosterone propionate (TP), testosterone enanthate (TE), testosterone cypionate (TC), testosterone benzoate (TB) were purchased from Sigma (St. Louis, USA). Testosterone isocaproate (TI), testosterone decanoate (TD), testosterone phenylpropionate (TPh) were a gift of Laboratorios Leo S.A. (Madrid, Spain), and testosterone undecanoate (TU) in oil solution was quantitatively taken from a pharmaceutical preparation (Androxon, Organon, Norway).

2.2. Materials

The derivatization reagent MSTFA (N-methyl-N-trimethylsilyl-trifluoroacetamide) was provided by Mackerey-Nagel (Düren, Germany), TFAA (trifluoroacetic anhydride) by Merck (Darmstadt, Germany), HFBA (heptafluorobutyric anhydride) and PFPA (pentafluoropropionic anhydride) by Supelco (Bellefonte, USA). Ammonium iodide GR from Merck and dithioerythritol 99+% from Aldrich-Chemie (Steinheim, Germany) were used as supplied. Diethyl ether from Carlo Erba (Milan, Italy) was redistilled over calciumhydride (Merck) before use. All other reagents were of analytical grade and used as supplied.

XAD-2 columns and the solid phase processor were from Biochemical Diagnostics (New York, USA).

The MEIA (Automated Microparticle Enzyme Immunoassay) kits for the measurement of LH were obtained from Abbott Laboratories (USA).

2.3. Sample preparation

Plasma

For plasma samples preparation, the method of *Baba S.,(1985)* with some slight modifications was used. Frozen plasma samples were allowed to stand at room temperature for thawing and then kept in an ice-bath. Methyltestosterone was added, at a final concentration of 4 ng/mL, as

internal standard. Immediately after adding 50 µl of 3 M potassium hydroxide the samples were extracted at 4°C with 2x4 mL of a n-hexane:ethyl acetate mixture (70/30) using a rocking mixer for 20 min.. The organic phase was pipetted out in a tube and washed with 1 mL of 5% acetic acid and then with 1 mL of distilled water. After evaporating the solvent mixture under a nitrogen stream, the residue was kept in a desiccator over potassium hydroxide and phosphorous pentoxide for at least 30 min. before derivatization

Urine

Urine samples were submitted to the routine doping extraction procedure used in our laboratory for the analysis of conjugated steroids as described elsewhere *(Segura J., 1993)*. Briefly, urine samples are extracted in XAD-2 columns previously washed with methanol and water and eluted with methanol. The dry residue in redissolved in 1 mL of phosphate buffer 0.2 M, pH 7, and the glucuronides are hydrolized for 1 hour at 50 °C with a ß-glucuronidase from *E. coli*. The pH is adjusted to 9-10 and the free steroids are then extracted with 5 mL of diethyl ether. The final residue is redissolved in the MSTFA/TMSI mixture to form the trimethylsilyl enol-ether derivatives.

2.4. Derivatization study for GC/MSD analysis

Due to the low amounts expected, the choice of an appropriate derivative for the analysis in GC/MSD and GC/MS/MS was studied.

a)*Acylation*: For this purpose different derivatives were studied: Pentafluoropropyl, trifluoroacetyl, and heptafluorobutiryl derivatives were prepared by dissolving a dried standard solution containing equal amounts of each standard in 100 µL of pentafluropropionic, trifluoroacetic and heptafluorobutyric anhydride respectively and heating at 70°C for 15 minutes. The reagent was then removed under a stream of nitrogen and the dry residue redissolved in 20 µL of n-heptane. 3µL of each solution were injected in the GC/MS.

b)*Silylation*: For the trimethylsilylation of steroids, the dry residue was dissolved in 20 µL of MSTFA/NH_4I/Dithioerythritol (1000:2:5) and heated at 60°C for 15 minutes.

3 µL of each solution were directly injected into the GC-MS system or 1 µL in the GC/MS/MS system.

2.5. GC/MSD analysis

A gas chromatograph mpdel 5890 series II (Hewlett-Packard (HP), Palo Alto, CA) fitted with a model 7673AA autosampler was connected to a mass selective detector 5971A (HP). The separation was carried out using a 5% phenylmethylsilicone fused silica capillary column (HP5 Ultra 2 ,12 m, 0.2 mm i.d., film thickness 0,33 µm).

The injector, operated in splitless mode (0.3 min.) and the interface were maintained both at 280°C. The oven temperature programs were: a) Program A1: initial temperature 190°C (0.5 min.), *rate 1:* 30°C/min to 280°C, *rate 2:* 40 °C/min to 310°C, and maintained for 3.50 min at 310°C; b) Program A2: conditions were as for program A1 except the final temperature of the oven program that was 315°C, the analites eluting ca. 1 minute before. Helium was used as a carrier gas at a flow rate of 0,8 mL/min. Mass spectra of reference standards were obtained in scan mode. For the analyses of plasma samples, the mass spectrometer was operated in the Selected Ion Monitoring (SIM) acquisition mode, the molecular ions (Table I) being selected for each ester.

2.6. GC/MS/MS analysis

GC/MS/MS analysis was performed with a Finnigan TSQ 700 triple quadrupole mass spectrometer equipped with a Varian 3400 gas chromatograph. The fused silica capillary column (Hewlett-Packard HP5 Ultra 2, 12 m x 0.2 mm x 0.33 µm film thickness) was programmed from 200°C (2 min initial isotherm) to 315°C at 40°C/min (7 min final isotherm). The injector (splitless, 2 min) was maintained at 290°C and the interface at 300°C. Helium was used as carrier gas, flowing at 0.8 ml/min. The mass spectrometer was operated in multiple selected reaction monitoring. At different time intervals, depending on the retention times of the analytes, the appropriate molecular ion, obtained by electron impact, was selected as parent mass in the first quadrupole and three daughter ions were monitored in the third quadrupole after collision of the molecular ion with argon at a cell pressure of 1.5 mtorr, and with a collision energy of -30 or -35

eV. (See Table I for selected ions).

2.7. Quantitative analysis:

Quantitative analyses of testosterone propionate and enanthate was performed using a calibration curve prepared by adding known amounts of testosterone propionate and testosterone enanthate to 1 mL of plasma at a final concentration of 0.2, 0.4, 0.8, 2, 4 and 8 ng/mL.
The quantitation of free testosterone was based on the response factor of an extracted sample from distilled water, containing 5 ng/mL of testosterone.
The quantitation of testosterone undecanoate by GC/MS/MS was based on the response factor of an extracted sample containing 1 ng/mL of the ester.
The spiked samples were then processed as real samples.

2.8. LH determination.

Plasma samples were assayed for LH by MEIA. Assay calibration was performed following manufacturer specifications and two quality controls were included in each run.

2.9. Sample collection.

2.9.1. Controlled studies

Two different studies were performed on a healthy male volunteer:
1) Intramuscular administration.

One male volunteer received an intramuscular administration containing 25 mg of *testosterone propionate* and 100 mg of *testosterone enanthate* (Testoviron Depot®, Schering AG, Germany). Heparinized blood samples (10 mL) were taken just before and at 6, 12 and 24 hours for the next four days and every 24 h until day 7 after dosing. Urine samples were collected following the same pattern of plasma until day 7 and the first morning miction was then collected until day 14. Blood plasma was separated by centrifugation at 4°C just after collection, and urines were kept at -20°C until analysis.

2) Oral administration.

Eight months after the intramuscular administration, the same male volunteer received orally 4 X 40 mg of testosterone undecanoate (Androxon®, Organon, Norway) every 8 hours.
Heparinized blood samples (10 mL) were collected just before and at 4, 8, 24, 26, 28, 32, 36, 48 and 72 hours after administration. Urine samples were taken at 2, 6, 10, 24, 26, 30, 34, 48, 72 and 96 hours.
Blood plasma was separated by centrifugation at 4°C, and urines were kept at -20°C until analysis.

2.9.2. Real samples

Blood samples (10 mL) from body-builders who volunteered for this study were also collected. Samples were centrifuged at 4°C in the following 1.5-2 hours after collection, plasma was separated, and frozen at -20°C until analysis. Urine samples from the same volunteers were also collected for the T/E determination, according to our routine procedure.

3. Results

Extraction

A practical improvement in regards to the extraction step proposed by *Baba S.* (13) is the addition of ethyl acetate to avoid the formation of emulsions that otherwise could retain part of the analytes. For the analysis of the samples after the intramuscular administration, the washing steps were maintained for the GC/MSD analysis in order to reduce biological background. They appeared not strictly needed for GC/MS/MS analysis because the selectivity of the technique
Kuokka L., (1986) described a method for the analysis of testosterone esters in plasma using a solid phase extraction procedure combining octadecyl and amino columns but only testosterone propionate could be detected without difficulties.
Regarding the stability of the esters, previous works *(Baselt RC, 1983; Isenschmid et al., 1989)* described the inhibition effect of the sodium fluoride (NaF) over plasmatic esterases. Blood samples were collected in tubes without NaF or with 40 µL of a saturated solution of NaF. The analyses demonstrated that when the samples are immediately frozen at -20°C the use of NaF is not necessary and no differences were observed.
To avoid any possible degradation of the esters, all the extraction steps of the procedure were

performed at 4°C.

Derivatization

According to the different fragmentation patterns presented by the differents derivatives (Figure 1), the TMS derivative was chosen for its better sensitivity even if other derivatives can be useful in confirmatory steps.

MS/MS analysis.

For the MS/MS analysis the presence of an intense molecular ion (in most cases the base peak) in the EI spectra of the TMS derivatives of testosterone and its esters offered the best conditions for tandem mass spectrometric analysis without switching to chemical ionization. The spectra of daughter ions obtained by collision of molecular ions with argon, however, are characterized by an extended fragmentation leading, in all cases, to the $[M-15]^+$ ion and to fragments originating from the testosterone moiety (Figure 2). Daughter ions at m/z 209 and 247 were chosen for their higher intensities (see Table I). Attempts to reduce fragmentation or to increase intensity of selected daughter ions by varying collision energy or cell pressure did not succeed. Nevertheless the adopted conditions allowed to reach the required selectivity and sensitivity. MS/MS spectra for testosterone and the studied esters are presented in Figure 3.

The GC/MSD method described permits the detection and quantitation of the trimethylsilyl testosterone esters in less than 9 minutes (a chromatogram of a standard mixture under GC/MSD analysis is shown in Figure 4). No interferences with the extracts of plasma are observed except a large peak co-eluting with testosterone isocaproate-TMS. Linearity was checked for the propionate and enanthate esters and the method was linear over the concentration range studied.

Intramuscular administration

As expected *(Alén M., 1985)*, plasma levels of testosterone increased after exogenous testosterone administration while LH levels decreased due to an inhibition of the hypothalamic-hypophisary axis. As a consequence there is a very significant increase of the T/LH ratio for

more than 7 days after the administration (Figure 5).

The analysis of the testosterone esters in plasma (Figure 6), permitted their detection during 3 days for the propionate and at least 7 days for the enanthate with a maximum concentration at 72 hours (Figure 7). The results obtained for the propionate with a maximum concentration between 6 to 12 hours after the administration are in agreement with those found by *Shinohara Y., (1988)*.

Urine analysis showed, as expected *(Donike M., 1983)*, an increase testosterone to epitestosterone ratio (based on peak areas) for at least 10 days after the administration of the drug. According to the regulations, a urine sample is considered positive for exogenous testosterone administration if the T/E ratios exceeds 6. The ratio observed in this study was above the proposed value of 6 from ca. 24 to 120 hours, and during this period, the detection of the unchanged testosterone enanthate was also possible (Figures 7-8).

Oral administration

Testosterone can also be administered orally as the undecanoate ester form. Even if testosterone undecanoate is not ideal for therapeutic purposes due to its relative serum short life and wide fluctuation serum levels *(Hermann MB., 1990)*, it is claimed that athletes could use it to maintain constant testosterone urinary excretion. We studied if a substantial unchanged fraction of the administered ester reached the blood stream and if its detection by GC/MS was possible.

In our experiment, we found no significant changes in the plasma testosterone and LH levels but in the other hand a marked increase in the urinary testosterone excretion and T/E ratio after the oral administration of testosterone undecanoate was observed (Figure 9).

The GC/MS plasma analysis, with a benchtop GC/MSD system, presented some indices of the detectability of unchanged plasma testosterone undecanoate but a more sensitive and selective detector was necessary (Figure 10).

The same samples were then analyzed in a GC/MS/MS system (Figure 11). These analysis confirmed GC/MS results. Serum testosterone undecanoate levels reached their maximum about 2 hours and were detectable until 10 hours after the administration. Also in this case unchanged testosterone undecanoate in plasma was detectable during the same time period in which T/E ratio was above 6 in urine (Figure 12).

Real body-builders samples

The GC/MS analysis of several plasma samples from body-builders was performed using the whole screening method including the 8 testosterone esters studied. Two positive cases (testosterone enanthate and testosterone cypionate) were detected. These samples were then confirmed by GC/MS/MS and in the enanthate case, testosterone propionate was also detected. In Table II the urine results of the same samples are shown. It can bee seen that only those samples with a simultaneous high T/E ratio in urine and a with high testosterone concentration are positive to esters in plasma. In the other cases, the high urinary T/E ratio is probably due to a suppression of epitestosterone excretion by the consumption of other anabolic steroids

In a previous work, it was described how the steroid profile of body-builders was changed after the administration of androgenic-anabolic steroids. Those subjects who were under stanozolol and methenolone treatment had urinary T/E ratios around 6, while those under only methenolone or not consuming steroids had values around 1 or even lower.

According to the present results, we could affirm that stanozolol can modify the T/E ratio in urine to values near the cut-off point of 6. Plasma analysis offers the possibility to disclose whether a combined administration of stanozolol and exogenous testosterone is present.

4. Discussion

The detection of unchanged testosterone esters by selective ion detection mass spectrometry after gas chromatographic separation is not optimal due to several factors. First, the relative low resolution of the underivatized esters obtained in the chromatographic step (Cairns et al. 1993) precludes to obtain very sharp peaks and therefore very high response in the mass spectrometric detector. Second, the fact that diagnostic ions for all esters appear at low mass ranges precludes the use of selective ion monitoring when studying biological extracts with high low molecular weight background. The use of chemical ionization mass spectromery can improve the capacity of the mass selective detector (Cairns et al. 1993). Nevertheless, for routine operation, the use of suitable derivatives appear a more promising approach. In this work, studies have been done on the possibilities of silyl and acyl derivatives for the screening and confirmation of testosterone esters obtained from biological extracts. The method is shown to be useful to detect the

administration of injectable testosterone esters.

The mass spectral characteristics of the silanized derivatives of testosterone esters make them highly valuable as compounds for screening in selective ion monitoring. Thus, high sensitivity is assured by the fact that molecular ion is nearly always the base peak. These molecular ions (range m/z 402-528) appear in a low biological background area of the spectrum. The ion $[M-15]^+$ is always present and adds selectivity to the molecular ion. Other diagnostic ions also exist for the respective ester side chain and for the TMS-testosterone structure giving additional specificity to the selective ion detection.

The fragmentation of the acyl derivatives of testosterone esters is more extensive than the one obtained with the silyl derivatives thus offering additional data to confirm the presence of one specific compound. Although the molecular ion is never the base peak, its relative abundance is usually high enough to assure easy detectability. The abundance of the molecular ion is higher with the trifluoroacetyl derivatives and decreases with the pentafluoropropionyl and, specially, with the heptafluorobutiryl derivatives. In all cases, the side chain is an important ion. The possibility of obtaining different acyl derivatives of the testosterone esters is highly relevant for confirmatory purposes of actual samples. It will be relatively easy to choose in each case the suitable derivative able to give a chromatographic peak whitout biological interferences and also a SIM or SCAN spectrum suitable for unambiguous confirmation.

The power of GC/MS/MS has also been evaluated and shown to be useful in real oral ingestions of testosterone esters.

The Medical Commission of the International Olympic Committee (IOC), based on population studies, considered in 1989 that urines with a T/E ratio above 6 should be considered as positives to an exogenous administration of testosterone. Since then, new cases, due to physiological or pathological conditions, with natural T/E above 6 have been observed *(Oftebro H. 1992)*. Today it is recommended to perform a set of endocrinological tests to evidence if the observed ratio is of physiological or pathological origin.

Blood sampling is a common practice to detect the presence of drugs in Clinical Chemistry and Therapeutic Drug Monitoring. In the sport world, the collection of blood samples for the detection of homologous blood transfusions was carried out in 1994 Olympic Winter Games as routinelly done since years by the International Sky Federation. Other major international sport Federations (i.e. track and field IAAF) have done also blood sampling on experiemntal basis. The possibility of using blood for the confirmation of other problematic drugs or doping practices does therfore exist.

Other markers than urinary T/E, like urinary T/LH *(Kicman A., 1990)*, or plasmatic T/17α-hydroxyprogesterone *(Carlström K., 1992)* had been proposed to confirm an exogenous testosterone administration. In these later cases the inter and intraindividual variability should be studied before excluding physiological or pathological reasons for the abnormal value.

A method for the screening of 8 of the most available testosterone esters in the market is presented. The TMS derivatives chosen for the analysis by GC/MSD offered a maximum sensitivity because the mass spectra present only one significant ion corresponding to the molecular ion. During an MS/MS analysis the possibility of selecting the base peak, in this case also the molecular ion, for its fragmentation in the second quadrupole is an ideal condition because of the selectivity and sensitivity of the technique. Obviously the use of an MS/MS instrument is not, at the moment, a routine practice in most laboratories, but the possibility to form other derivatives for GC/MSD analysis with a more selective fragmentation is also possible.

The possibility of detecting testosterone esters in plasma is of interest in doping control analysis if we can correlate the obtained values with the T/E in urine. The results obtained in our experiments with a single intramuscular or oral administration of testosterone esters shown that there is a very significant parallelism between T/E ratios in urine above the limit of 6 and the possibility to detect the unchanged esters in plasma.

In the case of real samples from body-builders, the urinary T/E values are extremely high but this is sometimes due to the suppression of epitestosterone excretion provoked by other androgenic-anabolic steroids consumed.

Consequently, the detection of the esters in plasma could be of interest for a better interpretation of the changes provoked on the steroid profile by the administration of anabolic steroids.

5. Conclusions

The detection of exogenous testosterone administration presents some difficulties because there is not an absolute and definitive method to distinguish synthetic testosterone to natural one, even if important steps have been done in this direction *(Becchi M, 1994)*.

The determination of testosterone esters in plasma by mass spectrometry allows a definitive confirmation of the administration of exogenous testosterone. The method here presented permit an unambiguous confirmation and could also be used for screening purposes for eight of the most available testosterone esters in the market.

Due to the low concentrations found, the choice of an appropriate derivative for the GC/MS analysis is of major importance. The trimethylsilyl derivatives used here offered a maximum sensitivity for the GC/MSD analysis and the best conditions for a GC/MS/MS analysis when a higher sensitivity is required without switching to chemical ionization.

The application of this method in antidoping analyses for the confirmation of exogenous testosterone administration, could be of relevant interest in the future.

6. Acknowledgements

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Table I

Absolute retention times, parent ions, and daughter ions selected for each analyte

COMPOUND	Absolute retention time (min) *	Parent ion (m/z) (1)	Daughter ions (m/z)
Testosterone-bisTMS	5.70	432($M^{+\cdot}$)	417,247,209
T-acetate-TMS	6.02	402($M^{+\cdot}$)	387,247,209
Methyltestosterone-bisTMS	6.05	446($M^{+\cdot}$)	301
T-propionate-TMS	6.37	416($M^{+\cdot}$)	401,247,209
T-isocaproate-TMS	7.52	458($M^{+\cdot}$)	443,247,209
T-enanthate-TMS	8.33	472($M^{+\cdot}$)	457,247,209
T-benzoate-TMS	9.87	464($M^{+\cdot}$)	449,247,209
T-cypionate-TMS	10.08	484($M^{+\cdot}$)	469,247,209
T-decanoate-TMS	10.93	514($M^{+\cdot}$)	499,247,209
T-phenylpropionate-TMS	11.53	492($M^{+\cdot}$)	477,247,209
T-undecanoate-TMS	12.09	528($M^{+\cdot}$)	513,247,209

* Retention times according to the oven program used in the GC/MS/MS analysis.

(1): ions selected for the analysis in the GC/MSD system and as a parent mass in the first quadrupole in the GC/MS/MS system.

Table II

Body-builders urine and plasma analysis

CODE (*)	T/E[1]	[E][1]ng/mL	[T][1]ng/m	Plasma
740758 (+)	7.2	2.0	14.4	-
741701 (+)	44	3.1	134.2	T-P, T-E
757193 (+)	10	3.0	30.0	-
741703 (+)	4	3.0	12.0	-
757188 (+)	5	9.4	46.8	-
741705 (+)	6	1.0	6.0	-
756715 (-)	100	3.6	360.0	T-Cyp
756722 (-)	10	1.5	15.0	-
756718 (+)	40	2.0	80.0	-
756724 (+)	4.6	5.3	24.2	-
757194 (-)	13	3.3	42.3	-
757195 (-)	7.2	1.6	11.5	-

(*) (+) stanozolol positive samples.
[1]: Urine analysis.
T-P: testosterone propionate, T-E: testosterone enanthate, T-Cyp: testosterone cypionate

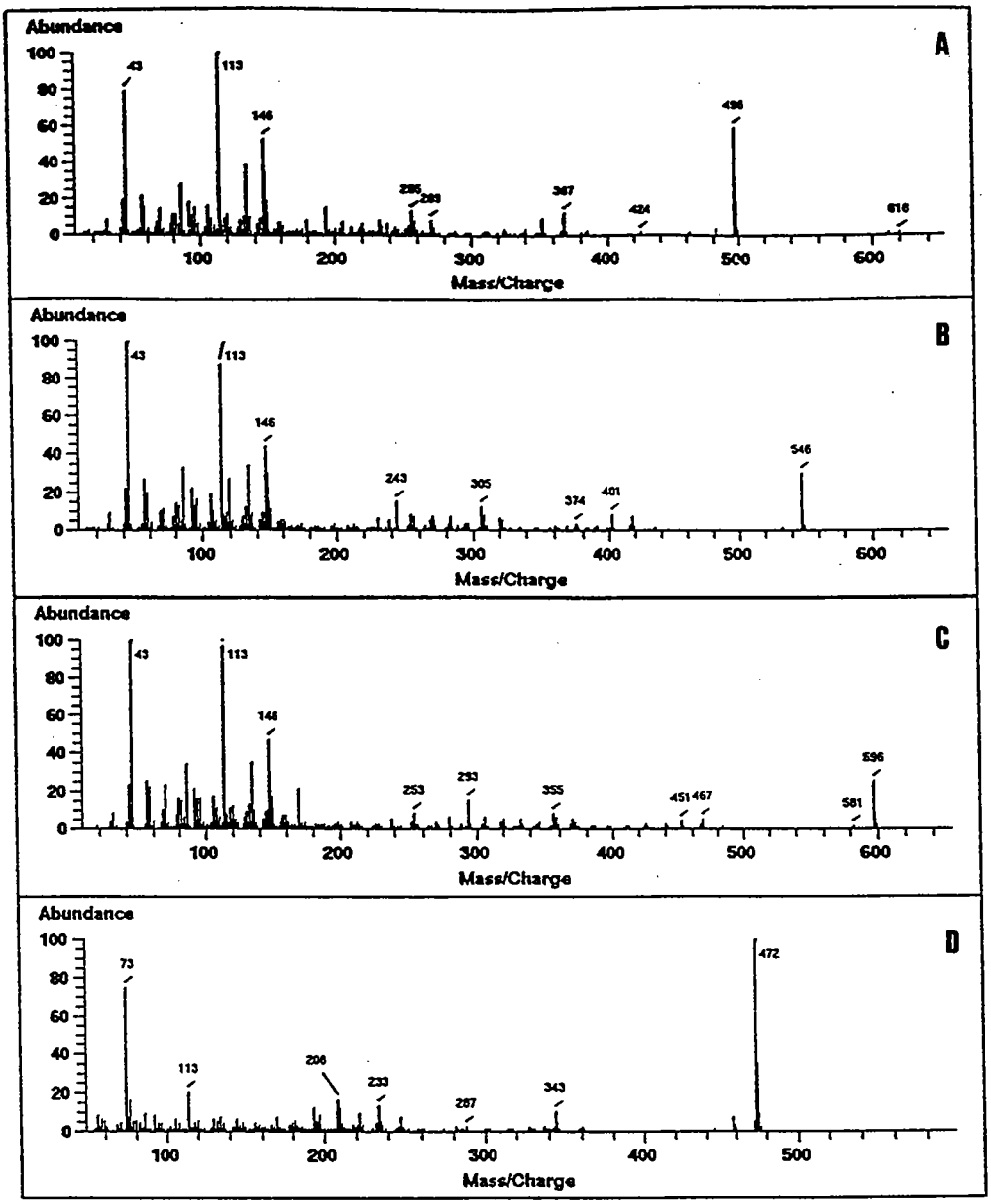


Figure 1. EI mass spectra of different testosterone enanthate derivatives
A, trifluoroacetyl; B, pentafluoropropionyl; C, heptafluorobutiryl and D, trimethylsilyl.

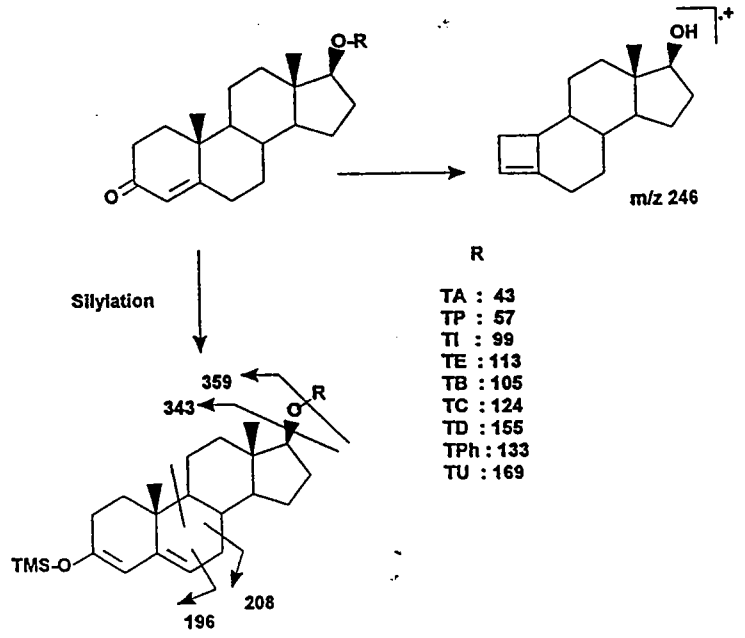


Figure 2. Fragmentation pattern of trimethylsilyl testosterone esters.

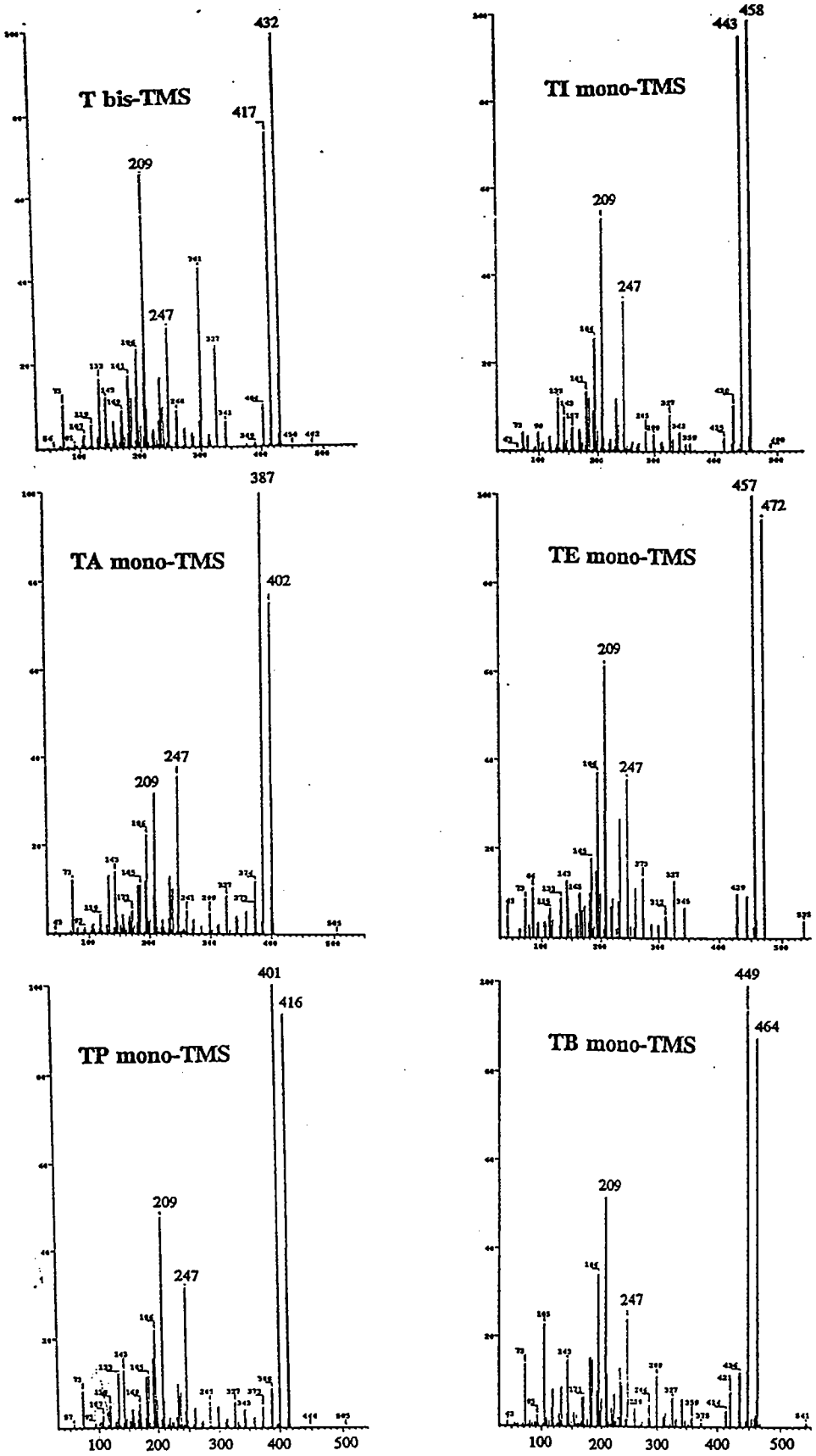


Figure 3. EI MS/MS mass spectra of testosterone and testosterone esters studied.

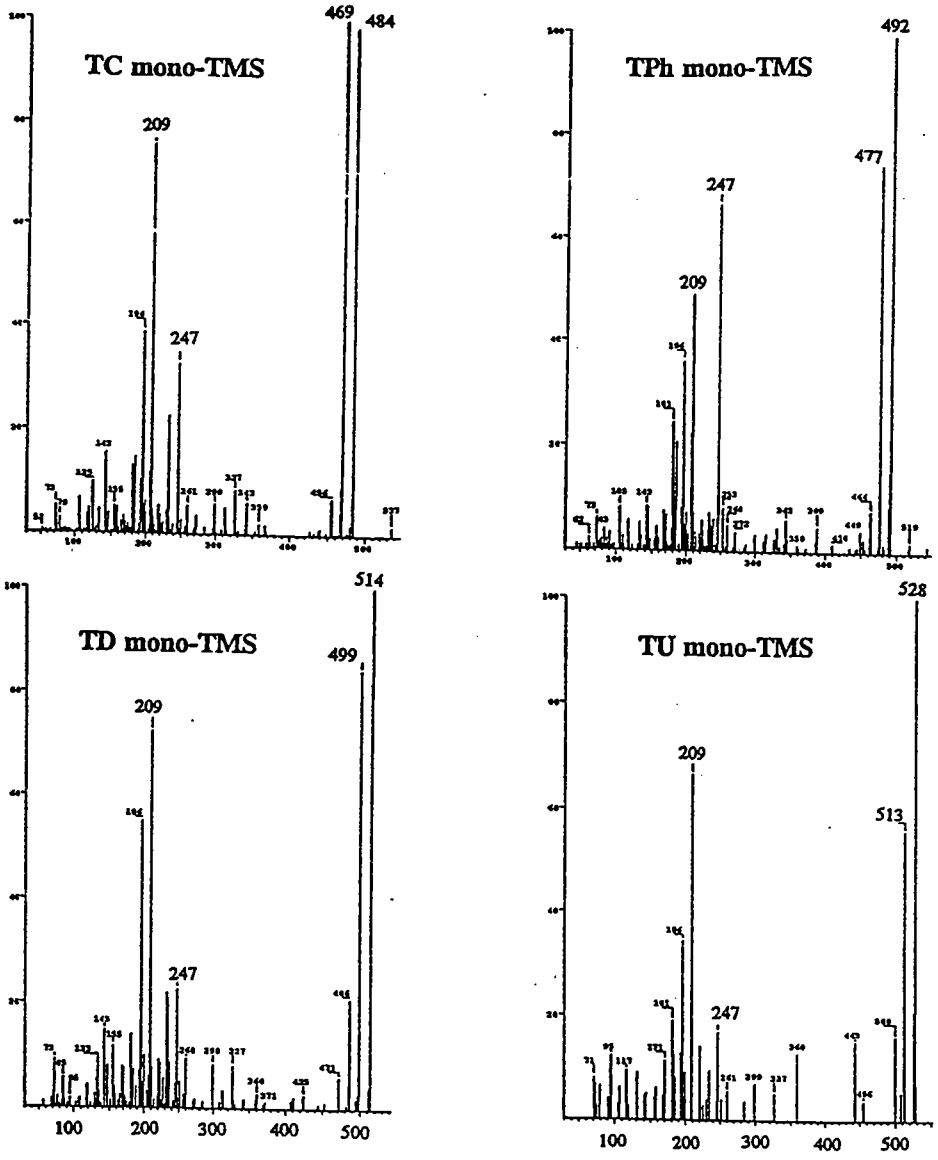
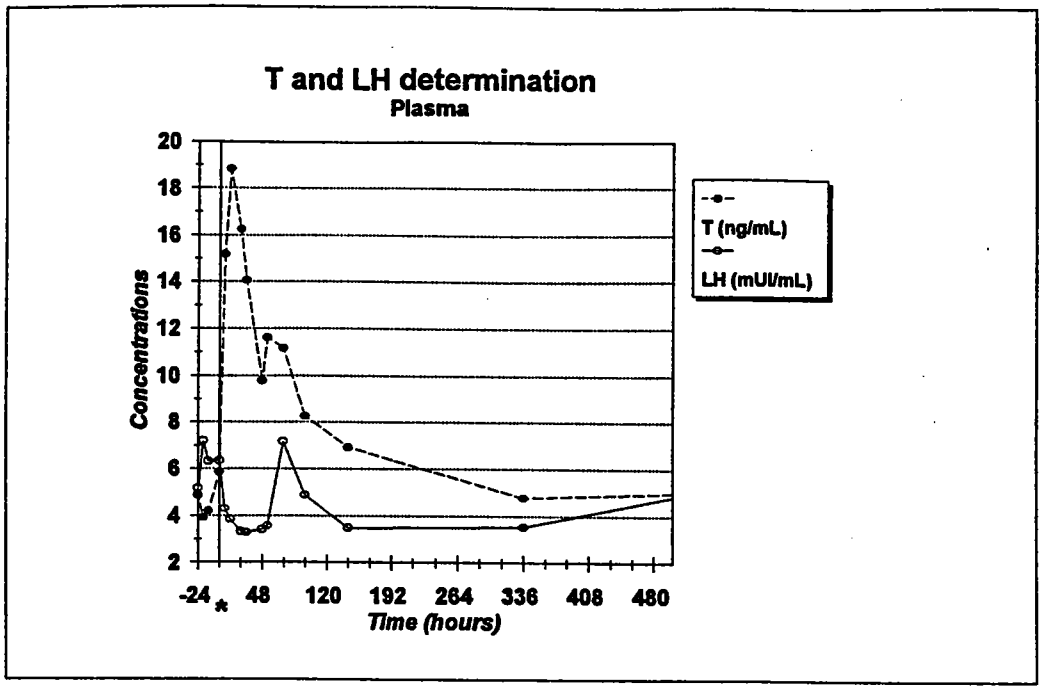


Figure 3 cont.



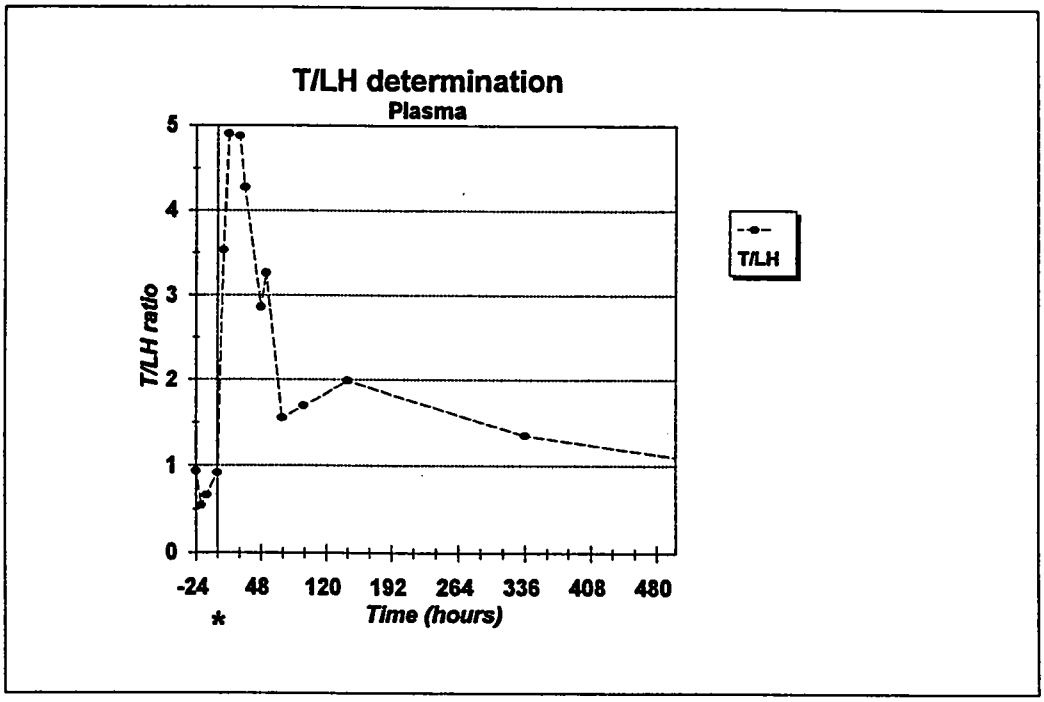


Figure 5: Testosterone, LH and T/LH determinations.
* Administration of Testosterone propionate (25 mg) and enanthate (100 mg)

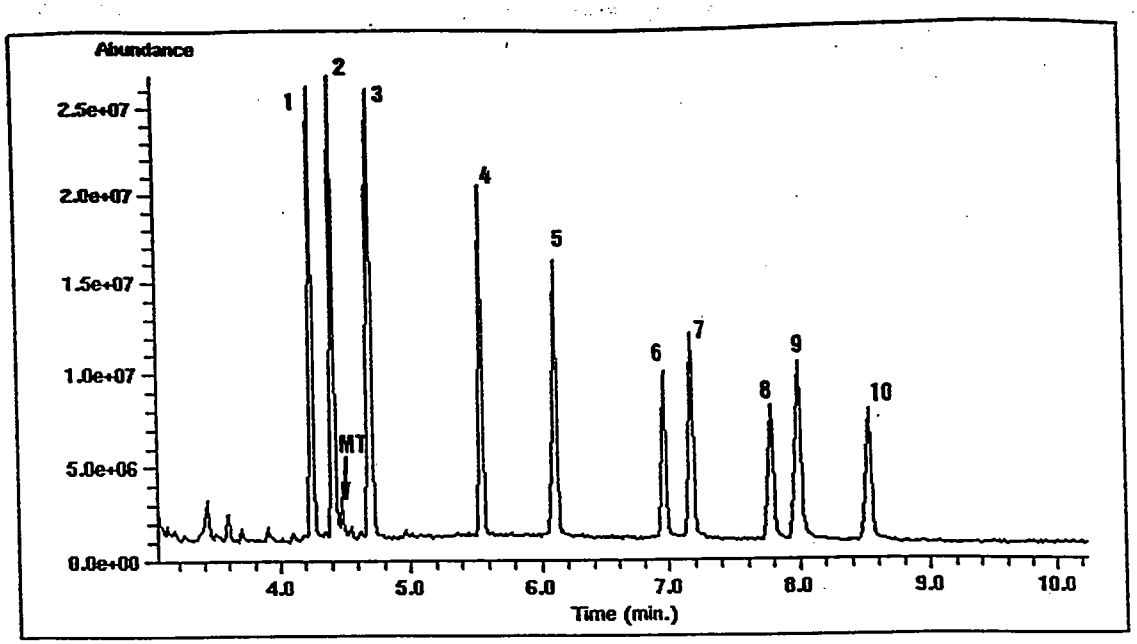


Figure 4. Chromatogram of a standard mixture containing the trimethylsilyl derivatives of: 1, T; 2, TA; 3, TP; 4, TI; 5, TE; 6, TB; 7, TC; 8, TD; 9, TPh and 10, TU.

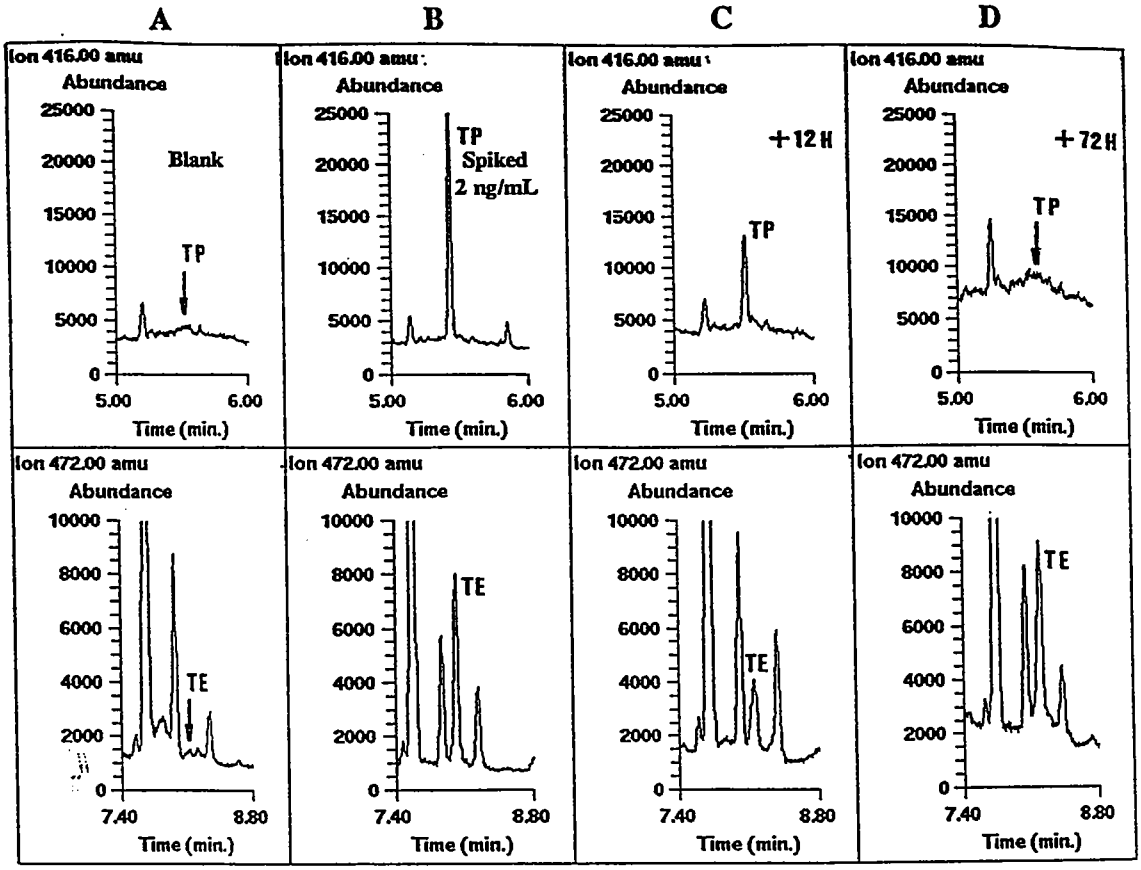


Figure 6. Chromatogram obtained after the GC/MSD analysis of samples collected after a single intramuscular dose of 25 mg of testosterone propionate (TP) and 100 mg of testosterone enanthate (TE) . A, exctracted ions for TP at m/z 416 and m/z 472 before the administration (elution times are signaled with arrows); B, exctracted ions of a plasma blank spiked with 2 ng/mL of each of testosterone ester; C, exctracted ions of a sample 12 h after the administration and D, extracted ions of a sample 72 h after the administration (note that the detection of TP is not possible at this time).

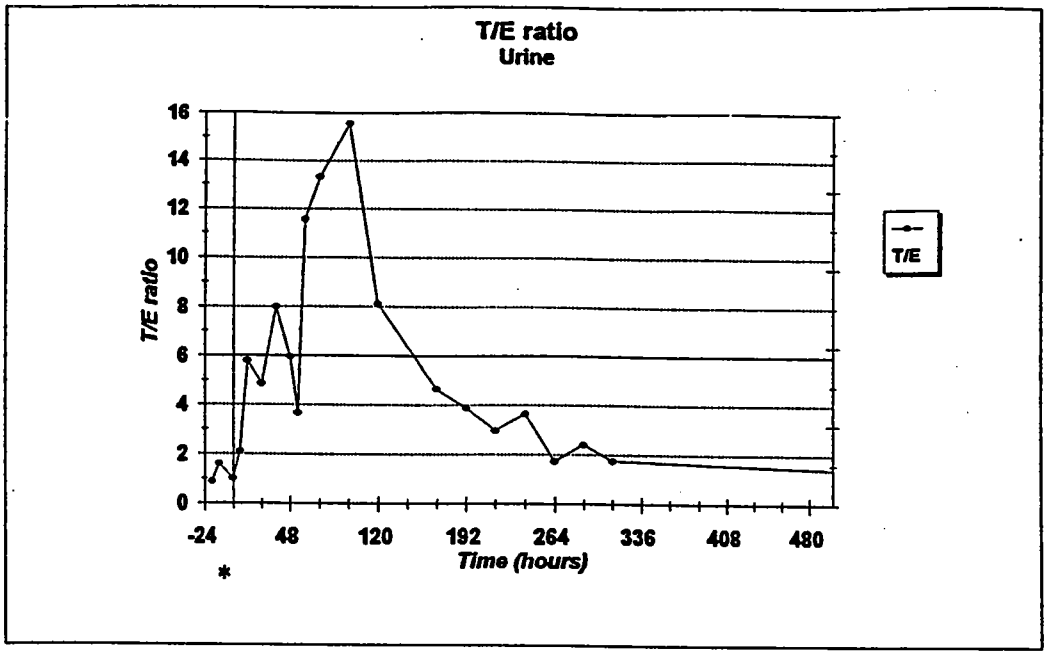


Figure 7: Urinary T/E ratio
* Administration of Testosterone propionate (25 mg) and enanthate (100 mg)

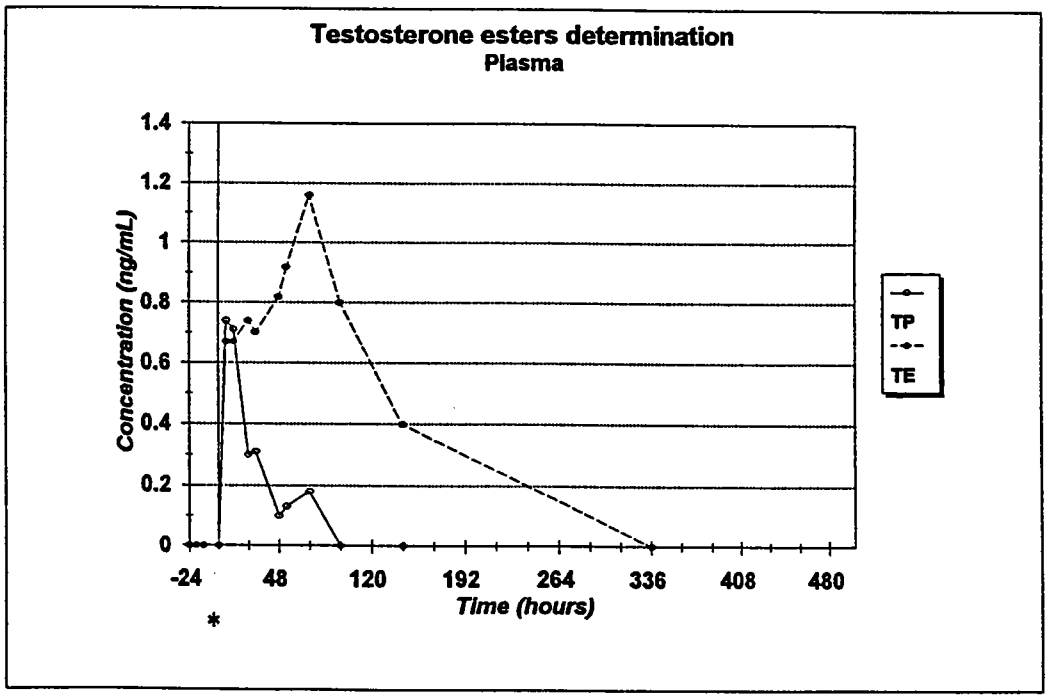


Figure 8: Plasma testosterone esters .
* Administration of Testosterone propionate (25 mg) and enanthate (100 mg)

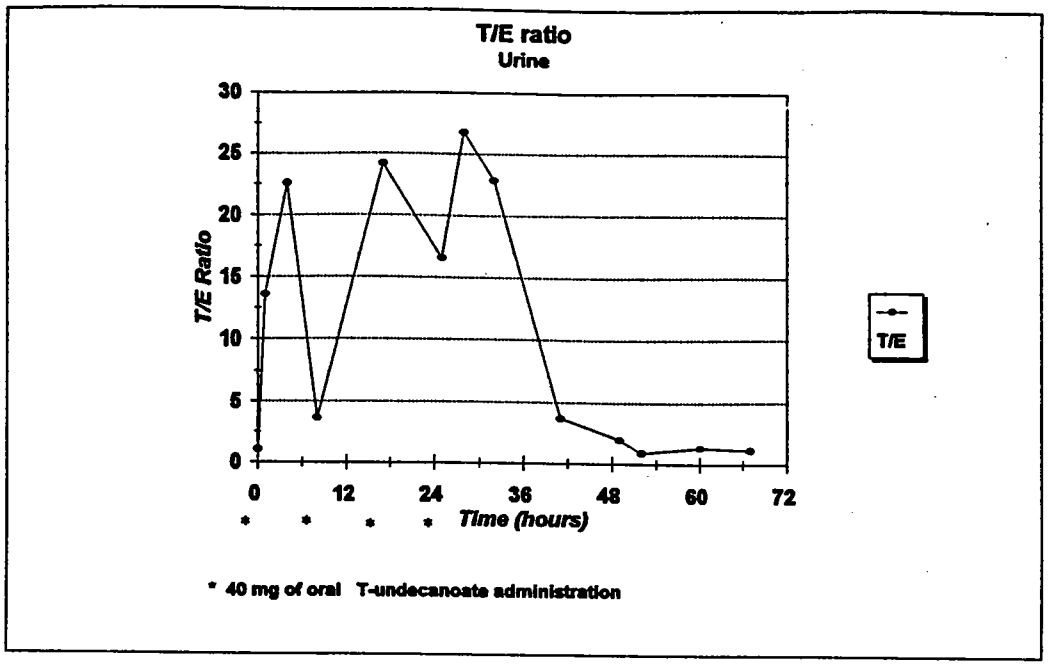


Figure 9: Urinary T/E after 4x40 mg of oral testosterone undecanoate.

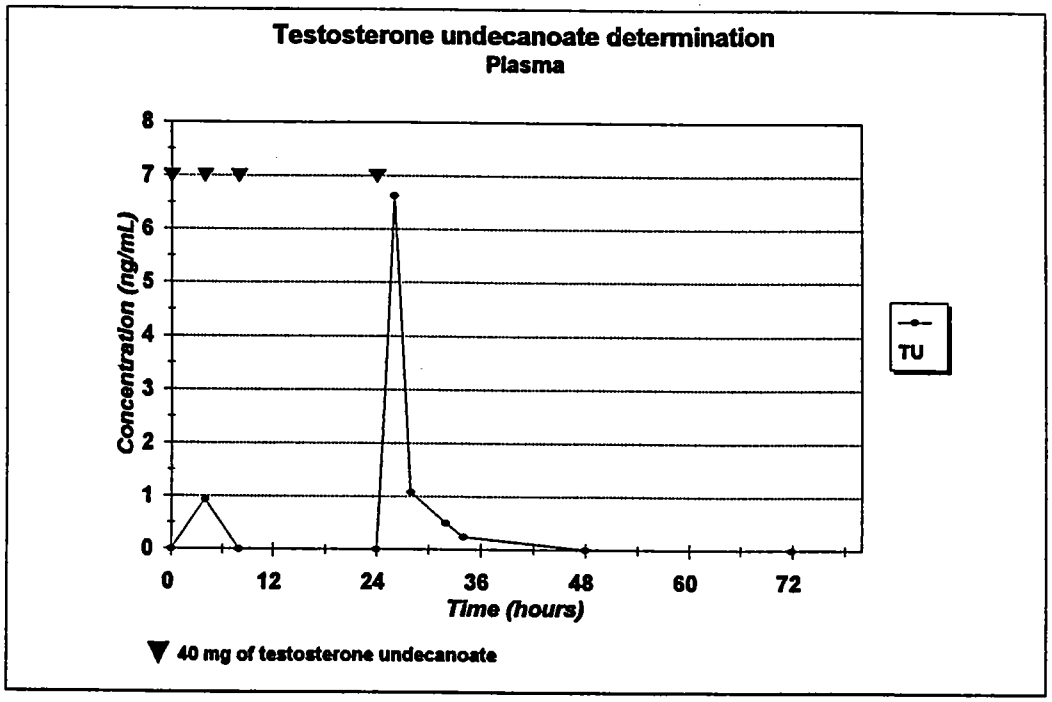


Figure 12: Plasma testosterone undecanoate after 4x40 mg of oral administration.

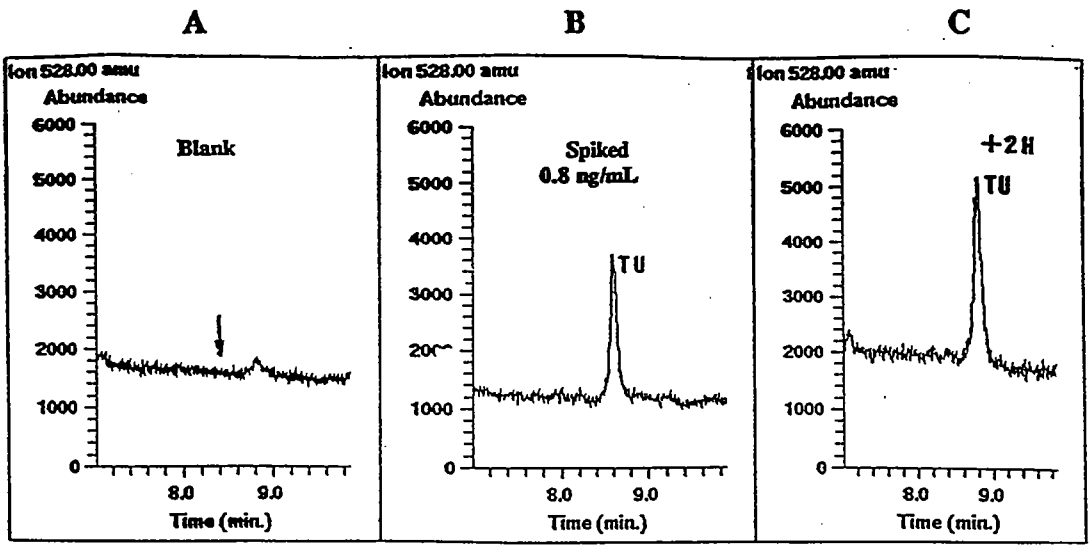


Figure 10. Chromatogram obtained after the GC/MSD analysis of samples collected after a 4x40 mg oral dose of testosterone undecanoate (TU).
A, exctracted ions for TU at m/z 528 before the administration (elution times are signaled with arrows); B, exctracted ions of a plasma blank spiked with 0.8 ng/mL of TU and C, exctracted ions of a sample 2 h after the last administration.

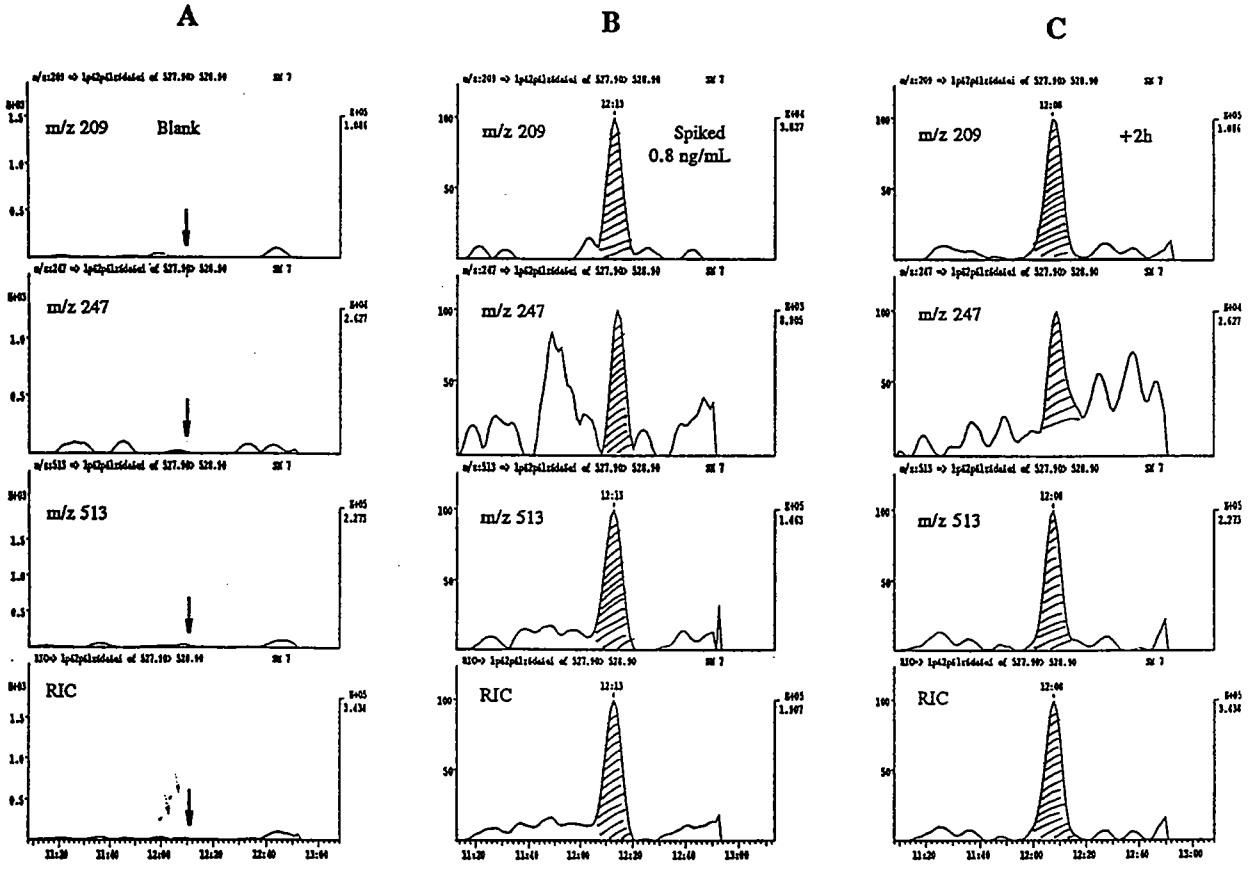


Figure 11. Exctracted ions chromatograms at m/z 209, 247 and 513 generated from ion m/z 528 (selected in the first quadrupole) obtained after the GC/M/MS analysis of samples collected after a 4x40 mg oral dose of testosterone undecanoate (TU).
A, before the administration (elution times are signaled with arrows); B, plasma blank spiked with 0.8 ng/mL of TU and C, sample 2 h after the last administration.