Nail Analysis in Forensic Toxicology for the Detection of Drug Misuse

A thesis submitted in part fulfilment of the requirements

for the Degree of Doctor of Philosophy

by

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献呈

私の３年余の海外留学を日本で待っていた妻、高市　寿美子と愛犬、ブッチ一、また今は亡き両親　高市　ツヤ子・源三郎にこの本を捧げる。

Dedication

To my wife, **Sumiko Takaichi** and my lovely dog, **Butchie** who were waiting for my homecoming from abroad study for more than three years in Japan, and to the memory of my beloved parents, **Tsuyako** and **Genzaburoh Takaichi**.

Acknowledgements

Japan developed remarkably from the ruin caused by **World War II** and became by the 1980's an internationally competitive power. The main factor in this might be the Japanese people's diligence, though there are various reasons. Everyone will also acknowledge the role of civil servants that were faithful in their obligation to develop the country, too.

I had been working hard in this same obligation for a long time as a forensic chemist and government official. My major responsibility was to introduce the latest, large scale, high performance analytical instruments and to make the best use of them for research and the development of forensic science.

I noticed nothing remains for long, when having looked back on my life by chance before I reached retirement age and I determined to study again in a foreign country.

One of my impressions when I came to **Scotland** was the very good character of **the people of Scotland**. Therefore, I have been able to study pleasantly throughout my stay.

I would like to express my gratitude to my supervisor, **Dr. Robert A. Anderson**, for his generous support and advice, along with having a warm heart. The word "**伯楽 = Hakuraku**" exists in Japan. It means an expert horse trainer who turns the hack into an excellent horse. It comes from the name of the person who often distinguished a horse's quality. That is, discovering a young person who has the nature, and raising them to become a good person. Of course, I am not an excellent horse or young person. He has an excellent nature, in the same way as "**伯楽, Hakuraku**", as an educator. Thanks cannot be told all in one word.

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The degree of **the Doctor of Philosophy** means one can work independently as a researcher. **Dr. Nikolas P. Lemos** was of great assistance at the beginning of my work. He has a generous mind, is zealous in research and was also part supervisor of my project at the same time. I deeply respect him as a good researcher and one of the pioneers of the cryogenic grinding method for fingernail analysis.

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Finally, I would like to thank **the Agency of Science and Technology**, attached to the **Ministry of Education, Japanese Government,** for giving me a short term overseas-abroad grant between May 2000 and April 2001.

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Abbreviations

Units of measurement and the technical terms are generally based on **the ACS Style Guide** (The ACS Style Guide, 2nd ed., J.S. Dodd, **American Chemical Society,** Washington, DC).

1) Volume

mL	millilitre
μL	microlitre

2) Mass

mg	milligram
ng	nanogram
pg	picogram

3) Length

m	metre
mm	millimetre
μm	micrometre

4) Time

h	hour
min	minute
sec	second

5) Other technical

°C	degrees Celsius
°C/min	degrees Celsius per minute
mg/mL	milligrams per millilitre
mL/min	millilitres per minute
ng/μL	nanograms per microlitre

rpm	revolutions per minute
v/v	volume per volume
v/w	volume per weight
v/w/v	volume per weight per volume

6) Electrical

eV	electronvolt
µA	microampere
V	volt

7) Chemistry

AH	alkaline hydrolysis (sodium hydroxide)
EPXMA	electron probe X-ray microanalyser
GC	gas chromatography
GC-MS	gas chromatography - mass spectrometry
HFB	heptafluorobutyryl
HPLC	high performance liquid chromatography
I. Std (I.S)	internal standard
LN	liquid nitrogen
LOD	limits of detection
MALDI	Matrix Assisted Laser Desorption Ionisation
MC	mass chromatogram
MF	mass fragment
MS	mass spectrum
MW	molecular weight
m/z	mass-to-charge ratio
Py-GC-MS	pyrolysis gas chromatography - mass spectrometry

R.I	retention index
RRT	relative retention time
Rt	retention time
SIM	selected ion monitoring
SPE	solid phase extraction
TIC	total ion chromatogram
TMS	trimethylsilyl
TOF-MS	Time of Flight - Mass Spectrometry

8) Chemical compounds

AAS	anabolic androgenic steroid
BHT	butylated hydroxytoluene
BSTFA	N,O-bis(trimethylsilyl)trifluoroacetamide
CBD	cannabidiol
CHCH	α-cyano-4-hydroxycinnamic acid
DBP	dibutyl phthalate
DHC	dihydrocodeine
DHT	dihydrotestosterone
DMCS	dimethylchlorosilane
DOP	dioctyl phthalate
DTE	1,4-dithio-erythritol
EDDP	2-ethylidene-1,5-dimethyl-3,3-diphenylpyrroline
EMDP	2-ethyl-5-methyl-3,3-diphenylpyrroline
Heroin	diacetylmorphine (diamorphine)
HFBA	heptafluorobutyric anhydride
11-OH-Δ^9-THC	11-hydroxy-Δ^9-tetra-hydrocannabinol

6-MAM	6-monoacetylmorphine
MSTFA	N-methyl-N-(trimethylsilyl)trifluoroacetamide
PFBO	O-(pentafluorobenzyl)hydroxyamine
SDS	sodium dodecyl sulfate
TFAA	trifluoroacetic anhydride
Δ^9-THC	Δ^9-tetrahydrocannabinol
Δ^9-THC-COOH	11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid
TMCS	trimethylchlorosilane
TMSI	trimethylsilyl iodide

9) Other general

EDAS	Edinburgh Drug Addiction Study
IOC	International Olympic Committee
USADA	United States Anti Doping Agency
WADA	World Anti-Doping Agency

Summary

Forensic toxicology is concerned with the analysis of biological specimens to detect the presence of drugs or poisons that have been previously administered. Traditional samples used for this purpose have been blood, urine and body organs, but also in the last decade, other (**alternative**) samples have become widely used. One notable example is hair, which provides a wide detection window for prior drug use.

This thesis examines the use of nail as an alternative biological specimen in forensic toxicology. Nail is a difficult analytical matrix from which to extract drugs because of its tough physical composition, based on keratin. Previously the keratin had been compulsorily dissolved with an alkaline solution. However, alkaline hydrolysis of the fingernail often chemically changed the target analytes, for example esters were hydrolysed to alcohols and carboxylic acids.

The initial part of the project investigated the use of a cryogenic grinding method for fingernail clippings. Grinding at liquid nitrogen temperatures was found to be an effective procedure and the conditions were optimised to a two or three cycle programme of freezing and grinding.

Small particle sizes were obtained of approximate size 1 μm. It was established that drugs could be extracted directly from nail powder with a range of solvents without the need for alkaline hydrolysis. Methanol was found to be the most effective extraction solvent, which also gave the lowest number of co-extracted interfering compounds.

This procedure was subsequently used with nail samples from different types of forensic cases, including cannabis, heroin and steroid abusers.

Cryogenic grinding of nail was evaluated as an extraction method for cannabinoids in nail clippings from chronic cannabis smokers. This method was also compared to the alkaline hydrolysis method. Fingernail clippings were collected with prior informed

consent from volunteers attending the **Edinburgh Drug Addiction Study (EDAS)** clinic. The collected nail clippings were decontaminated and divided into two groups: the first group was extracted with methanol after pulverisation in a liquid nitrogen cryogenic mill; the second was extracted with ethyl acetate after hydrolysis in sodium hydroxide. In both groups deuterated cannabinoids were used as internal standards. Both sets of extracts were derivatised with BSTFA before being analysed by gas chromatography - mass spectrometry (**GC-MS**).

Cannabidiol, Δ^9-tetrahydrocannabinol and **11-hydroxy-Δ^9-tetrahydrocannabinol** were quantified in both sets of extracts. **11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid** was only identified and quantified in the extracts resulting from the cryogenic grinding method.

Cannabinoid concentrations were very low, in the range 0–4 ng/mg. These results strongly support the use of nail as a biological specimen for the detection and quantification of past exposure to cannabis, and secondly, they indicate that grinding with a cryogenic mill is a useful procedure, which yields simultaneous results for the primary psychoactive cannabinoid and its metabolites.

Cryogenic grinding was then evaluated for the extraction of opioids in nail in comparison with the conventional alkaline hydrolysis method. Finger and toe nails were collected from donors with informed consent.

As before, these nails were decontaminated and divided into two groups. The first group was extracted with methanol after cryogenic grinding and the second was extracted by solid phase extraction after hydrolysis in sodium hydroxide solution.

Deuterated methadone and opioids were used as internal standards and the extracts were analysed by GC-MS as trimethylsilyl derivatives. **Morphine, codeine,** methadone, **papaverine** and **noscapine** were detected and quantified in both sets of extracts. In

addition, **heroin, 6-monoacetylmorphine** and **6-acetylcodeine** could be quantified in methanolic extracts from the cryogenic extraction method but not from the alkaline hydrolysis method.

Concentrations were also very low, in the range 0–25 ng/mg of nail. This is the first time **heroin** was detected in nail, along with **codeine, 6-acetylcodeine, papaverine** and **noscapine**. The presence of these components suggests that the specimen donors took **street heroin. Papaverine** and **noscapine,** alkaloids in opium, have not previously been detected in urine or blood.

Cryogenic grinding was subsequently used for extraction of endogenous and anabolic steroids from nail clippings of doping abusers to show that both can be detected, as in hair. This method was compared to blood **(plasma)** and urine samples analysed at the same time.

Fingernail clippings from users of **anabolic steroids** (including testosterone esters, stanozolol and methenolone acetate) were obtained from the **Institute of Doping Analysis/Sports Biochemistry,** Kreischa. Blank nail and urine samples were obtained from volunteers.

The nail samples were decontaminated, pulverised and extracted with methanol/ ethyl acetate (7:3, v/v). Deuterated anabolic steroids (5α-estran-3ß-ol-17-one-d_3, testosterone-d_3, and stanozolol-d_3) and medroxyprogesterone were used as internal standards.

The extracts were converted to TMS derivatives with MSTFA/NH_4I/2-mercaptoethanol and analysed by GC-MS in the EI + full scan and SIM modes. Endogenous steroids were identified and quantified in the nail samples from both steroid users and non-users.

Concentrations in nail were low, in the pg/mg range: (**androsterone** (1.5–12.8

pg/mg), **etiocholanolone, dehydroepiandrosterone** (1.4–25.3 pg/mg), **epiandrosterone** (0.7–14.3 pg/mg), **epitestosterone** (0.6–11.0 pg/mg) and **testosterone** (0.6–25.0 pg/mg).

Also, elevated concentrations of testosterone in nail were positively associated with high concentrations in plasma and urine. However, although the analytical results provided evidence for the presence of anabolic steroids in the samples from steroid users, including testosterone and its isocaproate and enanthate esters, at low concentrations, it has not yet been possible to confirm this due to interference from other endogenous substances.

Nail remains a potential, but still to be confirmed, alternative biological specimen to hair for the detection of past exposure to doping steroids.

With permission of **the Faculty of Science,** part of the work for this thesis was carried out at an external institution, **the Identification Research Laboratory, National Research Institute of Police Science,** Tokyo.

Pyrolysis GC-MS (**Py-GC-MS**) and MALDI-TOF mass spectrometry (**MALDI-TOF-MS**) were evaluated for the direct identification of illicit drugs within the nail matrix and in very small fingernail samples. Opium alkaloids and opioids were used as test compounds.

The results showed that opium alkaloids could be detected amidst a large amount of impurity resulting from the thermal degradation of samples by Py-GC-MS analysis.

Moreover, target analytes could be detected easily in the MALDI-TOF-MS analysis by a simple analytical procedure.

Papers in Support of This Thesis

Conference presentations:

(a) Evaluation of Alternative Extraction Procedures for Cannabinoids from Nail Clippings of Chronic Cannabis Smokers.

Nikolaos P. Lemos, Kenichi Takaichi, Robert A. Anderson and J. Roy Robertson,

Presented at **the American Academy of Forensic Sciences Meeting**, Seattle, February, 2001.

(b) Analysis of Opiates in Nail Clippings from Chronic Heroin Abusers.

Kenichi Takaichi, Nikoloas P. Lemos, Robert A. Anderson,

Presented at **the 39th International TIAFT Meeting**, Prague, August, 2001.

(c) Doping Steroid Analysis in Nail Clippings.

Kenichi Takaichi, R.A. Anderson and D. Thieme,

Presented at **the 42nd SOFT/TIAFT Meeting,** Washington D.C., August-September, 2004.

1. Introduction

Forensic toxicology is the study of poisons and of cases of poisoning for the purposes of a medico-legal enquiry [1, 2]. In different jurisdictions, the scope of the subject varies and can include fatalities, non-fatal poisoning, drugs in sport, road traffic safety, drug abuse monitoring in different contexts such as drug clinics, prison inmates and employment screening.

In the field of forensic toxicology, it is extremely important to determine promptly and accurately whether a person has used an illicit drug. Blood, urine, and internal organs have traditionally been used as samples to detect the presence of illicit drugs. As many new analytical methods were developed with scientific advancement through the ages, we have reached the stage when even amounts of an illicit drug can be analysed by high sensitivity in a small amount of blood and urine.

Blood, urine, and internal organs are considered to be the first generation of samples in the study of forensic toxicology. The second generation of samples appeared in the 1980's when hair, saliva, and sweat, amongst others began to be used as alternatives to blood.

Drugs taken into the human body are promptly metabolised and excreted out of the body. Therefore, an examination of the components of drugs in blood and urine should collect the samples at an early stage after administration. The feature of the first generation samples is that obtaining them is easy.

On the other hand, the identification of drugs becomes difficult as time passes. Moreover, metabolites and interfering substances increase as the amount of the target drug in blood decreases because of metabolism. Therefore, the extraction and identification of the drug become difficult.

It has been known for a long time that drugs are accumulated in hair, as hair - a

second-generation sample - became a subject of study [3].

As a result, there is a possibility that the existence of the drug can be proven because the drug concentration of each part of a hair differs even if time has passed since the drug was used.

Hair consists of the protein keratin. Therefore, the extraction of drug from hair becomes difficult compared with samples of the first generation.

There are chiefly two analytical methods used in the extraction of drugs from hair. One is a method of extraction after hair is dissolved with alkali (**alkaline digestion method**). The second is a method of extraction with solvent after the hair is cut with scissors as small as possible or pulverised with a mortar or other device (**soaking method**). The first method raises the fear that target analytes might be hydrolysed, and the second has the disadvantage that the extraction time becomes long.

As previously described, the advantage of hair as a second generation sample is to be able to prove the existence of the drug even if some time has passed since the drug was used. The disadvantage of hair samples is that the amount of sample available for the analysis can be very little.

The fingernail has been ignored for a long time as a sample in the field of forensic toxicology. For one reason, fingernails are harder than hairs. Therefore, the fingernail cannot be pulverised easily. The method of hydrolysing fingernail samples with alkali and the extraction method by cryogenic grinding method have been reported in the 1990's.

Therefore, the fingernail can be called the new sample of the third generation when considered from a forensic toxicological viewpoint. Drugs in the fingernail, where the cryogenic grinding method is applied, can be extracted without hydrolysis of a target drug compared with the alkali hydrolysis method.

Aims

The aims of the work carried out in this project were as follows:

1. To develop and assess improved methods of sample processing, drug extraction and analysis for nail samples when used as an alternative matrix in forensic toxicology. Earlier work used methods based on alkaline hydrolysis but this is unacceptable in many cases because of analyte degradation, especially when drugs of abuse are analysed. Both the opiate group and the cocaine alkaloids are easily hydrolysed under alkaline conditions.
2. To apply and evaluate this improved methodology for the analysis of samples relevant to the field of forensic toxicology, including nail specimens from controlled drug users and from doping steroid users. In this context, nail would be an alternative specimen to hair for detection of drug use over a long window of detection period. The most commonly abused drugs in the United Kingdom are cannabis and heroin and these were selected as the target drug groups in this project.
3. To investigate instrumental methods of analysis which might be suitable for direct analysis of target analytes in the nail matrix and which might enable this type of analysis to be carried out with very small samples. If very small samples can be directly analysed then nail could be analysed *in situ* or else nail scrapings rather than nail clippings could be analysed.

2. Evaluation of Alternative Extraction Procedures for Cannabinoids and Opiates in Nail Clippings.

2.1. Introduction

In forensic toxicology, if nail is considered as a biological sample, then it belongs to the relatively unexplored field of third generation biological samples.

The first generation samples were urine, blood, and internal organs. The second generation samples were hair, sweat and oral fluid (**saliva**) which had started to be used in the 1990's. The chemical compounds taken into nail have been actively researched in the fields of cosmetics, environmental medicine and dermatology, to name but a few.

In the cosmetics field, solvents and other ingredients of manicures (**nail polish**) have chiefly been researched [4, 5, 6, 7]. These investigations concern the permeation of chemical compounds from the dorsal nail plate of the nail. For this reason, these studies are unrelated to the work described in this thesis.

In environmental medicine, heavy metals such as arsenic [8], germanium [9], selenium [10, 11], nickel [12, 13, 14] and mercury [15] have been studied in the interests of environmental pollution or occupational disease. As for cosmetics, the work in this thesis has little to do with this research and these fields, because mainly the heavy metals have been studied.

In the dermatological field, in particular toenail onychomycosis, there are a lot of papers on the antimycotics in fingernail and toenail, for instance, itraconazole [16, 17, 18], ketokonazole [18], miconazole [19, 20], fluconazole [18, 21], and oxiconazole [22].

It is reported that the distribution of these antifungal agents to the nail following oral administration is prompt. Detailed analytical methods for the drugs are not generally described though the extraction methods of antifungal agents can be applied to the

extraction of illicit drugs. Moreover, the extraction methods were conventional methods.

Extremely little research has been done on illicit drugs in nail in the forensic toxicological field. The nail is composed of the hard protein that is called keratin, which changes the ***stratum corneum*** of the skin in the same way as the surface of an animal's skin is modified to hair, nail, scale, feather, bill, horn and hoof. Therefore, the extraction of drugs from nail is difficult. A detailed review was recently reported by **Palmeri** et al [23].

The conventional extraction of analytes in nail is a method involving soaking for a long time in solvent after the nail is cut into as small pieces as possible **(soaking method)** [24, 25, 26, 27, 28]. This method is subject to several drawbacks including low extraction efficiency and possible hydrolysis of the target analytes when exposed to solvents for extensive periods of time. The second method is an extraction method after the nail is dissolved with an alkaline solution such as sodium hydroxide **(alkaline hydrolysis method)** [29]. In both methods heroin is easily hydrolysed, especially in the latter method. Therefore, the presence of morphine and 6-monoacetylmorphine, which are the hydrolysis products of heroin, have been used instead to prove the administration of heroin. In the same way, the confirmation of 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (**Δ^9-THC-COOH**) and other metabolites is important as it proves the consumption and metabolism of Δ^9-tetrahydrocannabinol (**Δ^9-THC**) in a biological system and could help disprove claims of environmental contamination.

In the present study, cannabinoids, heroin and related opiates have been extracted directly from the nail by an extraction method that uses cryogenic grinding. This method was carried out under mild conditions, with little hydrolysis of heroin compared to the conventional extraction methods.

2.2. Experimental

2.2.1. Nail samples

Fingernail clippings for cannabinoid analysis were collected with informed consent from nine (9) male chronic cannabis smokers participating at **the Edinburgh Drug Addiction Study.**

The nine volunteers produced their own fingernail clippings using commercially available nail clippers under the supervision of a researcher. The nail clippings were placed in a transparent plastic bag and transported to the laboratory for analysis. The volunteers did not receive any remuneration for providing these specimens. The procedures followed were in accordance with the ethical standards of the responsible committees on human experimentation (institutional and regional).

Fingernail and toenail clippings for opioid analysis were collected from 17 people at **the Glasgow Drug Problem Service in Glasgow** (Scotland, U.K.) after informed consent had been obtained from the donors.

Samples were anonymised but relevant biographical details were noted when the clients were interviewed including age, sex, a history of what they have ingested (drug types, quantities and frequency of usage on average), an average amount of money spent per week on drugs, quantities of methadone a week which were prescribed in their methadone substitution programme.

2.2.2. Reagents

1) Standards and deuterated standards

Cannabidiol (**CBD**), Δ^9-tetrahydrocannabinol (**Δ^9-THC**), 11-hydroxy-Δ^9-tetra-hydrocannabinol (**11-OH-Δ^9-THC**), and 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (**Δ^9-THC-COOH**) were purchased from **Sigma** (Saint Louis, USA).

Δ^9-tetrahydrocannabinol-d_3 (Δ^9-**THC-d_3**) and 11-hydroxy-Δ^9-tetrahydrocannabinol -d_3 (**11-OH-Δ^9-THC-d_3**) used as internal standards were also purchased from **Sigma**.

11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid-d_3 (Δ^9-**THC-COOH-d_3**) was purchased from **Radian International** (Austin, USA).

Diacetylmorphine (Diamorphine, **heroin**) was purchased from **Sigma** (Saint Louis, USA).

6-monoacetylmorphine (**6-MAM**), morphine, codeine, dihydrocodeine (**DHC**), 2-ethyl-5-methyl-3,3-diphenylpyrroline (**EMDP**) and 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrroline (**EDDP**) were purchased from **Radian International** (Austin, USA).

6-Acetylcodeine was synthesised by acetylation in which codeine was heated at 60 °C with acetic anhydride and pyridine as catalyst for 30 min. The purity of the synthesised 6-acetylcodeine was confirmed with gas chromatography - mass spectrometry (**GC-MS**).

Methadone was purchased from **High Standard Products Corporation** (CA, USA).

Deuterated internal standard compounds of **6-MAM-d_3**, **morphine-d_3**, **codeine-d_3**, **methadone-d_3** and **EDDP-d_3** were obtained from **Radian International** (Austin, USA).

Stock solutions were purchased from suppliers at a concentration of 100 µg/mL in methanol and were diluted to give working standard solutions at a concentration of 1 µg/mL methanol.

2) Solvents and reagents

Methanol was high performance liquid chromatography (**HPLC**) grade (**BDH Laboratory Supplies**, U.K.). 2-Propanol was analytical reagent grade (**Fischer Science International Co.**, U.K.). Dichloromethane was from **Sigma-Aldrich**. Ethyl acetate was of analytical reagent grade from **Fischer Science International Co.**

N,O-bis(trimethylsilyl)trifluoroacetamide (**BSTFA**) with 1% of trimethylchlorosilane (**TMCS**) was from **Regis Technologies Inc** (Il, USA).

Solid phase extraction (**SPE**) cartridges were **Worldwide Monitoring Clean Screen** cartridges, CSDAU, ZSDAU020 (**United Chemical Technologies Inc.** Bristol, U.K.). Other solvents and reagents used were all analytical or HPLC grade.

2.2.3. Instruments

Cryogenic grinding was carried out using a **SPEX CertiPrep Model 6750 Freezer Mill** from **Glen Creston U.K. Ltd.** Essentially this device is a ball mill, which is operated in an insulated container containing liquid nitrogen. Samples are ground inside vials, which are available in both large and micro sizes. The micro-vial set, suitable for small samples, was used throughout this study.

Qualitative and quantitative analysis were carried out using a **Thermo Finnigan Trace GC-MS** equipped with an **AS 2000 autosampler** and an **RTX-5MS** column (15 m x 0.25 mm i.d.; film thickness 0.25 μm).

The column temperature was programmed from 150 °C to 300 °C (held for 2 min) at 10 °C/min, and the MS detector (70 eV) was operated in the selected ion monitoring (**SIM**) mode or repetitive full scan mode at a source temperature of 220 °C.

In the latter mode, scans were acquired at approximately 3 scans per sec, giving 12–18 data points per GC peak in reconstructed ion chromatograms, depending on peak width, for quantification of analytes.

2.2.4. Optimisation of cryogenic grinding method

Optimisation of the cryogenic grinding cycle (cooling time, number of grinding periods and length of each) was performed by **Lemos** and was not repeated for the work

described here. The optimum routine involved three cycles with 1 min pre-cooling period, 1 min grinding period and 1 min between cycles.

Six different solvents (acetonitrile, acetone, chloroform, ethyl acetate, hexane and methanol) were evaluated as extraction solvents.

2.2.5. Cleaning of micro-vials for re-use

To remove any residual contamination of the cryogenic grinder micro-vials before they were used again for another sample, the micro-vials were washed well with tapwater. Subsequently, they were washed in de-ionised water in a beaker for 15 min with ultrasonication. The micro-vials were finally washed with methanol for 15 min with ultrasonication.

To confirm that there was no residual contamination of the micro-vials, decontaminated blank nail was used to provide a procedural blank sample. As shown in **Figure 2.3 (f)**, no cannabinoids were detected. Similar results were obtained for **opioids** and **anabolic steroids,** described in subsequent sections.

Re-use of micro-vials is necessary because they are not disposable items. Routine analysis of large numbers of nail samples would best be carried out with multiple sets of micro-vials in order to avoid time delays caused by recycling of the vials.

2.2.6. Sample preparation

1) Decontamination of nail clippings

The nail clippings were washed with 10 mL of 0.1% sodium dodecyl sulfate (**SDS**) for 15 min using ultra-sonication.

The samples were similarly washed three times with 10 mL of de-ionised water and 10 mL of methanol, respectively. The methanol wash solutions were preserved for later

analysis, in order to establish the complete decontamination of the nails.

The washed nail clippings were dried at room temperature. The samples were approximately divided into half for the cryogenically ground method with **liquid nitrogen (LN method)** and the **alkaline hydrolysis** method **(AH method)**, respectively. Each sample was accurately weighed at each stage of preparation.

2) Methanolic extraction method with cryogenic grinding

The washed nail clippings (3.4–109.4 mg) were pulverised with the cryogenic grinder under freezing with liquid nitrogen.

The cryogenic grinder was equipped with a micro-vial set capable of handling small weights of samples. The grinding routine involved three cycles with 1-min pre-cooling period, 1 min grinding period and 1 min between cycles. The powdered samples were rinsed out of the grinding tubes with 10 mL of methanol into clean screw cap tubes and the suspensions were sonicated for 15 min.

Internal standard solutions (50 μL of each of the deuterated cannabinoids or deuterated opiates) were added to the methanolic extracts. The methanol extracts were centrifuged for 10 min at 3,000 rpm after vortex mixing for 30 sec. The supernatant was collected as the liquid nitrogen (**LN**) portion.

3) Alkaline hydrolysis method with sodium hydroxide

The washed nail clippings (3.3–133.0 mg), to which had been added each of the internal standards, were hydrolysed with 1 mL of 1 M sodium hydroxide solution at 90 °C for 30 min in a 15 mL test tube.

a) Cannabinoids

The samples were allowed to cool to room temperature and 5 mL of ethyl acetate was added to each alkaline hydrolysate.

Extraction was performed using a mechanical Rock and Roll extractor (**Luckham Ltd**, U.K.) for 30 min. The samples were centrifuged for 10 min at 3,000 rpm and the ethyl acetate layer was removed from the mixture and used for analysis by GC-MS after derivatisation.

b) Opioids

After the sample had been cooled, the sample solution pH was adjusted with 1 M hydrochloric acid using **the Accumet Model 10** pH meter within the range pH 6.0–6.5.

Potassium dihydrogen phosphate buffer solution (0.1 M, 2 mL) was added to the hydrolysates prior to SPE extraction.

The SPE extraction was carried out using an **IST Vac/Master** device (**International Sorbent Technology**, U.K.). The SPE cartridge was conditioned with 3 mL of methanol. Subsequently, the SPE cartridge was washed with 3 mL of de-ionised water and 1 mL of 0.1 M potassium dihydrogen phosphate buffer solution.

The alkaline hydrolysate sample was added to the SPE cartridge at flow of 2 mL/min after vortex mixing.

The SPE cartridge was washed with 2 mL of de-ionised water, 2 mL of 0.1 M hydrochloric acid and 3 mL of methanol, respectively. The SPE cartridge was dried for 10 min under full vacuum.

Opiates were eluted with 4 mL of dichloromethane/propane-2-ol/ammonium hydroxide (78:20:2, v/v/v) solution. The eluate was used for the analysis as the sodium hydroxide portion.

4) Derivatisation for GC-MS analyses

Both the LN portion and AH portions were evaporated to dryness at 60 °C under a stream of nitrogen and the residues were derivatised with 50 µL of BSTFA with 1% TMCS for 20 min at 70 °C. Derivatised samples were transferred to autosampler vials for

GC-MS analysis.

2.2.7. Gas chromatography - mass spectrometric analyses

Qualitative and quantitative analysis were carried out with the **Finnigan Trace GC-MS** with **AS 2000 autosampler.**

A 1 μL aliquot of derivatised analytes were analysed on an **RTX-5MS** column (15 m x 0.25 mm i.d.; film thickness 0.25 μm) with He (0.9 mL/min) as carrier gas, temperature programming from 150 °C to 300 °C (held for 2 min) at 10 °C/min.

Interface and ion source temperature, detector voltage, emission current were 250 °C, 200 °C, 500 V and 350 μA, respectively and the instrument was operated in the EI+ selected ion monitoring (**SIM**) mode with an electron energy of 70 eV.

2.2.8. Quantitation of cannabinoids in nail extracts

1) Preparation of standard samples for calibration curve

All standard cannabinoid stock solutions were prepared at a concentration of 1 mg/L in methanol.

For each cannabinoid analysed, a series of solutions was prepared in order to construct a standard calibration curve. In small test tubes 10, 25, 50, 100 and 250 μL of each cannabinoid standard were added together with 50 μL of internal standard solution.

These samples were then derivatised and analysed by GC-MS in order to produce proper calibration curves.

2) Calibration curve

The quantitative analysis of each cannabinoid used the SIM mode of GC-MS. One μL of each standard solution was injected into the GC-MS and the peak area ratio of the

sample to the internal standard sample was used for the preparation of the calibration curve.

3) Fragment ions used for the SIM mode

Table 2.1 shows the selected ions and retention times used for the identification and quantification of the cannabinoids.

CBD (*m/z* 390) and Δ^9-THC (*m/z* 371, 386) used Δ^9-THC-d_3 (*m/z* 389, 374) as internal standard. 11-OH-Δ^9-THC (*m/z* 371, 474) used 11-OH-Δ^9-THC-d_3 (*m/z* 374) as internal standard. Finally, Δ^9-THC-COOH (*m/z* 371, 473, 488) used Δ^9-THC-COOH-d_3 (*m/z* 374) as internal standard.

2.2.9. Quantitation of opioids in nail extracts

1) Preparation of standard samples for calibration curve

All stock standard opiates and methadone were prepared at concentrations of 1 mg/L with methanol and diluted prior to use. Each of the standard sample solutions prepared was separately collected in small test tubes in aliquots of 5, 15, 50, 100 and 250 µL, respectively. Internal standard solutions (50 µL) were added to each test tube at the same time. Unextracted standards were used as it was not possible to introduce drugs into the nail matrix. These samples were derivatised and prepared for the calibration curves by the same method as the sample preparation from the nail.

2) Calibration curve

The quantitative analysis of each drug used SIM mode of GC-MS. One (1) µL of each of the standard solutions at concentrations of 0.1, 0.3, 1.0, 2.0 and 5.0 mg/L were introduced into GC-MS respectively. The peak area ratios of the drug standards to

internal standards were used for the preparation of the calibration curve.

3) Fragment ions used for the SIM mode

The quantified compounds and selected ions were as shown in **Table 2.1.** Methadone (*m/z* 294) used methadone-d_3 (*m/z* 297) as an internal standard. Codeine (*m/z* 371), DHC (*m/z* 373), and 6-acetylcodeine (*m/z* 341) used codeine-d_3 (*m/z* 374) as an internal standard, respectively. Morphine (*m/z* 429) used morphine-d_3 (*m/z* 432) as an internal standard. Heroin (*m/z* 327) and 6-MAM (*m/z* 399) used 6-MAM-d_3 (*m/z* 402) as an internal standard.

Table 2.1. Retention times and selected ions for GC-MS analysis of nail extracts

No.	Compound name	R. Time (min)	Selected Ions (*m/z*)	MW	TMS (number)
1	CBD	7.91	301, 337, 390	488	di
2	**Δ^9-THC-d_3**	8.81	374, ***389***	389	mono
3	Δ^9-THC	8.83	371, ***386***	386	mono
4	**Δ^9-THC-COOH-d_3**	11.79	374	491	di
5	Δ^9-THC-COOH	11.81	371, 473	488	di
6	**11-OH-THC-d_3**	10.78	374	477	di
7	11-OH-THC	10.80	371, 372, ***474***	474	di
8	EMDP	5.36	208, 193	263	0
9	**EDDP-d_3**	6.14	***280***, 279	280	0
10	EDDP	6.17	***277***, 276	277	0
11	**Methadone-d_3**	7.06	297	312	0
12	Methadone	7.08	294	309	0
13	Dihydrocodeine	9.01	***373***	373	mono
14	**Codeine-d_3**	9.59	***374***, 346	374	mono
15	Codeine	9.61	***371***, 343	371	mono
16	**Morphine-d_3**	10.01	***432***, 417	432	di
17	Morphine	10.03	***429***, 414	429	di
18	6-Acetylcodeine	10.17	***341***, 282	341	0
19	**6-MAM-d_3**	10.53	***402***, 343	402	mono
20	6-MAM	10.54	***399***, 340	399	mono
21	Heroin	11.14	327, ***369***	369	0
22	Papaverine	12.64	N/Q	339	0
23	Noscapine	14.86	N/Q	413	0

Notes: N/Q = Not quantified, TMS (number) = number of trimethylsilyl group.

Bold, italic and underline = molecular ion (*m/z*).

2.3. Results and Discussion

2.3.1. Optimisation of the cryogenic grinding method

The extraction method of drugs in nail used **Lemos's method** [30]. The conditions with respect to the time needed to cool the grinder with liquid nitrogen and time intervals to pulverise the frozen nail with the cryogenic grinder were examined respectively. As a result, the conditions that were found to be effective were a cooling time of 1 min and grinding time of 2 min., with the cycle being repeated twice.

The electron probe X-ray microanalyser (**EPXMA**) was used to compare the size of the particulates in the powdered nail samples after extraction with cryogenic grinding. The minimum grain size in a powdered nail sample had a diameter of less than about 0.6 μm (**Figures 2.1** and **2.2**).

The kind of solvent used to extract drugs from the pulverised nail was also examined. The solvents tested were acetone, acetonitrile, chloroform, ethyl acetate, hexane, and methanol. Methanol was selected based on the low background interference obtained, the easiness of the solvent to handle and the solubility of the analytes (**Figures 2.3a–f**).

Impurities detected from methanol blank were mainly phthalate esters (dibutyl (**DBP**) and dioctyl (**DOP**)), aliphatic fatty acid esters (palmitic and stearic) and butylated hydroxytoluene (**BHT**). These components originate chiefly in plastic additives that contaminate the environment.

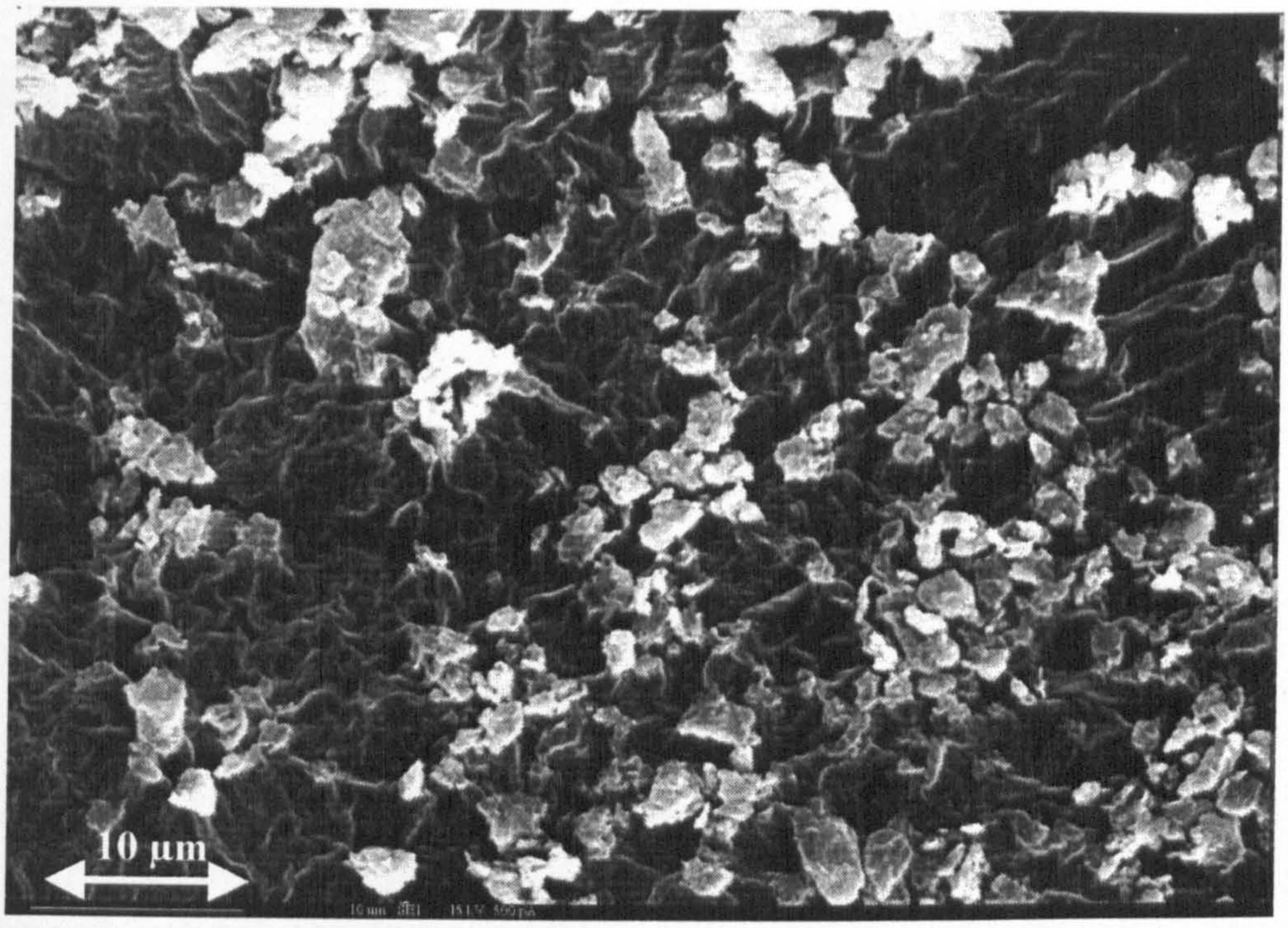


Figure 2.1. Pulverised nail after extraction with ethyl acetate

The diameter of a nail particle is less than about 0.6 µm.
(Courtesy of **Mr. Syuji Saitoh**, NRIPS, Japan).

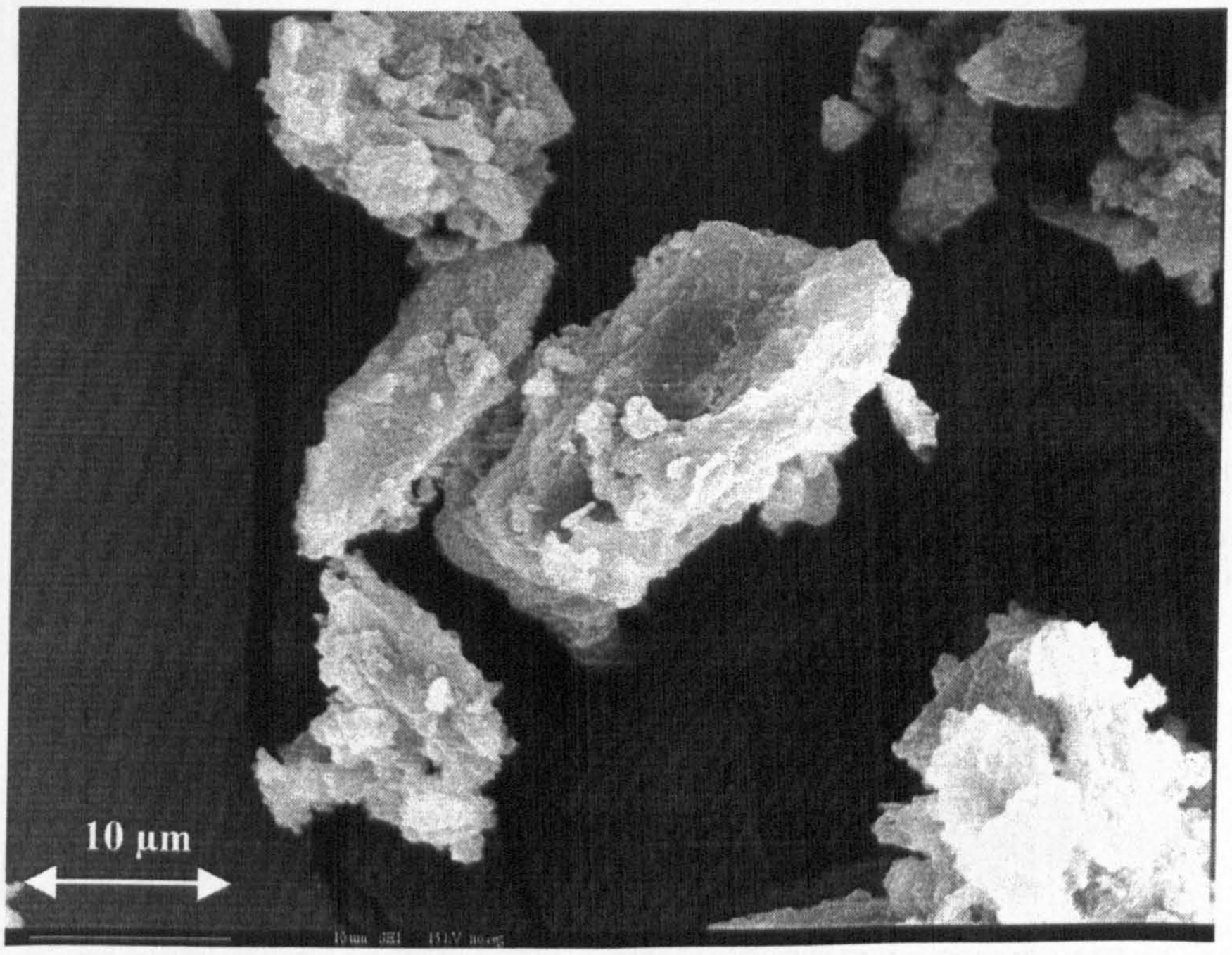


Figure 2.2. Pulverised nail after extraction with ethyl acetate

(Courtesy of **Mr. Syuji Saitoh**, NRIPS, Japan).

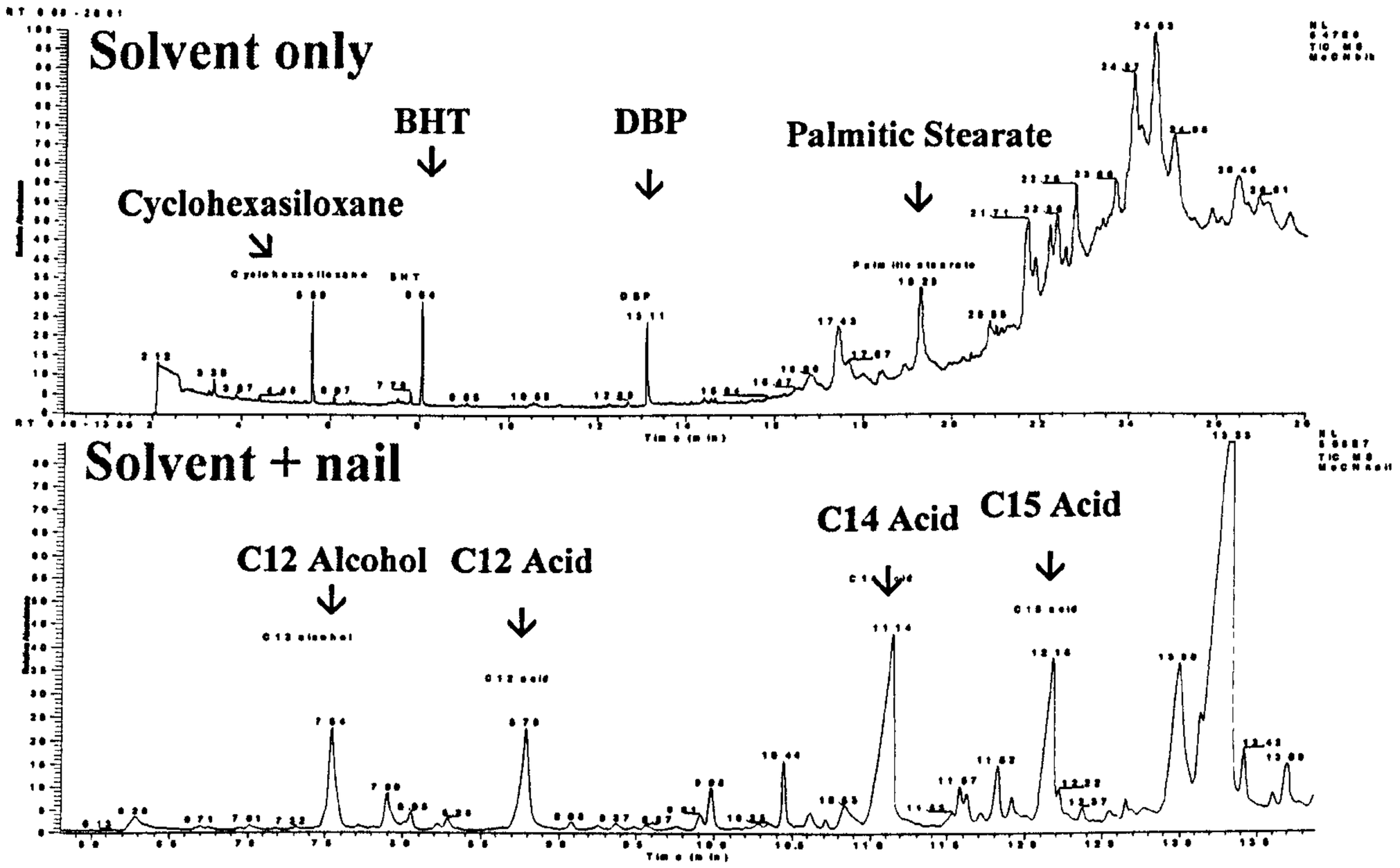


Figure 2.3. Evaluation of solvents for extraction of powdered nail: GC-MS analysis of solvent blanks and blank nail extracts

(a) Acetonitrile

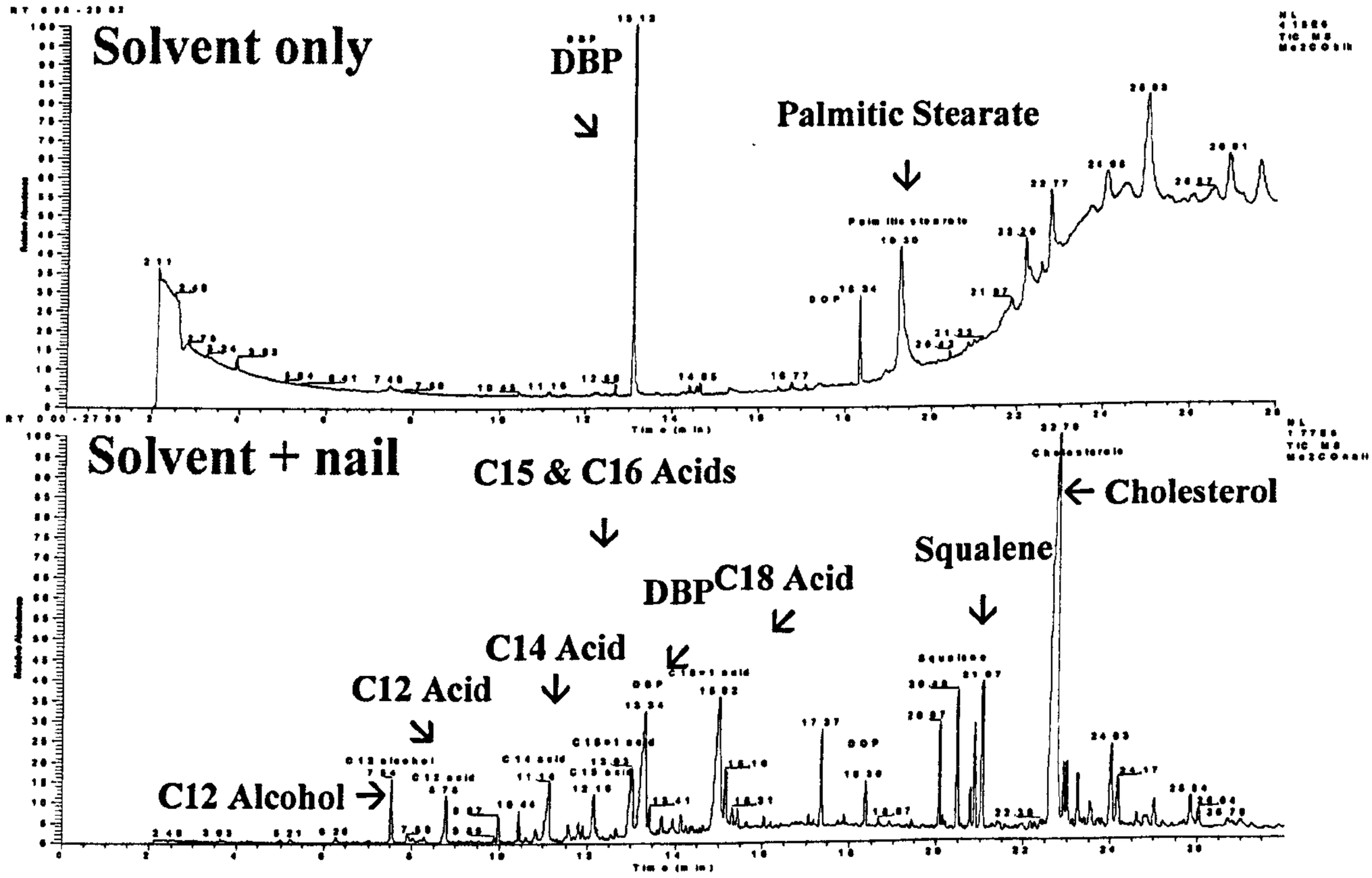


Figure 2.3. (b) Acetone

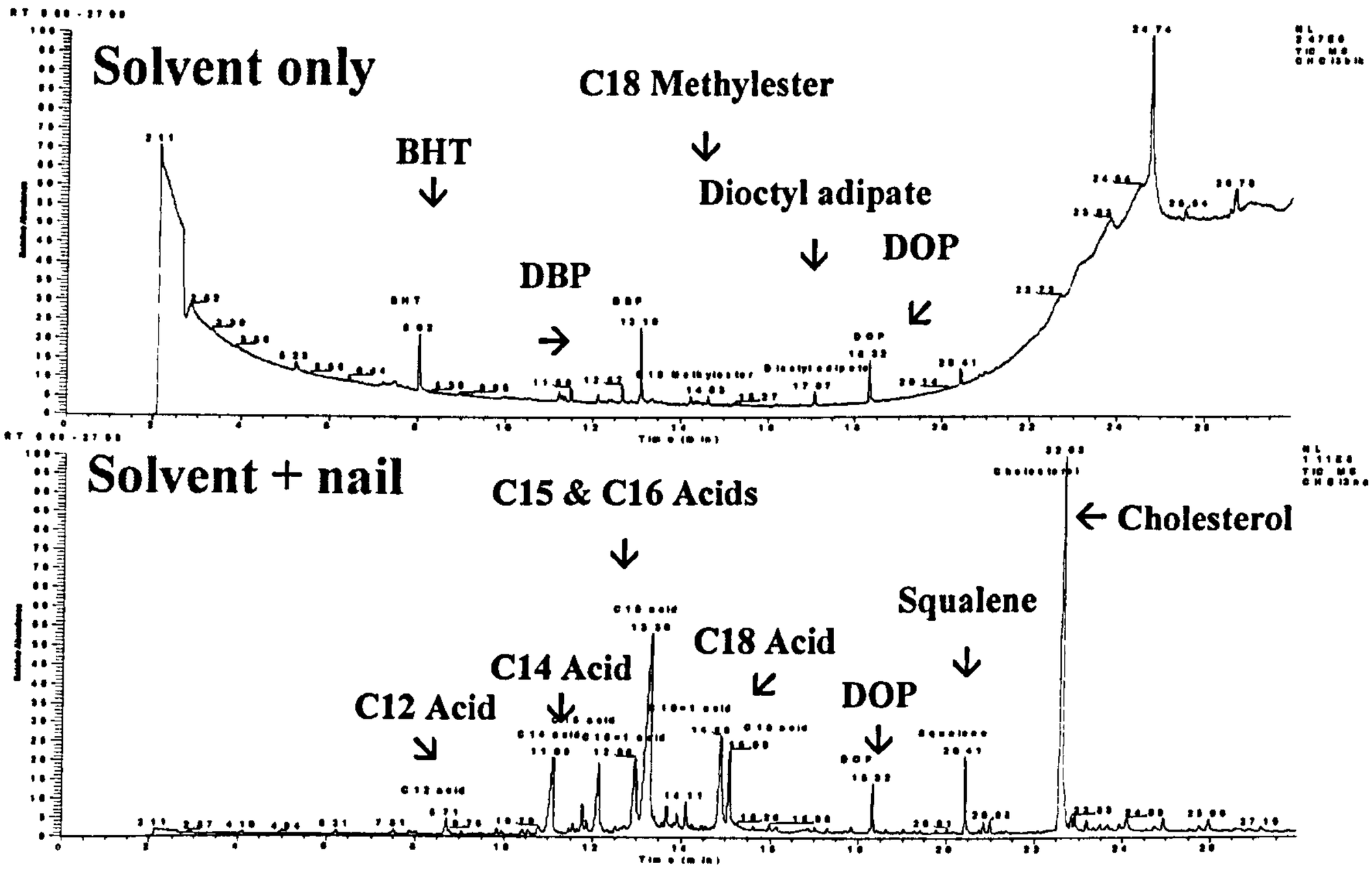


Figure 2.3. (c) Chloroform

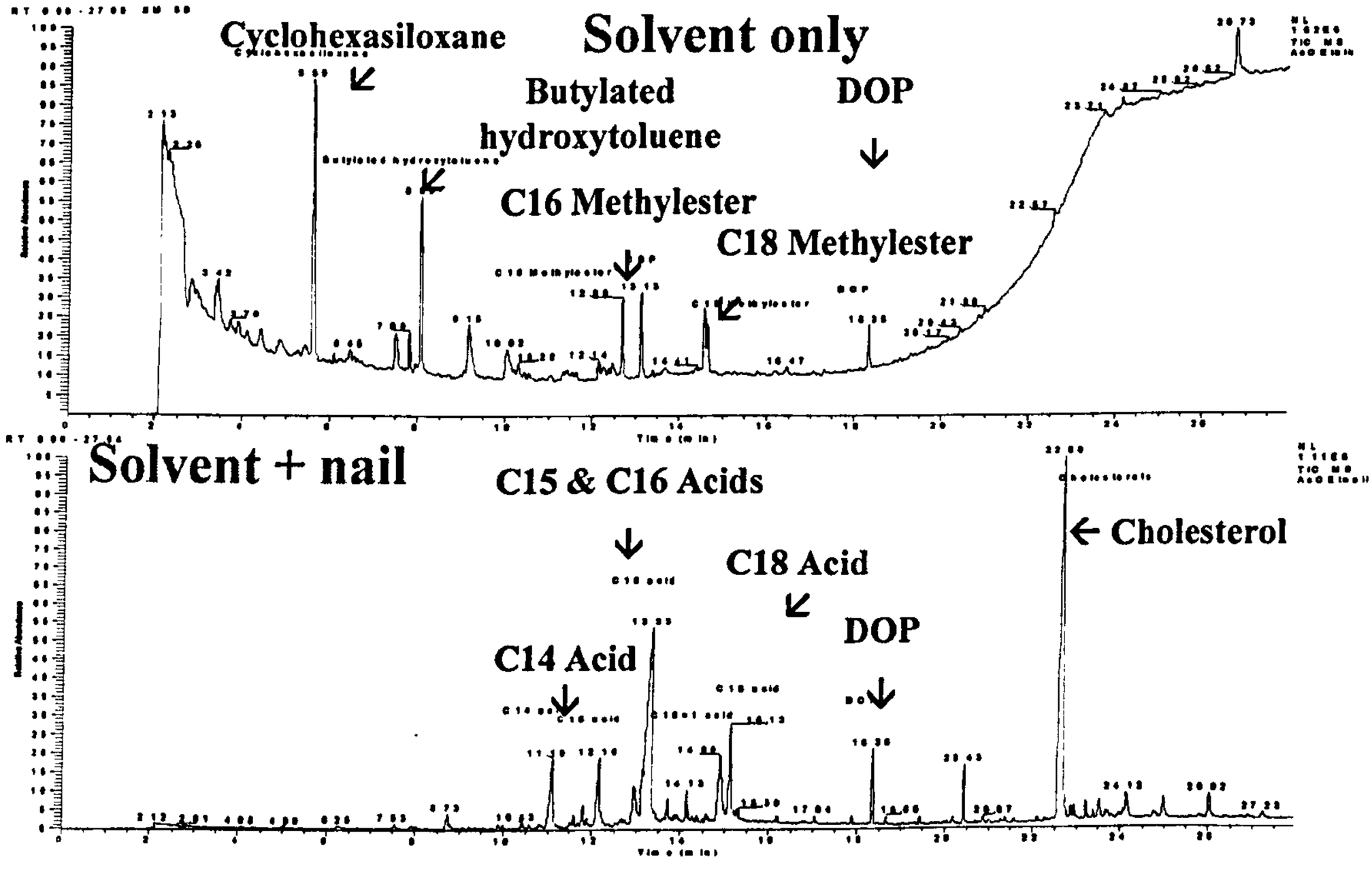


Figure 2.3. (d) Ethyl acetate

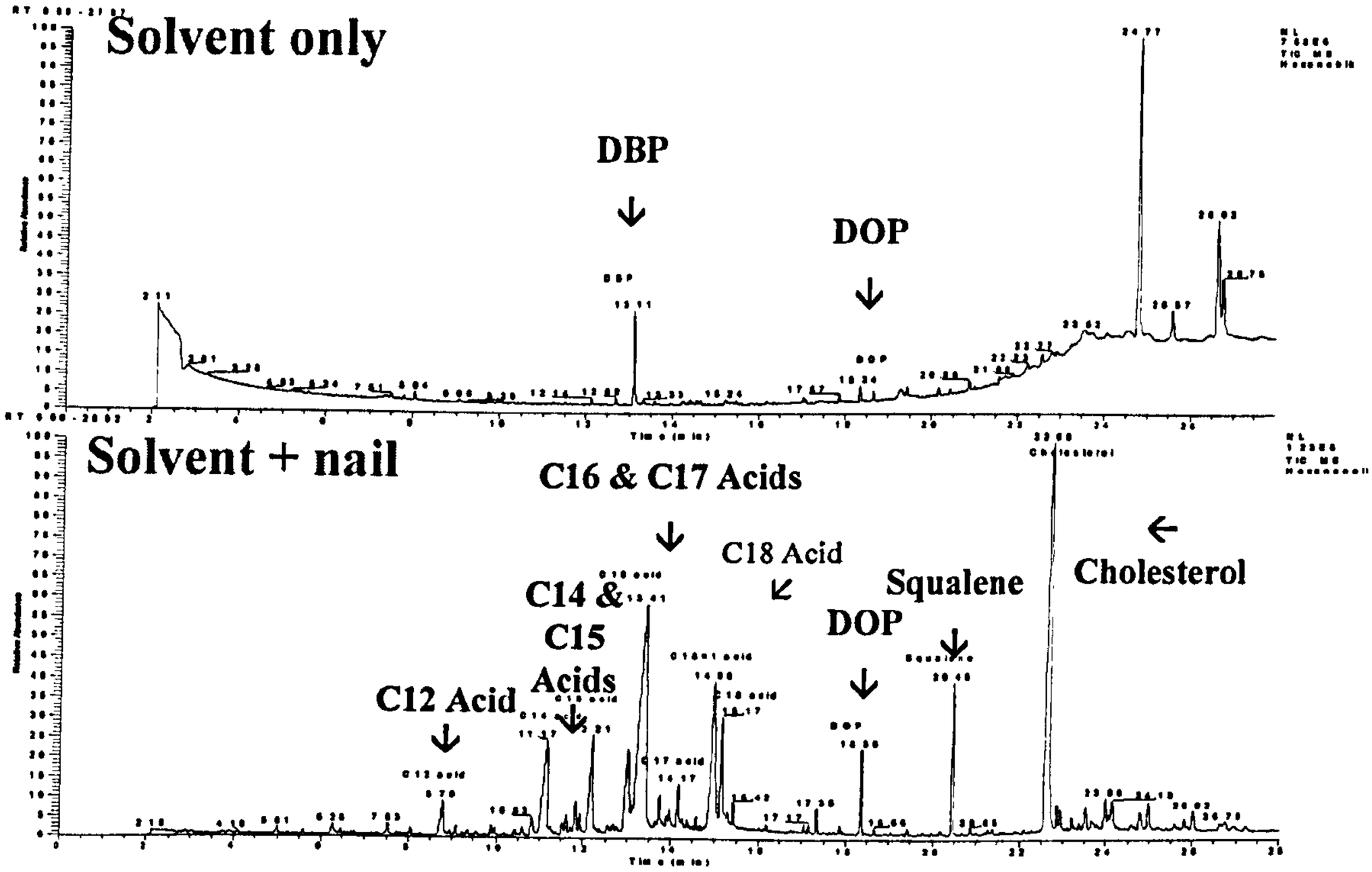


Figure 2.3. (e) Hexane

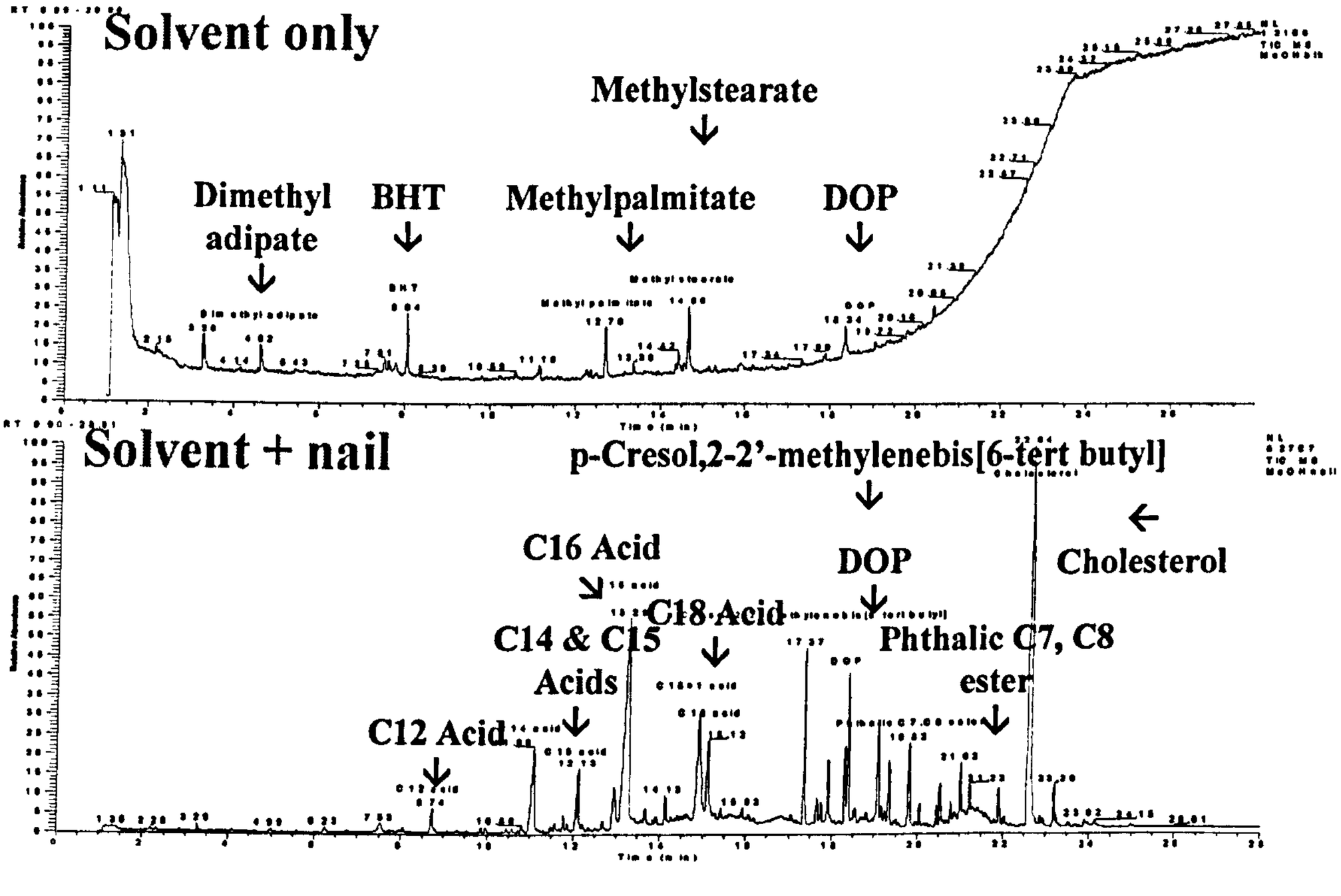


Figure 2.3. (f) Methanol

2.3.2. Cannabinoid analysis

The alkaline hydrolysis of the nail corresponded to **Lemos's method** [31]. In this method, nail clippings dissolved promptly in alkaline solution, and a pale yellow, transparent liquid was easily obtained.

CBD, 11-OH-Δ^9-THC and Δ^9-THC were identified and quantified in both the cryogenic grinding (**LN**) and alkaline hydrolysis (**AH**) portions.

Moreover, Δ^9-THC-COOH was detected in the LN portion [**Figures 2.4–2.6**]. All of these compounds were determined in the full scanning mode except in the case of 11-OH-Δ^9-THC and Δ^9-THC-COOH where the use of the SIM mode was necessary. All compounds eluted within the range of 7.5 to 12.0 min as shown in **Table 2.1**.

The number of peaks in the chromatogram for the AH portion was less than for the LN portion. A possible reason for this is that the nail sample is hydrolysed under extreme alkaline conditions and more endogenous materials are subsequently recovered in the extract. **Figure 2.7** summarises the compounds detected in each specimen using the two methods of extraction.

The calibration curves prepared showed linearity from 0.2 ng to 5.0 ng for all cannabinoids. **Figure 2.7** shows the concentrations of cannabinoids per one milligram of nail in each of the case samples from the **Edinburgh Drug Addiction Study (EDAS)**.

The concentration of Δ^9-THC was similar for our specimens in both the LN and the AH portions. Δ^9-THC-COOH could not be detected by the AH method, though this cannabinoid was clearly identified and quantified by the LN method. The reason is that Δ^9-THC-COOH is readily soluble in aqueous alkali and the recovery of this analyte is low.

Earlier work had shown that pH adjustment prior to solvent extraction permitted some recovery of the metabolite but at the expense of interference from co-extracted

interferences. Also of interest was the presence of cannabidiol at concentrations, on average, higher than either tetrahydrocannabinol or its carboxylic metabolite. **Figure 2.8** shows a comparison of the average values for the cannabinoids obtained by each method.

Various authors have reported the presence of minor cannabinoid components in seized cannabis or biological samples, such as Δ^8-THC [32], cannabichromene [33], cannabigerol [32, 33] and propyl [34, 35] homologues of Δ^9-THC [36, 37, 38]. None of these minor components were detected in either the cryogenic grinding or alkaline hydrolysis portion.

It can be concluded that nail analysis is useful for monitoring of past cannabis use and that for this purpose, the hydrolysis and cryogenic grinding methods produce comparable results. However, cryogenic grinding has some advantages. It is a milder chemical treatment, suitable for drug screening of alkali-sensitive substances, and, as shown in this study, Δ^9-THC-COOH can be detected. The applicability of this technique was subsequently evaluated for opioids in nail.

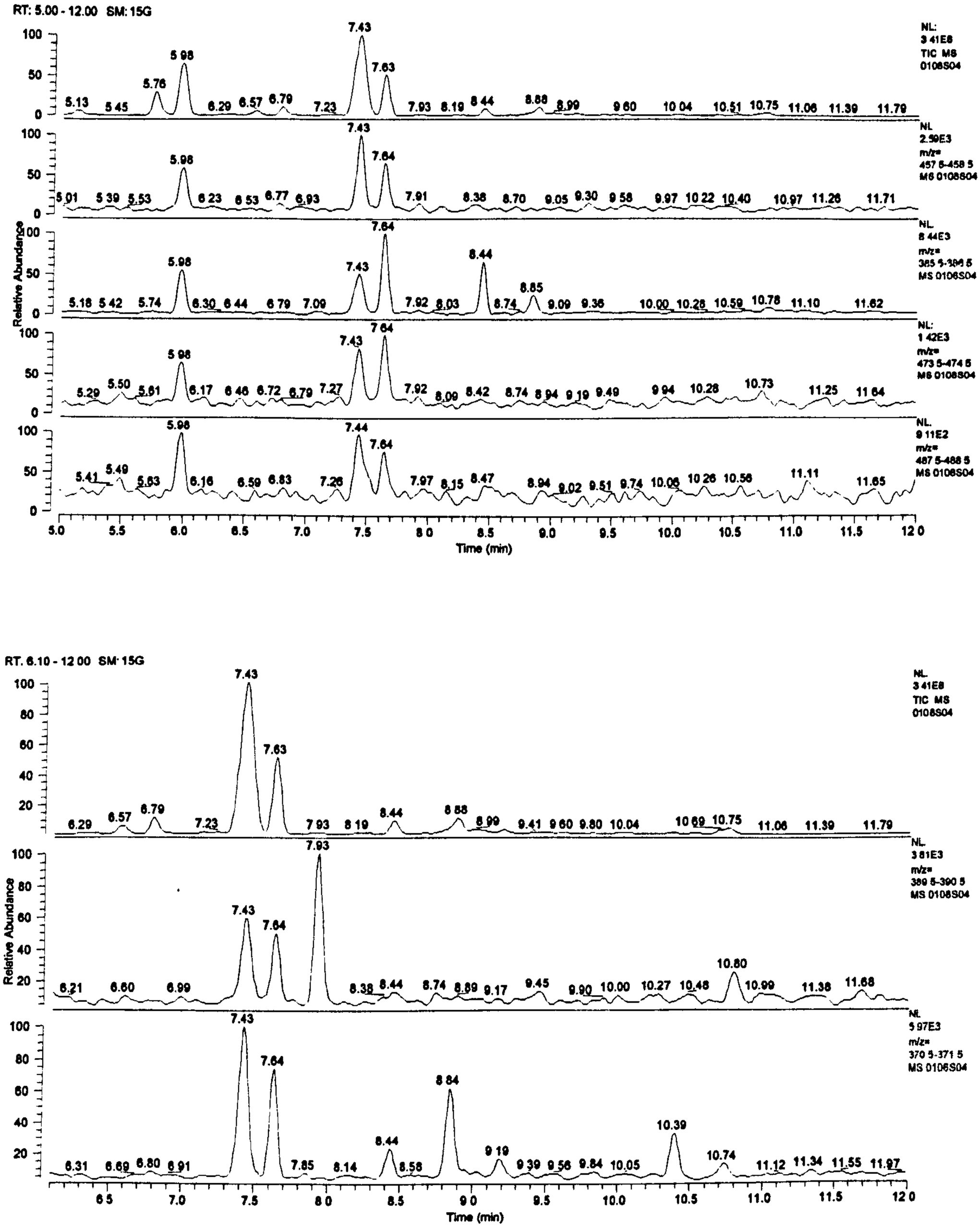
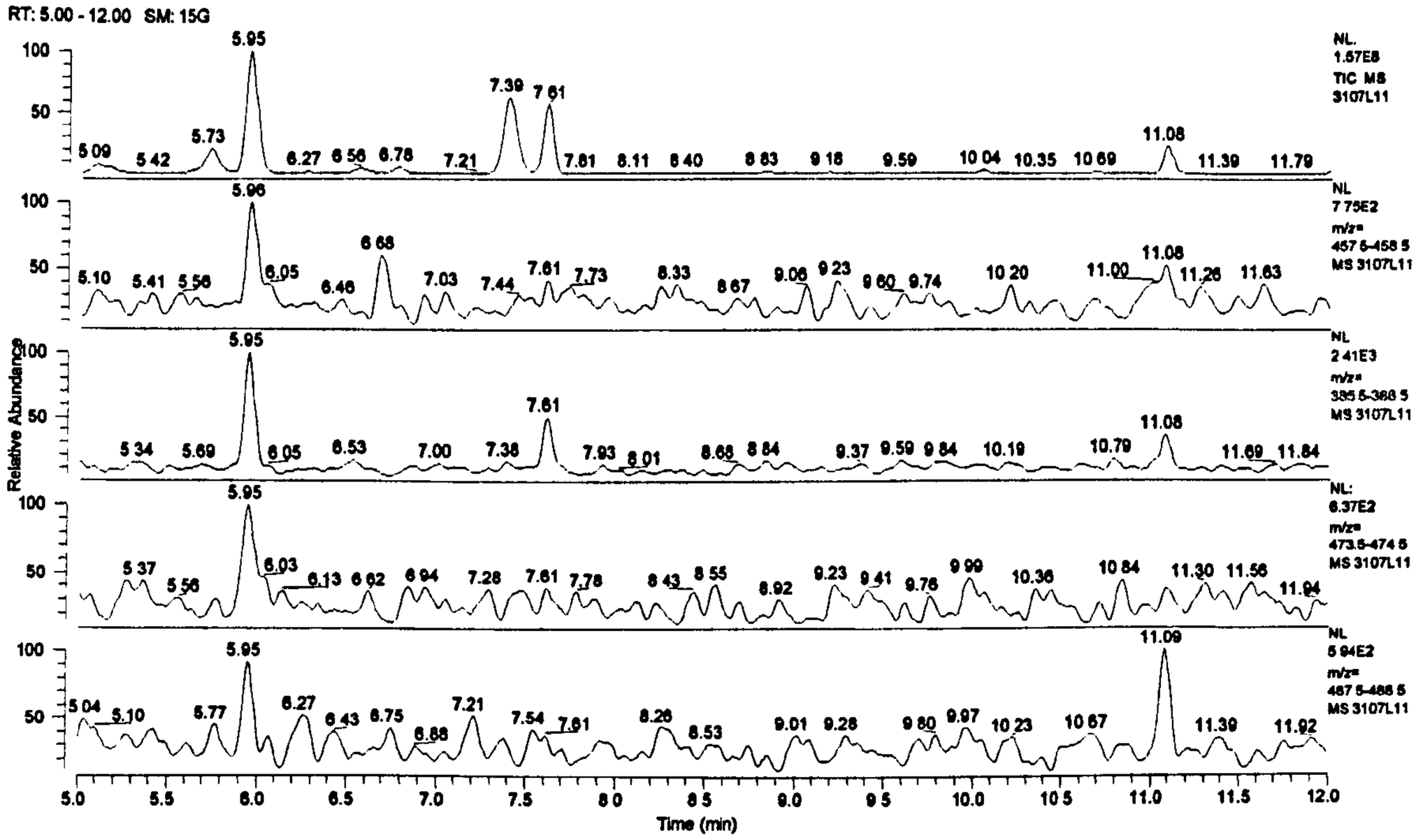


Figure 2.4. GC-MS analysis of cannabinoids in nail after hydrolysis with sodium hydroxide

Compounds detected were: CBD (7.93 min, *m/z* 390; THC, 8.84 min, *m/z* 386, 371; 11-HO-THC, 10.80 min, *m/z* 474, 371).



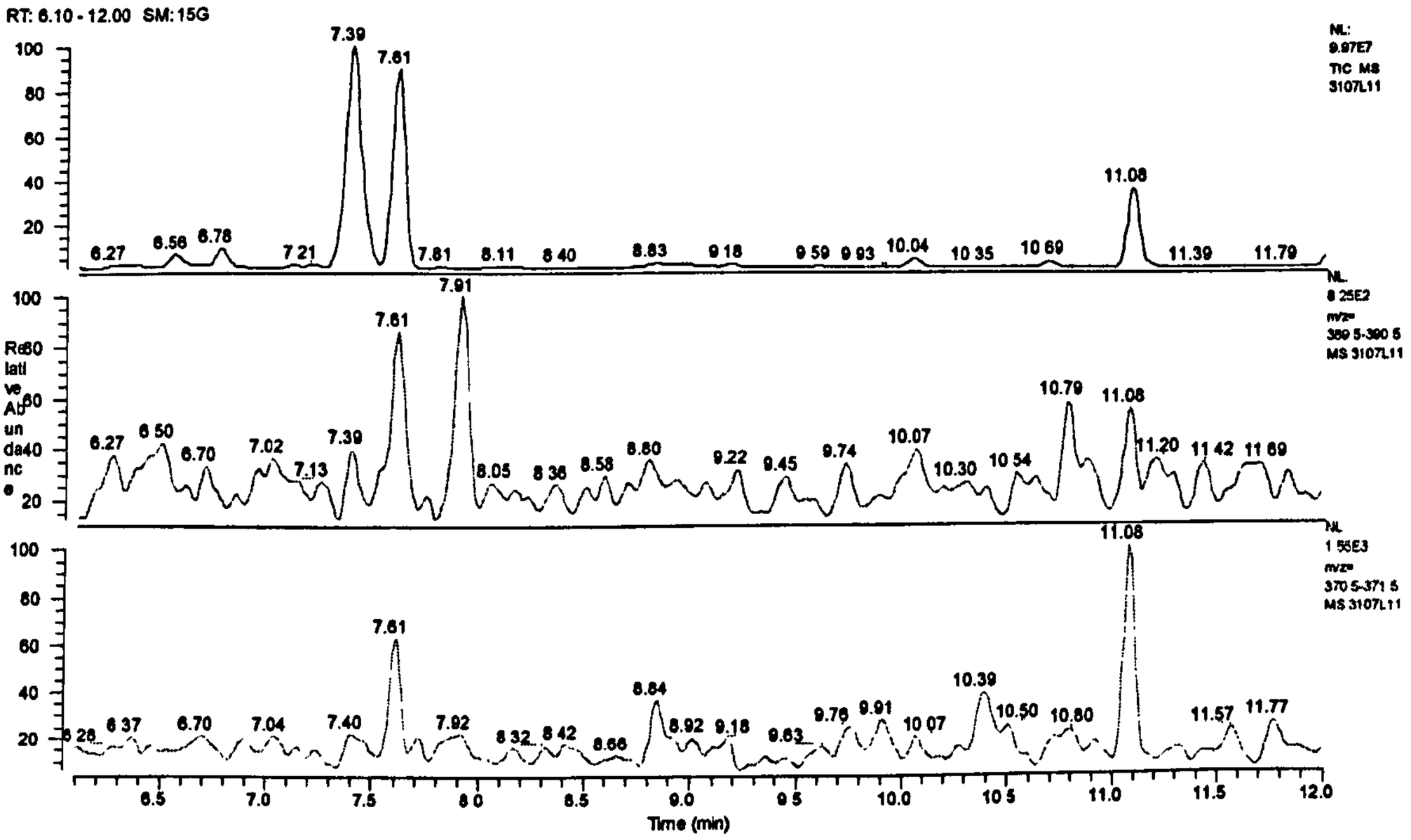


Figure 2.5. GC-MS analysis of cannabinoids in nail after cryogenic grinding and extraction with methanol

Compounds detected were: CBD (7.91 min, *m/z* 390; THC, 8.84 min, *m/z* 386, 371; 11-HO-THC, 10.80 min, *m/z* 474, 371; THC-COOH, 11.81 min, *m/z* 473, 371).

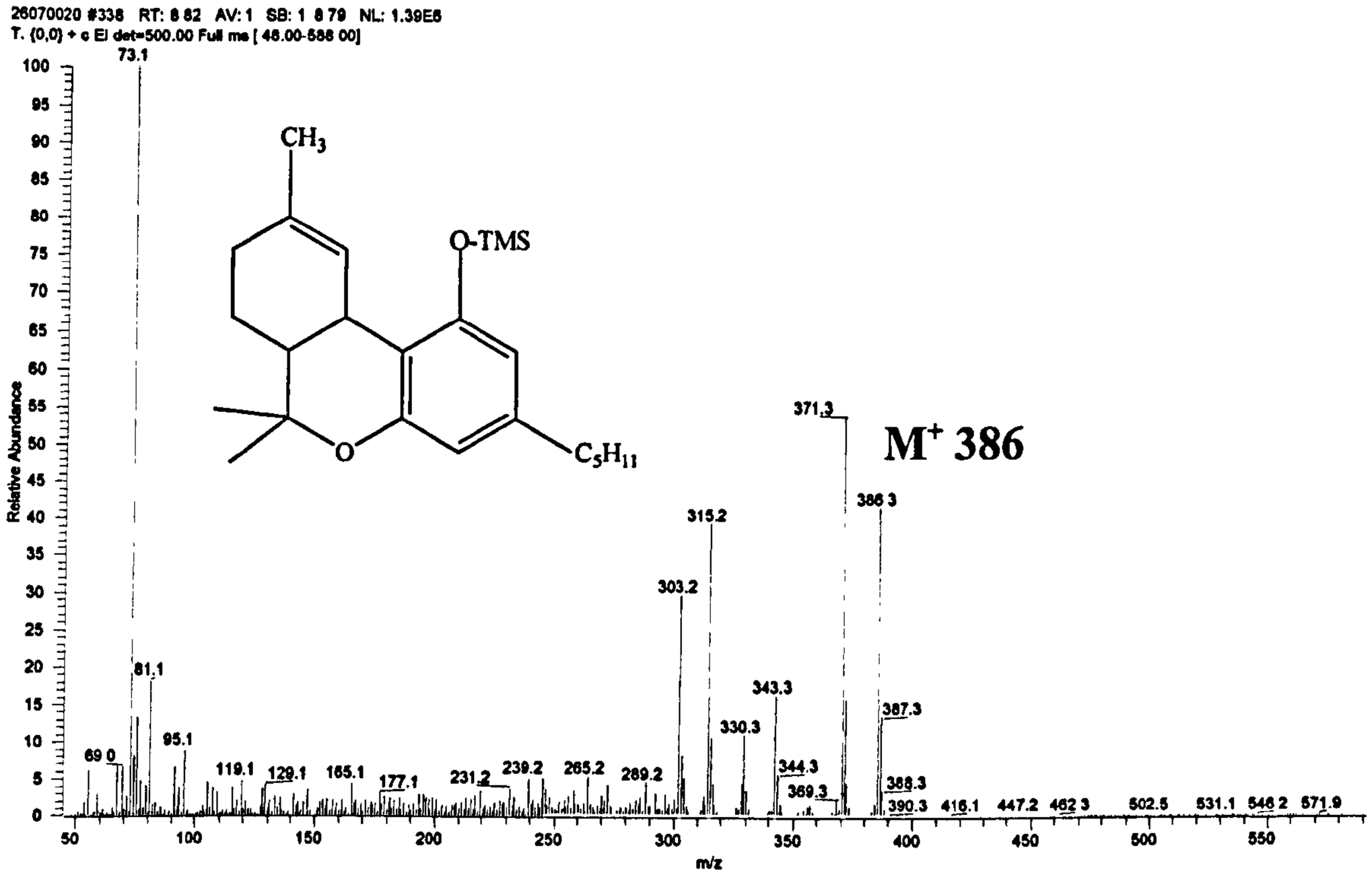


Figure 2.6. Mass spectra of cannabinoids identified in nail clippings from regular cannabis users.
(a) THC-mono-TMS

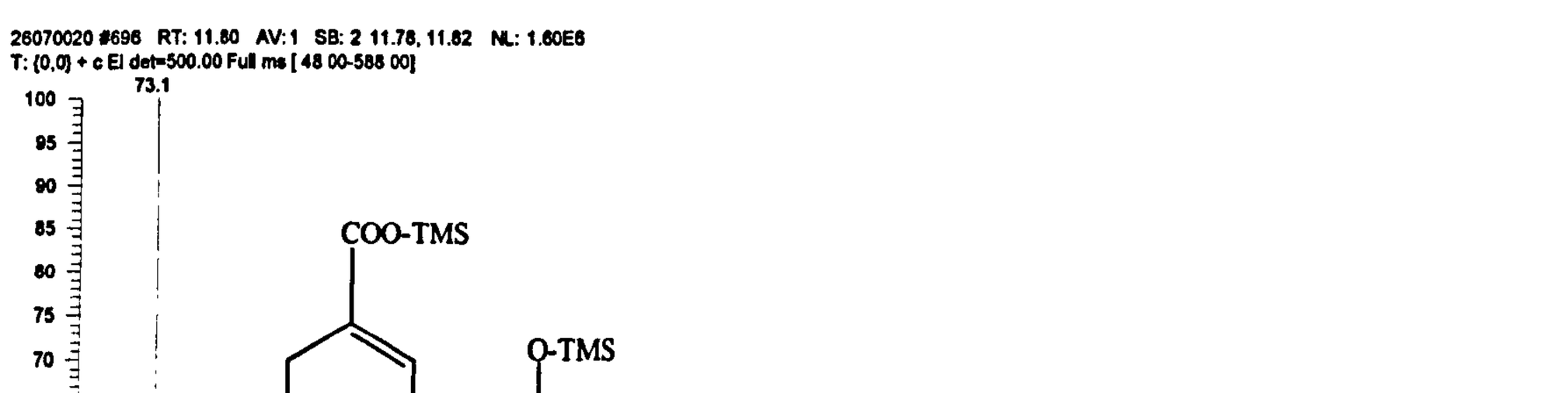


Figure 2.6. (b) THC-COOH-di-TMS

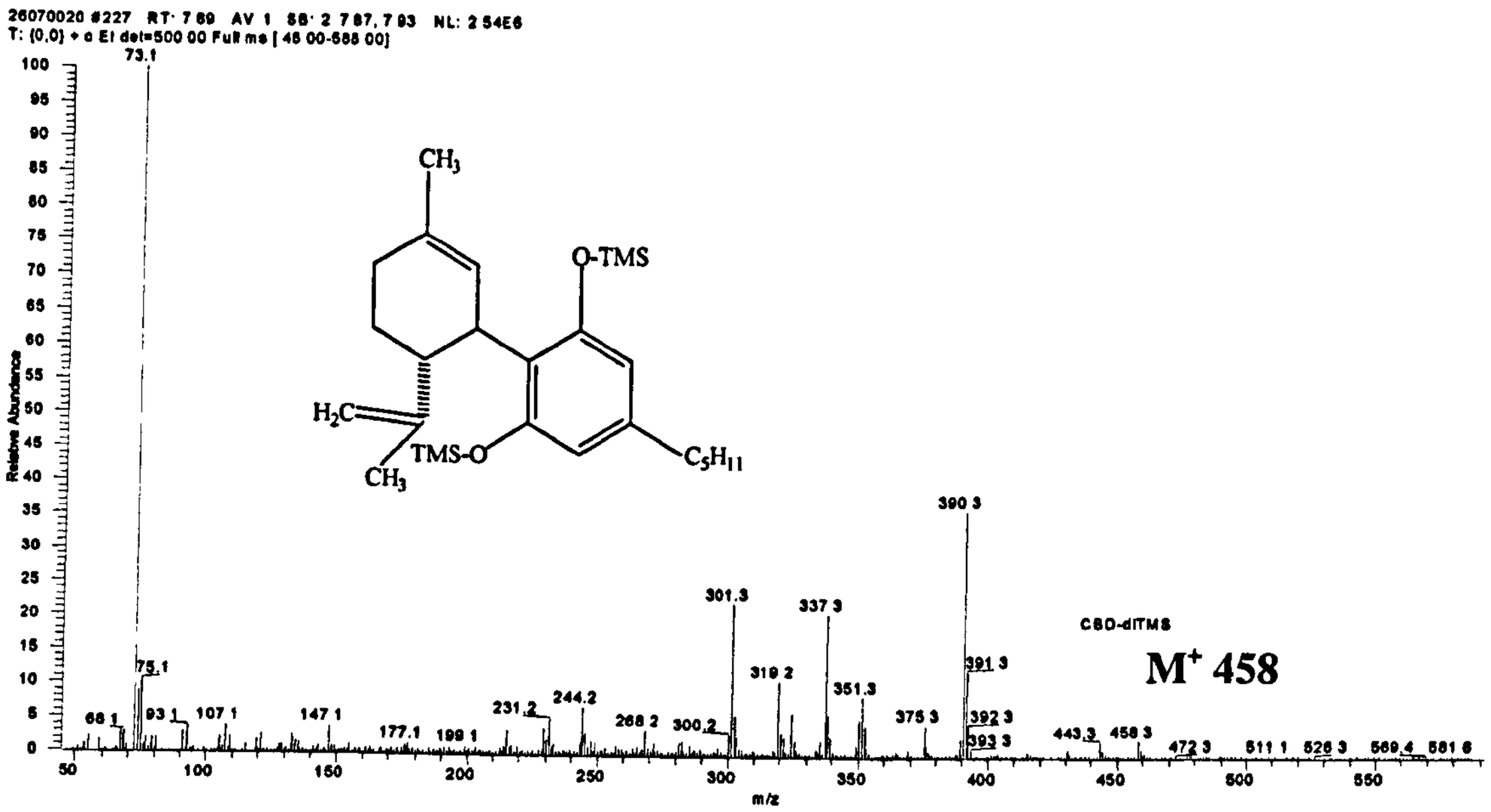


Figure 2.6. (Continued) (c) Cannabidiol-di-TMS

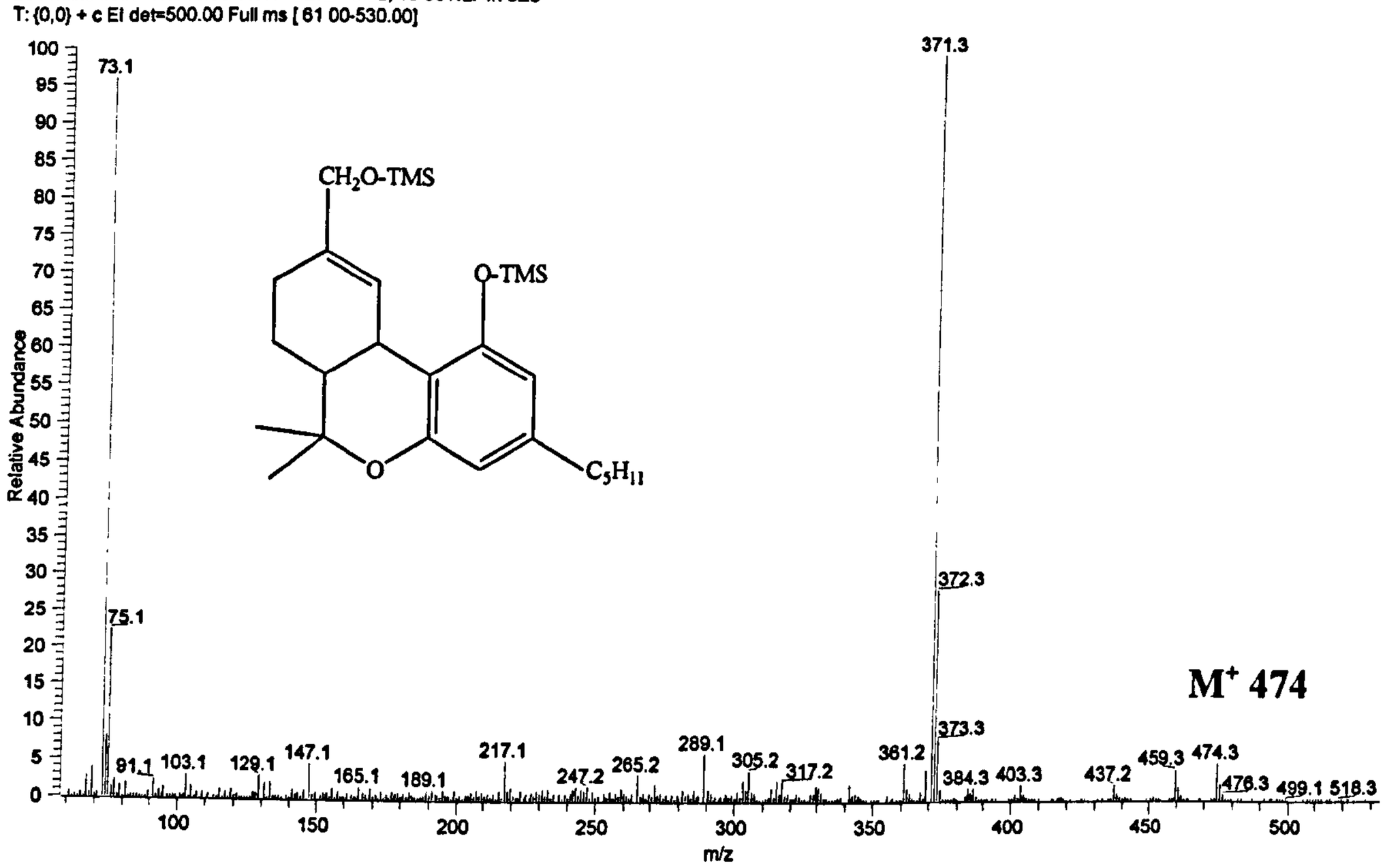
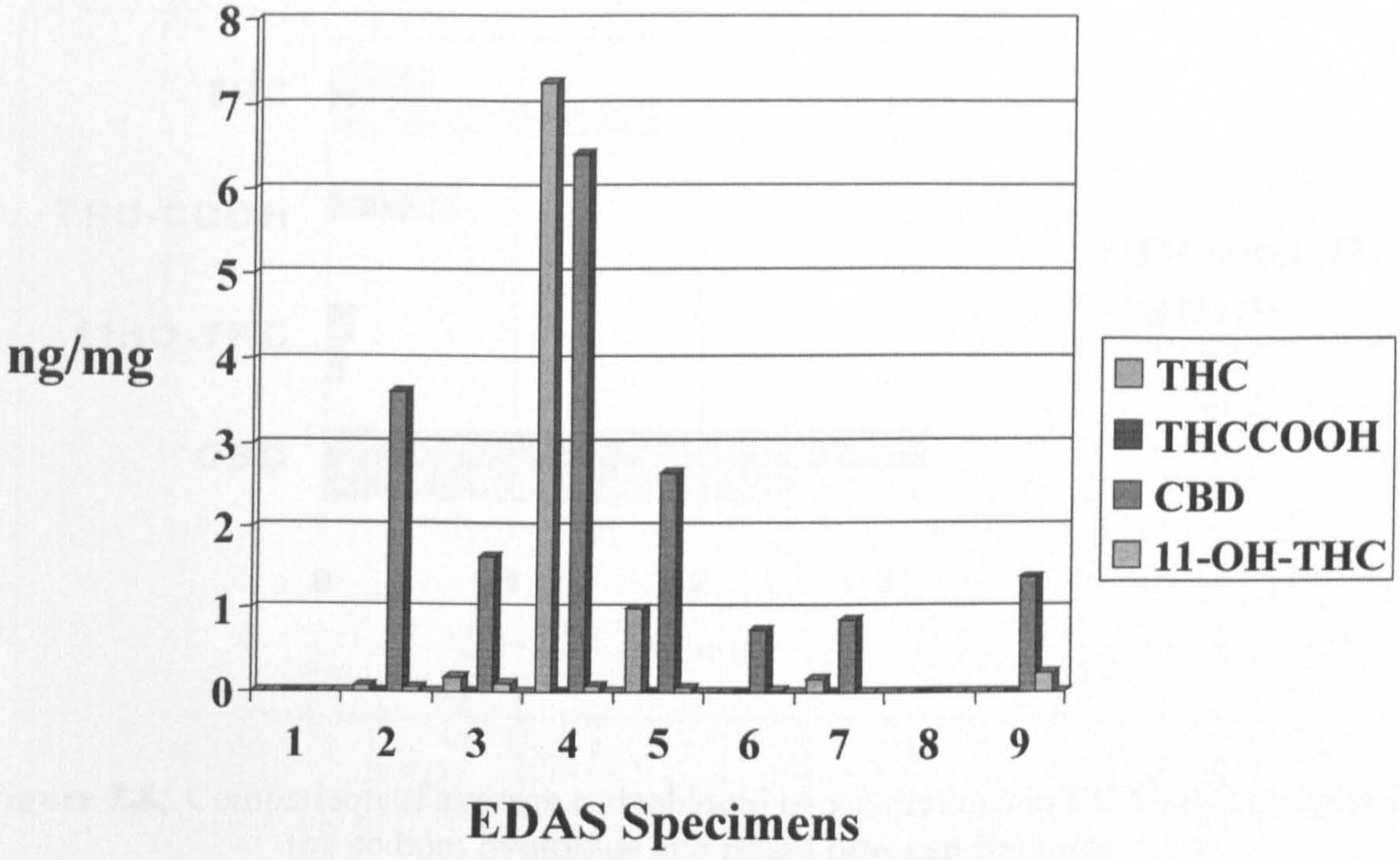
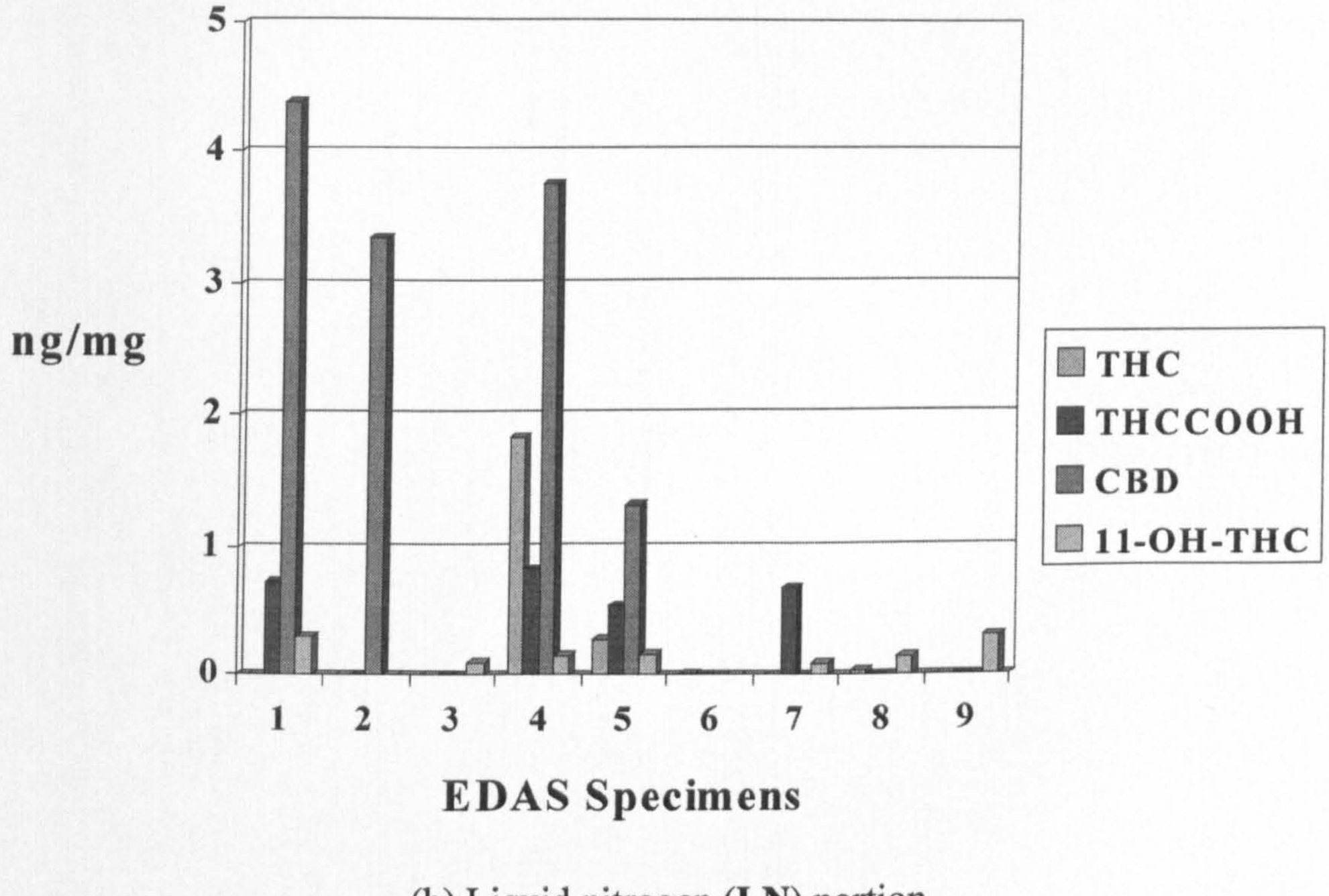


Figure 2.6. (Continued) (d) 11-hydroxy-Δ^9-tetrahydrocannabinol-di-TMS



(a) Sodium hydroxide (**AH**) portion



(b) Liquid nitrogen (**LN**) portion

Figure 2.7. Concentrations of cannabinoids detected in nail clippings from chronic cannabis users.

In the AH portion, THC-COOH is not detected because of an extraction at alkaline pH. In general, in the AH portion, the concentration of cannabidiol (CBD) was higher than in the LN portion except sample No. 1.

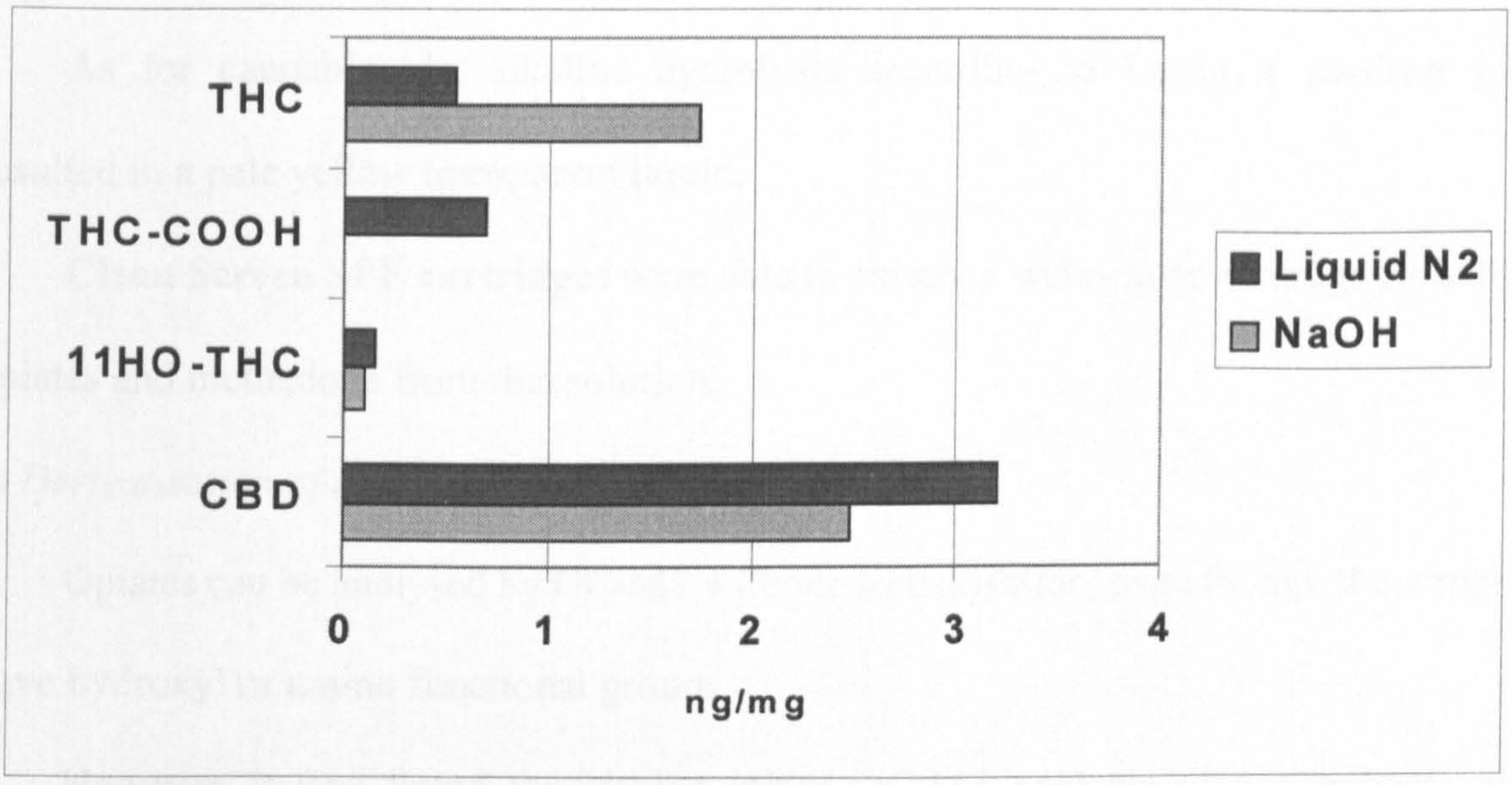


Figure 2.8. Comparison of average cannabinoid concentrations in **EDAS** nail samples in the sodium hydroxide and liquid nitrogen fractions

2.3.3. Opioid analysis

As for cannabinoids, alkaline hydrolysis according to **Lemos's method** [31] resulted in a pale yellow transparent liquid.

Clean Screen SPE cartridges were able to extract a wide-range of drugs including opiates and methadone from this solution.

1) Derivatisation of analytes

Opiates can be analysed by GC-MS without derivatisation, even though the samples have hydroxyl or amino functional groups.

However, it was found that during initial GC-MS analysis of nail extracts, the analytes were detected as TMS derivatives even though the samples were not treated with BSTFA, because the GC-MS used had already been contaminated with the silylation reagent and an on-column reaction occurred with the residual reagent. Therefore, all extracts were subsequently analysed by GC-MS after reaction with BSTFA reagent.

2) Qualitative and quantitative analyses of the GC-MS

a) Qualitative analysis

The opium alkaloids morphine, codeine, noscapine and papaverine and the opioid methadone were detected in both LN and AH portions. Moreover, heroin, 6-MAM and 6-acetylcodeine were only detected in the LN portion [**Figures 2.9** and **2.10**].

All compounds eluted within the range of 5–15 min, as shown in **Table 2.1**. The number of peaks of the AH portion was less than that of the LN portion [**Figures 2.11** and **2.12**]. The reason for this is that the sample is purified by SPE after hydrolysis in the AH method.

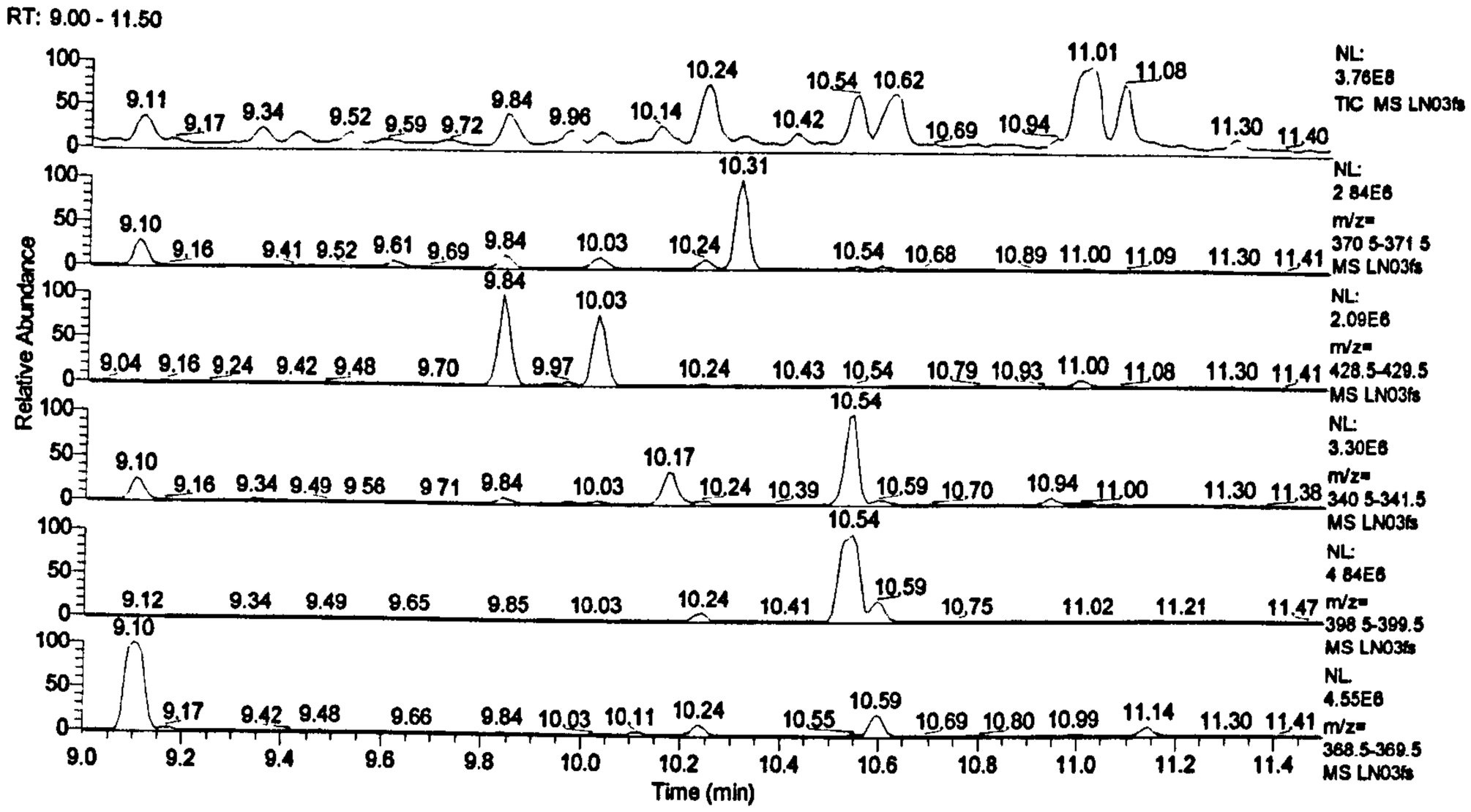


Figure 2.9. Mass chromatograms of sample number LN03 (LN portion)

Mass chromatograms of Codeine (*m/z* 371), Morphine (*m/z* 429), 6-Acetylcodeine (*m/z* 341), 6-MAM (*m/z* 399), and Heroin (*m/z* 369). Retention times were 9.61, 10.03, 10.17, 10.54, and 11.14 min, respectively.

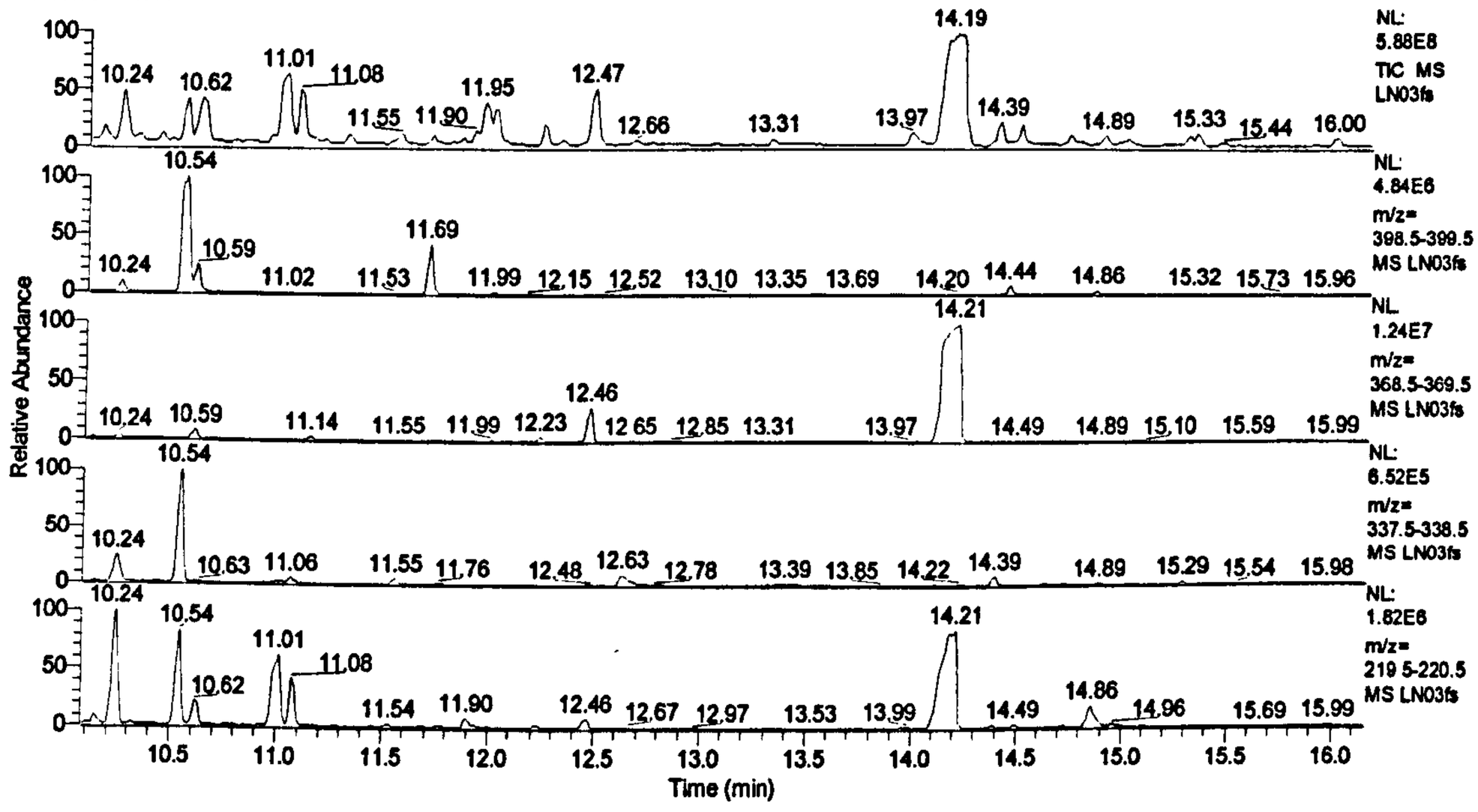


Figure 2.10. Mass chromatograms for sample number LN03 (LN portion)

Mass chromatograms of 6-MAM (*m/z* 399), Heroin (*m/z* 369), Papaverine (*m/z* 338), and Noscapine (*m/z* 220).

Retention times were 10.54, 11.14, 12.63, and 14.86 min, respectively.

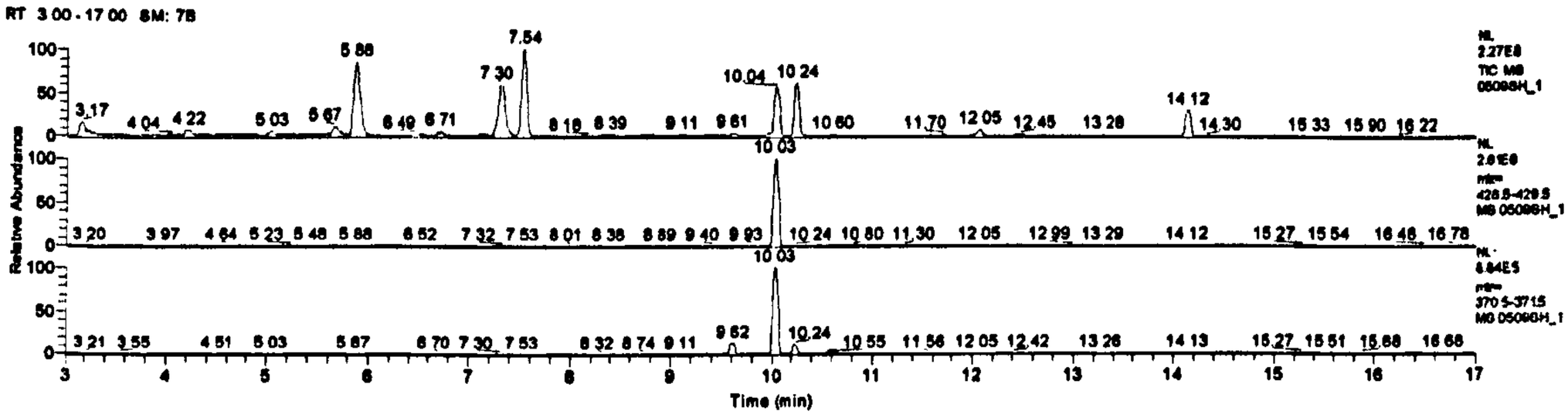


Figure 2.11. GC-MS analysis of sample number AH03 (AH portion)

Mass chromatograms (*m/z* 429 for Morphine-di-TMS, *m/z* 371 for Codeine-mono-TMS). Retention times were 9.62 (Codeine-mono-TMS) and 10.03 min (Morphine-di-TMS), respectively (upper). Mass spectrum of Codeine-mono-TMS (lower).

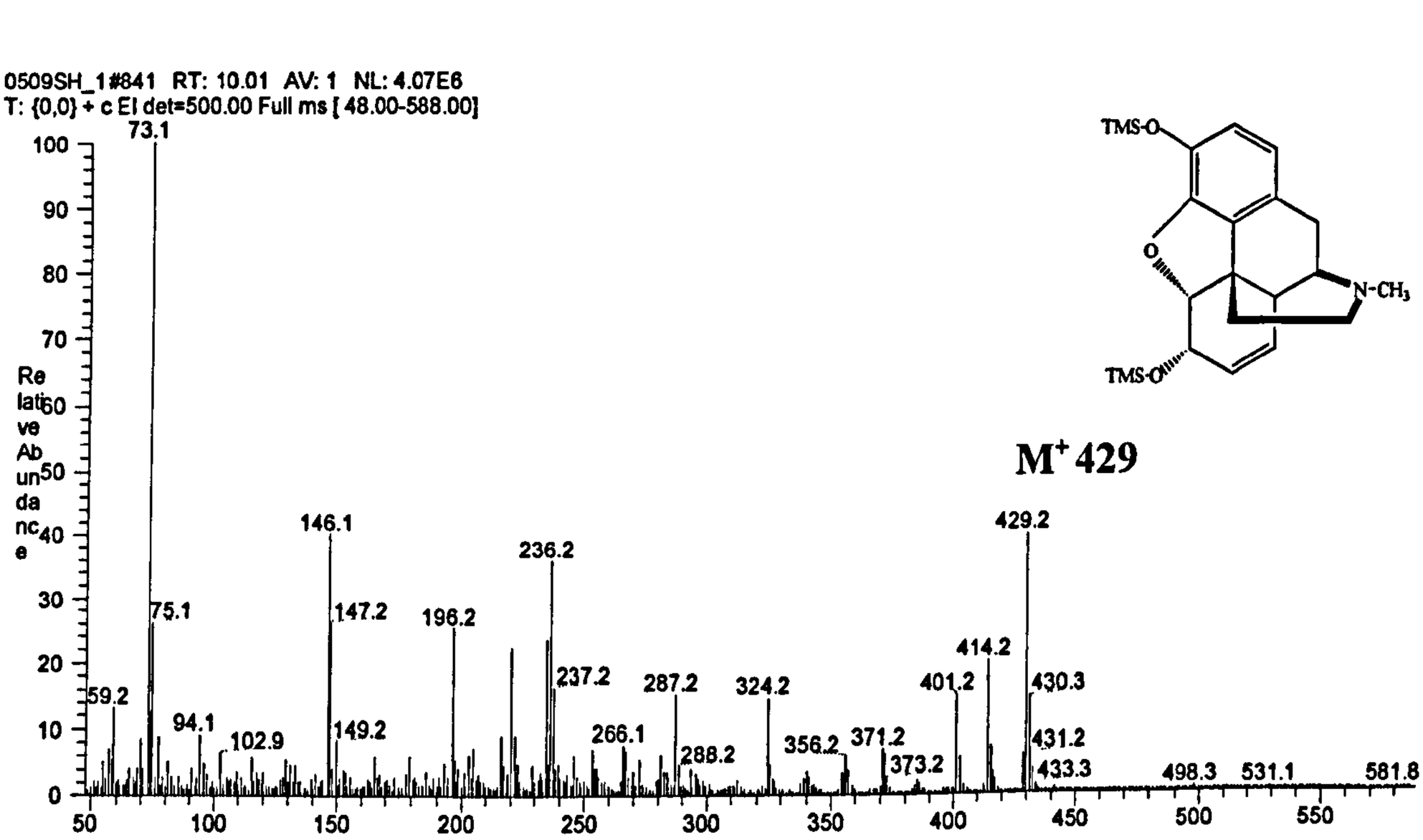
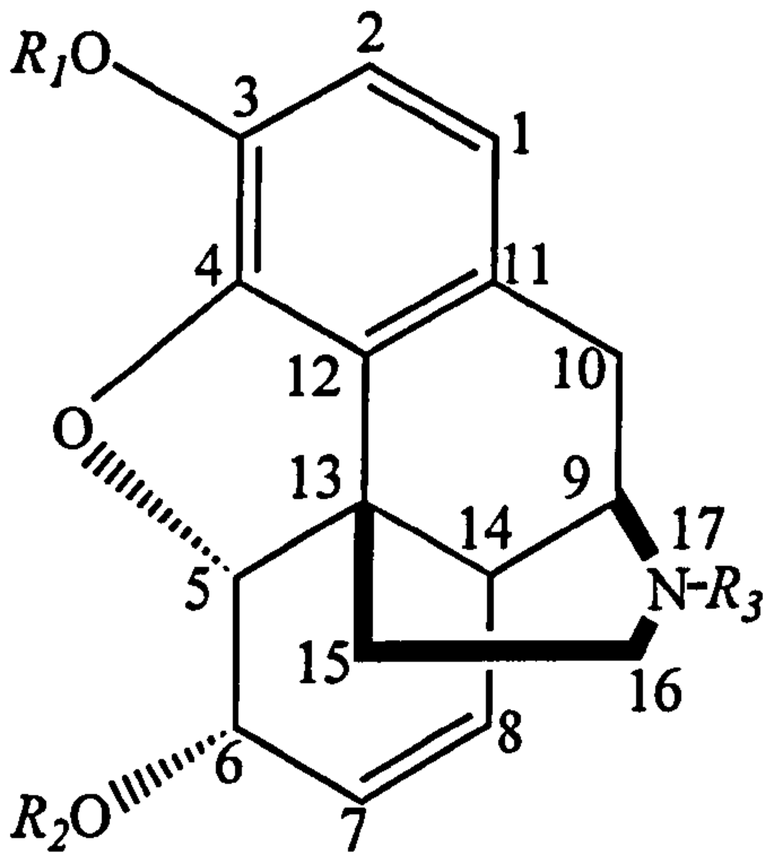


Figure 2.12. Mass spectrum of Morphine-di-TMS (Retention time 10.01 min)

Table 2.1 shows retention times for the detected compounds. These compounds could be determined in the full scanning mode.

Heroin, 6-MAM and 6-acetylcodeine with substituent groups at the C6-position of the morphine structure [**Figure 2.13**] could not be detected by the AH method, though these opiates could be identified with the LN method.

The reason is that the heroin is easily hydrolysed by alkaline conditions, which is a problem in the conventional alkaline hydrolysis method [**Figure 2.14**]. By contrast, opiates having substituent groups at the C6-position were determined easily with the LN method.



No.	Compound	R1	R2	R3
1	Morphine	H	H	CH_3
2	Normorphine	H	H	H
3	6-Monoacetylmorphine	H	$COCH_3$	CH_3
4	Diacetylmorphine (Heroin)	$COCH_3$	$COCH_3$	CH_3
5	Codeine	CH_3	H	CH_3
6	Norcodeine	CH_3	H	H
7	6-Acetylcodeine	H	$COCH_3$	CH_3

Figure 2.13. Chemical structure and numbering of the morphinan alkaloids.

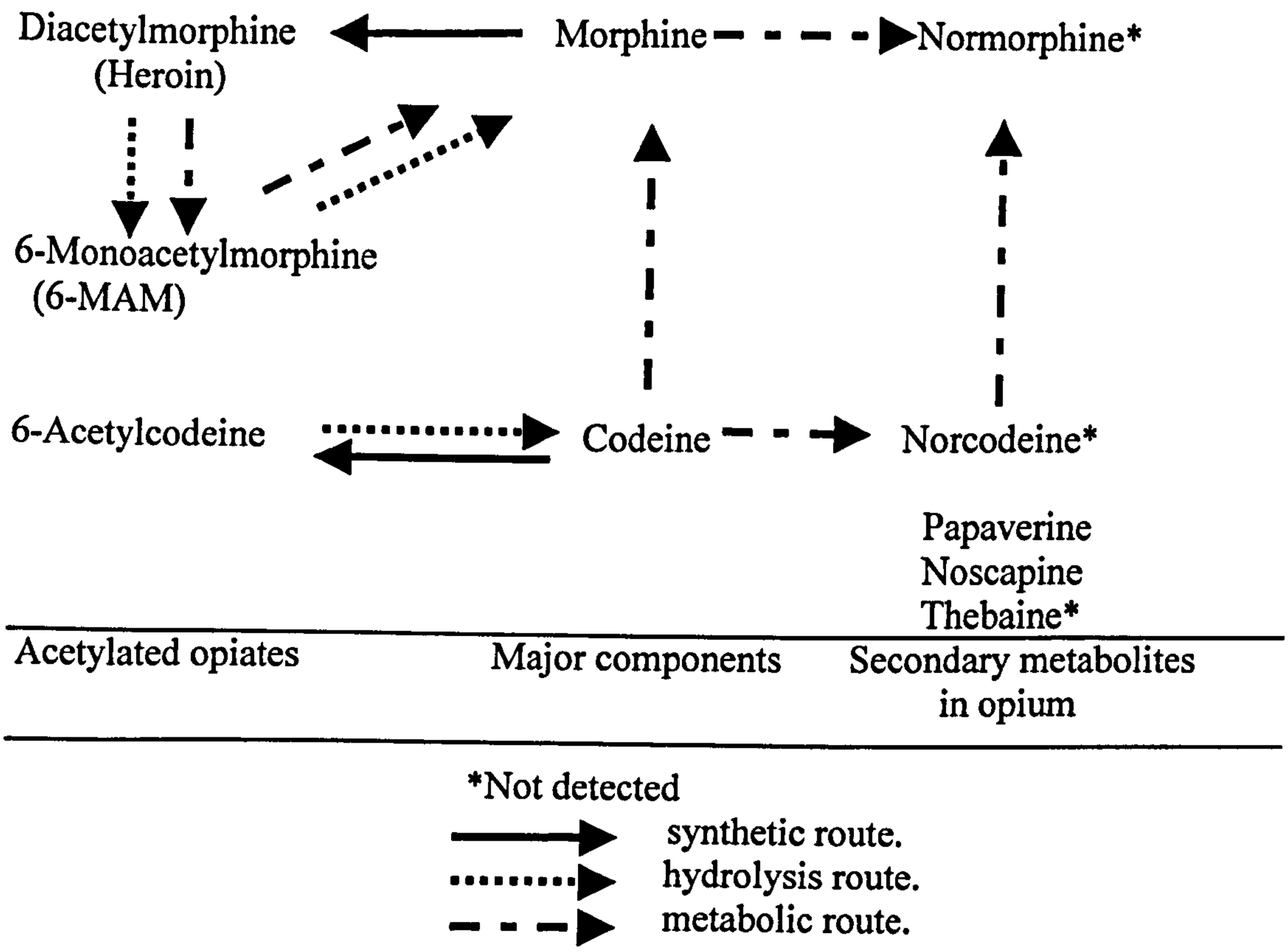


Figure 2.14. Synthesis (acetylation), metabolism, and alkaline hydrolysis of opiates

b) Quantitative analysis

The calibration curves showed straight lines from 0.1 ng to 5.0 ng for methadone and all opiates.

Correlation coefficients for heroin, 6-MAM, 6-acetylcodeine, morphine, DHC, and codeine were 0.9958, 0.9985, 0.9995, 0.9958, 0.9937 and 0.9995, respectively [**Figure 2.15**]. The calibration curve for codeine at low concentrations, from 0.025 to 0.10 ng, was also measured and showed a calibration curve with a particularly good linear correlation coefficient. It was concluded that little decomposition of the sample occurred, because the unstable morphine structure at the C3-position is occupied by the alkyl (methyl) ether group [**Figure 2.13**].

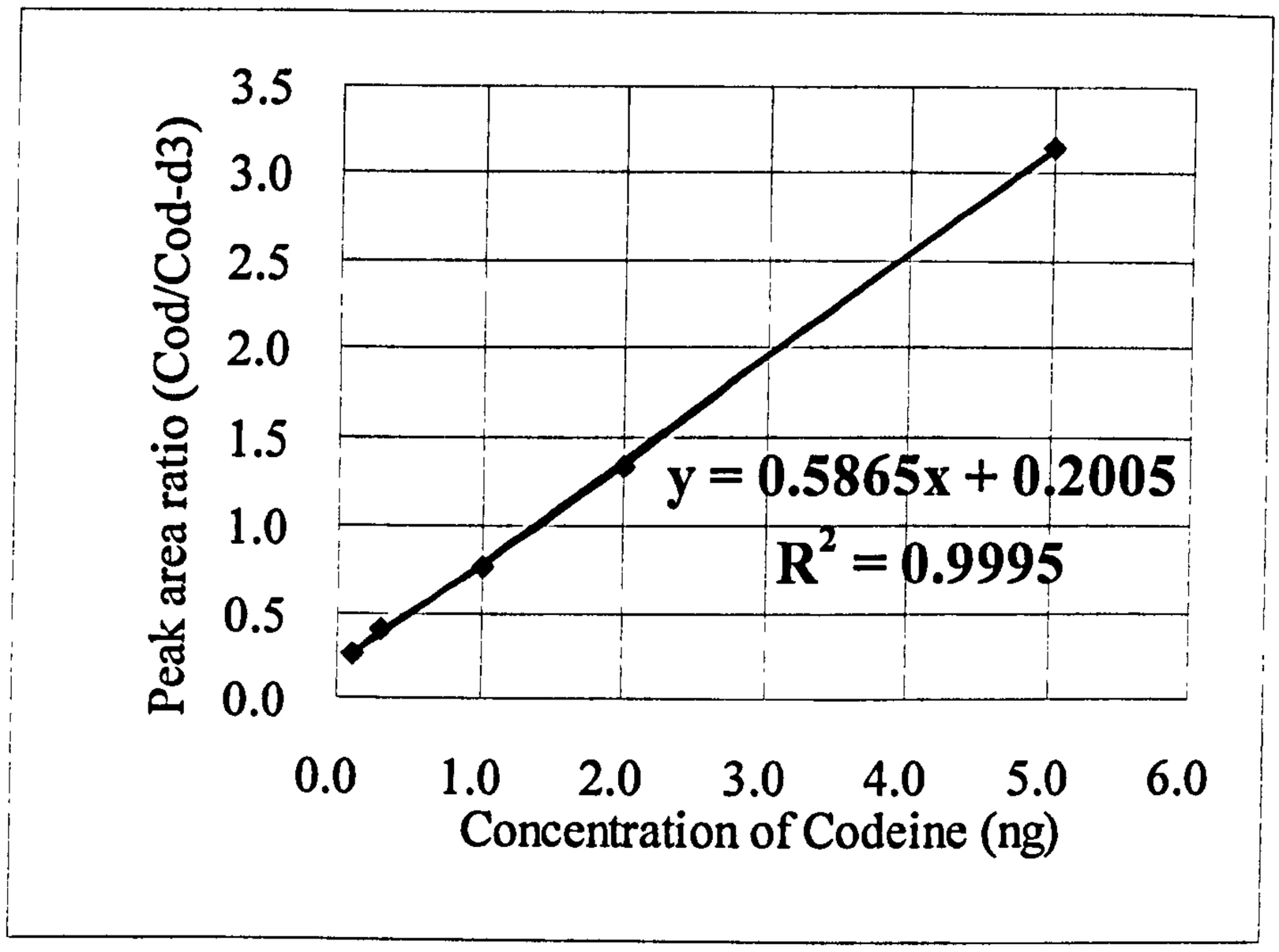


Figure 2.15. Calibration curve for Codeine-mono-TMS

Table 2.2 shows the concentration of opiates per one milligram in weight of the nail in the same.

In the AH portion, the morphine concentration was very high compared with the LN portion. This suggests that morphine formed by hydrolysis of heroin and 6-MAM has increased the apparent morphine concentration in the AH portion [**Figure 2.14**].

Similarly, the codeine concentration was higher in the AH portion because of hydrolysis of 6-acetylcodeine.

In general, it is not yet possible to attempt any correlation between the dose of administered opioid and the subsequent concentration of the opioid in nail. The doses administered by the donors of nail samples used in this study are not known. Controlled dose studies will be required in future to allow this type of correlation to be studied.

Table 2.2. Concentrations of opioids in nail samples (ng/mg nail)

Sample	Heroin		6-MAM		6-AcCodeine		Morphine		Codeine		Methadone	
No.	LN	AH	LN	AH	LN	AH	LN	AH	LN	AH	LN	AH
1	*0.45*	0.00	*2.92*	0.00	0.00	0.00	0.00	*9.96*	*0.04*	*0.63*	*1.87*	0.00
2	0.00	0.00	0.00	0.00	0.00	0.00	*0.08*	0.00	*0.13*	*0.15*	*0.78*	*2.07*
3	*1.14*	0.00	*15.91*	0.00	*1.97*	0.00	*7.62*	*25.37*	*0.86*	*7.66*	*0.09*	*0.50*
4	*0.21*	0.00	*10.35*	0.00	*0.32*	0.00	*5.42*	*9.69*	*0.52*	*2.55*	*0.42*	*0.72*
5	0.00	0.00	0.00	0.00	0.00	0.00	*0.21*	*0.33*	*0.49*	*0.89*	*1.16*	*1.66*
6	0.00	0.00	*0.52*	0.00	0.00	0.00	0.00	*0.07*	0.00	0.00	0.00	0.00
7	0.00	0.00	*1.54*	0.00	0.00	0.00	*0.42*	*9.95*	*0.24*	*0.83*	0.00	0.00
8	*2.38*	0.00	*5.87*	0.00	*0.03*	0.00	*1.39*	*2.93*	*0.37*	0.00	*0.14*	*3.61*
9	0.00	0.00	*0.23*	0.00	0.00	0.00	0.00	*1.02*	0.00	0.00	0.00	0.00
10	0.00	0.00	*0.36*	0.00	0.00	0.00	*0.07*	*0.26*	*0.05*	0.00	*0.11*	0.00
11	0.00	0.00	*0.14*	0.00	0.00	0.00	0.00	*3.44*	*0.04*	0.00	*1.05*	0.00
12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	*0.03*	0.00	0.00	0.00	0.00
13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	*0.19*	0.00	0.00	0.00	0.00
14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	*0.50*	0.00	0.00	*1.01*	0.00
15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	*0.25*	0.00	0.00	*1.10*	0.00
16	0.00	0.00	*1.11*	0.00	0.00	0.00	*0.33*	*2.88*	*0.45*	0.00	*0.36*	0.00
17	0.00	0.00	*0.30*	0.00	0.00	0.00	*0.06*	*1.15*	0.00	0.00	*1.58*	0.00

Notes: LN = liquid nitrogen method, AH = alkaline hydrolysis method, 6-AcCodeine = 6-acetylcodeine.

3) Consideration of individual opioids

a) Methadone and related compounds

Methadone is a drug supplied as a replacement for heroin to heroin abusers. Methadone was detected in 12 nail samples out of the 17 obtained from donors at **the Glasgow Drug Problem Service.** EDDP and EMDP are metabolites of methadone.

The quantification of these compounds was attempted at the same time as methadone, using deuterated internal standard, EDDP-d_3 but the chromatographic peak shape was poor and no useful results were obtained.

b) DHC

As shown in **Figure 2.13**, codeine has a methyl ether substituent group at the C3-hydroxyl position. In DHC, the double bond in codeine between the C7- and C8-positions is saturated with hydrogen. DHC is known as a strong anti-tussive semi-synthetic morphinoid drug and is restricted by the law.

Because DHC is frequently detected in the analysis of opiates in biological samples [39, 40, 41, 42, 43, 44], it was decided to include it in the list of target analytes in nail.

During quantitative analysis using the SIM mode, DHC was quantified using the chromatograms for *m/z* 373, 358, and 315. However, peaks at the retention time for DHC in the sample extracts had different relative intensity ratios compared to those of the qualifier ions in the DHC standard. Also, DHC was not detected easily in the analysis by full scan mode, though peaks were detected at the correct retention time in the SIM mode analysis.

Therefore, DHC was considered not to have been reliably detected, because it is a semi-synthetic drug and the quantitative and qualitative analyses were performed without addition of DHC-d_3 as an internal standard, which would have assisted in confirming the presence of DHC.

c) Heroin, 6-MAM and morphine

Heroin, 6-MAM and morphine were identified and quantified in the LN portions as shown in **Figures 2.9–2.10** and **Figures 2.16–2.18**. However, they were not detected in the AH portion, in which only morphine was detected [**Figures 2.11–2.12**].

In general, it is difficult to identify heroin directly in biological samples (urine, blood, internal organs and hair etc.). The acetyl group at the C3-position of heroin is easily hydrolysed during sample preparation and/or metabolism in human body and the heroin becomes 6-MAM (**Figure 2.14**). Therefore, to prove the existence of heroin in biological samples, 6-MAM was identified as a biomarker of heroin [45, 46, 47, 48].

Similarly, the acetyl group at the C6-position of 6-MAM is easily hydrolysed under alkaline conditions and 6-MAM becomes morphine. Therefore, the detection of heroin was quite impossible in the conventional alkaline hydrolysis method for nail samples.

Heroin is detected only by using the conventional soaking method when the analyte concentration is high or rapid sample preparation was performed to avoid solvolysis of the analyte.

Because the LN method was a soft extraction procedure, heroin could be directly detected in nail for the first time.

d) 6-Acetylcodeine and codeine as specific markers for illicit heroin

In the LN portion, 6-acetylcodeine and codeine were both detected (**Figures 2.9, 2.19, 2.20**). However, 6-acetylcodeine could not be detected with the alkaline hydrolysis method, as expected. The reason is that 6-acetylcodeine converts to codeine by alkaline hydrolysis [**Figure 2.14**].

Though identification and quantification of morphine and its derivatives were described above, the correlation between the LN and AH methods were considered.

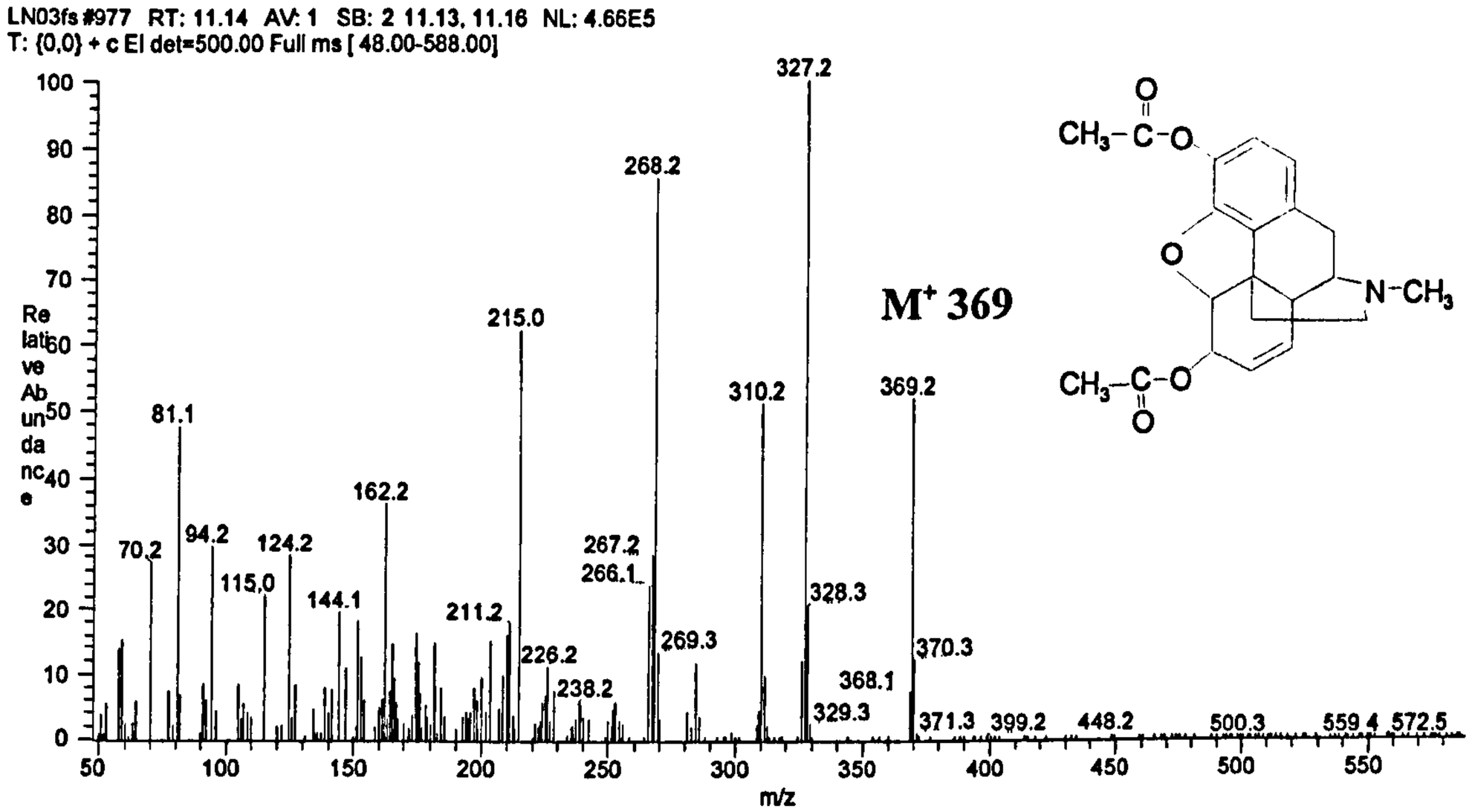


Figure 2.16. Mass spectrum of Heroin (Retention time 11.41 min)

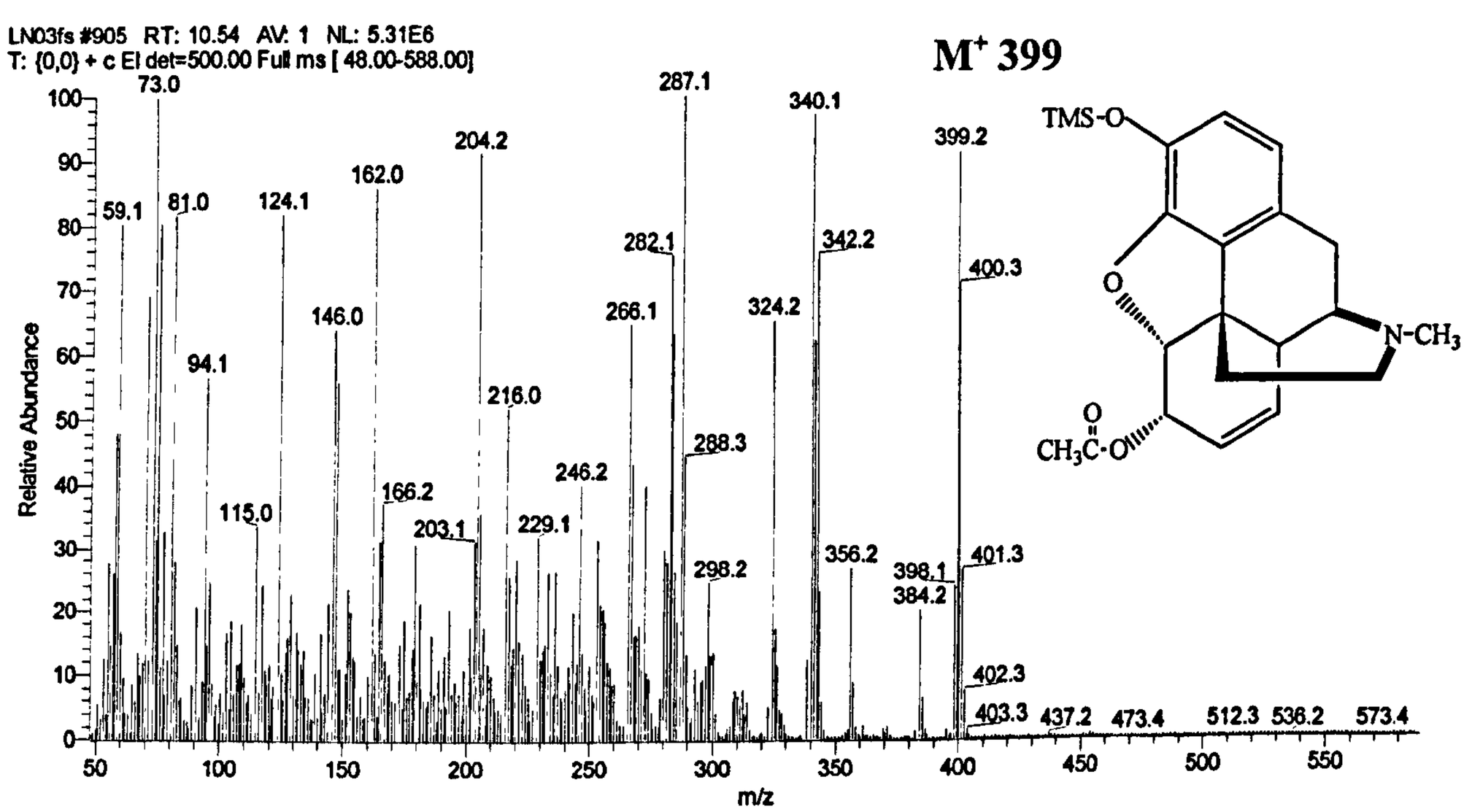


Figure 2.17. Mass spectrum of 6-MAM-mono-TMS
(Retention time 10.54 min)

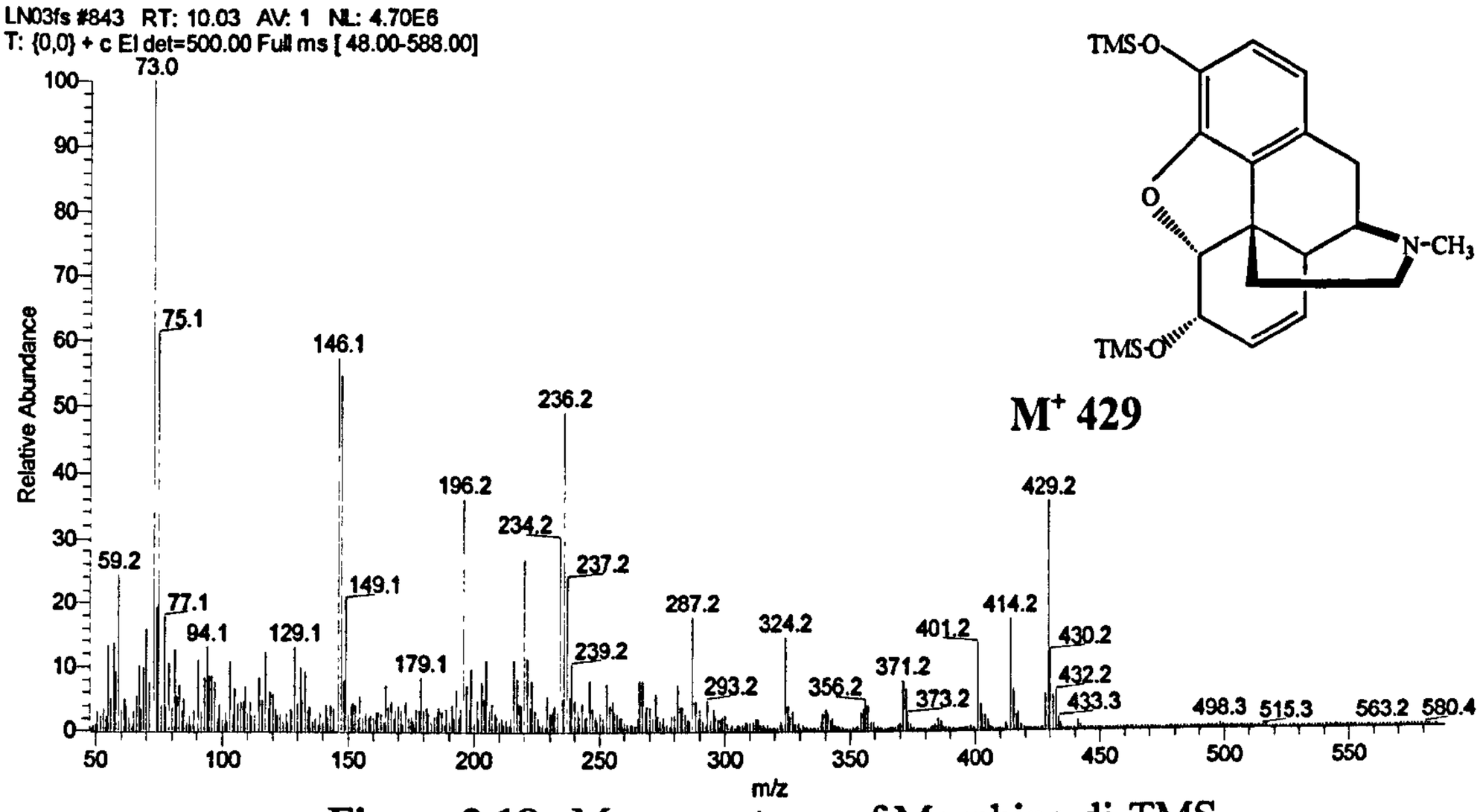


Figure 2.18. Mass spectrum of Morphine-di-TMS
(Retention time 10.03 min)

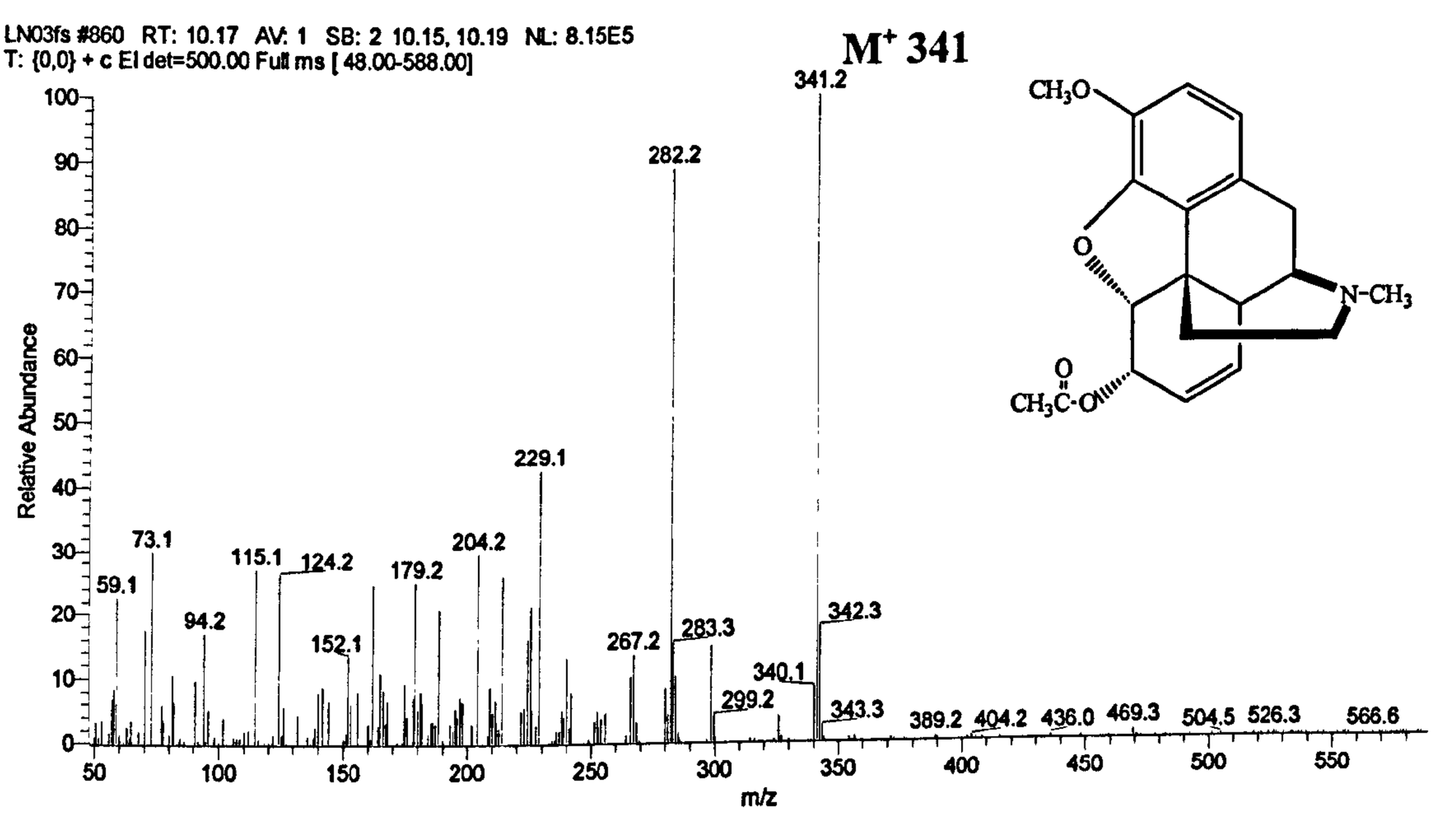


Figure 2.19. Mass spectrum of 6-Acetylcodeine
(Retention time 10.17 min)

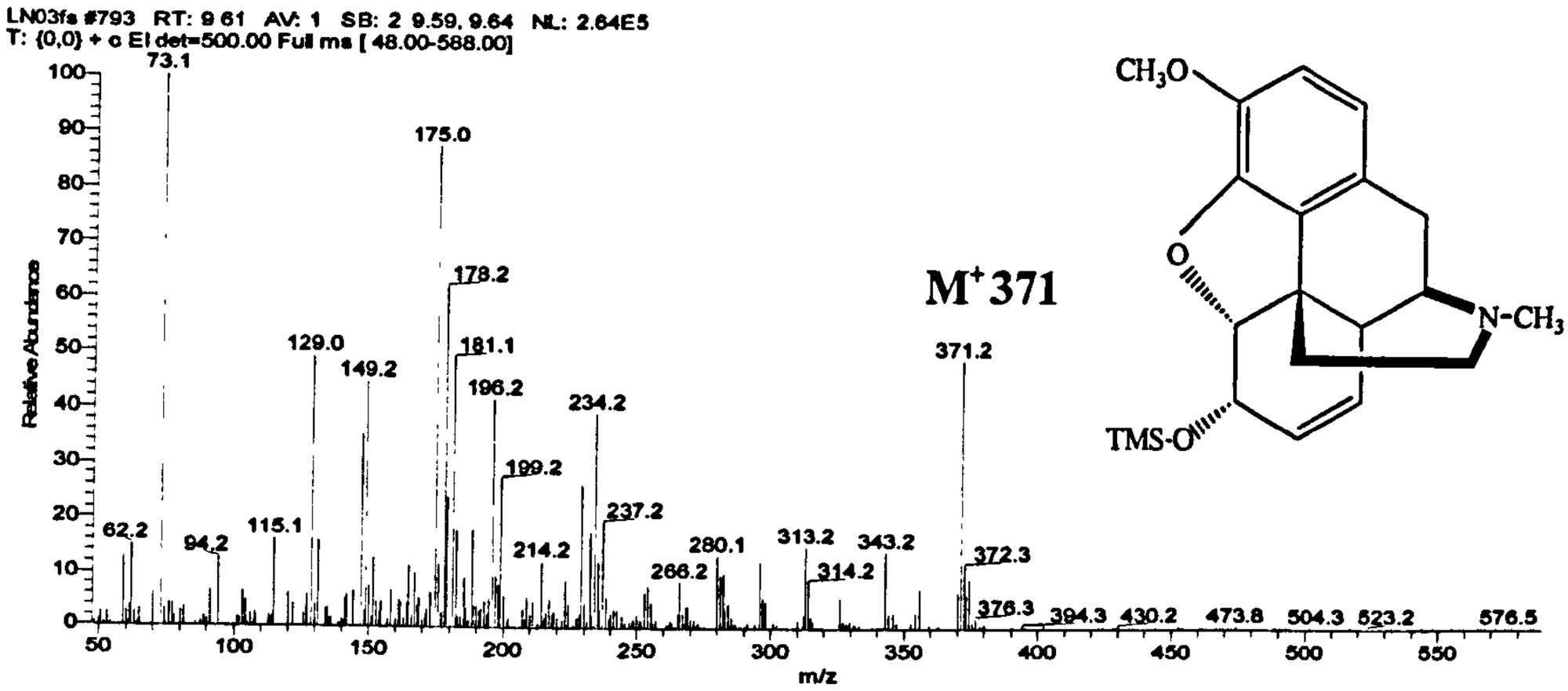


Figure 2.20. Mass spectrum of Codeine-mono-TMS (Retention time 9.61 min)

Table 2.3. Concentration of each opioid in nail sample No. 3 (ng/mg nail)

Opioid Name	LN ng/mg	AH ng/mg
Heroin	1.14	0.00
6-MAM	15.91	0.00
6-AcCodeine	1.97	0.00
Morphine	7.62	25.37
Codeine	0.86	7.66
Methadone	0.09	0.50

Notes: LN = liquid nitrogen method. AH = alkaline hydrolysis method.

When the total number of nanomoles of morphine and its derivatives in each LN and AH portion were calculated, the number in the LN portion was due to morphine (7.62 ng/MW 285) + heroin (1.14 ng/MW 369) + 6-MAM (15.91 ng/MW 327) = 0.079.

Similarly, the nanomoles of morphine in the AH portion from morphine alone (25.37 ng/MW 285) = 0.079. As a result, molar content of morphine determined by the LN and the AH methods was almost the same value [**Table 2.3**]. Therefore, it can be considered

that the LN method does not have any recovery problem in the extraction compared with the AH method.

There are many reports in which 6-acetylcodeine is identified as the major impurity in seized street heroin [49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60], therefore 6-acetylcodeine has been used as a marker of illicit heroin.

Papaverine, noscapine, thebaine are also well known as major impurities in seized heroin. By contrast, very few reports have been published concerning 6-acetylcodeine in biological samples such as hair [61, 62] or urine [63, 64] and there is no previous report for nail at all.

In the analysis of hair, it was reported that 6-acetylcodeine was not suitable as a biomarker of illicit heroin use as the concentration in hair is low [65]. In future, 6-acetylcodeine will be able to serve as a biomarker of illicit heroin in nail samples if a lot of analytical data were accumulated using either new methods or the LN method which was used in this work.

e) Papaverine, noscapine, and thebaine as major impurities in illicit heroin.

Both papaverine and noscapine were detected in the LN and the AH portions though thebaine could not be detected [**Figures 2.10, 2.21–2.22**]. This is the first report until the present that papaverine and the noscapine were detected in biological samples along with heroin.

According to **the Merck Index** (12th edition, drug number 6986), papaverine and noscapine, which have the isoquinoline structure, are minor components in opium. By contrast, another minor component, thebaine, has the morphinan structure. These minor components nevertheless constitute the quantitatively largest impurities. The number and the composition of the alkaloids are described as follows: about 20 alkaloids, constituting

about 25% of the opium; meconic acid, some lactic and sulfuric acids, sugar, resinous and waxy-like substances; 12–25% water.

Morphine is most important alkaloid and occurs to the extent of 10–16%, noscapine 4–8%, codeine 0.8–2.5%, papaverine 0.5–2.5% and thebaine 0.5–2%.

The question arises with respect to how many types of alkaloid in opium are known at this moment in time, though the number of alkaloids has been described as 20 kinds or more. More than 100 types of alkaloid exist in opium [66]. However, the best known alkaloids of interest might be the 20 compounds listed in **Table 2.4**. Actually, the number of opium components reported in articles using instrumental analysis is no more than about ten types.

Illicit heroin mostly contains only major impurities. There are many reports concerning the papaverine and the noscapine contained in illicit heroin [50,53, 57, 59, 67, 68, 69, 70, 71, 72, 73, 74]. However, few articles have been published which report on the thebaine content of opium.

However, no previous studies have been reported concerning the detection of papaverine and noscapine along with illicit heroin in biological samples. Moreover, there have been no previous studies in which papaverine and noscapine were detected in nail.

Studies in the literature concerning papaverine and the noscapine in biological samples do not relate these compounds to heroin use [75, 76].

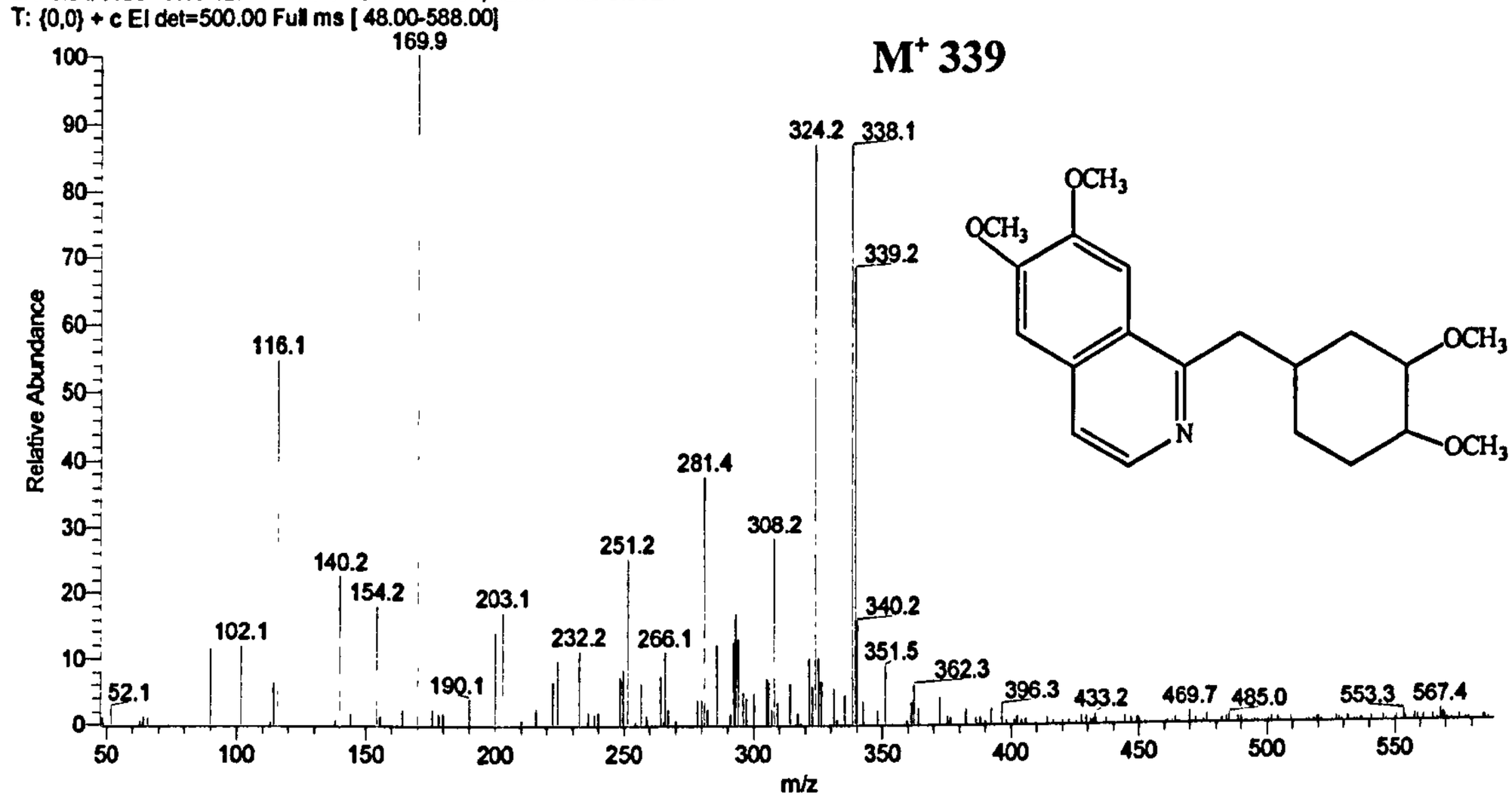


Figure 2.21. Mass spectrum of Papaverine (Retention time 12.63 min)

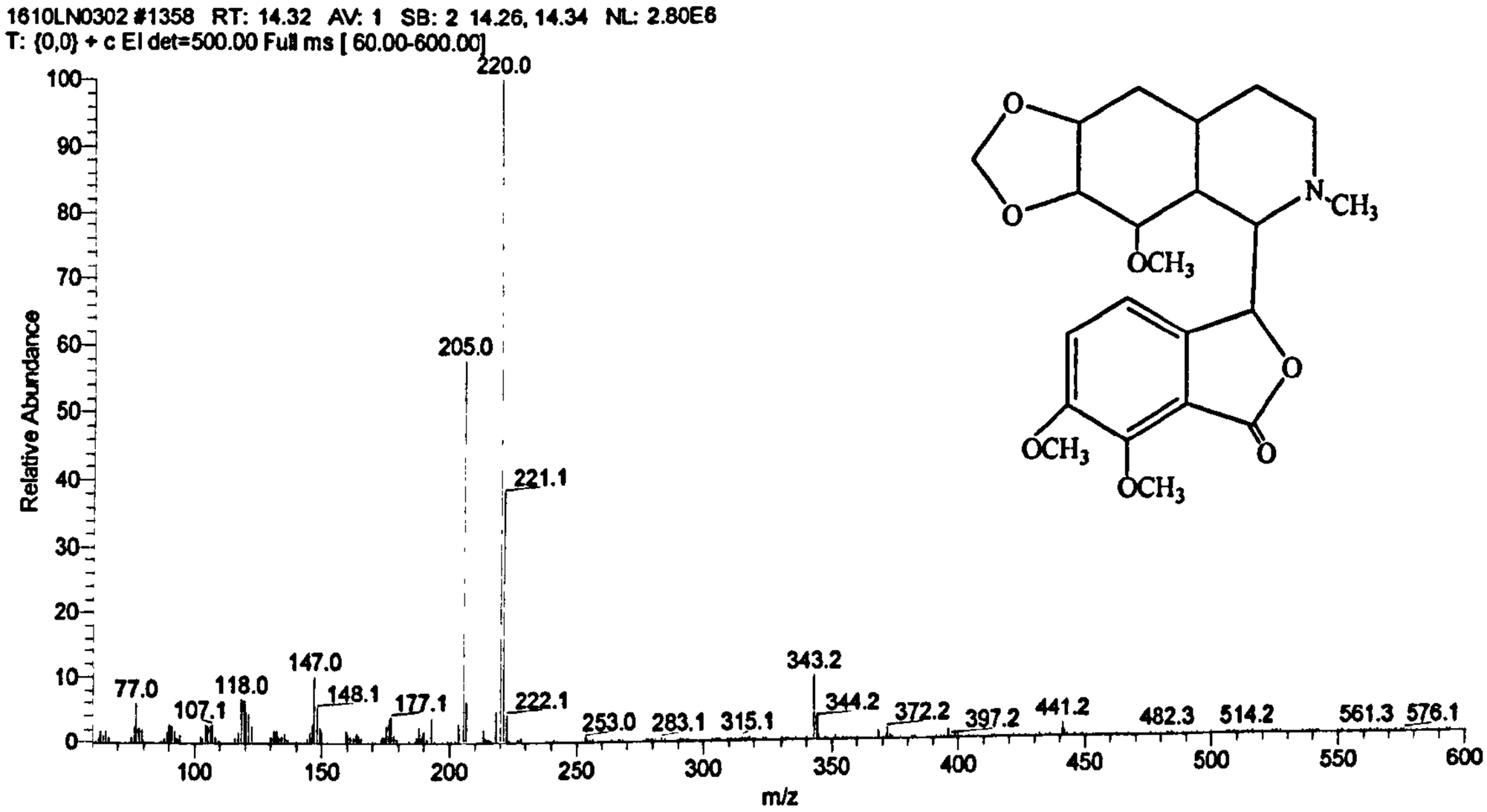


Figure 2.22. Mass spectrum of Noscapine

(Base peak *m/z* 220, MW = 413, Retention time 14.32 min)

Table 2.4. Opium alkaloids and their discoverers
(a) *from* "**Science of Opiates**", **Yoshiyuki Ichinoe** (**Kenseisya**, Japan, 1982), p. 58

No.	Structure Group	Alkaloid Name	Mol. Formular	MW	Discoverer	
					Name	Year
1	Morphinan	Morphine	C17H19NO3	285.33	C. Derosne	1804
2		Pseudomorph.	(C17H18NO3)2	568.67	J. Pelletier	1903
3		Codeine	C18H21NO3	299.36	M. Robiquet	1832
4		Thebaine	C19H21NO3	311.37	J. Pelletier, Thiboumery	1832
5		Neopine	C18H21NO3	299.36	T. & H. Smith	1911
6		Porphyroxine	C19H23NO4	329.38	J. N. Rakshit	1919
7	Benzyl-isoquinoline	Papaverine	C20H21NO4	339.38	G. Merck	1848
8		Pseudopapav.	----------	-------	O. Hesse	1903
9		Codamine	C20H25NO4	343.41	O. Hesse	1870
10		Laudanine	C20H25NO4	343.41	O. Hesse	1870
11		Laudanosine	C21H27NO4	357.43	O. Hesse	1871
12		Papaveraldine	C20H19NO5	353.36	T. & H. Smith	1893
13		Laudanidine	C20H25NO4	343.43	O. Hesse	1894
14		l-Laudanidine	C20H25NO4	343.43	E. Kauder	1890
15	Phthalide-isoquinoline	Noscapine	C22H23NO7	413.43	C. Derosne	1804
16		Narceine	C23H27NO8	445.47	J. Pelletier	1832
17		Gnoscopine	C22H23NO7	413.43	O. Hesse	1871
18		Oxynarcotine	C22H25NO8	431.44	G.H. Beckett, C.R.A. Wright	1875
19		Narcotline	C21H21NO7	399.39	Wrede	1937
20	Protopine	Cryptopine	C21H23NO5	369.40	T. & H. Smith	1864
21		Protopine	C20H19NO5	353.36	O. Hesse	1871
22	Tetrahydro-isoquinoline	Hydrocotarnine	C12H15NO3	221.25	O. Hesse	1871
23	Other	Rheadine	C21H21NO6	383.39	O. Hesse	1866
24		Aporeine	C18H17NO2	343.43	Pavesi	1907
25		Lantopine	----------	-------	O. Hesse	1870
26		Meconidine	----------	-------	O. Hesse	1870
27		Papaveramine	----------	-------	O. Hesse	1903
28		Sanguinarine	C20H14NO4	332.34	Schmidt	1893

Table 2.4. (b) *from* **Matthias Unger** et al., **J. Chromatogr., A 767 (1997) 263-276**

No.	Group (Structure)	Name	Molecular Formular	MW	Discoverer	
					Name	Year
2		Coptisine-Cl	C19H14ClNO4	355.80	Kitasato	1927
3		Berberine-HCl	C20H18ClNO4	299.36	Wasicky Joachimowitz	1917
4		Palmatine	C21H22NO4	352.41	Feist, Dschu	1925
5		Chelidonine	C20H19NO5	353.37		
6		Columbamine	C20H20NO4	339.40		
7		Jatrorrhizine	C20H21NO4	323.36		
8		Stylopine	C19H17NO4	323.36		
9		Conadine	C20H21NO4	339.40		
10		Scoulerine	C19H21NO4	327.39		
11	β-Carboline	Norharmane	C11H8N2	168.21		
12		Harman	C12H10N2	182.22		
13		Harmaline	C13H14N2O	214.27		
14		Harmine	C13H12N2O	212.25		
15		Harmalol	C12H12N2O	200.24		
16		Harmol	C12H10N2O	198.23		

f) Miscellaneous minor impurities in illicit heroin.

The minor impurities in illicit heroin such as thebaol, acetylthebaol, hydrocotarnine and 3,6-dimethoxyphenanthrene-4,5-epoxide were not detected in either the LN or AH portions, though thebaol [77, 78], acetylthebaol [78, 79, 80, 81], hydrocotarnine [82] and 3,6-dimethoxyphenanthrene-4,5-epoxide [77] have been reported as minor impurities in seized illicit heroin.

g) Normorphine and norcodeine as secondary metabolites of illicit heroin

Normorphine and norcodeine were not detected in either the LN or AH portions. Sample number 3 contained large amounts of morphine and codeine and it was therefore presumed that it would contain the metabolites (normorphine and norcodeine) if these were formed, but neither compound was detected.

Many opium alkaloids were targeted in this GC-MS analysis. However, these compounds could not be detected easily, because analytical conditions had not settled for the analysis of the secondary metabolites.

2.4. Conclusions

THC and its metabolites 11-OH-THC and THC-COOH as well as CBD were detected and quantified in nail clippings from cannabis users using the cryogenic grinding method followed by GC-MS analysis. By contrast, THC-COOH was not readily detected in nail clippings by alkaline hydrolysis. Extraction of cannabinoids from nail clippings using the cryogenic grinding method was found to be a simple procedure that could be applied effectively to a wide range of analytes without the need to consider their different polarities.

Similarly, heroin and the related opiates in nail clippings were extracted with the cryogenic grinding method and the results were evaluated in comparison with the conventional alkaline hydrolysis extraction method. Both the AH and LN methods gave the same total concentration of morphine (0.079 nano-moles/milligram of nail), when the result of the analyses were expressed as equivalents of morphine.

Heroin, 6-MAM, and acetylcodeine could not be detected by the conventional alkaline hydrolysis method, but could be detected with the cryogenic grinding method. In addition, papaverine and noscapine were identified together with heroin from nail samples for the first time. This is also the first report in which these analytes have been analysed by GC-MS at the same time in any biological sample.

6-acetylcodeine, having an acetyl group at the C6-position of the morphinan structure, and opium alkaloids (papaverine, noscapine etc.) might be used in the near future as biomarkers for the administration of impure street heroin.

In contrast, only morphine and codeine were identified and quantified with the alkaline hydrolysis method. These opioids could also be determined with the cryogenic grinding method.

Heroin is a synthetic opioid compound having acetyl groups at both the C3- and

C6-positions of the morphine structure. The acetyl substituent group of heroin at the C6-position is easily hydrolysed, and heroin is converted to 6-MAM in the soaking method, which uses solvent exposure over a relatively long period of time. Moreover, both substituent groups are completely hydrolysed to produce morphine in the alkaline hydrolysis method.

In summary, cryogenic grinding has been shown to be a suitable sample preparation method for the routine analysis of nail specimens, which can therefore be used as an alternative analytical sample in cases of forensic toxicological interest. The cryogenic grinding method has significant advantages over the alkaline hydrolysis method with respect to sample degradation and extends the range of accessible analytes to include several markers of heroin use. The practical application of the method requires the use of liquid nitrogen and the provision of multiple sets of micro-vials for the grinder, if large numbers of samples are to be analysed, but these costs are offset by the improved utility of the method. The use of nail, as for hair samples, extends the potential window of detection of drugs beyond what is possible with more conventional samples such as blood and urine. However, unlike hair analysis, analysis of drugs in nail is a relatively new subject and much additional work is required to establish the extent of its usefulness in forensic toxicology.

3. Analysis of Anabolic Steroids in Nail Clippings from Steroid Users

3.1. Introduction

3.1.1. Doping steroids

It is difficult to define *doping steroid* in a single phrase. In the dictionary, dope means a drug used to enhance the performance of an athlete, racehorse, or greyhound [83].

According to the dictionary of the **Microsoft Encarta** [84], the word ***dope*** is probably derived from the Dutch word ***dop***, the name of an alcoholic beverage made of grape skins used by **Zulu** warriors in order to enhance their prowess in battle [85]. The term became current around the turn of the 20th century, originally referring to illegal drugging of racehorses. The practice of enhancing performance through foreign substances or other artificial means, however, is as old as competitive sport itself.

It is surmised that "**dope**" naturally became synonymous with various kinds of drugs, and came to be used in the context of playing a game or sport illegally. A doping steroid should meet at least the following three requirements:

1) a doping steroid must have basically **the chemical structure of testosterone.**

2) a doping steroid must be an **anabolic androgenic steroid (AAS).**

3) a doping steroid must be registered in the prohibited list of the **World Anti-Doping Agency (WADA).**

3.1.2. Testosterone

Doping steroids comprise testosterone (17ß-hydroxyandrost-4-en-3-one, $C_{19}H_{28}O_2$ = 288.43) and its related steroid compounds with the testosterone nucleus, shown in **Figure 3.1.**

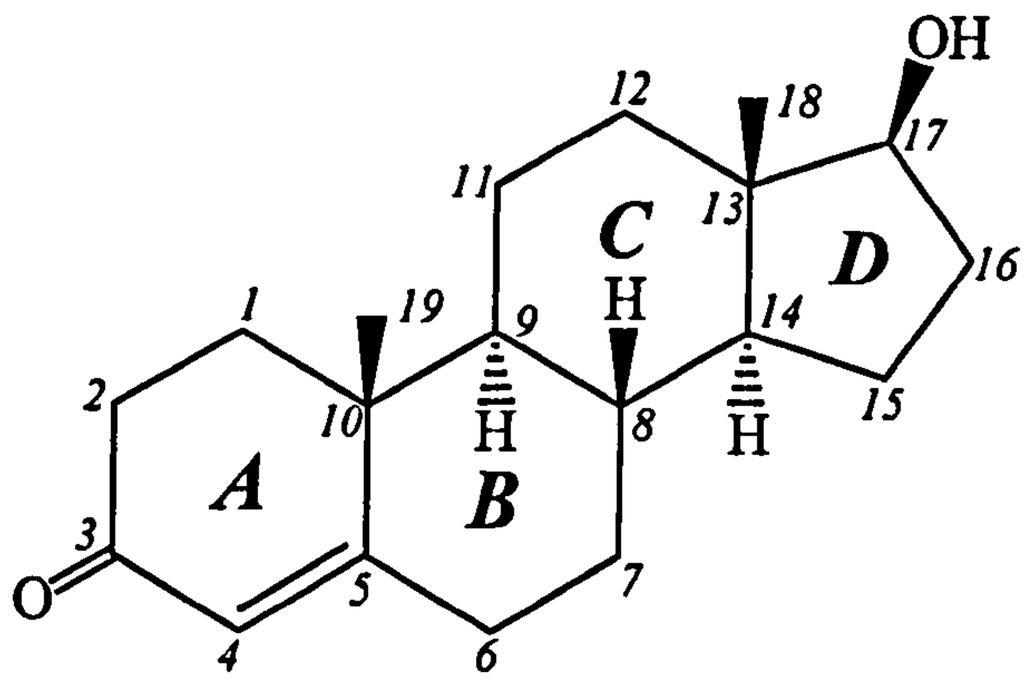


Figure 3.1. Chemical structure and numbering system of Testosterone

Testosterone is an anabolic steroid, which forms the basic framework of doping steroids. Testosterone is described according to **Martindale** [86] as follows:

"Testosterone is the main androgenic hormone formed in the interstitial (**Leydig**) cells of the testes. A small proportion of circulating testosterone is also derived from the metabolism of less potent androgens secreted by the adrenal cortex and ovaries."

In many target tissues testosterone is then converted to the more active hormone, dihydrotestosterone (**DHT**) by 5α-reductase. Some testosterone also undergoes peripheral conversion to estradiol.

Testosterone controls the development and maintenance of the male sex organs and the male secondary sex characteristics. It also produces systematic anabolic effects, such as increased retention of nitrogen, calcium, sodium, potassium, chloride and phosphate.

This leads to an increase in water retention and bone growth. The skin becomes more vascular and less fatty and erythropoiesis is increased. The androgens generally possess anabolic activity and were formerly used to increase weight in patients suffering from emaciation or debilitating diseases but their effectiveness was doubtful. The anabolic steroids were developed in order to enhance the ability to build proteins and diminish the virilising and masculinising effects of the natural androgens, but all

anabolics retain some androgenic activity [87]. The anabolic steroids have, like the androgens, been used in an attempt to produce weight gain in cachexia (weakness and wasting of the body due to severe chronic illness) and wasting diseases.

Anabolic androgenic steroids have been subject to much misuse and abuse by athletes, sports persons and body builders in an attempt to increase muscle mass and body-weight but such use cannot be justified.

As shown in the description in **Martindale** [86], the illegal abuse of testosterone and related compounds is more prominent than normal use. However, only doping steroids (illegal use of anabolic steroids) in humans will be the subject described here.

3.1.3. Biosynthesis and metabolism of testosterone

As indicated in **Martindale** [86], testosterone is formed in the interstitial (**Leydig**) cells of the testes. The starting point for its biosynthesis is cholesterol, which itself is derived from the terpenoid precursor, squalene. In general, cholesterol is synthesised in the body by the acetyl CoA metabolic pathway as shown in **Figures 3.2–3.4.**

The intermediate C_{21} steroid, pregnenolone, becomes testosterone through various metabolic pathways. Estradiol, which is a type of female hormone, is generated by metabolism of testosterone ***in vivo***, though testosterone is considered to be a male hormone. Is this a mysterious phenomenon of life? In fact both male and female produce both sets of steroids, but usually in different amounts.

When steroid extraction from biological samples and analysis is carried out a complex mixture of C_{18}, C_{19} and C_{21} steroids is encountered. For this reason, estrogens and progestagens as well as the metabolites of anabolic steroids were included in this study.

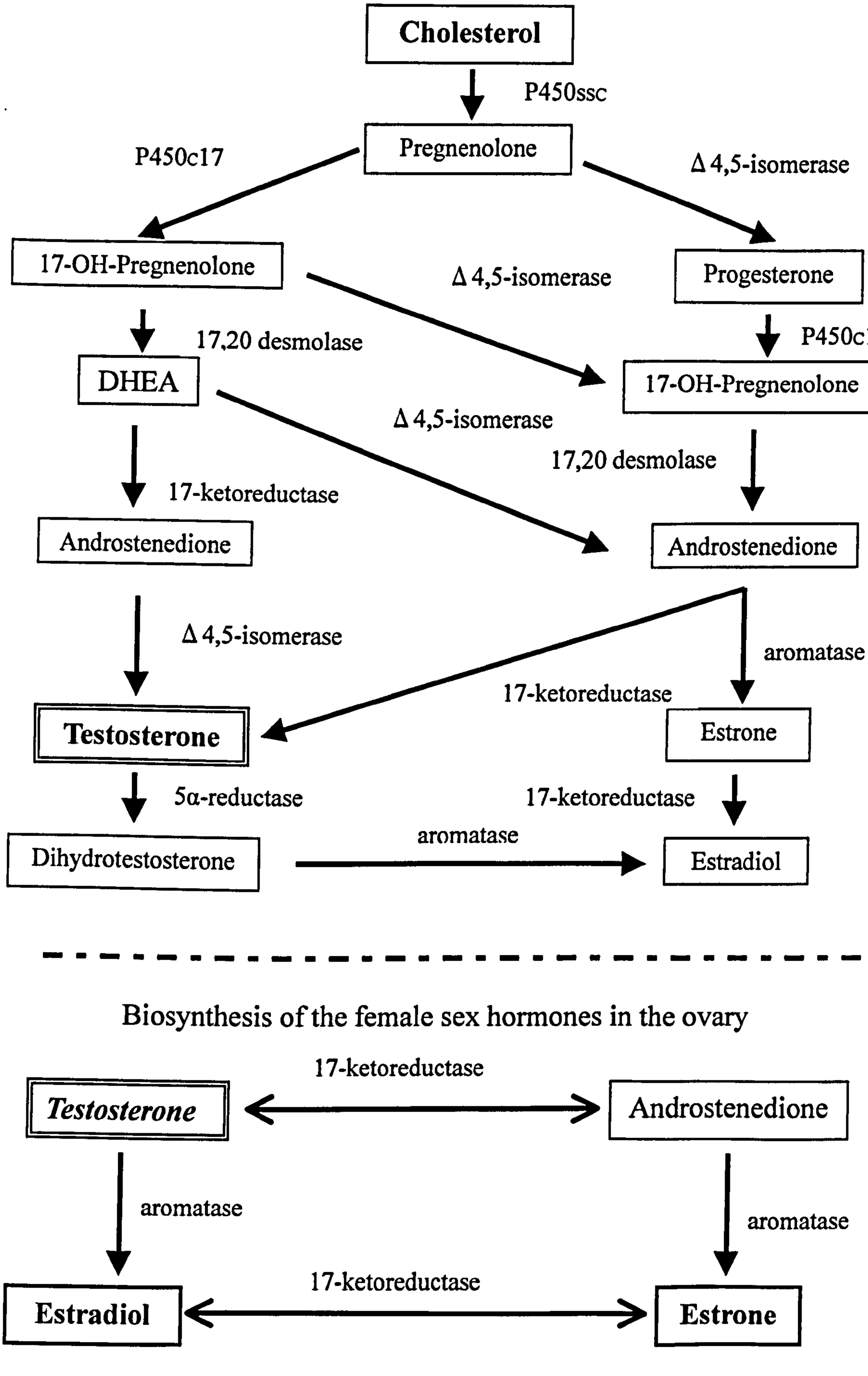


Figure 3.2. Biosynthesis and metabolism of Testosterone

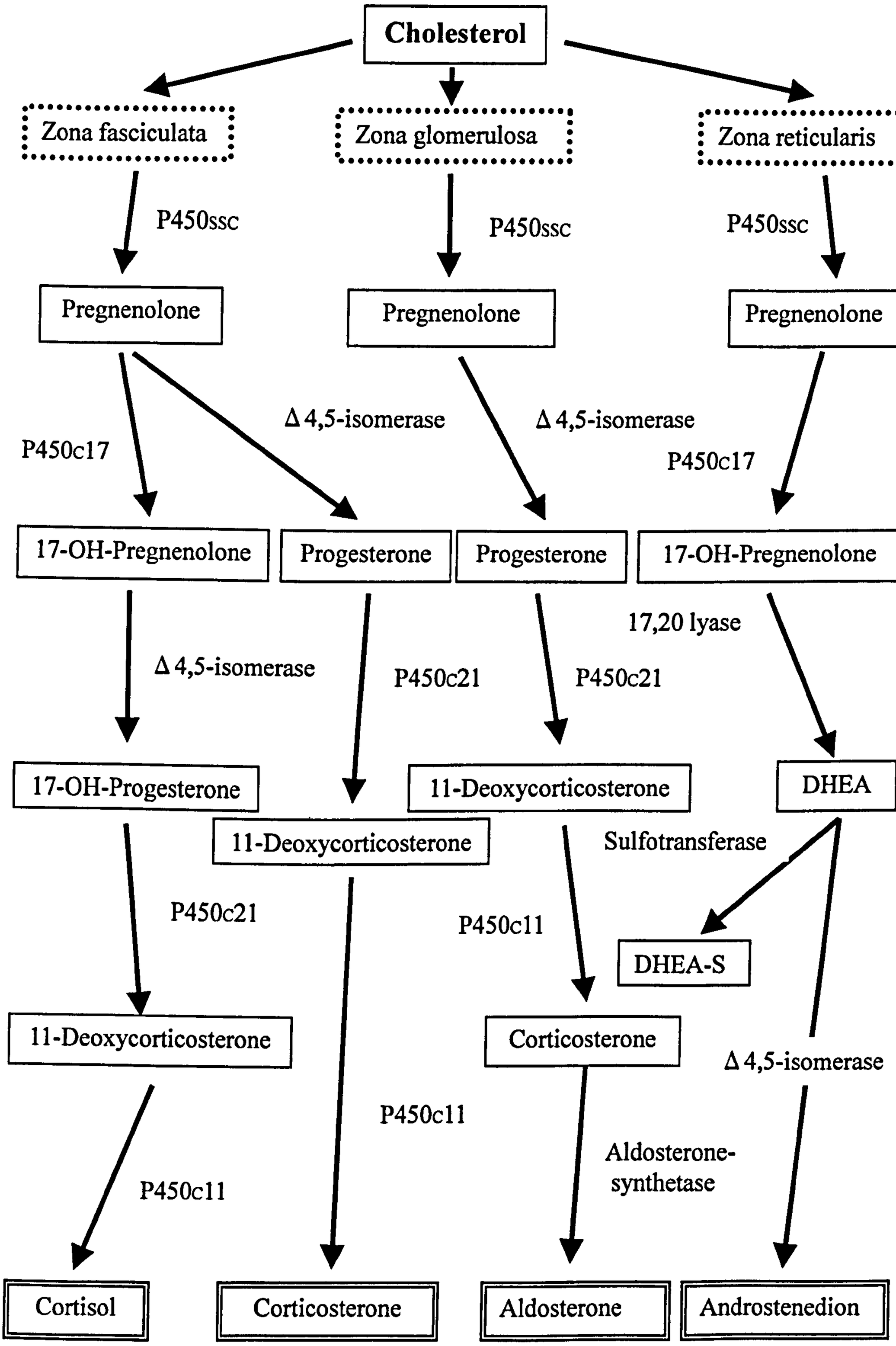
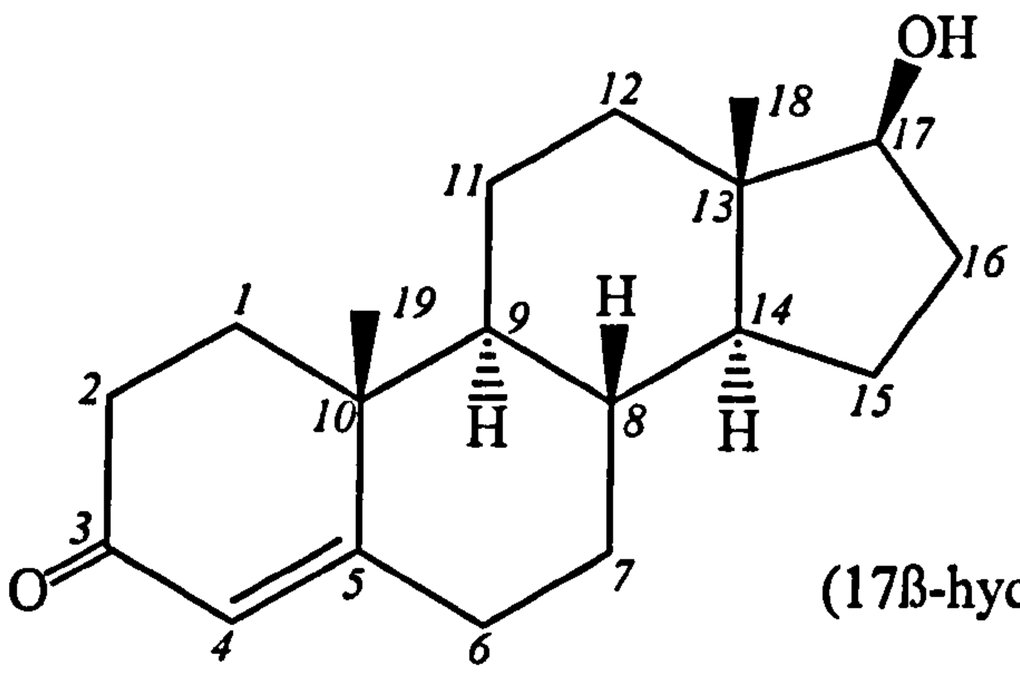


Figure 3.3. Biosynthesis and metabolism of Adrenal Cortex Steroids



Testosterone
(17ß-hydroxyandrost-4-en-3-one)

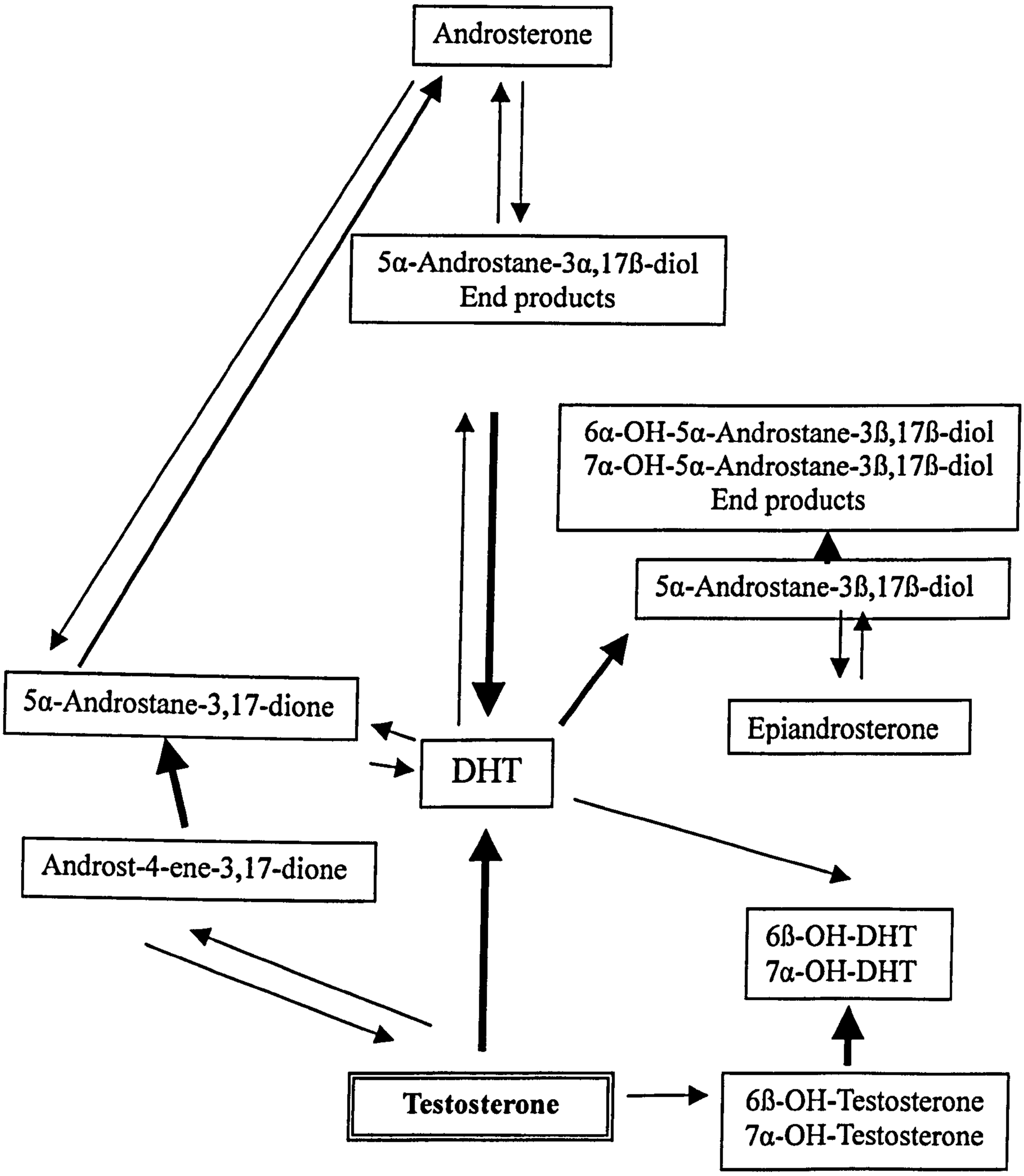


Figure 3.4. Metabolism of Testosterone

3.1.4. World Anti-Doping Agency

World Anti-Doping Agency (WADA) is an international organisation concerned with the control of doping in sport. WADA was formed in November 1999, as a result of an initiative of the **International Olympic Committee (IOC)**, to support and promote fundamental values in sport.

According to the **WADA Internet web site** [85], a large number of prohibited medical substances were found by police in a raid during the **Tour de France** in 1998. The scandal led to a major reappraisal of the role of public authorities in anti-doping affairs.

The Tour de France scandal highlighted the need for an independent international agency, which would set unified standards for anti-doping work and co-ordinate the efforts of sports organisations and public authorities.

The World Conference on Doping in Sport was held in **Lausanne** in February 1999 and subsequently the World Anti-Doping Agency was established in Lausanne on 10 November 1999.

The WADA doping control code is revised every year [**Table 3.1**]. Nevertheless, the abuse of doping steroids in the sporting world has been a regular topic. Eight kinds of prohibited substances including stimulants, narcotics, cannabinoids, peptide hormones, beta-2-agonist, agents with anti-estrogenic activity, masking agents, and glucocorticosteroids as well as anabolic steroids are regulated under the WADA code. The controlled steroids and related compounds in the WADA doping steroid group basically include about 40 kinds as of 2004.

Doping control by various sports groups that are signatory subsidiaries of WADA set an original doping restriction in accordance with this WADA code. For instance, the content is almost the same in the United States of America and Britain as shown in the

United States Anti-Doping Agency (**USADA**) guide list [**Table 3.2**] and the list of new controls in the supply of anabolic steroids issued by the **Home Office in the United Kingdom [Table 3.3]**.

Similarly, the list of controlled substances in the steroid group used in sporting events hardly differs from the WADA charter. Therefore, it is not an exaggeration to say that the content of doping control in various sports groups is the same as the content of the WADA code.

3.1.5. Types of doping steroids

A review of doping steroids based on related books, journals and **Internet Web sites** gave a collection of about 600 types of steroid, including anabolic steroids and their related metabolites as well as progestagens and estrogens.

Roughly speaking, when doping steroids are analysed, about 50 or 60 substances should be considered. The reason is that a number of endogenous anabolic steroids are added to the number of exogenous (**synthetic**) anabolic steroids for which WADA provides (**Table 3.1** and **Table 3.4**).

3.1.6. Contexts in which doping steroids are used

As time went on, dope came to be used illegally not only in man but also in various animals. The abuse of doping steroids has been reported in horseracing [88, 89, 90, 91, 92, 93, 94], dog racing [95], and pigeon racing [96], amongst others. No papers have been published dealing with abuse of doping steroids in blood sport games such as bull, dog and cockfighting. However, the illegal use of dope is easily speculated in gambling sports that use these animals.

Serious problems concerning illegal use of doping steroids result from their use in

various human sports and as growth promoters for livestock and poultry [97, 98, 99, 100, 101, 102, 103, 104, 105]. Additionally, there are data regarding naturally occurring steroids in fish, including sex hormones [105, 106].

Humans have indirectly taken synthetic anabolic steroids into their body from meat, chicken and fish polluted with anabolic steroids, though they did not know of the presence of the steroids in these foodstuffs.

Obtaining doping steroids is actually difficult because of legal restrictions. However, the spread of Internet Web sites has promoted illegal acquisition of doping steroids. Therefore, doping steroids have been increasingly abused nowadays.

3.1.7. Analytical samples

The analytical specimens for doping steroids have included internal organs, blood and urine as conventional biological samples.

There are innumerable theses and publications concerning these samples. As interesting examples, studies of faeces [96, 107, 108] and hair of domestic animals [99, 109, 110, 111] were reported as samples used for the detection of promoters in livestock.

In the human, hair has been used as a substitute for these biological samples [112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131].

However, hair samples have not yet been officially adopted in doping control in regular sport. A hair sample has the advantage that information is obtained on doping steroids accumulated in the past. Though a fingernail sample has a similar advantage, the number of papers concerning the use of human fingernail clippings is extremely low. For example, **Choi** and co-workers analysed testosterone and pregnenolone in nail[132].

3.1.8. Difficulties in the analysis of doping steroids in nail

The problems are basically brought together by the following two points. Firstly, the fingernail is composed of hard keratin. Therefore, it is difficult to extract doping steroids directly without degradation. **Choi** used alkaline digestion method for the analysis of testosterone and pregnenolone in nail [132]. However, if an alkaline digestion method were to be used for testosterone esters, the steroid esters could not be detected and quantified because of the decomposition of the esters by hydrolysis.

The second reason is that, even if doping steroids are extracted from the fingernail, the amount of doping steroid in nail is extremely low just as are the endogenous sex hormones. Moreover, the extracts usually contain many interfering substances.

3.1.9. Method of direct extraction of doping steroids from fingernail

Cryogenic grinding was described in **Chapter 2,** using liquid nitrogen as a method of overcoming the difficulty of extracting drugs from fingernail clippings.

Application of the cryogenic grinding method to analysis of cannabinoids [30] and opioids [133] in nail was extremely effective, and the analytes were extracted without degradation of morphine esters. A similar procedure was expected to be applicable for the analysis of steroid esters.

3.1.10. Aims of this study

The first objective was to apply the cryogenic grinding method to the extraction of doping steroids in the fingernail samples, and to verify the presence of steroids in nail.

The second was to evaluate this alternative steroid extraction procedure for nail clippings from anabolic steroids abusers.

Table 3.1. WADA prohibited list

S4. ANABOLIC AGENTS

1. Anabolic Androgenic Steroids (**AAS**)

a. Exogenous AAS including but not limited to:

androstadienone, bolasterone, boldenone, boldione, clostebol, danazol, dehydrochloromethyltestosterone, Δ^1-androstene-3,17-dione, drostanolone, drostanediol, fluoxymesterone, formebolone, gestrinone, 4-hydroxytestosterone, 4-hydroxy-19-nortestosterone, mestanolone, mesterolone, methandienone, metenolone, methandriol, methyltestosterone, mibolerone, nandrolone, 19-norandrostenediol, 19-norandrostenedione, norbolethone, norethandrolone, oxabolone, oxandrolone, oxymesterone, oxymetholone, quinbolone, stanozolol, stenbolone, 1-testosterone (Δ^1-dihydrotestosterone), trenbolone and their analogues.

b. Endogenous AAS including but not limited to:

androstenediol, androstenedione, dehydroepiandrosterone (DHEA), dihydrotestosterone, testosterone and their analogues.

* Note: Extracts from the WADA prohibited list, 2004

Table 3.2. USADA guide list (EXAMPLES OF ANABOLIC AGENTS)		
No.	Generic Name	Pharmaceutical Preparations Examples
1	Androstenediol	Androstederm, 4-Androstenediol, 5-Androstenediol
2	Androstenedione	Andro Stack850, Andro-Gen, Androsten, Androstene 100, Testro Rx
3	Bolasterone	Dimethyltestosterone, Myagen
4	Boldenone	Equipoise, Vebonol
5	Clostebol	Steranabol
6	Danazol	Cyclomen, Danatrol, Danocrine, Danokrin, Danol, Ladogar, Win 17757, Winobanin
7	Dehydro-Cl-Me-testosterone	Turinabol
8	Dehydroepiandrosterone	DHEA
9	Dihydrotestosterone	Stanolone
10	Dimethyltestosterone	Myagen
11	Dromostanolone	Drolban, Masteril
12	Fluoxymesterone	Android F, Halotestin, Ora-Testryl, Ultradren
13	Formebolone	Esiclene, Hubernol
14	Gestrinone	Tridomose
15	Mesterolone	Androviron, Proviron
16	Metandienone	Danabol, Dianabol
17	Metenolone	Primoboran, Primonabol-Depot
18	Methandriol	Stenediol, Trofomone
19	Methyltestosterone	Android, Estratest, Metandren, Oreton Methyl, Testred
20	Mibolerone	Cheque Drops
21	Nandrolone	Deca-Durabolin, Durabolin, Kabolin, Nandrobolic
22	19-Norandrostenediol	19-Nor Diol, 19-Norandrobol, Norandrodiol, Norandronate
23	19-Norandrostenedione	19-Nor Androstene, 19-Nora Force, Anabolic Stack, Androbolic, Androdyne, Androstat Poppers, Androstat Pro 6, Ultimate Release 24
24	Norbolethone	
25	Norethandrolone	Nilevar
26	Oxandrolone	Anavar
27	Oxymesterone	Oranabol 10
28	Oxymetholone	Adroyd, Anadrol, Anapolon
29	Stanozolol	Stromba, Winstrol
30	Testosterone	(T:E >6:1) Delatestryl, Malogen, Malogex
31	Trenbolone	Finajet, Parabolan

*Notes: extracts from the USADA guide

Beta-2 agonists are included in the class of anabolic agents.

Authorised in the aerosol or inhalant forms only to prevent and/or treat asthma and exercise-induced asthma (EIA and EIB).

According to the USADA guide, methandrostenolone (Dianabol) is repeated.

Table 3.3. UK list 1996

New controls on the supply of anabolic steroids
Home Office 082/96, 21 march 1996

ANABOLIC AND ANDROGENIC STEROIDS

Atamestane Bolandiol Bolasterone Bolazine Boldenone Bolenol Bolmantalate

Calusterone 4-Chloromethandienone Clostebol Drostanolone Enestebol Epitiostanol

Ethyloestrenol Fluoxymesterone Formebolone Furazabol Mebolazine Mepitiostane

Mesabolone Mestanolone Mesterolone Methandienone Methandriol Methenolone

Methyltestosterone Metribolone Mibolerone Nandrolone Norboletone Norclostebol

Norethandrolone Ovandrotone Oxabolone Oxandrolone Oxymesterone Oxymetholone

Prasterone Propetandrol Quinbolone Roxibolone Silandrone Stanolone Stanozolol

Stenbolone Testosterone Thiomesterone Trenbolone

Polypeptide Hormones Chorionic Gonadotrophin (**HCG**) Non-human chorionic

gonadotrophin Somatrotopin Somatrem Somatropin

Andrenoceptor stimulant Clenbuterol

*Note: Extracted from documents

3.2. Materials and Methods

3.2.1. Nail samples

Nail clippings from anabolic steroid users were obtained from **the Institute of Doping Analysis/Sports Biochemistry, Kreischa**. Blank nail samples were obtained from volunteers.

3.2.2. Blood and urine samples

Blood and urine samples were collected from volunteers for validation of the steroid analytical methods and to compare with the steroid concentrations in the nail samples.

3.2.3. Reagents

3.2.3.1. Standard steroid samples

Standards were obtained from the following suppliers:

Steraloids (RI, USA);

Testosterone acetate, testosterone undecanoate, and testosterone benzoate.

Testosterone homologues that are banned by the World Anti-Doping Agency **(WADA) [Table 3.4]**, bolandiol, bolasterone, calusterone, canrenone, clostebol, dromostanolone, 11-ketotestosterone, mibolerone, and norgestrel.

Sigma-Aldrich (Dorset, U.K.);

Androsterone (**AND**), dehydroepiandrosterone (**DHEA**), epiandrosterone (**epiAND**), and epitestosterone (**epiTEST**).

Stanozolol (**STN**), fluoxymesterone, oxandrolone, 4-androstene-3,17-dione, 19-nor-4-androstene-3,17-dione, 19-nor-testosterone-17-decanoate (nandrolone decanoate), testosterone-17ß-esters, propionate, isocaproate, heptanoate (enanthate), decanoate, cypionate, and phenylpropionate

IKAPHARM (Ramat-Can, Israel);

Etiocholanolone (**ETIO**).

Cerilliant (Texas, USA);

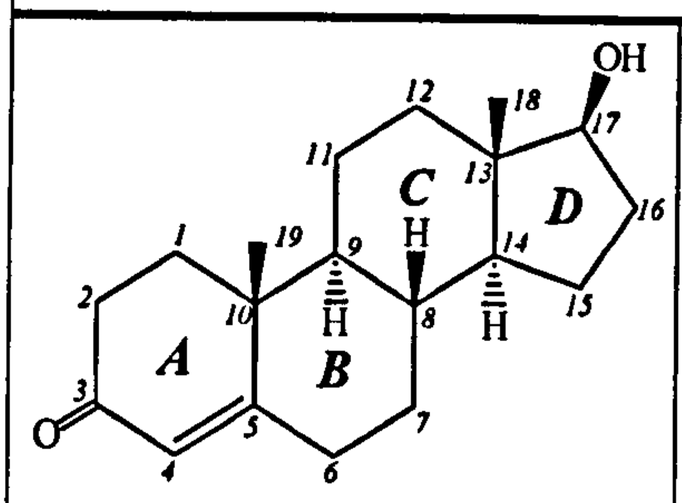
Oxymesterone, methenolone, Anabolic metabolites, 16ß-hydroxystanozolol, 3'-hydroxystanozolol, 6ß-hydroxyfluoxymesterone, 6ß,11ß-dihydroxyfluoxymesterone, 6ß-hydroxy-methandienone, 5α-estran-3α-ol-17-one (Norandrosterone), and 5ß-estran-3α-ol-17-one (Noretiocholanolone).

Research Biochemicals International (MA, USA) ;

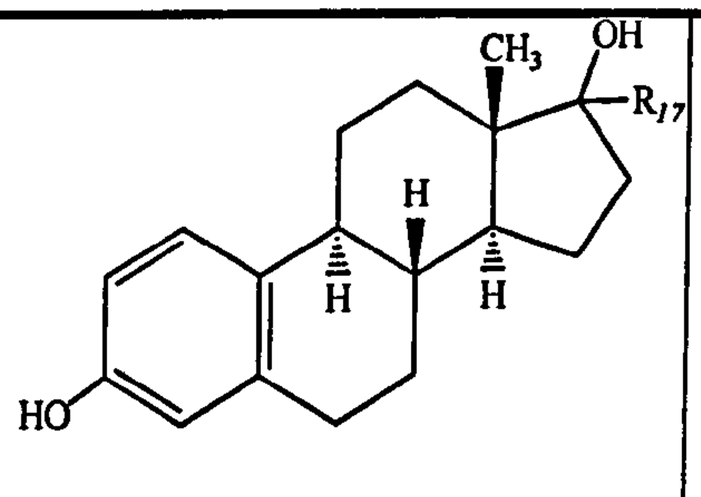
Testosterone propionate.

Table 3.4. Chemical structures of typical exogenous anabolic steroids homologues

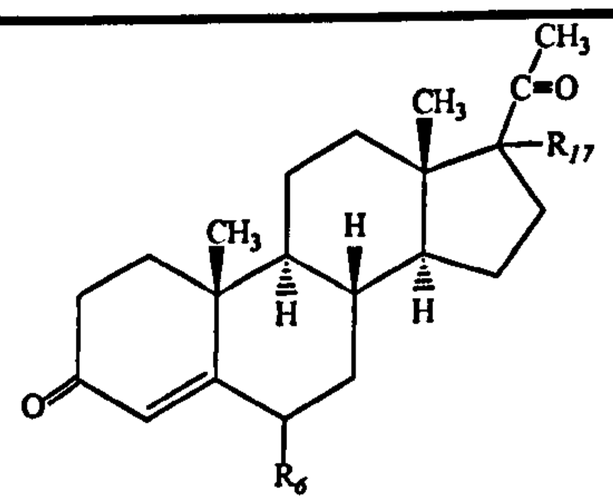
* prohibited list from the WADA



Testosterone (numbering)



Estrogens



Progestagens

Testosterone homologues

Steroid	Subst. at **C4**	Subst. at **C17α**	Substituent groups at **Cn** position
Testosterone			17ß-hydroxyandrost-4-en-3-one
Bolasterone		CH_3	7α-Me-
Boldenone			1,2-dehydro-
Calusterone		CH_3	7α-Me-
Clostebol	Cl		
Dehydro-Cl-Me-testosterone	Cl	CH_3	1,2-dehydro-
Dihydrotestosterone			4,5-dihydro-

Dromostanolone			2α-Me-, 4,5-dihydro-
Fluoxymesterone		CH_3	9-F-, 11ß-OH-
Formebolone		CH_3	1,2-dehydro-, 2-formyl-, 11α-OH-
Gestrinone		CH≡C	9,10,11,12-dehydro-, 18α-Et-, 19-nor-
Mestanolone		CH_3	4,5-dihydro-
Mesterolone			1α-Me-, 4,5-dihydro-
Methandienone		CH_3	1,2-dehydro-
Methenolone			1-Me-, 1,2-dehydro-, 4,5-dihydro-
Methyltestosterone		CH_3	
Mibolerone		CH_3	7α-Me-
Nandrolone			19-nor-
Norbolethone		C_2H_5	13ß-Et-, 19-nor-
Norethandrolone		C_2H_5	
Oxabolone	OH		19-nor-
Oxymesterone	OH	CH_3	
Oxymetholone		CH_3	2-CH_2OH-
Quinbolone			1,2-deH-,17ß-(1-cyclopenten-1-yloxy)-
Stenbolone			2-Me-, 1,2-dehydro-, 4,5-dihydro-
Trenbolone			9,10,11,12-dehydro-, 19-nor-
Danazol*		CH≡C	(2,3-d)isoxazol-
Oxandrolone*		CH_3	2-oxa-, 4,5-dihydro-
Stanozolol*		CH_3	2-eno(3,2-c)pyrazol-, 4,5-dihydro-

* Heterocyclic anabolic steroid attached on the A ring.

3.2.3.2. Steroids for use as internal standards

Deuterated testosterone (17ß-hydroxyandrost-4-en-3-one-16,16,17-d_3, **TEST-d_3**) was from **Sigma-Aldrich**.

Deuterated 5α-estran-3ß-ol-17-one (19-nor-5α-androstan-3ß-ol-17-one-3α,4,5α-d_3, norepiandrosterone-d_3, **NEA-d_3**) and stanozolol (17ß-hydroxy-17α-methylandrostano [3,2-c]pyrazole-20,20,20-d_3, **STN-d_3**) were from **Cerilliant** (Texas, USA).

Medroxyprogesterone (17α-hydroxy-6α-methylpregn-4-ene-3,20-dione, **MEDR**) was from **Steraloids**.

3.2.3.3. Other anabolic steroid alkanoic acid esters

These steroids were synthesised by reaction of anabolic steroids with the corresponding alkanoic acid anhydrides or acyl chlorides using pyridine or 4,4'-bipyridyl as catalysts.

These steroid acyl derivatives are shown in **Tables 3.5–3.7** and Appendix (**Table 3.8**) in bold and with double underscore.

*3.2.3.4. Hydrocarbon reagents for **Kovat index** (Retention index)*

Docosane ($C_{22}H_{46}$), octacosane ($C_{28}H_{58}$), triacosane ($C_{30}H_{62}$), dotriacosane ($C_{32}H_{66}$), tetratriacosane ($C_{34}H_{70}$), hexatriacosane ($C_{36}H_{74}$), octatriacosane ($C_{38}H_{78}$), and tetratetra-contane ($C_{44}H_{90}$) were purchased from **Sigma-Aldrich**.

Tetracosane ($C_{24}H_{50}$) was from **Ralph N Emanuel** (Wembley, England).

Hexacosane ($C_{26}H_{54}$), tetracontane ($C_{40}H_{82}$), and dotetracontane ($C_{42}H_{86}$) were from **Fluka Chemie** (Buchs, Switzerland). These hydrocarbons were dissolved in toluene (about 10 ng/μL) and used for measuring retention indices of anabolic steroids.

3.2.3.5. Solvents and reagents

Methanol and ethyl acetate used were both high performance liquid chromatography (**HPLC**) grades from **BDH Laboratory Supplies**, U.K.

N-methyl-N-(trimethylsilyl)trifluoroacetamide (**MSTFA**), 2-mercaptoethanol, and ammonium iodide were from **Sigma-Aldrich**.

Other solvents and reagents used were all analytical reagent or HPLC grade.

3.2.3.6. Solid phase extraction cartridges

Solid phase extraction (**SPE**) cartridges were **Worldwide Monitoring Clean Screen** cartridges, CSDAU (200 mg, 10 mL, **United Chemical Technologies Inc.**, Bristol, U.K.), **Varian Bond Elut** LRC C_{18} cartridges, (200 mg, 10 mL, Varian, CA, USA), or **Isolute-XL** (200 mg, 10 mL, **International Sorbent Technology Limited**, MidGlamorgan, U.K.).

Table 3.5. GC-MS Analysis of Testosterone derivatives; in relative retention time order

No.	Compound Name	RT (min)	I.Std. (min)	RRT	R.I (Found)	Mol. Form.	MW	Mass Spectrum (Found, *m/z*)	Mass Spectrum (Literature, *m/z*)
t0	dioctyl phthalate	10.57	11.98	0.882	2551.05	C24H38O4	390.57	149, 71, 167, 279, free-	149, 57, 167, free-
t2	***testosterone-D3***	11.96	11.98	0.998	2704.49	***C19D3H25O2***	291.40	73, ***435***, 436, 75, 209, di-	***435***, 73, 420, 209, di-
t1	testosterone	11.98	11.98	1.000	2706.60	C19H28O2	288.43	***432***, 73, 433, 75, di-	73, ***432***, 209, 417, 195, di-
t3	**testosterone-17ß-acetate**	12.68	11.94	1.062	2780	C21H30O3	330.50	73, ***402***, 75, 403, mono-	387, ***402***, 247, 209, mono-
t10	**testosterone-17ß-propionate**	13.84	12.04	1.150	2888	C22H32O3	344.50	***416***, 73, 75, 401, 91, 208 , mono-	401, ***416***, 209, 247, mono-
t8	**testosterone-17ß-isocaproate**	17.22	12.29	1.401	3175.88	C25H38O3	386.60	73, ***458***, 81, 99, 208, mono-	***458***, 443, 209, 247, mono-
t7	**testosterone-17ß-enanthate**	18.79	12.19	1.541	3329	C26H40O3	400.60	73, ***472***, 113, 473, mono-	457, ***472***, 209, 247, mono-
t4	**testosterone-17ß-benzoate**	21.07	12.19	1.728	3520	C26H32O3	392.50	105, 73, 77, ***464***, mono-	449, ***464***, 209, mono-
t5	**testosterone-17ß-cypionate**	21.40	12.15	1.761	3551	C27H40O3	412.60	73, 79, 81, 125, mono-	469, ***484***, 209, mono-
t6	**testosterone-17ß-decanoate**	22.61	12.04	1.878	3648	C29H46O3	442.70	***514***, 71, 73, 155, 75, mono-	***514***, 499, 209, mono-
t9	testosterone-17ß-phenylpropionate	23.05	11.94	1.930	3687	C28H36O3	420.60	91, 105, 73, 104, 107, ***492***, mono-	***492***, 477, 247, 209, mono-
t11	**testosterone-17ß-undecanoate**	24.57	12.02	2.044	3768	C29H48O3	444.70	***528***, 73, 343, 169, mono-	***528***, 513, 209, 343, mono-
x1	testosterone isobutyrate					C23H34O3	358.53		
x2	testosterone phosphate					C19H29O5P	368.41		
x3	testosterone furoate					C24H30O4	382.51		
x4	testosterone nicotinate					C25H31NO3	393.53		
x5	testosterone hexahydrobenzoate					C26H38O3	398.59		
x6	testosterone hexahydrobenzyl carbonate					C27H40O3	412.62		
x7	testosterone cyclohexylpropionate					C28H42O3	426.64		
x8	testosterone chloral hemiacetal acetate					C23H31Cl3O4	477.78		
x9	testosterone ketolaurate					C31H48O5	500.73		
X10	testosterone hexyloxyphenylpropionate					C34H46O4	518.74		

Notes: Internal standard (I. Std) = Testosterone; * **Bold and underline** = Purchased steroids

** **Bold and double underline** = Synthesised steroids by author

*** ***Bold, italic and underline*** = Deuterated steroids. Symbol "***D***" for deuterium is put on the deuterated steroids behind the compound name.

Table 3.6. GC-MS Analysis of Anabolic Steroids

No.	Compound Name	R.T (min)	I.Std. (min)	RRT	R.I (Found)	R.I (Liter.)	File Name (Xcalibur)	Mol. Form.	MW	Mass Spectrum (Found, *m/z*) TMS derivative	Mass Spectrum (Literature, *m/z*) TMS derivative
45	**norandrosterone**	10.22	12.47	0.820	2464	2430	22080345	C18H28O2	276.42	75, 73, 147, 169, 405, ..***420***, di-	405, ***420***, 315, 73, di-
46	**noretiocholanolone**	10.66	12.46	0.856	2516	#2510	22080346	C18H28O2	276.42	73, 75, 147, 169, 91, ..***420***, di-	41, 55, 67, 81, 79, free-
26	***estran-3ß-ol-17-one-D3, 5α-***	10.47	11.95	0.876	2541	-----	18120226	***C18D3H25O2***	279.40	***423***, 318, 407, 169, 228, di-	-----
7	androsterone	10.89	12.39	0.879	2550	2506	16010307	C19H30O2	290.45	73, 419, 75, ***434***, 329, 169, di-	419, ***434***, 73, 329, 169, di-
999	dioctyl phthalate	10.93	12.39	0.882	2554	-----	1040487	C24H34O4	390.56	149, 71, 167, 279, 113, free-	149, 167, 113, 279, free-
12	etiocholanolone	10.96	12.40	0.884	2557	2515	16010312	C19H30O2	290.45	73, 419, 167, ***434***, 181, di-	419, ***434***, 73, 329, 75, di-
10	androstane-3α,17ß-diol, 5α-	11.07	12.41	0.892	2567	2522	16010210	C19H32O2	292.45	241, 73, 129, 75, 256, ..***436***, di-	75, 241, 129, 257, 215, 331, di-
19	androstane-3α,17ß-diol, 5ß-	11.09	12.41	0.894	2568	2529	16010319	C19H32O2	292.45	256, 241, 129, 73, ..***436***, di-	256, 241, 73, 129, 75, di-
37	**bolandiol**	11.43	12.52	0.913	2597	2590	31050337	C18H28O2	276.42	73,75, 129, 91, ..***420***, di-	75, ***420***, 74, 73, 240, di-
48	androst-5-ene-3ß,17a-diol	11.46	12.50	0.917	2597	2590	17080348	C19H30O2	290.45	73, 129, 240, 215, ..***343***, di-	254, 305, 215, ***343***, 249, di-
15	dehydroepiandrosterone (DHEA)	11.30	11.98	0.943	2632	2589	14120215	C19H28O2	288.43	73, 129, 75, ***432***, 169, 417, di-	73, ***432***, 417, 327, di-
8	epiandrosterone	11.34	11.98	0.947	2637	2578	14120208	C19H30O2	290.45	73, 419, 75, ***434***, 169, di-	419, ***434***, 73, 329, 239, di-
32	androst-4-ene-3ß,17ß-diol	11.63	12.27	0.948	2639	2500	13020332	C19H30O2	290.45	143, 75, 73, ***434***, 129, 127, di-	***434***, 143, 142, 434, di-
34	**estr-4-ene-3,17-dione**	11.66	12.27	0.950	2644	-----	24020334	C18H24O2	272.39	73, ***416***, 75, 194, 234, di-	-----
33	androst-5-ene-3ß,17ß-diol	11.72	12.29	0.954	2649	2596	13020333	C19H30O2	290.45	73, 129, 75, 239, 215, ..***434***, di-	239, 305, 254, 344, 329, 215, di-
47	**androstane-3,17-dione, 5α-**	11.56	12.05	0.959	2654	2550	21100347	C19H28O2	288.41	73, 275, 75, 417, 290, ***432***, di-	73, 275, ***432***, 417, di-
9	androstane-3ß,17ß-diol, 5α-	11.45	11.92	0.961	2652	2597	06010309	C19H32O2	292.45	75, 73, 129, 81, 241, ***436***, di-	129, 73, 75, 241, 421, 256, di-
3	epitestosterone, 17α-	11.56	12.01	0.963	2657	2599	14120203	C19H28O2	288.43	73, 129, ***432***, 75, 91, 433, di-	73, ***432***, 433, 417, di-
14	nandrolone	11.53	11.93	0.966	2662	2612	06010314	C18H26O2	274.41	73, ***418***, 75, 117, 194, 419, di-	***274***, 110, 91, free-
24	**dihydrotestosterone (DHT)**	11.68	11.97	0.976	2675	2610	14120224	C19H30O2	290.45	142, 73, ***434***, 127, 129, 105, di-	73, ***434***, 143, 75, 142, di-
29	**androst-4-ene-3,17-dione**	12.13	12.29	0.987	2692	2633	13020329	C19H26O2	286.41	73, ***430***, 75, 169, 209, 431, di-	***430***, 431, 73, 75, 432, 75, di-
17	mesterolone	11.87	12.01	0.988	2690	W2530	14120217	C20H32O2	304.47	73, 141, 157, 156, 75, ..***448***, di-	141, 73, 157, 433, ***448***, di-
4	boldenone	11.94	12.05	0.991	2694	2694	21100304	C19H26O2	286.41	206, 191, ***430***, 229, 325, di-	73, 206, ***430***, 325, 415, di-

203	androst-4-ene-3,16-dione	12.00	12.07	0.994	2700	-----	201003203	C19H26O2	286.41	***430***, 73, 85, 71, 415, 169, di-	-----
1	testosterone	11.98	11.98	1.000	2707	2642	141202t2	C19H28O2	288.43	73, ***432***, 75, 143, 433, 129, di-	73, ***432***, 209, 417, 195, di-
40	**dromostanolone**	12.56	12.51	1.004	2716	W2625	31050340	C20H32O2	304.47	141, 157, 73, 156, ..***448***, di-	***448***, 73, 141, 405, 157, di-
5	trenbolone	12.25	11.95	1.025	2734	-----	19120205	C18H22O2	270.37	73, 75, ***414***, 74, 415, 309, di-	***414***, 73, 283, 309, 193, di-
18	**methenolone**	12.27	11.96	1.026	2737	2694	14120218	C20H30O2	302.46	195, 208, 73, 179, 196, ..***446***	73, ***446***, 208, 193, 129, di-
36	methandriol	12.56	12.24	1.026	2740	2617	11030336	C20H32O2	304.47	129, 268, 253, 143, ***448***, di-	253, 213, ***304***, 43, 145, free-
16	mestanolone	12.52	11.95	1.048	2761	2611	14120216	C20H32O2	304.47	143, 73, 75, 144, 216, ***448***, di-	73, 143, ***448***, 216, 358, di-
42	**mibolerone**	13.12	12.51	1.049	2771	-----	03060342	C20H30O2	302.46	73, 75, 301, 143, 341, ***446***, di-	431, ***446***, 301, 341, 356, di-
11	methandrostenolone	12.76	12.01	1.062	2780	W2670	14120211	C20H28O2	300.44	73, 206, 75, 143, 129, ***444***, di-	73, 206, ***444***, 143, 339, di-
49	androstan-3ß-ol-7,17-dione, 5α-	12.89	12.12	1.064	2767	-----	21090349	C21H30O4	346.47	73, 430, 169, 75, ***520***, tri-	-----
41	**keto-testosterone, 11-**	13.36	12.51	1.068	2793	-----	03060341	C19H26O3	302.42	73, 75, 74, 169, ***518***, tri-	-----
6	methyltestosterone, 17α-	12.91	12.05	1.071	2794	2733	14120206	C20H30O2	302.46	73,75, 301, 74, ***446***, di-	73, ***446***, 301, 75, 447, di-
43	**dromostanolone-17ß-acetate**	13.41	12.49	1.074	2799	-----	26060343	C22H34O3	346.51	141, 73, 75, 72, 85, ***418***, mono-	55, 94, 93, 79, 149, free-
38	**bolasterone**	13.53	12.48	1.084	2813	2692	03060338	C21H32O2	316.48	73, 75, 143, 315, 445, ***460***, di-	43, 298, 124, 175, 55, free-
22	trenbolone-17ß-acetate	12.93	11.92	1.085	2804	-----	18120222	C18H22O2	312.41	73, 75, 77, 91, ***384***, 309, mono-	43, 252, ***312***, free-
39	**calusterone**	13.63	12.51	1.090	2820	-----	31050339	C21H32O2	316.48	73, 75, 315, ..***460***, di-	43, ***316***, 124, 71, 55, 135, free-
30	methenolone-17ß-acetate	13.17	11.91	1.106	2831	-----	18120230	C22H32O3	344.49	194, 179, ***416***, 208, 93, mono-	123, 136, 135, 91, 79, free-
35	**nandrolone-17ß-propionate**	13.69	12.30	1.113	2849	-----	24020335	C21H30O3	330.47	73, ***402***, 194, 182, 75, mono-	-----
202	androst-5-ene-3ß,16α-diol-17-one	13.78	12.11	1.138	2865	-----	220903202	C19H28O3	304.44	505, 73, ***520***, 506, 147, tri-	***304***, 213, 286, 271, 231, free-
27	norethandrolone	13.78	11.96	1.152	2889	2695	14120227	C20H30O2	302.46	287, 73, 75, ***446***, 288, di-	***302***, 231, 85, 91, 57, free-
23	**oxandrolone**	14.20	12.31	1.154	2897	2778	19020323	C19H30O3	306.45	143, 73, 130, 107, ***378***, mono-	291, 248, 43, 79, free-
44	**dromostanolone-17ß-propionate**	14.44	12.44	1.161	2905	-----	15070344	C23H36O3	360.54	141, ***432***, 73, 433, 157, mono-	286, 149, 271, 57, 94, free-
50	**chlorotestosterone, 4-** (Clostebol)	15.01	12.47	1.204	2955	2693	22080350	C19H27ClO2	322.87	73, 75, 147, 77, 69, ***466***, di-	73, 125, 358, 268, ***466***, di-
25	**fluoxymesterone**	15.03	12.28	1.224	2976	*2840	13020325	C20H29FO3	336.45	73, 75, 143, 74, 319,.***552***, tri-	73, ***552***, 462, 407, 319, tri-
31	**oxymesterone**	15.10	12.21	1.237	2989	2950	21030331	C20H30O3	318.46	73, 147, 75, ***534***, 143, tri-	***534***, tri-
13	danazol	15.07	11.96	1.260	3012	#3111	18120213	C22H27NO2	337.46	73, 75, 83, 91, 394, ***409***, mono-	***337***, 146, 91, 132, free-
21	oxymetholone	15.26	11.95	1.277	3027	2950	18120221	C21H32O3	332.49	72, 268, 281, 295, ***548***, tri-	***548***, 73, 490, 281, 405, tri-
201	**chlorotestosterone-17ß-acetate, 4-**	16.01	12.49	1.282	3045	-----	190803201	C21H29ClO3	364.96	***436***, 438, 401, 296, mono-	43, 147, 158, 91, free-
28	***stanozolol-D3***	16.81	12.06	1.394	3162	-----	11100328	***C21D3H29N2O***	331.50	146, 73, 147, 75, 168, ..***475***, di-	-----

2	stanozolol	16.80	12.04	1.395	3163	W3025	17100302	C21H32N2O	328.50	143, 73, 75, 144, 168, di-	143, ***472***, 168, 342, di-
20	**nandrolone-17ß-decanoate**	22.53	12.35	1.824	3613	-----	11010320	C28H44O3	428.66	***500***, 73, 501, 194, 155, mono-	274, 155, 110, 256, 147, 91, free-

Notes: Internal standard (I. Std) = Testosterone

* **Bold and underline** = Purchased steroids

** **Bold and double underline** = Synthesised steroids by author

*** ***Bold, italic and underline*** = Deuterated steroids. Symbol "***D***" for deuterium is put on the deuterated steroids behind the compound name.

Table 3.7. GC-MS Analysis of Other Steroids (Androgens, Progestagens, Estrogens, and its Metabolites etc.)

No.	Compound Name	R.T (min)	I.Std (min)	RRT	R.I (Found)	R.I (Literat.)	File Name (Xcalibur)	Mol. Form.	MW	Mass Spectrum (Found, *m/z*)	Mass Spectrum (Literature, *m/z*)
78	androstan-3α-ol, 5α-	8.32	12.16	0.684	2272	W2240	16040378	C19H32O	276.47	108, 148, 258, 75, ..***347***, mono-	258, 256, 75, 243, 95, mono-
77	androstan-17-one, 5α-	8.77	12.15	0.722	2327	-----	16040377	C19H30O	274.45	331, 73, ***346***, 169, 67, mono-	***274***, 109, 67, 230, free-
76	androstan-17ß-ol, 5α-	8.90	12.11	0.735	2344	W2320	16040376	C19H32O	276.47	148, 129, 244, 258, ..***348***, mono-	243, 75, 129, 67, 258, mono-
79	androstan-3-one, 5α-	9.23	12.13	0.761	2378	-----	18040379	C19H30O	274.45	142, 127,75, 73, 189, ***346***, mono-	202, ***274***, 41, 203, 55, free-
105	pregn-5-en-3ß-ol	10.60	12.09	0.877	2543	-----	210903105	C21H34O	302.50	129, 245, 284, 73, ..***374***, mono-	***302***, 284, 303, 287, 269, free-
999	dioctyl phthalate	10.93	12.39	0.882	2554	-----	1040487	C24H34O4	390.56	149, 71, 167, 279, 113, free-	149, 167, 113, 279, free-
102	pregnan-20-one, 5α-	10.77	12.05	0.894	2567	-----	221003102	C21H34O	302.50	360, 157, 73, 195, ***374***, mono-	43, 55, 41, 44, 67, 71, free-
91	estradiol-3-methyl ether, 17ß-	11.79	12.54	0.940	2635	-----	17080391	C19H26O2	286.42	227, ***358***, 129, 173, 174, mono-	***286***, 186, 160, 173, 227, free-
51	estrone	11.76	11.96	0.983	2684	-----	08110351	C18H22O2	270.37	73, 75, 74, 155, ***414***, 399, di-	***270***, 146, 185, 172, 213, free-
56	OH-etiocholanolone, 11ß-	12.26	12.36	0.992	2700	2683	11010356	C19H30O3	306.45	168, 73, 327, 256, ***522***, 283, tri-	73, 168, 75, 283, 256, tri-
52	estradiol, 17ß-	11.96	11.97	0.999	2705	W2680	08110352	C18H24O2	272.39	73, 75, 129, 285, ***416***, di-	285, ***416***, 218, 417, 75, di-
80	**DHEA-3ß-acetate**	12.60	12.55	1.004	2719	-----	14050380	C21H30O3	330.47	327, 73, 75, 91, ..***402***, mono-	270, 57, 173, 77, free-
75	methyl-5α-androstane-3ß,17ß-diol, 17α-	12.57	12.18	1.032	2748	-----	08040375	C20H34O2	306.49	143, 73, 75, 130, ..***450***, di-	-----
68	mestranol	12.68	12.19	1.040	2758	-----	18030367	C21H26O2	310.44	73, 75, 147, 174, ..***368***, mono-	227, ***310***, 174, 147, 159, free-
92	pregnan-3α-ol-20-one, 5ß- (Pregnanolone)	13.14	12.50	1.051	2772	-----	19080392	C21H34O2	318.50	447, 157, 75, 143, ..***462***, di-	43, 300, 41, 84, 81, free-
59	**boldenone-17ß-acetate**	12.93	12.29	1.052	2774	-----	24020359	C21H28O3	328.46	206, 73, 191, 93, ..***402***, mono-	-----
67	norethynodrel	12.92	12.18	1.061	2782	-----	18030367	C20H26O2	298.43	73, 75, 194, ***442***, 83, di-	91, 215, 79, 77, 105, free-
60	pregnane-3a,20a-diol, 5ß-	13.24	12.27	1.079	2805	2761	07030360	C21H36O2	320.52	117, 75, 73, 93, 105, ..***464***, di-	234, 216, 302, 233, free-
57	**androst-5-ene-3ß,17ß-diol, diacetate**	13.34	12.27	1.087	2816	2790	27020357	C23H34O4	374.53	121, 146, 91, 314, ..***374***, free-	43, 314, 121, 133, 107, free-
73	**methandriol-3ß,17ß-diacetate**	13.39	12.22	1.096	2821	-----	23030373	C24H36O4	388.55	143, 268, 73, 253, ..***388***, none	-----
69	ethinyl estradiol	13.37	12.20	1.096	2826	2780	18030369	C20H24O2	296.41	73, 75, 83, 196, 205, ..***440***, di-	285, 231, 425, 73, 300, di-
95	pregnanetriol (5ß-Pregnane-3α,17α,20α-triol)	13.78	12.55	1.098	2835	-----	17080395	C21H36O3	336.50	73, 255, 436, 211, ..***552***, tri-	255, 291, 273, 215, free-
103	pregn-5-ene-3ß,20ß-diol	13.52	12.10	1.117	2847	-----	210903103	C21H34O2	318.50	117, 73, 129, 372, ..***462***, di-	***318***, 45, 91, 189, 105, free-

87	**fluoxymesterone-M1-tetrol (metabolite)**	13.89	12.39	1.121	2858	2856	01040487	C20H29FO4	354.46	73, 143, 75, 182, ..***642***, tetra-	***642***, 552, tetra-
94	pregnenediol (Pregn-5-ene-3ß,20α-diol)	13.74	12.07	1.138	2865	-----	21100394	C21H34O2	318.50	117, 73, 129, 75, 118, ..***462***, di-	117, 372, 118, ***462***, di-
90	androst-5-ene-3ß,17ß-diol-16-one	14.19	12.45	1.140	2872	-----	11080390	C19H28O3	304.43	505, 73, 147, ***520***, 506, tri-	433, 73, 75, 434, ***448***, di-
53	pregnenolone (Pregn-5-en-3ß-ol-20-one)	13.65	11.95	1.142	2877	-----	14120253	C21H32O2	316.48	73, 157, 445, 117, ..***460***, di-	129, 308, 73, 259, ***388***, mono-
83	**norgestrel, levo-**	14.28	12.48	1.144	2888	-----	27080383	C21H28O2	312.45	73, 194 316, 147, ***456***, di-	91, 79, 245, 77, 110, free-
107	pregnane-3α,17α,20α-triol-11-one,5ß-(PT=on)	13.80	12.03	1.147	2883	-----	221003107	C21H34O4	350.50	117, 73, 147, 118, ..***638***, tetra-	-----
93	pregnane-3,20-dione, 5α-	14.47	12.50	1.158	2902	-----	19080393	C21H32O2	316.47	445, 157, 73, 446, ..***460***, di-	43, ***316***, 55, 246, 283, free-
89	**OH-methandienone, 6ß-**	14.67	12.47	1.176	2924	#2915	22080389	C20H28O3	316.44	73, 75, 147, 517, ..***532***, tri-	517, 518, 73, 229, 294, tri-
70	estriol	14.45	12.21	1.183	2925	2930	18030370	C18H24O3	288.39	73, 147, 129, 74, 311, ..***504***, tri-	297, 296, 73, 231, 75, tri-
74	**methandriol-3ß,17ß-dipropionate**	14.48	12.22	1.185	2933	-----	21030374	C26H40O4	416.59	268, 253, 143, 226, ..***416***, none	57, 342, 253, 268, 105, free-
54	progesterone	14.26	11.94	1.194	2938	-----	14120254	C21H30O2	314.50	157, ***458***, 443, 117, 158, di-	124, ***314***, 272, 229, mono-
98	**norgestrel-17ß-acetate, levo-**	15.17	12.53	1.211	2966	-----	27080398	C23H30O3	354.49	147, 73, 75, 77, ..***426***, mono-	91, 53, 55, 79, 67, free-
104	pregnane-3ß,17α-diol-20-one, 5α-	14.76	12.09	1.221	2966	-----	210903104	C21H34O3	334.51	230, 147, 73, 243, ***550***, tri-	43, 255, 55, 229, free-
96	pregnenetriol (Pregn-5-ene-3ß,17α,20α-triol)	14.93	12.06	1.238	2987	-----	21100396	C21H34O3	334.50	434, 73, 147, 253, ..***550***, tri-	289, 271, 253, ***334***, 213, free-
97	pregnane-3α,17,21-triol-20-one,5ß- (THS)	15.03	12.04	1.248	2995	-----	21100397	C21H34O4	350.47	332, 75, 169, 73, ..***638***, tetra-	-----
109	pregn-5-ene-3ß,16α-diol-20-one	15.61	12.37	1.262	3017	-----	290903109	C21H32O3	332.49	231, 73, 117, 147, ..***548***, tri-	-----
58	**androst-5-ene-3ß,17ß-diol, dipropionate**	15.81	12.39	1.276	3040	-----	27020358	C25H38O4	402.58	130, 215, 213, 328, ..***402***, free-	57, 328, 121, 133, 146, free-
82	**medroxyprogesterone**	16.31	12.51	1.304	3071	-----	31050382	C22H32O3	344.49	73, 75, 147, 74, 143, ..***560***, tri-	***560***, 330, tri-
88	**OH-fluoxymesterone, 6ß-**	16.47	12.54	1.313	3085	-----	27080388	C20H29FO4	352.45	***640***, 641, 143, 642, 74, tetra-	-----
61	tetrahydrocortisone	16.34	12.29	1.330	3095	-----	07030361	C21H32O5	364.48	403, 619, 313, 404, ..***724***, penta-	-----
62	tetrahydrocortisol (THF)	16.47	12.29	1.340	3108	-----	07030362	C21H34O5	366.50	331, 169, 243, 73, ..***726***, penta-	-----
106	pregnane-3α,21-diol-11,20-dione, 5ß-	16.20	12.07	1.342	3105	-----	201003106	C21H32O4	348.50	230, 231, 73, 531, ..***636***, tetra-	-----
55	proligestone	16.20	12.05	1.344	3114	-----	14120255	C24H34O4	386.54	73, 75, 246, 143, 72, 69, .***530***, di-	-----
101	OH-pregnenolone, 21-	16.52	12.09	1.366	3129	-----	210903101	C21H32O3	332.49	230, 73, 147, ***548***, 231, tri-	301, 255, ***332***, 91, 41, free-
998	cholesterol	17.32	12.52	1.383	3160	-----	31050382	C27H46O	386.67	73, 75, 129, ..***458***, mono-	329, 129, 368, 73, .***458***, mono-
100	deoxy-corticosterone, 11-	17.25	12.08	1.428	3192	-----	210903100	C21H30O3	330.47	73, ***546***, 147, 230, 301, tri-	299, 271, 253, 147, free-
85	**OH-stanozolol, 3'-**	18.51	12.54	1.476	3271	3218	27080385	C21H32N2O2	344.50	147, 73, 77, 75, 207, ..546, tri-	143, 545, ***560***, 254, tri-
81	**canrenone**	18.51	12.52	1.478	3267	-----	03060381	C22H28O3	340.46	73, 75, 111, ..***412***, mono-	267, 55, 107, 91, 131, free-

64	**dihydrotestosterone-17ß-enanthate**	18.50	12.18	1.519	3303	-----	16030364	C26H42O3	402.62	142, 73, 127, 203, ..***474***, mono-	-----
99	corticosterone	18.59	12.09	1.538	3305	-----	21090399	C21H30O4	346.47	73, 147, 230, ***634***, 75, tetra-	315, 269, 55, 41, 316, free-
84	**medroxyprogesterone-17a-acetate**	18.61	12.07	1.542	3315	-----	16090384	C24H34O4	386.54	73, 75, 133, ***530***, 131, di-	283, 301, 91, 207, 344, free-
108	pregn-4-ene-11ß,17a,21-triol-3,20-dione	19.04	12.12	1.571	3320	-----	220903108	C21H30O5	362.47	147, 632, 633, 243, ..***722***, penta-	123, 163, 41, 91, 79, free-
63	**methenolone-17ß-enanthate**	19.19	12.16	1.578	3368	-----	16030363	C27H42O3	414.61	195, 73, 208, 179, ..***486***, mono-	136, 43, 123, 135, free-
110	betamethasone	20.01	12.09	1.655	3434	-----	220903110	C22H29FO5	392.47	147, 206, 191, 207, ..***752***, penta-	122, 121, 41, 91, free-
86	**OH-stanozolol, 16ß-**	20.86	12.53	1.665	3471	3334	27080386	C21H32N2O2	344.50	218, 231, 117, 207, ..***560***, tri-	-----
66	**nandrolone-17ß-benzoate**	20.47	12.21	1.676	3472	-----	13030366	C25H30O3	378.52	73, ***450***, 77, 105, 194, mono-	-----
71	**dihydrotestosterone-17ß-benzoate**	20.43	12.07	1.693	3480	-----	21100371	C26H34O3	394.56	105, 143, 127, 142, ..***466***, mono-	-----
65	**boldenone-17ß-benzoate**	20.81	12.21	1.704	3500	-----	13030365	C26H30O3	390.53	105, 206, 73, 77, ..***462***, mono-	-----
72	**estradiol-3-benzoate**	21.04	12.21	1.723	3511	-----	20030372	C25H28O3	376.50	105, 73, 77, ***448***, 241, mono-	105, 77, ***376***, free-

Notes: Internal standard (I. Std) = Testosterone

* **Bold and underline** = Purchased steroids

** **Bold and double underline** = Synthesised steroids by author

3.2.4. Sample preparation

3.2.4.1. Nail samples

a) Decontamination of nail clippings

The nail clippings were washed with 5 mL of 0.1% sodium dodecyl sulfate (**SDS**) for 15 min using ultra-sonication. The samples were similarly washed three times with 5 mL of de-ionised water and 5 mL of methanol, respectively. These methanolic washes were preserved for later reference.

The washed nail clippings were dried at room temperature. Each sample was weighed accurately.

b) Extraction with cryogenic grinding method

The washed nail clippings (1.84–75.28 mg) were pulverised with a cryogenic grinder (**SPEX CertiPrep 6750 Freezer Mill**) under freezing conditions with liquid nitrogen (**LN**).

The powdered samples were extracted with 7 mL of methanol/ethyl acetate (7:3, v/v) solution. Each of the internal standard solutions (10 μL of 1.00 ng/μL solutions of deuterated steroids NEA-d_3 and TEST-d_3 and MEDR, respectively) was added to the solvent extracts and sonicated for 15 min.

The extracts were vortex mixed for 30 sec and then centrifuged for 10 min at 2,000 rpm. The supernatant was collected and evaporated to dryness at 60 °C under a stream of nitrogen.

80 μL of 0.1% ammonium iodide/methanol (w/v) solution was added to the residue as catalyst prior to trimethylsilyl derivatisation. The methanolic solvent was dried again under a stream of nitrogen.

c) Trimethylsilyl derivatisation for gas chromatography-mass spectrometric analyses

The residues were derivatised with 40 μL of MSTFA with 2-mercaptoethanol

(1,000:6, v/v) for 15 min at 60 °C. Derivatised samples were transferred to autosampler vials of the GC-MS instrument.

d) Gas chromatography-mass spectrometric (**GC-MS**) analysis

Qualitative and quantitative analysis were carried out with a **Finnigan Trace GC-MS** instrument equipped with an **AS 2000 autosampler.** Data was acquired using the **Xcalibur** software from **Finnigan** in full scan and selected ion monitoring (**SIM**) modes.

A 3–4 µL aliquot of derivatised analytes were analysed on an **HP-5** column (30 m x 0.32 mm i.d.; film thickness 0.25 µm) with helium (0.9 mL/min) as carrier gas, temperature programming from 180 °C to 240 °C at 3 °C/min, 240 °C to 300 °C at 5 °C/min (held for 10–15 min), and 70 eV electron energy in the SIM.

Interface and ion source temperature, emission current, and detector voltage, were 250 °C, 200 °C, 350 µA, and 750 V, respectively. Mass spectra and SIM data were acquired using the EI+ mode at 70 eV electron energy.

An alternative temperature programme was used to analyse all steroids such as anabolic, estrogens, progestagens steroids, and so on. The temperature programme was 150 °C to 250 °C at 10 °C/min, 250 °C to 300 °C at 5 °C/min (held for 7 min).

3.2.4.2 Blood (plasma) samples

a) Blood samples

The samples were centrifuged for 10 min at 2,000 rpm and the supernatant (plasma) was collected. The plasma was stored in refrigerator at 4 °C until analysed.

b) SPE of plasma

Solid phase extraction (**SPE**) was carried out using the **IST Vac/Master** vacuum work station from **International Sorbent Technology,** U.K.

One (1) mL of each plasma sample was loaded after the SPE cartridges (**Bond Elut**

C_{18}) had been conditioned with 2 mL of methanol, and 2 mL of de-ionised water, respectively.

The cartridges were washed with 1.0 mL of de-ionised water. Subsequently, the cartridges were dried under vacuum for 10 min using a **Millipore** (MA, USA) diaphragm vacuum pump.

Analytes were eluted with 2 mL of methanol. The eluates were collected and the extracts were evaporated to dryness under a stream of nitrogen.

80 μL of 0.1% ammonium iodide/methanol solution was added to the extracts as catalyst prior to trimethylsilyl derivatisation. The methanolic ammonium solutions were evaporated to dryness with nitrogen gas at 60 °C. The residues were dried under vacuum for 30 min.

The analytical samples contain moisture when the samples are extracted by the SPE method and when the SPE method was used, vacuum drying was applied to dry the samples.

c) Trimethylsilyl derivatisation for GC-MS analyses

The residues were derivatised with 40 μL of MSTFA with 2-mercaptoethanol (1,000:6, v/v) for 15 min at 60 °C. Derivatised samples were then transferred to autosampler vials for GC-MS analysis.

3.2.4.3. Urine samples

Steroids were extracted from urine samples by SPE. The extracts were then subjected to enzymatic hydrolysis and re-extracted. Details are as follows.

a) SPE extraction for conjugated and free steroids

The urine samples were not centrifuged because there were no precipitates.

Fifteen (15) μL of each internal standard solution (each containing 1 ng/μL of NEA-d_3, TEST-d_3, MEDR, apart from STN-d_3, which contained 2 ng/μL) were added to 5

mL of urine sample, respectively.

The samples were vortex-mixed for 30 sec and loaded on **Varian Bond Elut** LRC C_{18} SPE cartridges (200 mg) which had been conditioned with 3 mL of methanol and 3 mL of de-ionised water, respectively beforehand.

The cartridges were then washed with 3 mL of de-ionised water and subsequently dried for 10 min under vacuum using a diaphragm vacuum pump.

The analytes were eluted with 4 mL of methanol. The eluates were evaporated to dryness under a stream of nitrogen.

b) Enzymatic hydrolysis of conjugated steroids

The extraction residues were dissolved in 600 µL of 0.2 M potassium phosphate buffer at pH 6.8, 400 µL of ß-glucuronidase (***Escherichia coli***, 400 Units) were added and the solutions were incubated at 60 °C for 3 h.

c) SPE extraction of hydrolysates

The deconjugated and free steroids were extracted by SPE as follows.

The cartridges (**Varian Bond Elut** LRC C_{18} SPE cartridge, 200 mg) were activated with 2 mL of methanol and 2 mL of de-ionised water, respectively. The hydrolysed samples were loaded to the cartridges after the samples were vortex-mixed for 30 sec. Then, the cartridges were washed with 2 mL of de-ionised water.

Subsequently, the cartridges were dried for 10 min under vacuum using a diaphragm vacuum pump. The analytes were eluted with 3 mL of methanol.

The eluates were evaporated to dryness under a stream of nitrogen. One hundred and twenty (120) µL of 0.1% methanolic ammonium iodide solution was added to the residue as catalyst prior to trimethylsilyl derivatisation. The solution was dried under a stream of nitrogen.

The residue was then completely dried again under vacuum using a diaphragm

vacuum pump.

d) Trimethylsilyl derivatisation for GC-MS analyses

The extraction residues were derivatised with 60 µL of MSTFA with 2-mercapto-ethanol (1,000:6, v/v) as catalyst for 15 min at 60 °C.

Derivatised samples were transferred to autosampler vials for GC-MS analysis.

3.2.5. Qualitative analysis of endogenous steroids

To determine the retention time, relative retention time (**RRT**), retention index, and mass spectrum (**MS**) of each steroid under the GC-MS conditions described earlier, TEST and hydrocarbons were used as internal standard and retention index standards respectively.

The sample concentration of each steroid was in the range 1 to 5 ng/µL of reagent containing hydrocarbon for measurement of **Kovat indices (Retention Indices)**.

3.2.6. Qualitative analysis of exogenous steroids

To identify and determine exogenous (**synthetic**) steroids, NEA-d_3, TEST-d_3, MEDR, and STN-d_3 were used for GC-MS analysis as internal standards.

The temperature programmes used were:

- 150 °C to 250 °C at 10 °C/min, 250 °C to 300 °C at 5 °C/min (held for 7 min), and
- 180 °C to 240 °C at 3 °C/min, 240 °C to 300 °C at 5 °C/min (held for 10–15 min).

3.2.7. Quantitative analysis of endogenous steroids

3.2.7.1. Preparation of standard samples for calibration curves

All standard steroids were prepared to give stock solutions with a concentration of 1.0 mg/mL in methanol/ethyl acetate (70:30, v/v). Working solutions containing 0.04

ng/μL were then prepared by diluting the stock solutions.

The standard sample solutions prepared were separately collected in small vials containing each of the following volume: 0, 5.0, 12.50, 25.0, 125.0, 250.0 and 500.0 μL using working solution (0.04 ng/μL), respectively. Internal standard solutions (10 μL of 1 ng/μL of NEA-d_3 and TEST-d_3) were added to each of the vials at the same time.

These samples were evaporated, derivatised, and prepared for the calibration curves by the same method as described earlier for the sample preparation of nail.

3.2.7.2. Calibration curves for endogenous steroids in nail, plasma and urine samples

The SIM mode was used for the quantitative analysis of each steroid.

Four (4) μL of each standard solution containing 0.00, 0.005, 0.0125, 0.0250, 0.125, 0.250 and 0.500 ng/μL were injected into the GC-MS respectively. The peak area ratios of the standards in each sample to an internal standard were used for the preparation of the calibration curves for quantitative analysis of endogenous steroids.

3.2.7.3. Fragment ions used for SIM

The quantified compounds and selected ions were as follows. NEA-d_3 (*m/z* 423) was used for AND (*m/z* 434) and ETIO (*m/z* 434) as internal standard. Similarly, TEST-d_3 (*m/z* 435) was used for the DHEA (*m/z* 432), epiAND (*m/z* 434), epiTEST (*m/z* 432), and TEST (*m/z* 432) as internal standard

3.3. Results and Discussion

3.3.1. Methanolic ethyl acetate extraction with the cryogenic grinding method

A direct extraction method was developed for steroids in nail clippings which was almost the same as the method used previously for cannabinoids and opioids in nail [30].

However, the extraction solvent was different and the conditions with respect to the time used to cool the grinder with liquid nitrogen and interval times used to pulverise the frozen nail with the cryogenic grinder were examined and optimised.

As a result, effective conditions were found to be a pre-cooling time of 2 min and a grinding time for 2 min, which was carried out twice in the cycle with a 1-min re-cooling period between each grinding period.

The type of solvent used to extract the steroids from the pulverised nail was considered. The solvents tried were acetone, acetonitrile, chloroform, ethyl acetate, hexane, and methanol. As a result, a mixture of methanol/ethyl acetate (70:30, v/v) was selected in consideration of the low background, the easiness of the solvent to handle, and solubilities of the analytes.

Impurities (contaminants) detected in the methanol/ethyl acetate blank solution were mainly phthalic acid esters (dibutyl (**DBP**) and dioctyl (**DOP**)). These components originate chiefly in plastic additives derived from laboratory-wares.

3.3.2. Derivatisation of analytes

One of the most important factors in GC-MS analysis is what derivatisation to use to prepare the steroid extracts.

Anabolic steroids can be analysed by GC-MS without derivatisation though the sample steroids have either hydroxyl or amino functional groups or both [**Figure 3.1, Table 3.4**].

In GC-MS analysis of steroid samples, derivatisation is used to improve the analytical sensitivity and retention times, to increase the thermal and chemical stability of the analytes and their volatility, and to make identification of molecular ions easier (e.g. tertiary butyldimethylsilyl ether derivatives give prominent M-57 ions). In general, silylation, acylation and other methods have mostly been used for the derivatisation of steroids to improve the performance of the GC-MS analysis.

The advantages of silyl derivatives are that they are easy to prepare and the derivatives are volatile compounds. On the other hand, the disadvantage of silyl derivatives is that they react with moisture (water) and other hydroxylic solvents such as methanol.

While many different silyl ethers and esters have been used, trimethylsilyl (**TMS**) derivatives have been most widely accepted as the preferred silyl derivatives for steroids for GC-MS analysis. The literature concerning TMS derivatisation of anabolic steroids up to 1996 was reviewed in detail by **Opfermann** [134]. New derivatisation methods have been successively reported as the chemistry develops.

Ayotte [135], **Wolthers** [136] and **Segura** [137] evaluated recent analytical methods for doping steroids in detail in their reviews. The merits and weak points of the steroid derivatives in GC-MS analysis are described in more detail below.

3.3.2.1. Silyl derivatives

There are several kinds of silylating method. In general, TMS derivatives of testosterone are roughly classified into three types. The first is testosterone-17-mono-TMS derivative in which only the hydroxy group at C17-position is derivatised as showing in **Figure 3.5**.

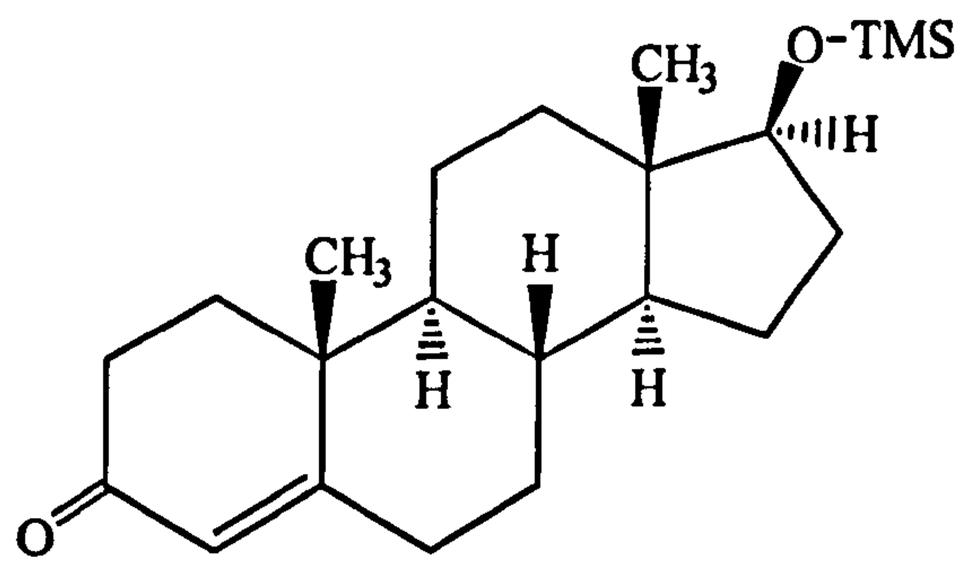


Figure 3.5. Chemical structure of Testosterone-17-mono-TMS

The second type is testosterone-3-methyloxime-17-TMS derivative in which the ketone group at C3-position reacts with methoxyamine and TMS reagents consecutively as shown in **Figure 3.6** [92, 104, 138, 139, 140, 141].

One disadvantage of methyloxime derivatives of 3-keto-Δ-4-steroids is that two geometric isomers (***syn*** and ***anti***) are obtained which differ according to the orientation of the methoxy group.

The two isomers do not interchange readily and as a result two peaks are usually obtained during GC analysis. Replacement of the methyl group of the oxime with a larger substituent, such as butyl or benzyl, results in a marked stereo-chemical preference for one of the two isomers and, subsequently, in a single GC peak. However, these heavier substituents also result in a correspondingly longer retention time, particularly if two ketone groups are initially present leading to a double oxime derivative.

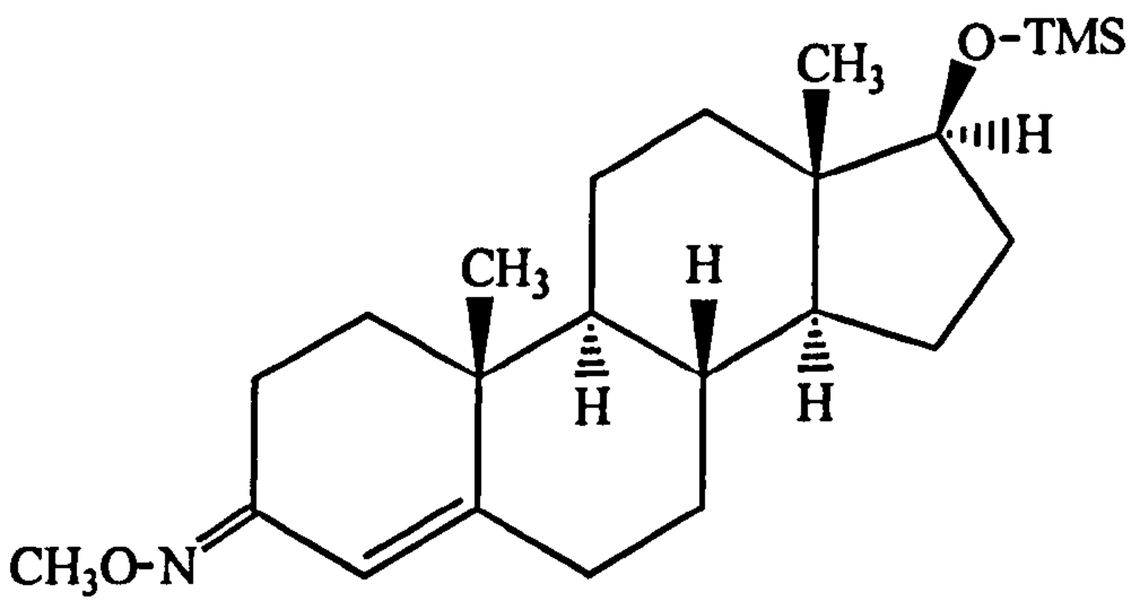


Figure 3.6. Chemical structure of Testosterone-3-N-methyloxime-17-mono-TMS

The third testosterone TMS derivative is testosterone-3,17-di-TMS derivative in which both an enolic hydroxyl group at the C3-position and the hydroxy group at the C17-position are derivatised as shown in **Figure 3.7.**

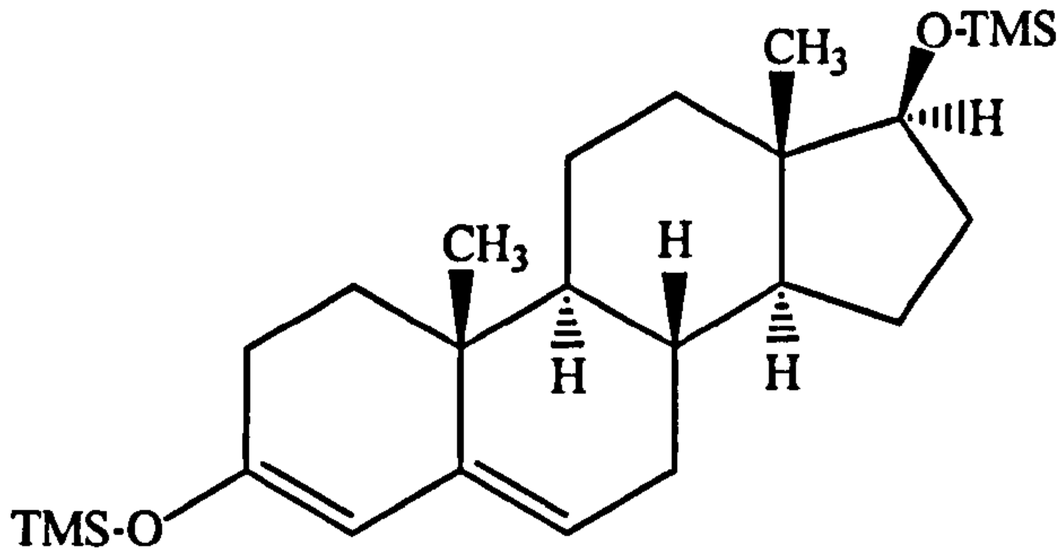


Figure 3.7. Chemical structure of Testosterone-3,17-di-TMS

In addition, there is another TMS derivative in which the ketone group at the C3-position is reacted with hydroxylamine and then with TMS reagent in succession, such that both the oxime and at C17-hydroxyl groups are derivatised at the same time, as shown in **Figure 3.8** but this type of derivative is very rarely used [142].

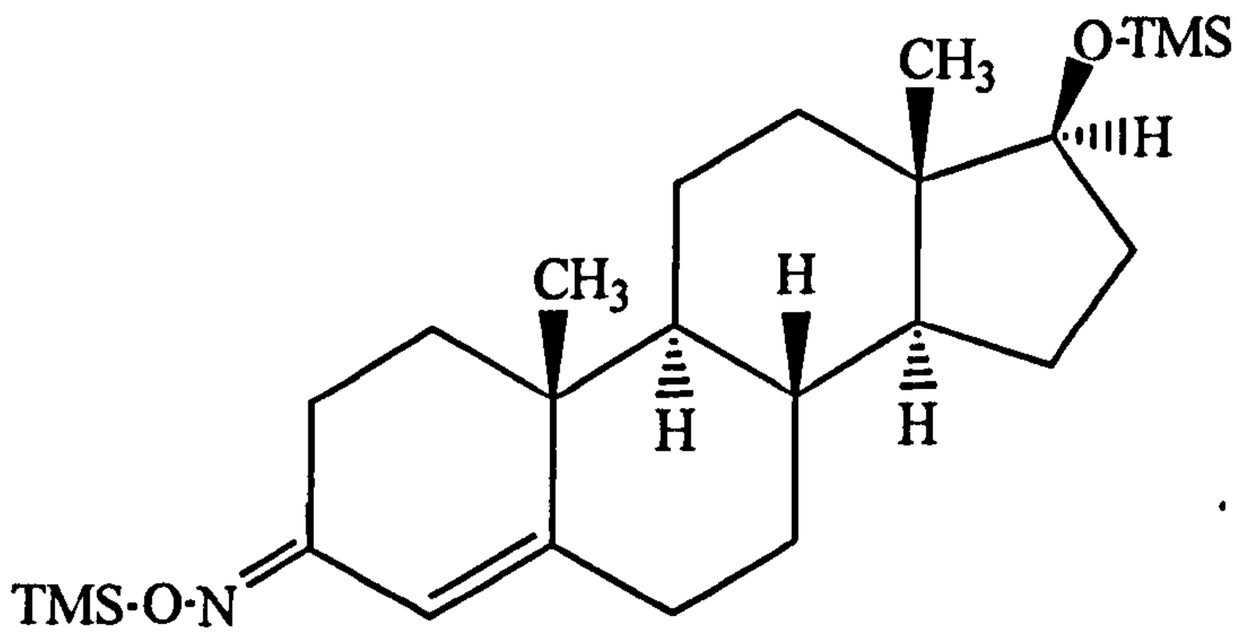


Figure 3.8. Chemical structure of Testosterone-3-oxime,17-di-TMS

To prepare testosterone-3,17-di-TMS derivative, the enolic hydroxyl group does not readily form and react in the presence of the TMS reagent alone and it is necessary to add a catalyst. Iodine compounds such as ammonium iodide and trimethylsilyl iodide are often used as the catalyst for derivatising testosterone and its analogues. However, a reducing agent is also needed together with TMS reagent.

N-Methyl-N-(trimethylsilyl)trifluoroacetamide (**MSTFA,** see **Figure 3.9**) has been

widely used as the TMS reagent for silylation of steroids together with a catalyst and reducing agent. A number of different reagents were evaluated for the present study and the combinations of TMS derivatising reagents are described in detail below.

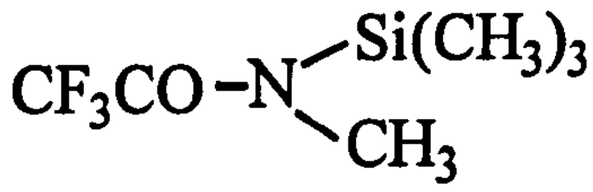


Figure 3.9. Chemical structure of MSTFA

1) TMS reagent #1: MSTFA/NH_4I/1,2-Ethanedithiol (1,000:2:6, v/w/v), 60 °C, 15 min

Silylation using this combination of TMS reagents has been widely used [143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153,154]. However, 1,2-ethanedithiol ($C_2H_6S_2$ = 94.20) is difficult to use in practice because of its strong odour.

As for the relative proportions of the MSTFA, NH_4I, and 1,2-ethanedithiol reagents, the ratios of 1,000:2:6 (v/w/v) are generally used. Moreover, the temperature and the reaction time are commonly set at 60 °C for 15 min for sample derivatisation.

2) TMS reagent #2: MSTFA/NH_4I/2-Mercaptoethanol (1,000:2:6, v/w/v), 60 °C, 15 min

It is easy in practice to handle 2-mercaptoethanol (C_2H_6OS = 78.13) because, although it also has a strong smell, it is considerably less than that of 1,2-ethanedithiol. The conditions for sample derivatisation are also almost the same as the above-mentioned method [111, 116, 155].

In this thesis, the above method was used for trimethylsilylation of steroids. The advantage of this method is that MSTFA, as well as the by-products **N-methyl-trifluoro-acetamide** ($C_3H_4F_3NO$ = 127.07) and **2-mercaptoethanol-di-TMS** ($C_8H_{22}OSSi_2$ = 222.50), are very volatile liquids and do not interfere in the GC-MS analysis. The silylation reaction mixture can be injected directly into the GC and there is no need to use solvent for reconstituting the derivatised analytes for GC-MS analysis.

However, when this method was applied to steroids having an enolic hydroxy group, the TMS derivative was sometimes not obtained. One reason is that because ammonium iodide is a solid, it is not easy to dissolve it in MSTFA. An additional reason is that it was difficult to prepare a reagent with an exact ratio of TMS reagent to ammonium iodide of 1,000:2. Therefore, a 0.1% methanolic ammonium iodide solution was prepared and this solution plus twice the amount of MSTFA reagent was added to the steroid samples. As a result, it was ascertained that the steroids were completely derivatised.

The greatest advantage of MSTFA reagent is that it can easily derivatise functional groups such as the enolic ketone group at the C3-position as well as the sterically hindered hydroxy group at 17α-position of steroids.

When hydrocortisone (pregn-4-ene-11ß,17α,21-triol-3,20-dione, $C_{21}H_{30}O_5$ = 362.47) was tried as an example, all functional groups of the hydrocortisone were completely converted to TMS derivatives (MW = 722) **[Figure 3.10]**.

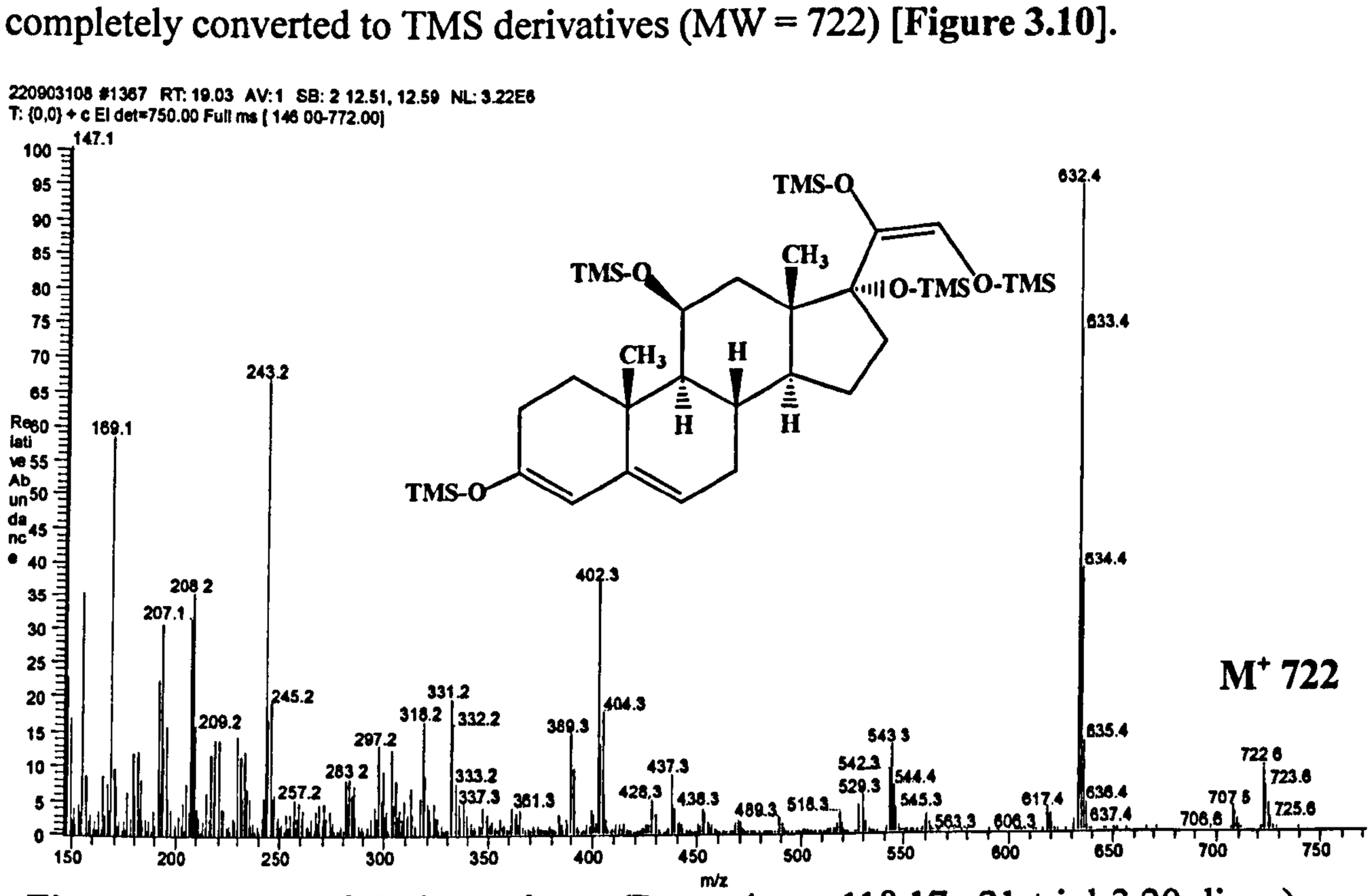


Figure 3.10. MS of Hydrocortisone (Pregn-4-ene-11ß,17α,21-triol-3,20-dione) -penta-TMS

The enolic ketone (C3), sterically hindered hydroxy (C17), and ketol (C20) groups were completely derivatised.

Therefore, when the TMS derivative is prepared with MSTFA reagent, the selection of the reducing agent and the catalyst is just as important as the choice of the silylating reagent itself.

3) TMS reagent #3: MSTFA/NH_4I/Propanedithiol (1,000:2:3, v/w/v), 60 °C, 60 min

A method using propanedithiol ($C_3H_8S_2$ = 108.23) as a replacement for 1,2-ethanedithiol has been reported [156].

4) TMS reagent #4: MSTFA/NH_4I/1,4-Dithioerythritol (1,000:2:4, v/w/w), 60 °C, 15 min

This TMS reagent has been widely used [141, 157, 158, 159, 160, 161, 162, 163, 164, 165]. The 1,4-dithio-erythritol (**DTE**, $C_4H_{10}O_2S_2$ = 154.26) reagent is a solid, and this catalyst has been used as a replacement for the above-mentioned alkyl thiol reducing agents. There is no offensive smell, and it is easy to handle, though it is expensive.

However, it is difficult to prepare the silanising reagent mixture containing DTE in exactly the specified ratio, as previously described. This problem is described in detail below.

5) TMS reagent #05: MSTFA/TMSI/DTE (1,000:5:5; v/v/w), 60 °C, 40 min

This TMS reagent uses trimethylsilyl iodide (**TMSI**, C_3H_9ISi = 200.10) as a replacement for ammonium iodide as catalyst [97, 104, 166, 167].

As for the ratios of the reagents MSTFA, TMSI, and DTE in the mixture, 1,000:5:5 is used. For the conditions of the derivatisation reaction, the temperature is 60 °C but the time required is a little long reaction.

With respect to the weight of DTE used, DTE is a solid and present in trace amounts compared to MSTFA. Therefore, there are some reports in the literature in which the

ratios of the TMS reagents are vaguely expressed, for example:

(1) a spatula tip of the reductant DTE in MSTFA [168]

(2) approximately 2 mg [169]

(3) MSTFA/TMSI/DTE (1,000/2/1 mg) [170]

It is usually difficult to weigh a trace amount (1 mg) of DTE accurately compared with the large amount of MSTFA (1,000 μL). Many authors prepared a large volume of reagent and stored it in the refrigerator until used.

6) TMS reagent #06: MSTFA/Trimethylsilyl iodide (**TMSI**)/DTE or Ethanedithiol

In this reagent, TMSI is used as a replacement for ammonium iodide as a catalyst. DTE [162] or ethanedithiol [135] is used as a reducing agent.

7) TMS reagent #07: MSTFA/Imidazole (1,000:2, v/w), 60 °C, 15 min

According to the **Schanzer**'s report, this TMS reagent is equivalent to a mixture of MSTFA/NH_4I/ethanedithiol (1,000:2:6, v/w/v) reagent [144, 145, 171].

8) TMS reagent #08: Hydroxylamine/Pyridine (0.3%), 60 °C, 60 min, followed by MSTFA/TMSI/DTE (1,000:5:5; v/v/w), 80 °C, 60 min

As shown in **Figures 3.6** and **3.8**, the ketone group at the C3-position of the steroid was derivatised with TMS reagent after derivatisation with hydroxylamine ($HONH_2$ = 33.03) or methoxyamine (CH_3ONH_2 = 47.06) [92, 104, 138, 139, 140, 141]. There is one report in which ethoxyamine ($C_2H_5ONH_2$ = 61.09) has been used as a replacement for methoxyamine [140].

3.3.2.2. Acyl derivatives

Acyl derivatives as well as TMS derivatives have been commonly used for GC-MS

analysis. In particular, heptafluorobutyric derivatives prepared with heptafluorobutyric anhydride (**HFBA**, $C_8F_{14}O_3$ = 410.10) have been reported.

As the molecular weight of the derivative increases, the sensitivity of detection rises and the acylation of steroids progresses as well as the trimethylsilylation reaction.

However, to get high sensitivity in the detection of mass fragment ions in the high mass range, more than *m/z* 600, in a conventional bench top GC-MS instrument can be difficult.

1) Acylation reagent #01: Heptafluorobutyric anhydride (**HFBA**) [158, 172, 173]

Williams [95] used this heptafluorobutyryl (**HFB**) derivative to analyse anabolic steroids in the racing greyhound. Moreov er, **Ferchaud** [97] reported on the HFB derivative to improve the sensitivity of GC-MS analysis of stanozolol in domestic animals.

Both alcoholic hydroxyl and amino groups of anabolic steroids react with HFBA reagent. HFB derivatives are stable compounds as they are either ester (-O-COC_3F_7) or the acid amide (N-COC_3F_7). The sensitivity of GC-MS analysis increases at the same time, too. The sensitivity is especially remarkable in the negative ion chemical ionisation -mass spectrometry (NCI-MS) mode.

2) Acylation reagent #02: Acetic anhydride

Acetic anhydride ($C_4H_6O_3$ =102.10) has generally been used for isotope ratio GC-MS of anabolic steroids [174, 175, 176, 177]. Anabolic acetate derivatives are shown in the Appendix **(Table 3.8)**.

3) Acylation reagent #03: Trifluoroacetic anhydride

This esterification method uses trifluoroacetic anhydride (**TFAA**, $C_4F_6O_3$ = 210.04)

reagent, giving a derivative with high cost-effectiveness which is obtained with a comparatively easy experimental procedure. **Choi** [174] applied the TFAA reagent to the analysis of anabolic steroids in equine plasma and urine.

4) Other reagents #01: Heptafluorobutyryl/Pentafluorobenzyloxime

Hold [114] and **De Boer** [175] used HFBA and O-(pentafluorobenzyl)hydroxy-amine (**PFBO**, $C_7H_4F_5NO = 213.12$) for derivatisation of anabolic steroids in the hair and urine of pregnant women.

HFBA and the PFBO reagents react with hydroxy group at the C17-position and enolised ketone at the C3-position of steroids, respectively [**Figure 3.11**].

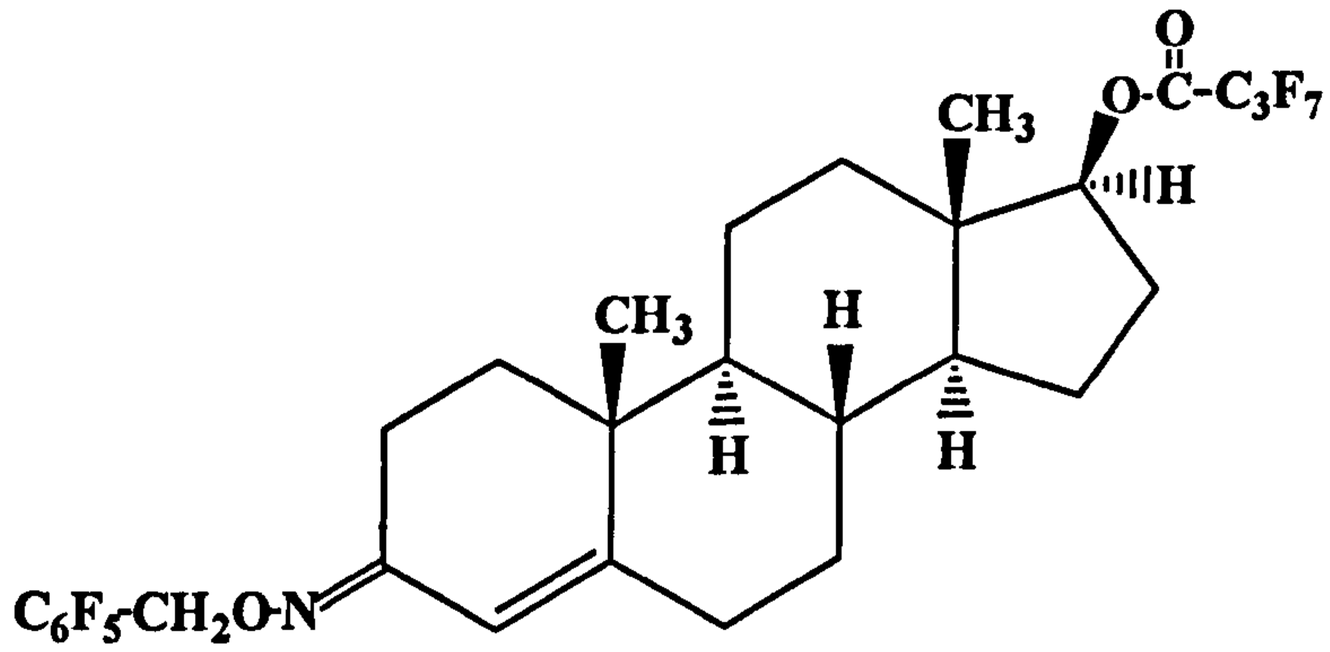


Figure 3.11. Chemical structure of HFB-PFBO ester/oxime derivative of Testosterone

5) Other reagents #02: Pentafluorophenyldimethylsilyl (**Flophemesyl**) ether - TMS enol ether ($MSTFA/NH_4I/DTE$) (1,000:4:5, v/w/w), 60 °C, 15 min,

This TMS reagent was used for the analysis of testosterone and pregnenolone in nail by **Choi** [132]. The alcoholic hydroxy group at the C17-position of anabolic steroids was derivatised with flophemesyl chloride ($C_8H_6ClF_5Si = 260.67$), following which an enolic hydroxy group at the C3-position was derivatised with the TMS reagent (**Figure 3.12**).

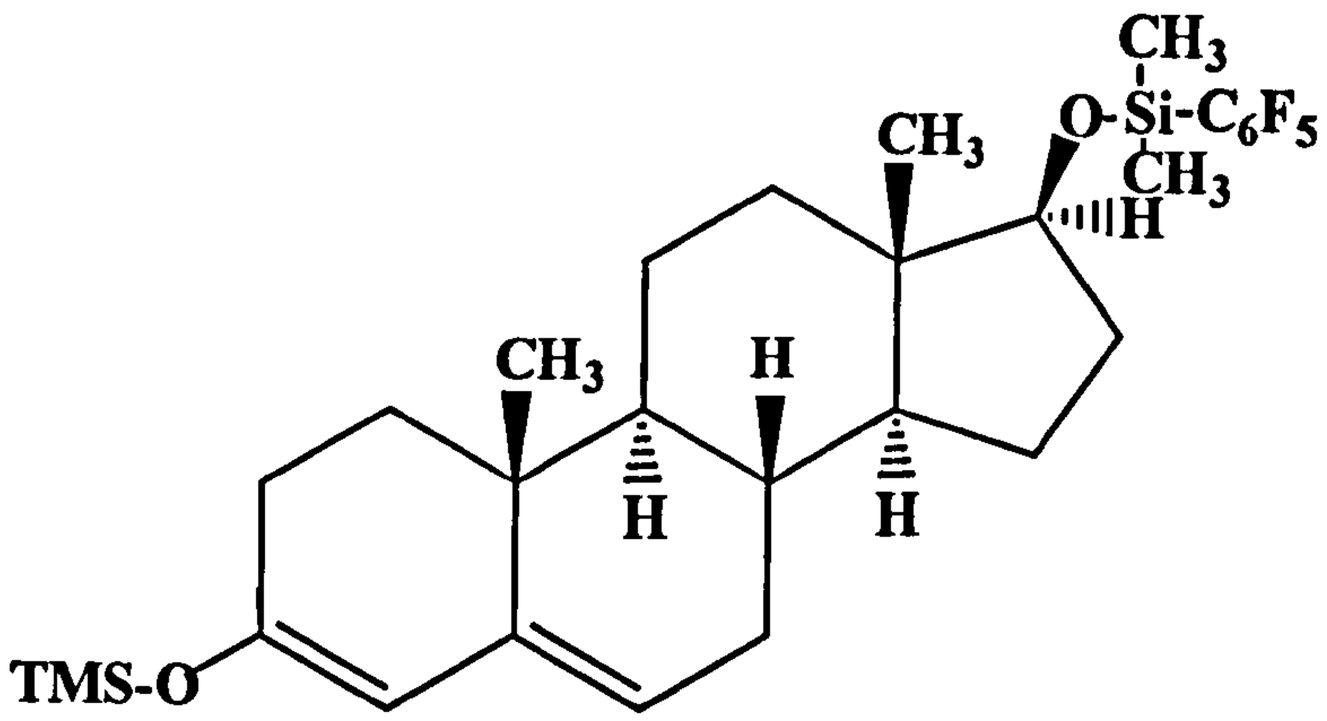


Figure 3.12. Chemical structure of Testosterone Flophemesyl-TMS ether derivative

This derivative, as well as **Hold's method**, gives good sensitivity, but the fragment ions of the analytes are in the high mass range. Therefore, a high resolution mass spectrometer is needed, although a bench-top quadrupole mass spectrometer was used.

6) Other reagents #03: N-methyl-N-trimethylsilyl-heptafluorobutyramide (**MSHFB**)/ trimethylchlorosilane/trimethylsilylimidazole (**TMSimidazole**) (100:5:2, v/v/v).

Kim [180] has reported this TMS reagent for the analysis of furazabol and its metabolites. This method is the same as the conventional TMS derivatisation methods and has no significant unusual features.

3.3.2.3. Formation of cyclic derivatives

Examples of these include methyl, butyl or phenylboronate derivatives. These derivatives containing boron are prepared by reaction of dihydroxy steroids that are metabolites of the anabolic steroids with alkyl boronic acids.

Methylboronic acid and butylboronic acid [140] or phenylboronic acid [176] were reported as derivatising reagents for stanozolol and fluoxymesterone, respectively.

3.3.2.4. Miscellaneous derivatives

One example is MSTFA/I_2. In general, GC-MS analyses of anabolic steroids containing a hetero-atom ring in the steroid skeleton (stanozolol, danazol) and anabolic

steroids having multiple double bonds in the steroid nucleus (e.g. trenbolone) are difficult because of thermal degradation and HPLC based methods have been used in preference.

This problem in GC-MS analysis of thermally labile compounds was improved by the derivative shown in **Figure 3.13** [167, 177].

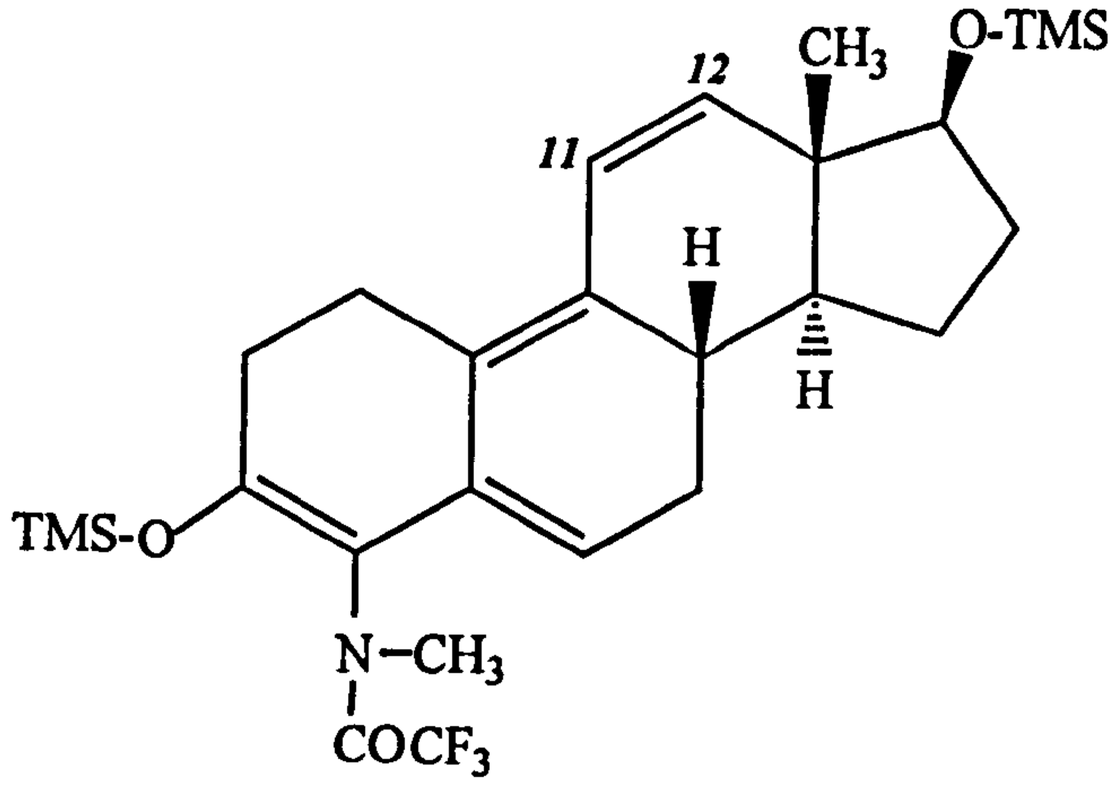


Figure 3.13. Chemical structure of Trenbolone-4-(N-methyl,N-trifluoroacetyl)-3,17-di-TMS

3.3.3. Qualitative and quantitative GC-MS analyses

3.3.3.1. Qualitative analysis

a) Gas chromatographic retention times and retention indices.

More than 600 anabolic steroid compounds and their metabolites, estrogens, progestagens and glucocorticosteroids were identified from the literature [Appendix **(Table 3.8)**], and, of these, 125 steroids were obtained for this project and analysed by GC-MS.

All of these steroid compounds eluted within the range of 10–25 min as shown in **Tables 3.5–3.7**. Relative retention times (**RRT**) were measured relative to the retention time of testosterone. Retention indices (**Kovats indices**) were also measured at the same time using *n*-hydrocarbon standards.

In general, long retention times were obtained for anabolic steroids having an alkyl

ester group at the C17-position. As examples, testosterone esters eluted in order of the carbon number of the alkyl substituent group, as shown in **Table 3.5** (page 67). Similarly, GC-MS analysis data for other anabolic steroids and for other types of steroid (metabolites, estrogens, progestagens, etc) are shown in **Tables 3.6** and **3.7** (pp 68-73), respectively.

Dioctyl phthalate (di-ethylhexyl phthalate, **DOP**, $C_{24}H_{34}O_{24}$ = 390.56) and cholesterol (**delta-5-cholesterol**, $C_{27}H_{46}O$ = 386.67) appeared as large, broad peaks.

The DOP was a contaminant compound derived from plastics as previously mentioned. To prevent or reduce interference by DOP, plastic screw caps used for sample preparation tubes were washed with methanol beforehand.

As a result, the peak intensity of DOP was decreased, but nevertheless was still large. Cholesterol was mainly derived from biological samples such as urine and plasma samples. Both DOP and cholesterol were interfering compounds though they played a role as markers of retention times (**Rt**).

b) Mass spectra

TMS derivatives of exogenous (**synthetic**) anabolic steroids controlled by WADA gave mass spectra that showed that all functional groups that could be silylated were completely derivatised with TMS reagent, except for oxandrolone. For example, danazol has one active hydrogen group (**proton**) on the 17β hydroxy group and the number of TMS substituent groups in the TMS derivative of danazol is naturally one [**Figure 3.14**].

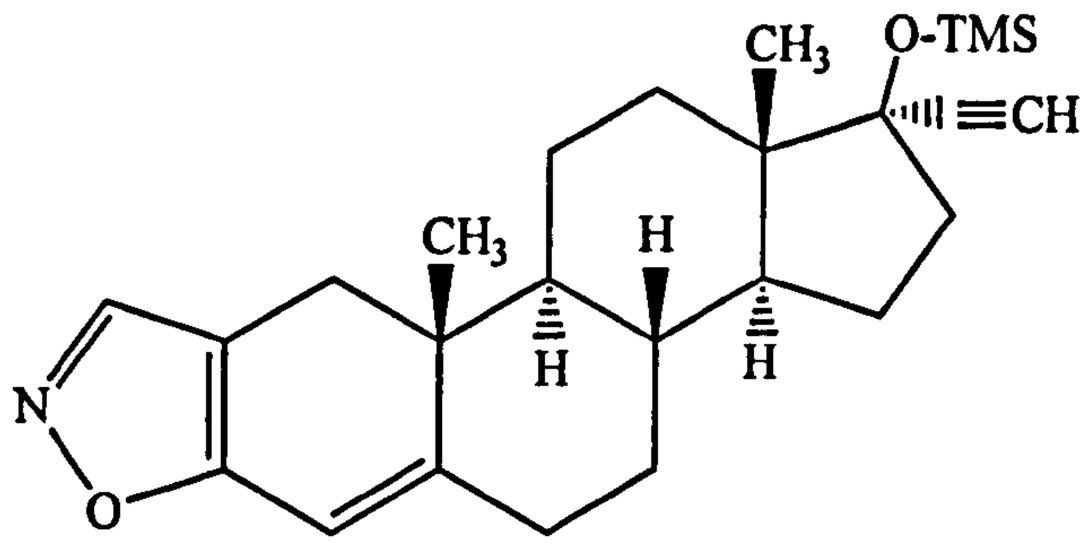


Figure 3.14. Chemical structure of Danazol-mono-TMS derivative

The TMS derivative of oxandrolone gave a mass spectrum corresponding to a mono-TMS derivative (MW = 378) whereas **Heunerbein** [146] had reported that the oxandrolone TMS derivative has two TMS substituent groups. However, this report is incorrect because the ketone group at the C3-position of oxandrolone forms a lactone ring with the steroid skeleton **[Figure 3.15]** and the ketone group of a lactone is not amenable to enolisation.

Therefore, the enol group cannot be formed, and the ketone group of the oxandrolone cannot be derivatised with TMS reagent.

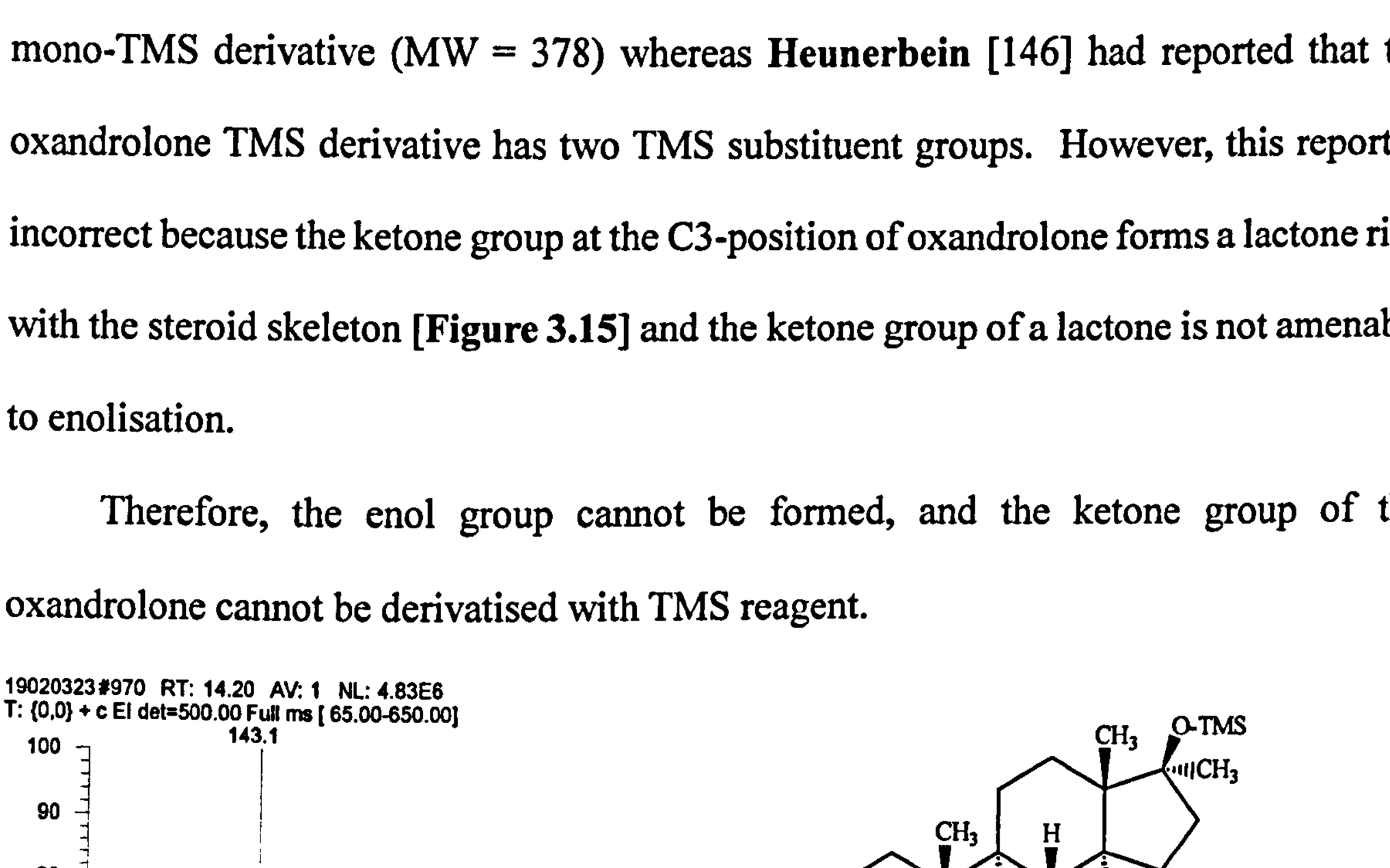


Figure 3.15. Mass spectrum of Oxandrolone-mono-TMS
The molecular ion and M-15 ion appear at *m/z* 378 and *m/z* 363 respectively.

The mass spectra of TMS derivatives usually gave the **M-15** ion along with the molecular ion (M^+), and the identification of the TMS derivative was easy by comparing it with a standard, excluding isomers, which tend to have similar spectra. The mass spectrum of the TMS derivative of testosterone is shown in **Figure 3.20**.

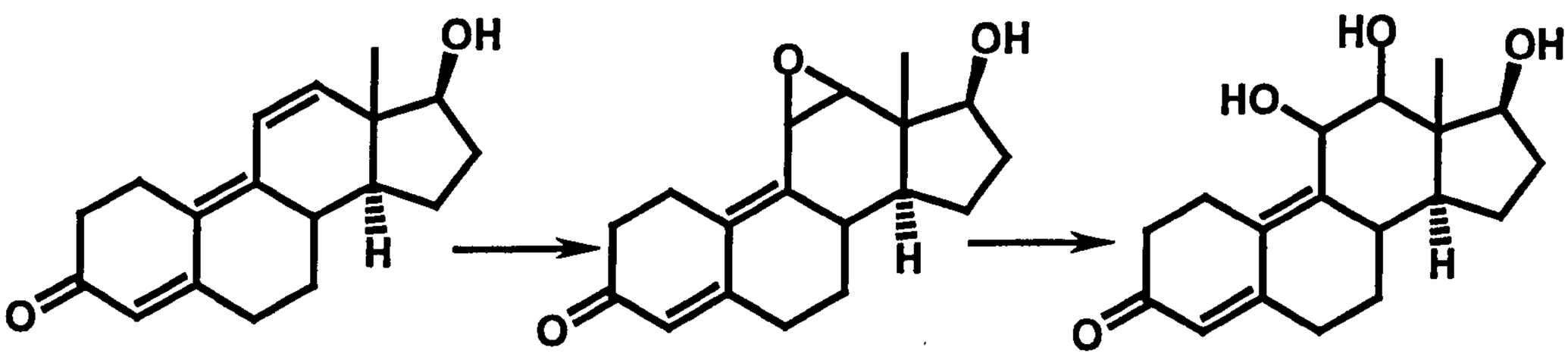
c) Steroids that are difficult to analyse by GC-MS

In the GC-MS analysis of stanozolol or danazol, an acceptable chromatogram was obtained only when a new septum and liner were used in the injection port of the GC.

The chromatograms were not reproducible. In particular, stanozolol did not give good results because of pyrolysis, which occurred under the usual GC-MS conditions.

Many workers have reported methods to improve the GC-MS analysis of stanozolol [97, 107, 130, 131, 144, 146, 178, 179, 180, 181, 182].

A similar result was obtained with trenbolone. However, judging from the results of GC-MS analysis, the data showed that the derivative had two hydroxy groups at the C11 and C12-positions of the anabolic steroid skeleton, probably resulting from an intermediate epoxide. Therefore, it seemed likely that trenbolone, which had been stored for a long time, had been oxidised (degraded) [see **Figure 3.16**].



Trenbolone Trenbolone -11,12-epoxide 11,12-Dihydroxytrenbolone

Figure 3.16. Proposed oxidation of Trenbolone

3.3.3.2. Identification of endogenous anabolic steroids in nail samples.

Identification of endogenous steroids was carried out based on the data in **Tables**

3.5–3.7 and **Table 8** in Appendix. As a result, the retention times of the endogenous anabolic steroids were chiefly between internal standard steroid, nor-epiandrosterone-d_3 (15.84 min) and testosterone-d_3 (19.54 min) **[Figure 3.17]**.

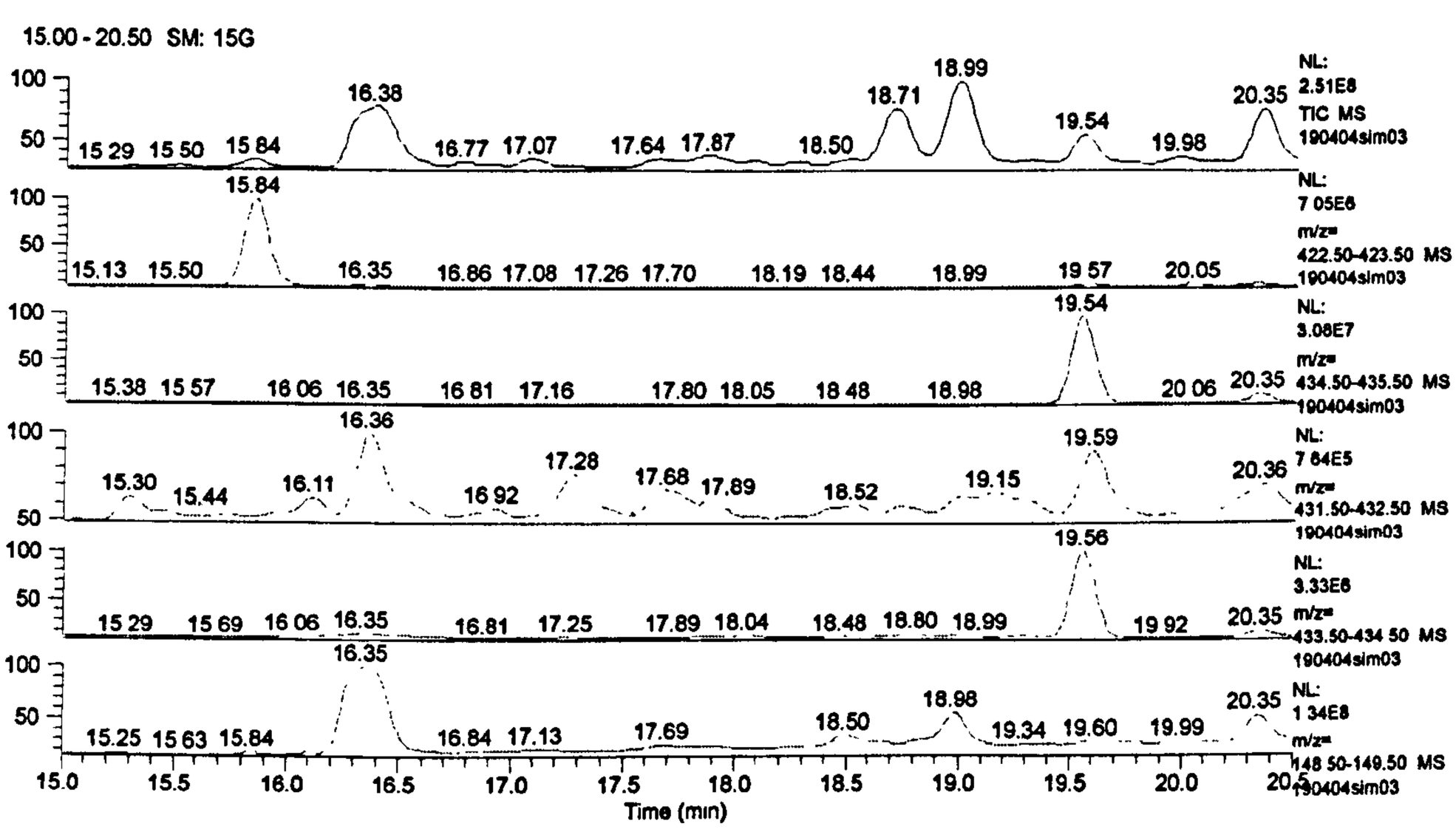


Figure 3.17. Mass chromatograms (**MC**) of nail (Sample No. 190404sim03)

Androsterone (16.06 min), etiocholanolone, dehydroepiandrosterone (17.89 min), epiandrosterone (18.04 min), epitestosterone (18.52 min) and testosterone (19.59 min) were detected in nail samples using retention times, mass chromatograms (**MC**), and standard samples **[Figure 3.18–3.19]**.

The retention times of endogenous steroid standards and DOP were: NEA-d_3 (15.90 min), AND (16.11 min), ETIO (16.27 min), DOP (16.38 min), epiAND (18.10 min), epiTEST (18.58 min), TEST-d_3 (19.60 min), and TEST (19.64 min) **[Figure 3.18]**. Note that the dates of **Figures 3.17** and **3.18** are different. Mass chromatograms of standard endogenous steroids, recorded on a different date of analysis, are shown in **Figure 3.18**.

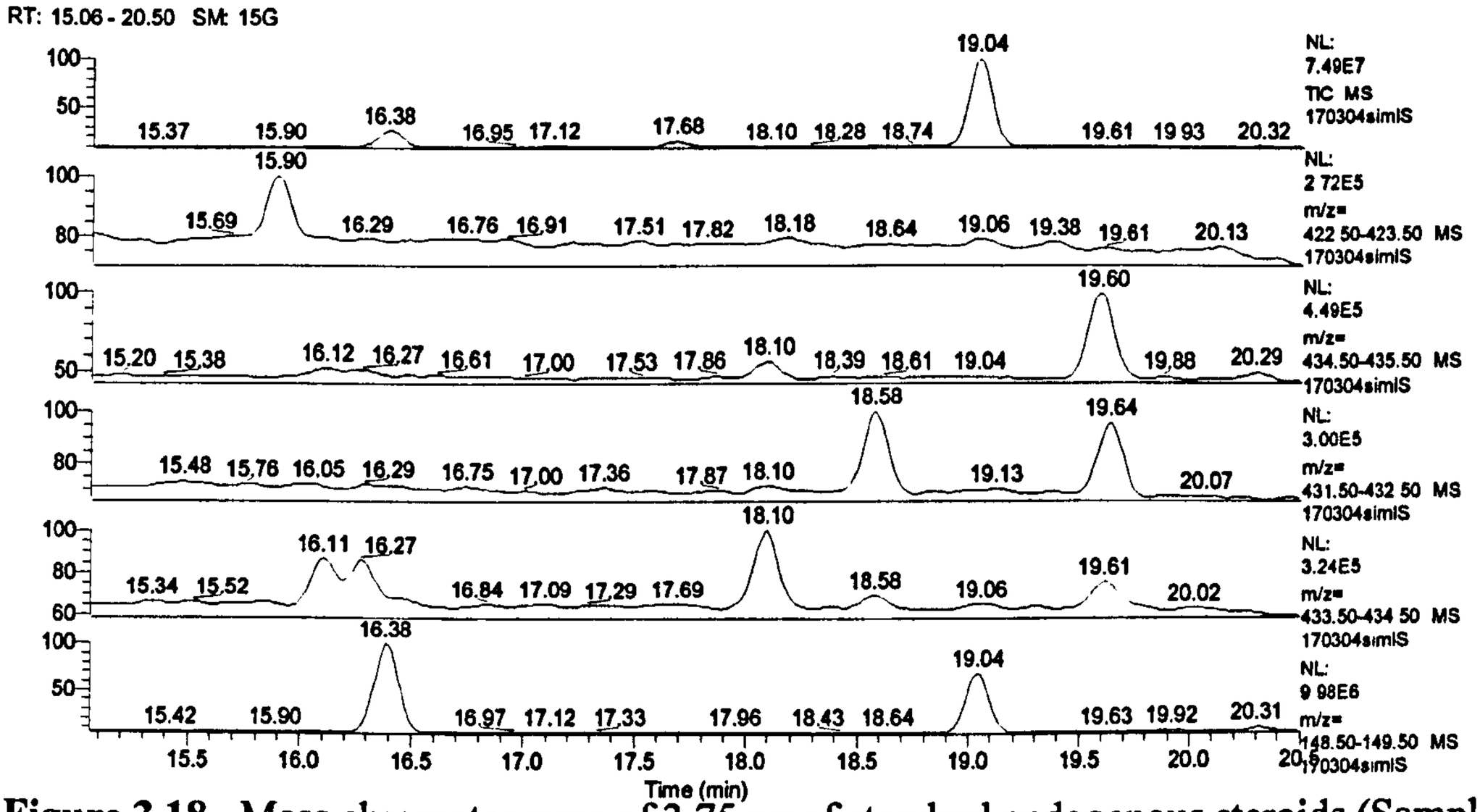


Figure 3.18. Mass chromatograms of 3.75 ng of standard endogenous steroids (Sample No. 170304simIS)

The retention times of endogenous standard steroids and DOP in **Figure 3.17** were: NEA-d_3 (15.90 min), androsterone (16.11 min), etiocholanolone (16.27 min), DOP (16.38 min), epiandrosterone (18.10 min), epitestosterone (18.58 min), testosterone-d_3 (19.60 min), and testosterone (19.64 min).

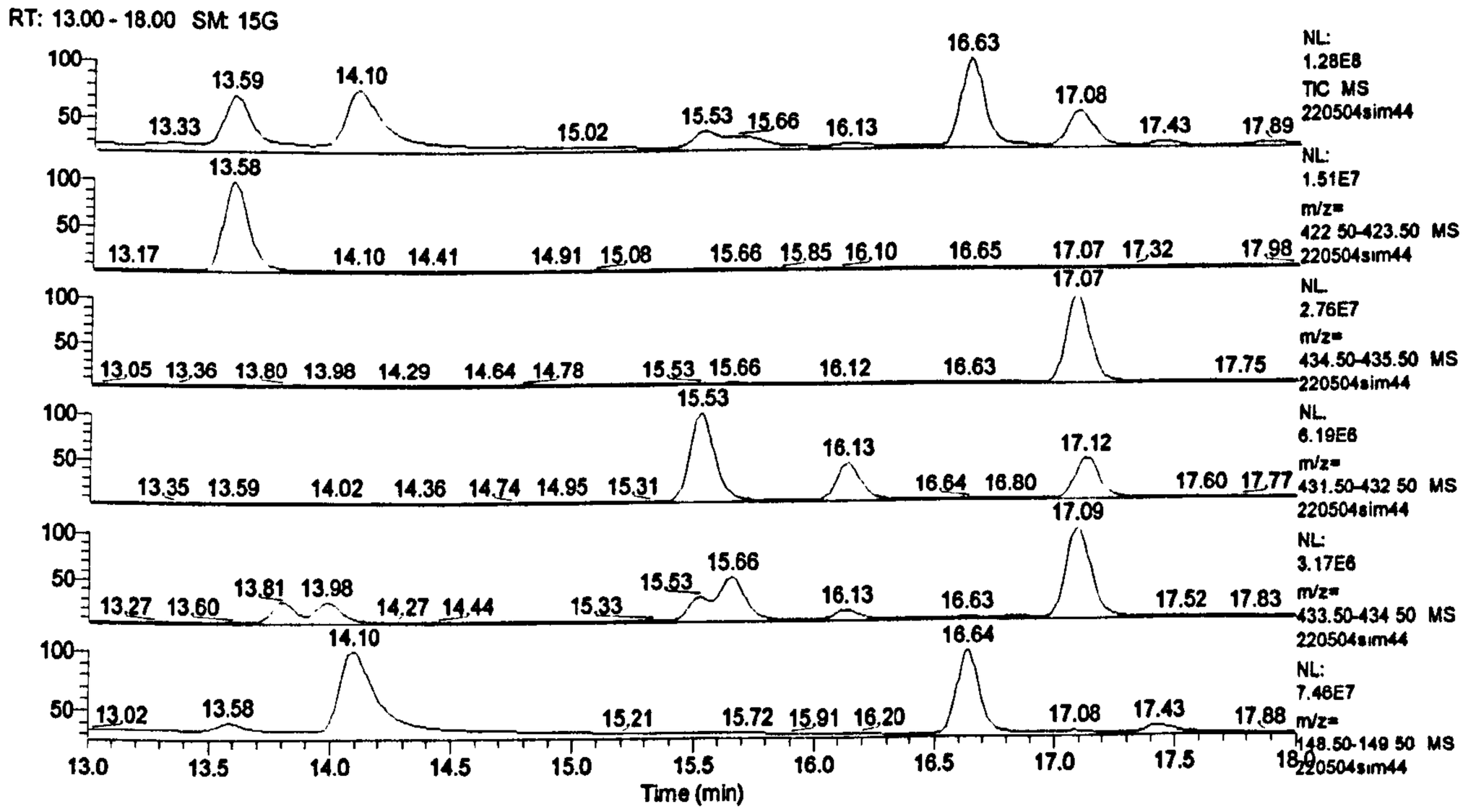


Figure 3.19. MC of 0.10 ng of standard endogenous steroids (Sample No. 220504sim44)

The retention times of the endogenous standard steroids and DOP in **Figure 3.19** were: NEA-d_3 (13.58 min), androsterone (13.81 min), etiocholanolone (13.98 min), DOP (14.10 min), DHEA (15.53 min), epiandrosterone (15.66 min), epitestosterone (16.13 min), testosterone-d_3 (17.07 min) and testosterone (17.12 min).

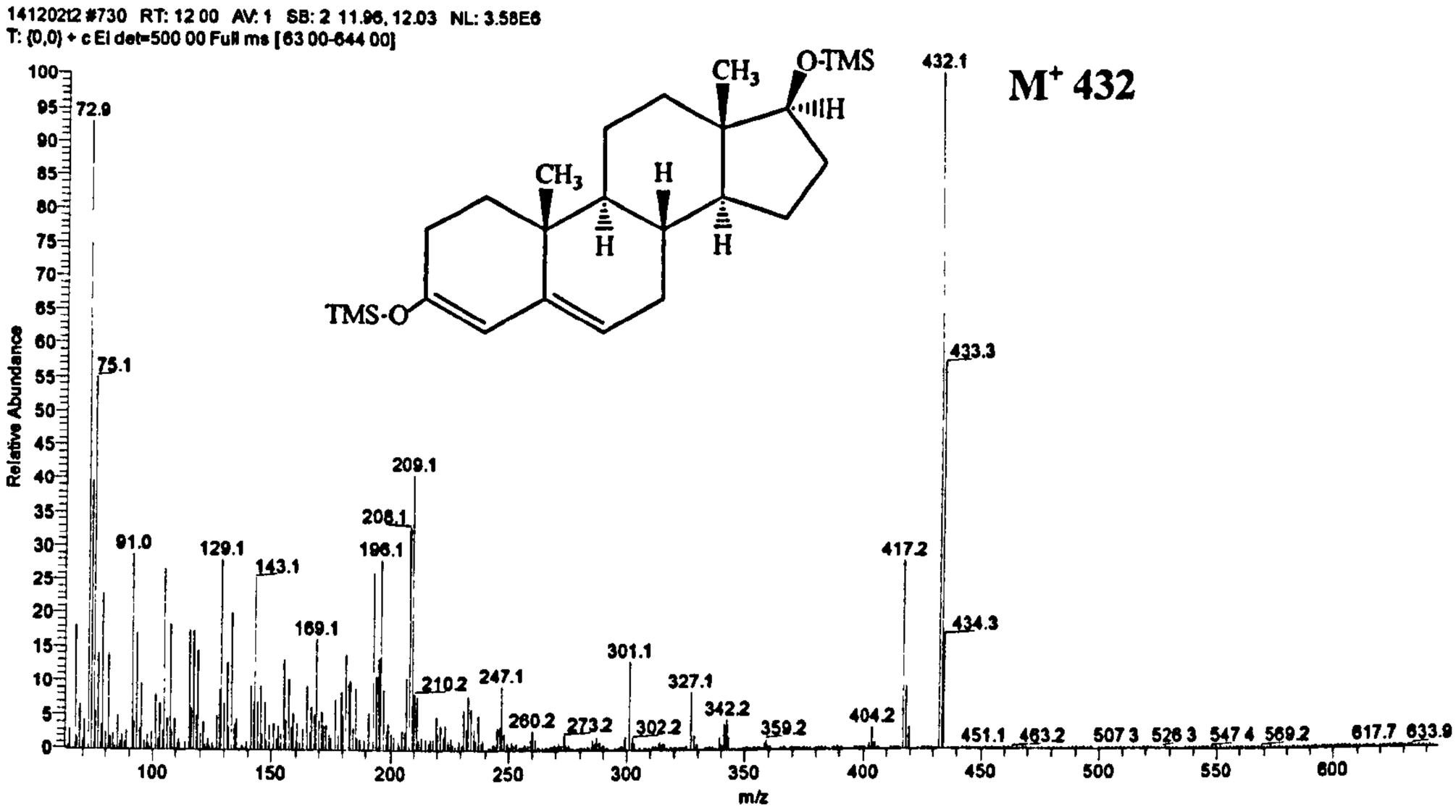


Figure 3.20. Mass spectrum of Testosterone-di-TMS

The molecular ion and M-15 ion appear strongly at (*m/z* 432) and (*m/z* 417) respectively.

3.3.3.3. Identification of exogenous anabolic steroids in nail samples

Concerning the nail samples from the steroid abusers, the concentrations of each steroid in hair and urine are shown in **Table 3.9**. All exogenous anabolic steroids present in the hair and urine samples were screened in the hair samples. Mass fragment ions **(MF)** used are shown in **Table 3.9**. The Retention index **(R.I)** of the steroids, measured with an initial programme temperature of 150 °C, are also shown in the **Tables 3.5–3.7**. TEST-d_3, MEDR, and STN-d_3 were used as internal standards for the analysis of exogenous steroids that eluted after testosterone. STN-d_3 was not suitable because of thermal degradation. Therefore, MEDR was used as the internal standard. Moreover, cholesterol, which was a major contaminant in steroid extracts of biological samples, was used as an additional marker of retention times. The exogenous steroids expected to be present on the basis of the urine and hair analysis could not all be detected because of interfering compounds and only testosterone isocaproate **[Figures 3.21** and **3.22]** and testosterone enanthate **[Figures 3.28** and **3.29]** were tentatively identified.

(1) Testosterone isocaproate.

In the mass chromatogram **(MC)** of **Figures 3.21** and **3.22**, the internal standard peak for medroxyprogesterone (*m/z* 560) appears at 26.55 min. Retention times of steroid TMS derivatives were: cholesterol (27.78 min), 4-cholesten-3ß-ol (27.83 min), other isomers of cholesterol (27.92 min), testosterone isocaproate (28.16 min), 7-cholesten-3ß-ol (28.64 min), respectively. The mass spectrum and retention time of the peak at 28.16 min in the **Figures 3.21** and **3.22** are in agreement with those of standard testosterone isocaproate **(T-isocaproate)** (see **Figure 3.26**). However, the presence of T-isocaproate remains tentative because of interference in the mass spectra, even though the retention time in the gas chromatogram matched that of the standard.

Table 3.9. Nail samples from suspected steroid abusers

No.	Compound	Urine	Hair (pg/mg)	RRT	R.I	MF (*m/z*)	Nail Wt (mg)
B1	Clenbuterol	+	250	------	2164	86, 262	8.32
	Testosterone (T/E)	53		1.000	2707	***432***	
	Testosterone enanthate		145	1.541	3329	***472***	
	Methenolone	+		1.026	2737	***446***, (194)	
	Methenolone acetate		385	1.106	2831	***416***, (194)	
	Stanozolol	+	n.a	1.408	3165	***472***	
	Oxandrolone	+	n.a	1.154	2897	143, ***378***	
	Fluoxymesterone	+	n.a	1.224	2976	***552***, (143)	
B2	Testosterone (T/E)	36		1.000	2707	***432***	15.10
	Testosterone enanthate		23	1.541	3329	***472***	
	Nandrolone	+		0.966	2661	***418***, (194)	
	Nandrolone decanoate		810	1.824	3613	***500***	
	Methandienone	+	86	1.063	2781	***444***	
	Mesterolone	+	10	0.987	2687	***448***	
	Tamoxifen	+	n.a	------	------	371, 72	
B3	Clenbuterol	negative	35	------	2164	86, 262	1.84
	Testosterone (T/E)	292		1.000	2707	***432***	
	Testosterone enanthate		20	1.541	3329	***472***	
	Testosterone decanoate		18	1.878	3648	***514***	
	Stanozolol	+		1.408	3165	***472***	
B4	Testosterone (T/E)	30		1.000	2707	***432***	4.24
	Testosterone enanthate		25	1.541	3329	***472***	
	Testosterone phenylpropionate		425	1.930	3687	***492***	
	Testosterone decanoate		220	1.878	3648	***514***	
	Testosterone isocaproate		340	1.401	2975	***458***	
	Methenolone	+		1.026	2737	***446***	
	Methenolone acetate		155	1.106	2831	***416***	
	Methandienone	+	97	1.063	2781	***444***	
	Stanozolol	+	n.a	1.408	3165	***472***	
	Furosemide	+	n.a	------	------	81	
B5	Norandrost-4-ene-3ß,17ß-diol			????	?2590	***420***	35.91
	Norandrost-4-ene-3,17-dione			0.950	2644	***416***	
Internal standard (I. Std)							
C	5a-Estran-3ß-ol-17-one-D3			0.876	2541	***423***	
	Testosterone-D3			0.998	2704	***435***	
	Medroxyprogesterone			1.304	3070	***560***	
	Stanozolol-D3			1.392	3163	***475***	

Notes: Analytical data for urine (ratio of testosterone/epitestosterone), RRT = Relative retention time (Testosterone = 1.000), R.I = Retention index, MF = mass fragment ions for quantitation (*m/z*), Wt = weight (mg) of decontaminated nail.

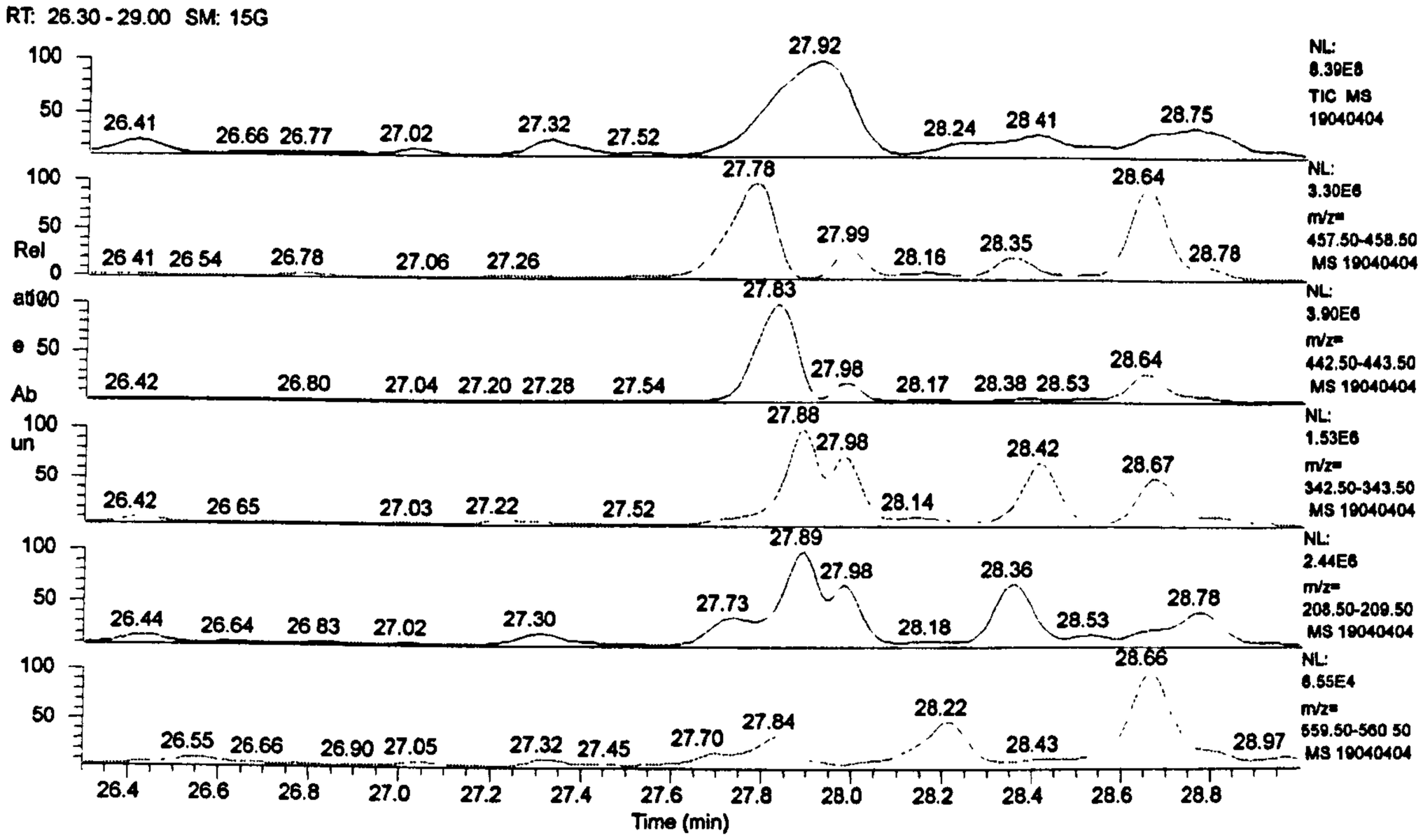


Figure 3.21. Mass chromatogram of nail #04 from anabolic steroid abuser (for Testosterone-17-isocaproate-mono-TMS)

The peak at retention time 28.16 min is tentatively identified as Testosterone-isocaproate.

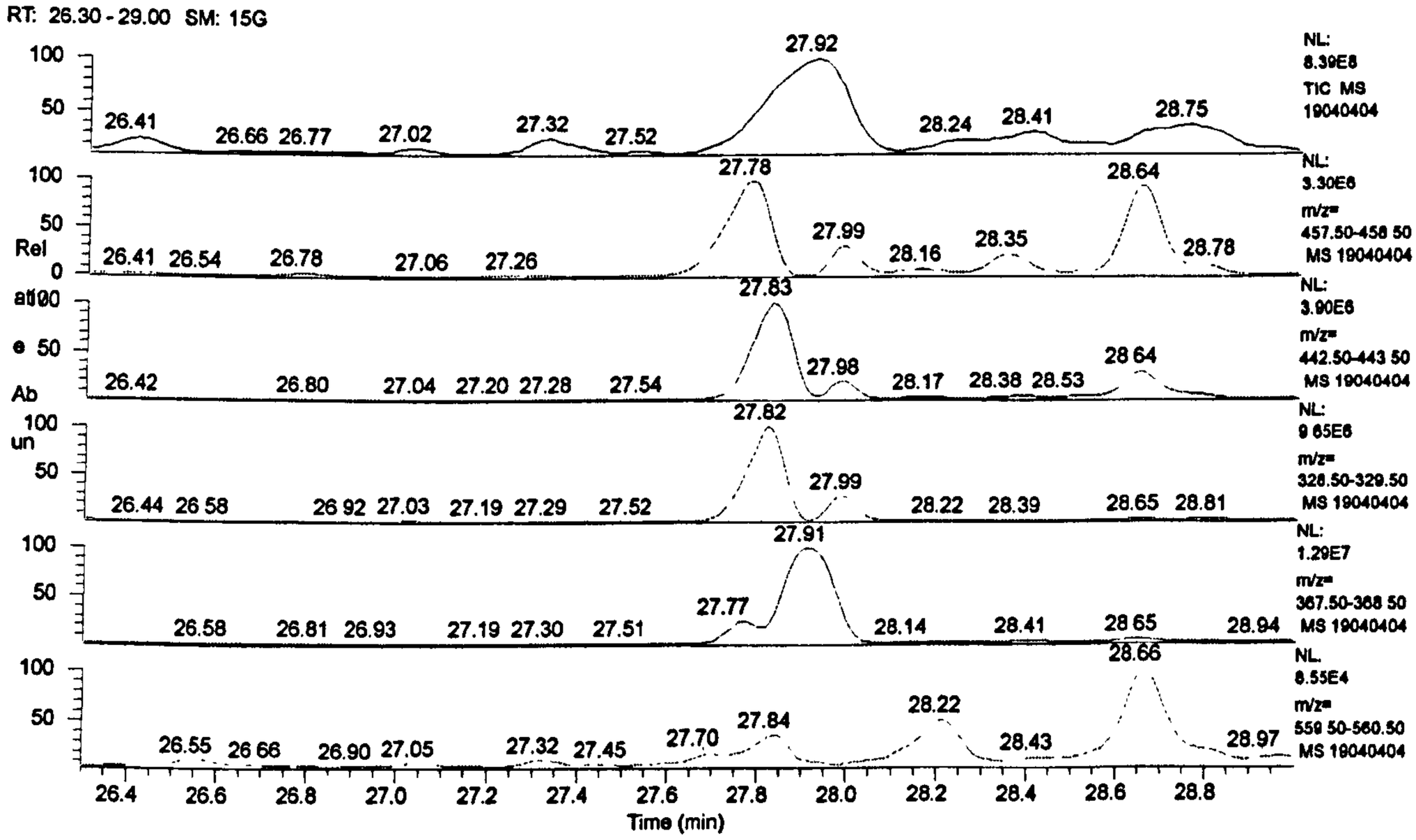


Figure 3.22. MC of nail #04 from anabolic steroid abusers (For Cholesterol-mono-TMS)

Mass chromatograms (**MC**) using significant fragment ions for cholesterol-5-en such as *m/z* 458, 443, 329, and 368.

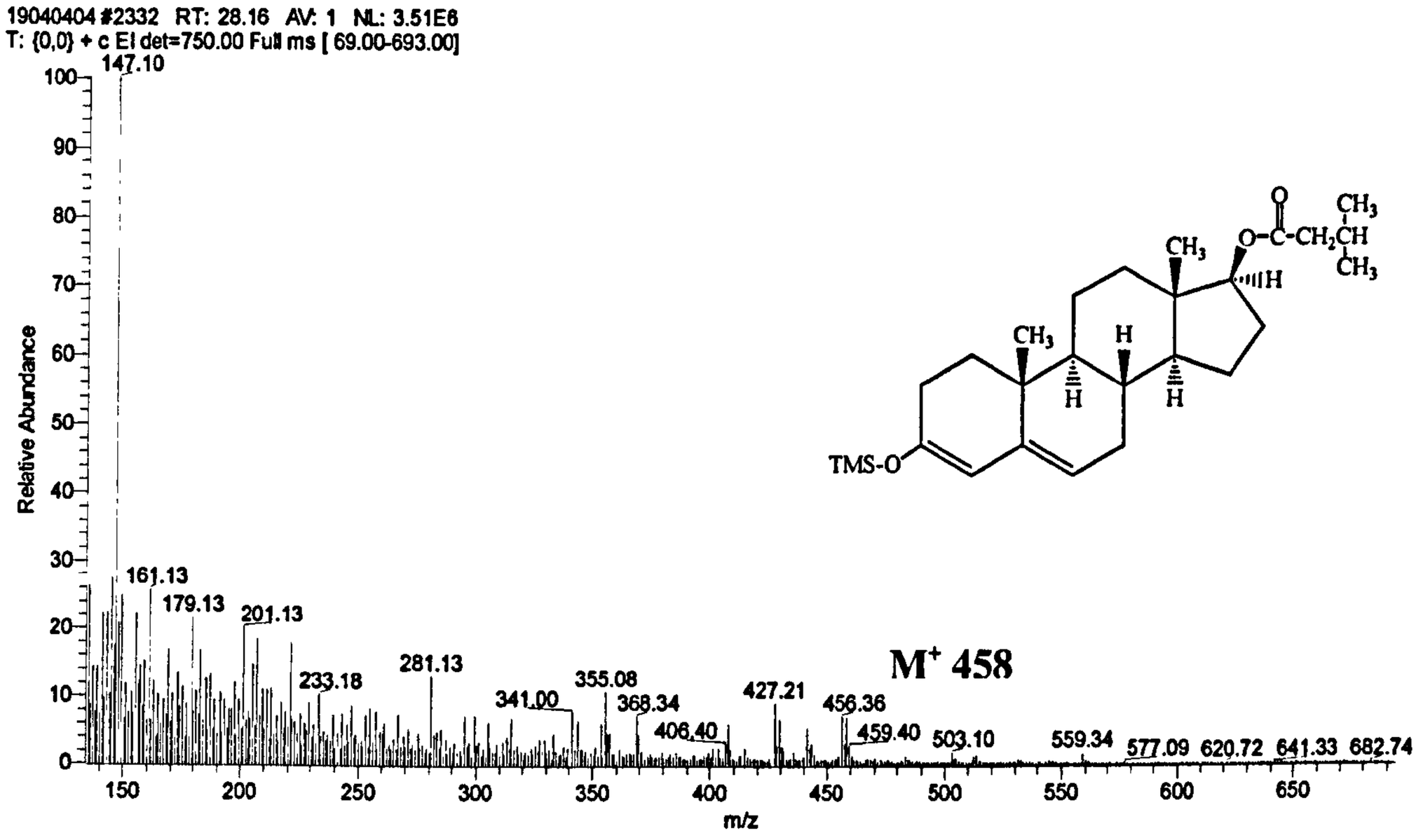


Figure 3.23. MS of Testosterone isocaproate-mono-TMS (Rt = 28.16 min)

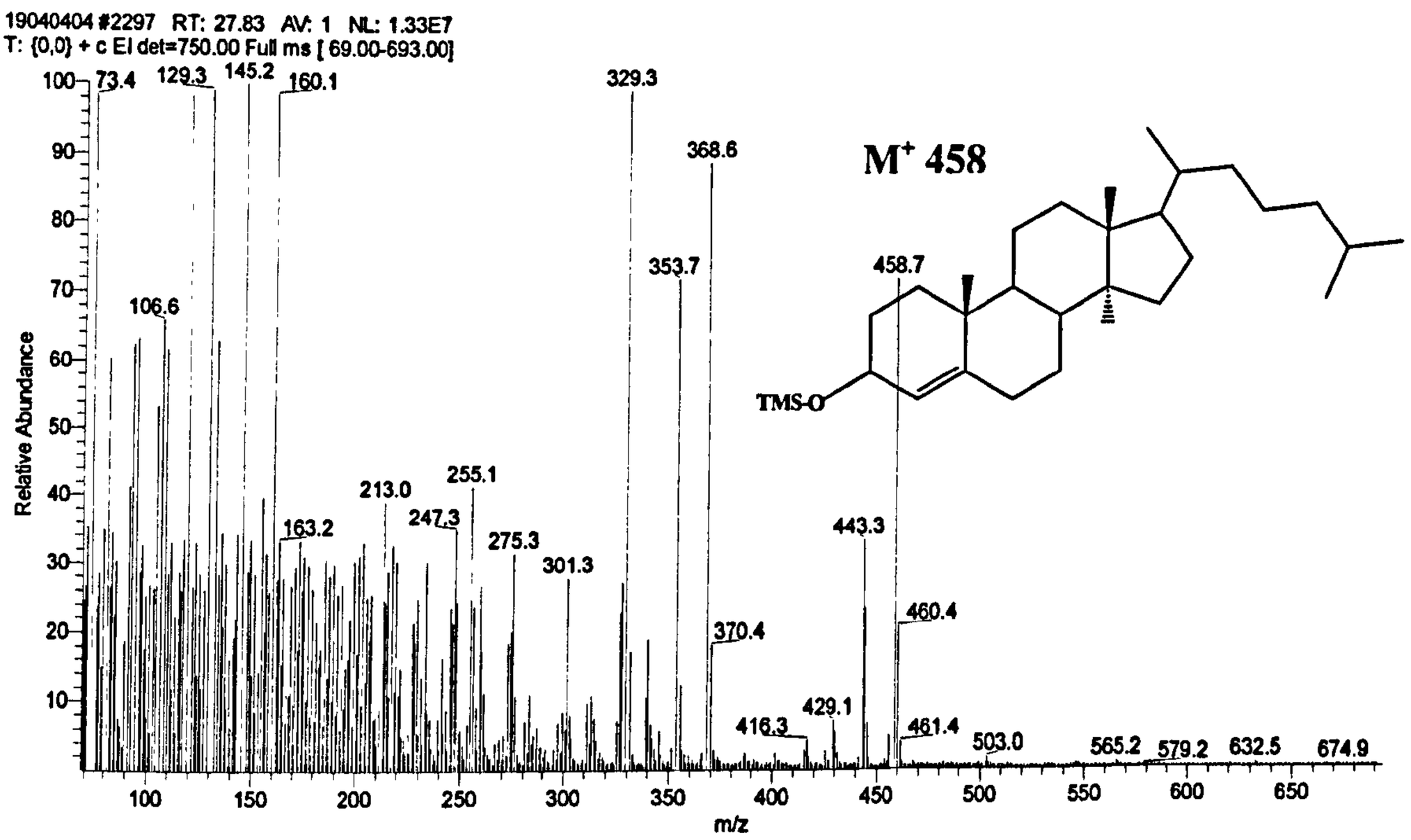


Figure 3.24. MS of nail #04 from anabolic steroid abusers (Rt = 27.83 min)
(Interfering steroid, cholesterol-4-en-mono-TMS)

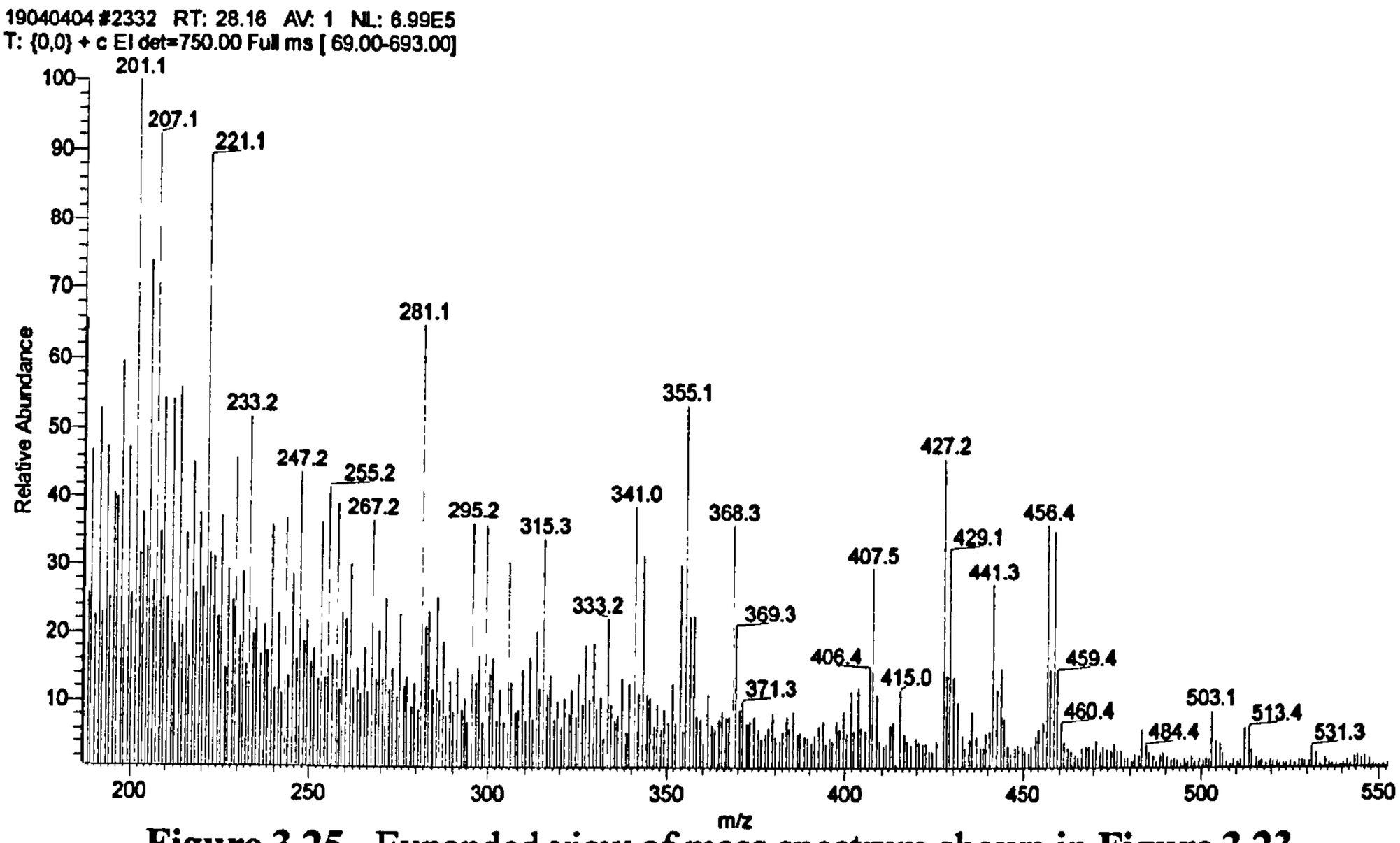


Figure 3.25. Expanded view of mass spectrum shown in **Figure 3.23** (Rt = 28.16 min) (Specific fragment ions, *m/z* 458, 443, 343, 207 were detected)

(2) Testosterone enanthate

Testosterone enanthate (T-enanthate) was tentatively identified but could not be confirmed, although both T-enanthate and T-isocaproate were presumed to be present, based on the urine and hair analysis results.

Data obtained from GC-MS analysis of sample 4 and the data for the standard T-enanthate are shown in **Figures 3.28, 3.29** and **3.30** respectively.

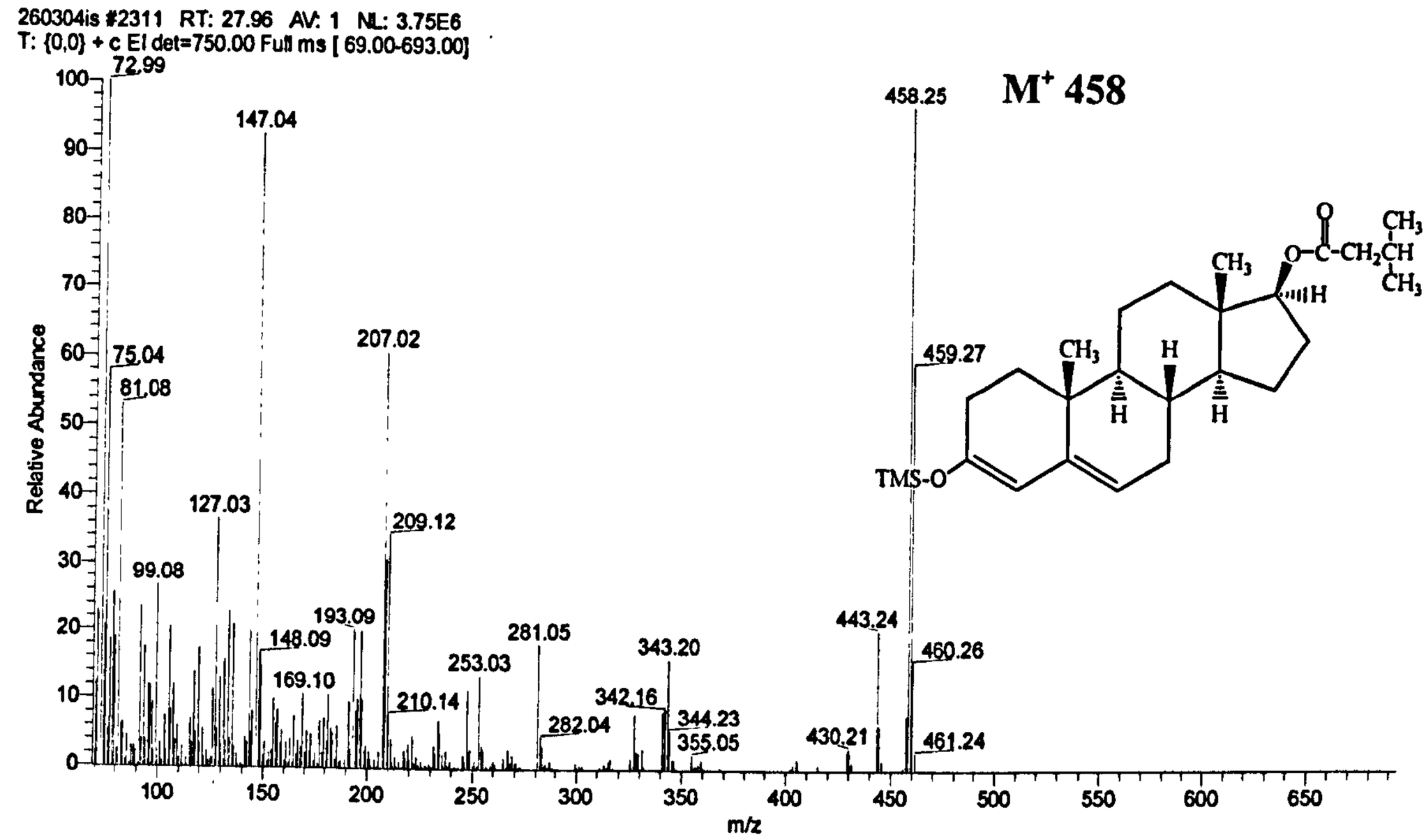


Figure 3.26. Mass spectrum of standard T-isocaproate-mono-TMS (MW = 458)

The molecular ion (*m/z* 458) and M-15 ion (*m/z* 443) are prominent ions in the spectrum.

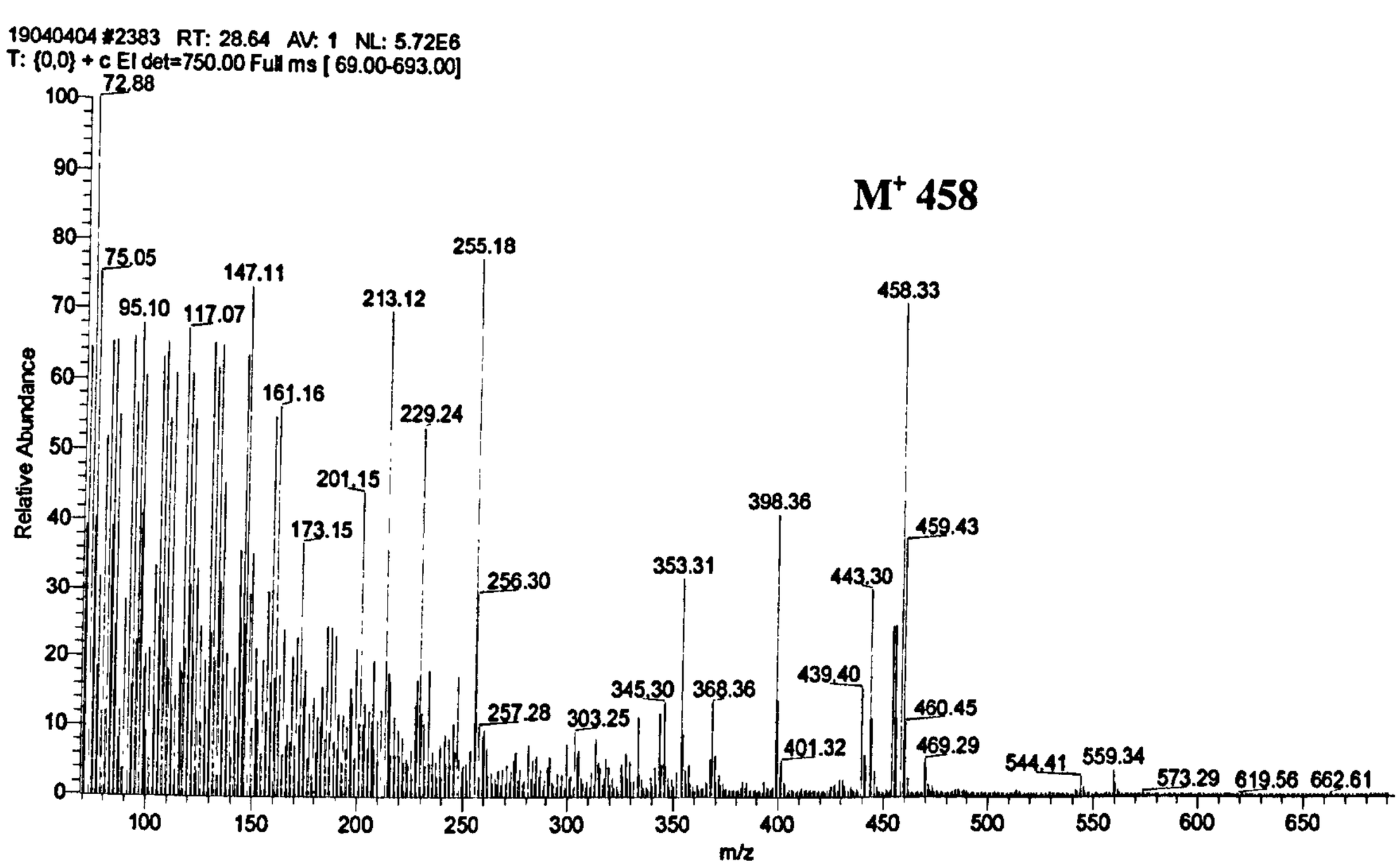


Figure 3.27. MS of nail #04 from anabolic steroid abusers (Rt 28.64 min)

(Interference mass spectrum, that corresponds to cholesterol-7-en-mono-TMS)

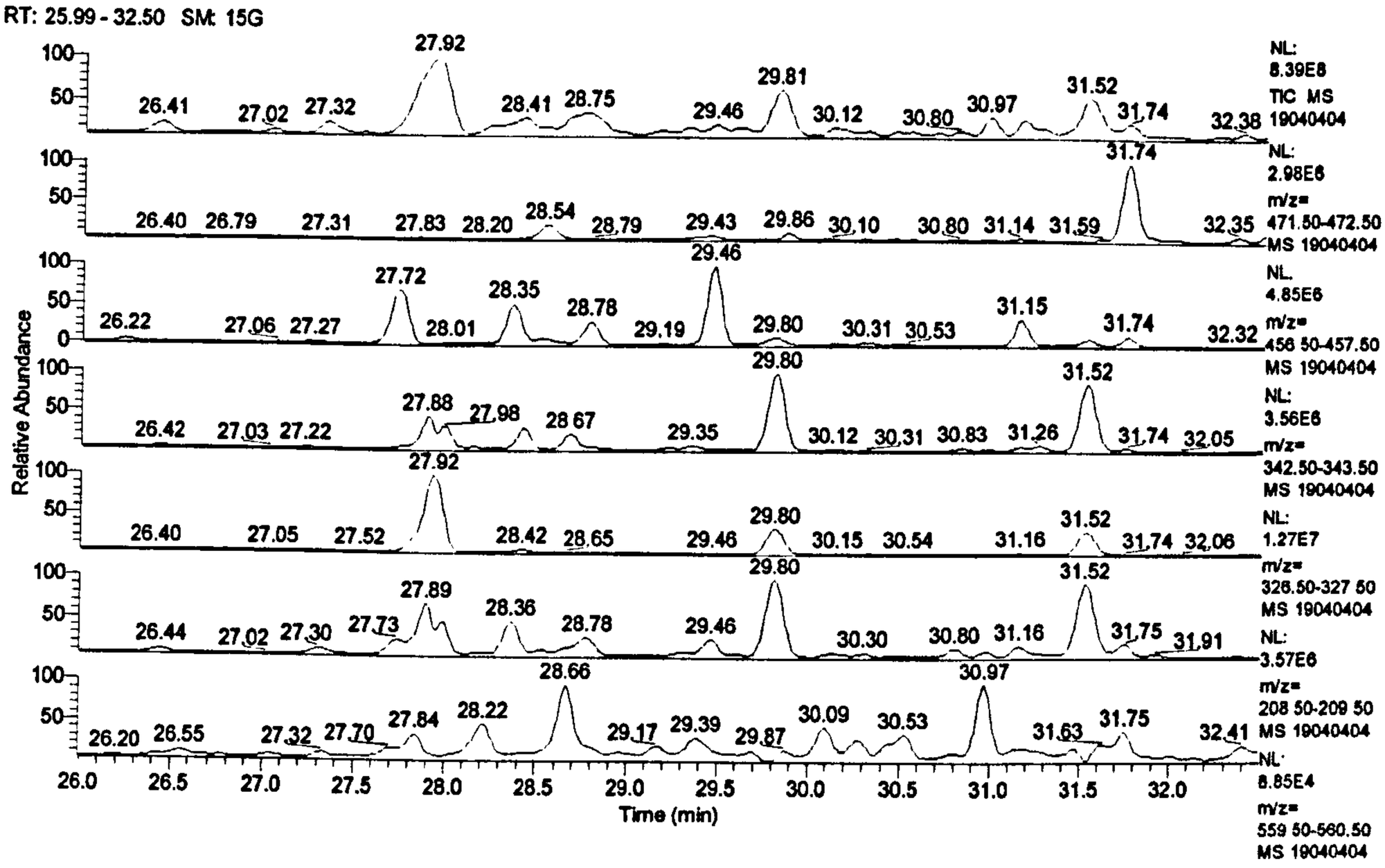


Figure 3.28. MC of nail #04 from steroid abuser for T-enanthate-mono-TMS

The mass spectrum of the peak at 31.74 min looks very similar to that of T-enanthate. However, the peak should be detected at a retention time of about 30.20 min under the GC-MS conditions used.

The retention time of the peak at 31.74 min is too long. Moreover, the same compound was detected in all of the fingernail samples. It appeared to be a compound with a molecular weight of 472 having a methyl functional group at the C23-position of cholesterol.

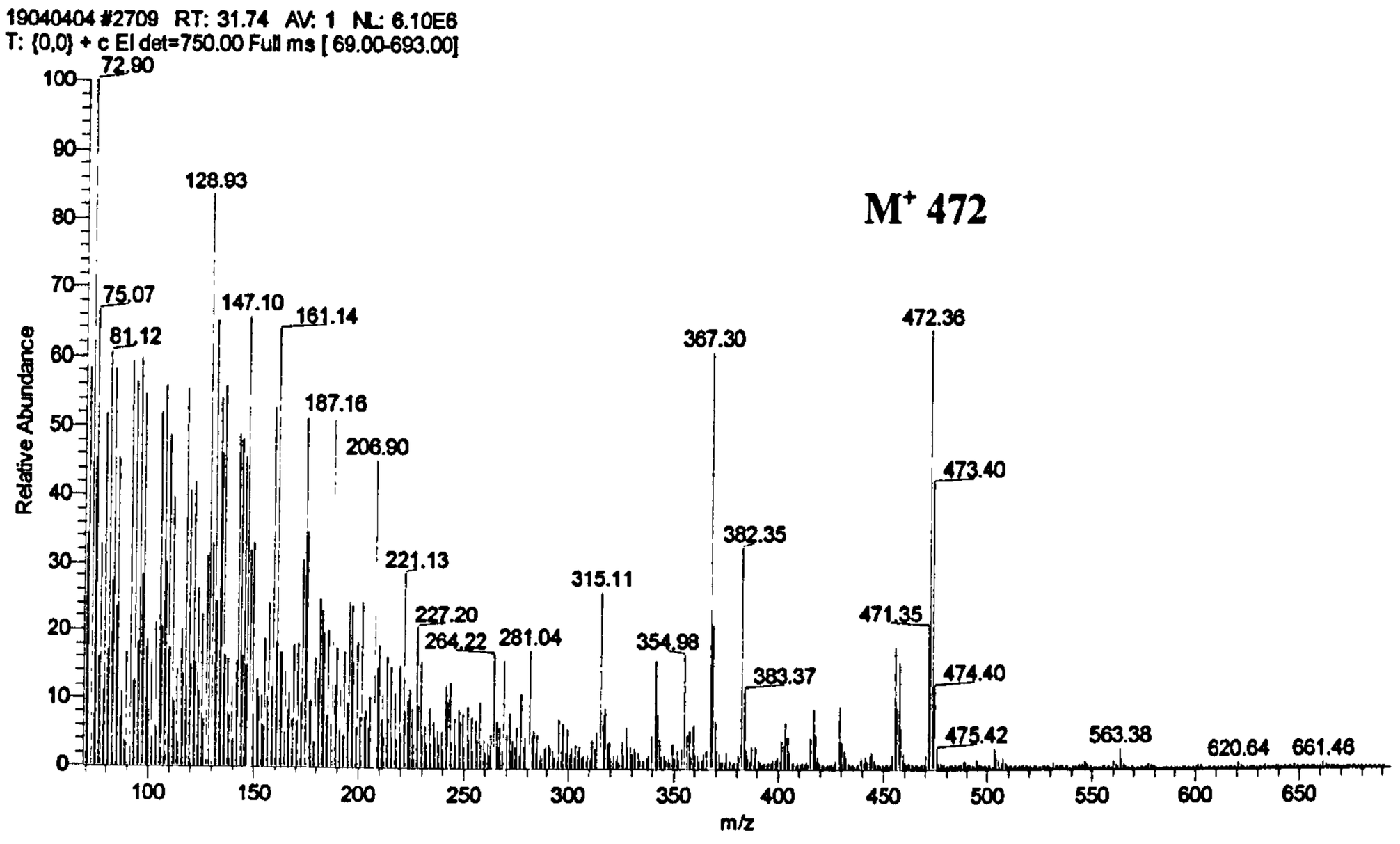


Figure 3.29. MS of nail #04 from anabolic steroid abusers for T-enanthate-mono-TMS (Rt = 31.74 min)

The molecular ion and M-15 ion appear at (*m/z* 472) and (*m/z* 457) respectively. This mass spectrum is very similar to that of the standard (**Figure 3.30**).

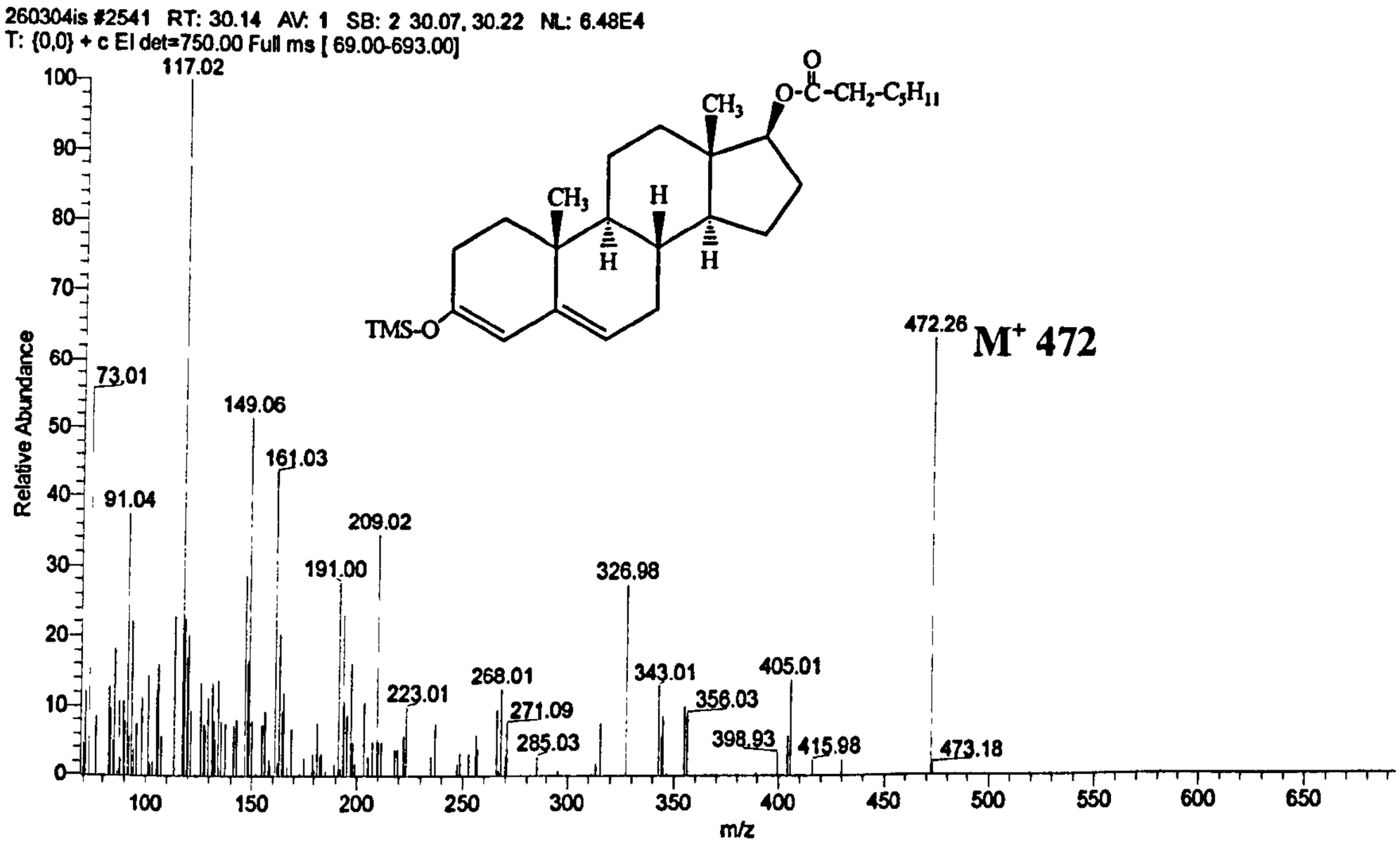
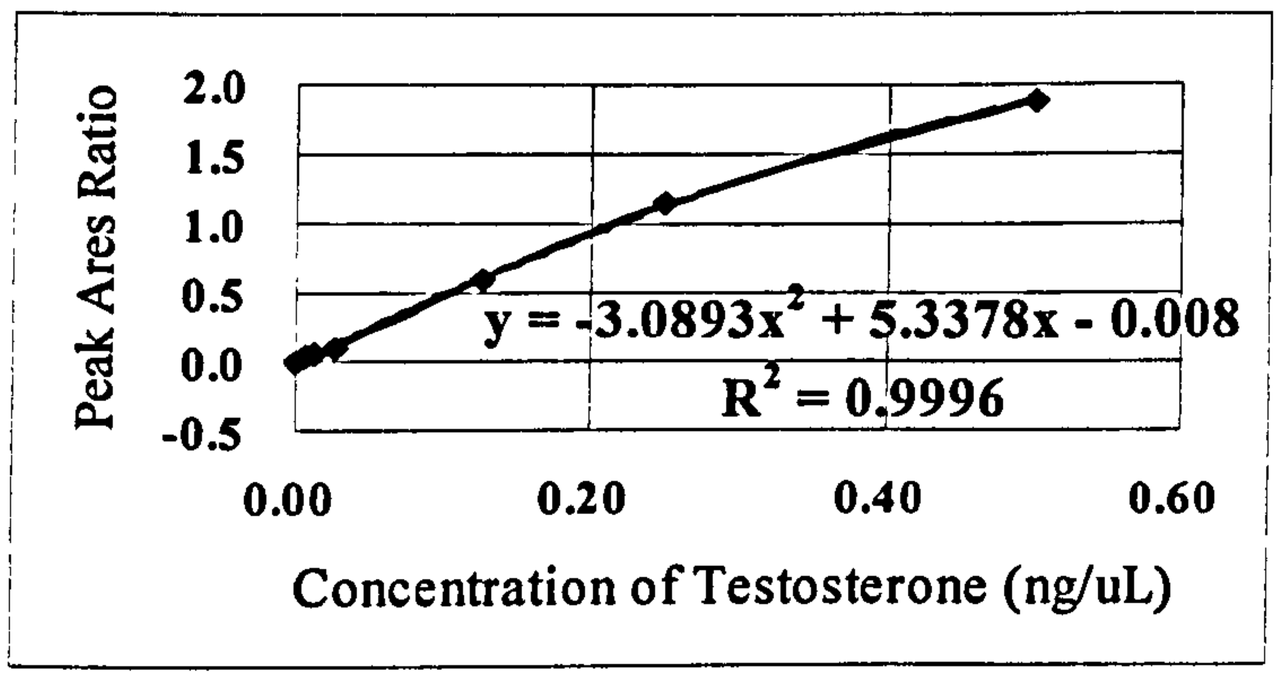


Figure 3.30. Mass spectrum of T-enanthate-mono-TMS standard (MW = 472)

A prominent molecular ion appears at *m/z* 472.

3.3.3.4 Quantitative analysis of endogenous steroids

The calibration curves were prepared with unextracted standards and were nonlinear curves, which were fitted using **polynomial regression** (statistical application package of **Microsoft Excel**) within the range from 0.000 to 0.500 ng/μL for AND, ETIO, DHEA, epiAND, epiTEST, and TEST **[Figure 3.31]**.



No.	Conc (ng)	Conc (ng/μL)	Test/d3-T
1	0.00	0.0000	0.0058
2	0.02	0.0050	0.0302
3	0.05	0.0125	0.0569
4	0.10	0.0250	0.1035
5	0.50	0.1250	0.5926
6	1.00	0.2500	1.1505
7	2.00	0.5000	1.8854

Figure 3.31. Calibration curve and GC-MS data for Testosterone-di-TMS

The correlation coefficients of the curves for AND, ETIO, DHEA, epiAND, epiTEST and TEST were 1.0000, 0.9999, 0.9805, 0.9971, 0.9988 and 0.9996, respectively. When linear regression was used initially instead of polynomial regression, the regression line did not fit the data well and the correlation coefficient was much lower.

The limits of detection (**LOD**, calculated using the statistical application package of **Microsoft Excel**) for AND, ETIO, DHEA, epiAND, epiTEST and TEST were 5.4, 13.9,

7.2, 0.4, 1.4 and 0.8 pg on column, respectively.

3.3.3.5. Nail samples

Five (5) nail samples from anabolic steroid abusers and two (2) nail samples of healthy persons were analysed for the identification and determination of endogenous anabolic steroids. Norepiandrosterone-d_3 (d3-NEA) and testosterone-d_3 (d3-TEST) were used as internal standards for the quantitative GC-MS analysis of endogenous steroids.

As shown in **Table 3.10**, the concentrations of the steroids in nail were extremely low compared to plasma and urine. As exceptions, the concentrations of endogenous steroids in nail samples 3 and 4 were high compared with other nail samples [**Table 3.10**].

Table 3.10. Concentrations of endogenous steroids in nail samples (pg/mg), plasma (pg/mL), and urine (pg/mL)

Analyte	AND	ETIO	DHEA	epiAND	epiTEST	TEST
AAS Abusert #1(Nail	4.1	0.0	5.6	3.3	3.0	4.3
AAS Abuser #2(Nail)	4.7	0.0	3.0	1.9	1.5	3.9
AAS Abuser #3(Nail)	**9.6**	**0.0**	**25.3**	**14.3**	**11.0**	**10.6**
AAS Abuser #4(Nail)	**12.8**	**0.0**	**12.0**	**6.2**	**10.9**	**25.0**
AAS Abuser #5(Nail)	1.7	0.0	1.8	0.8	0.6	0.6
Healthy #1(Nail)	1.5	0.0	1.4	0.7	0.6	6.5
Healthy #2(Nail)	8.4	0.0	4.5	1.7	2.6	1.1
Healthy #1(Plasma)	30322.9	54222.5	11218.3	Trace	222.2	3583.8
Healthy #2(Plasma)	9923.3	2966.7	100.6	Trace	200.2	196.0

Note: The concentration of endogenous steroids in nail samples #3 and #4 are high compared with other nail samples. AAS = Anbolic Androgenic Steroids

Tables 3.11-1 – 3.11-3 show the peak areas of selected ion chromatograms of each endogenous steroid in nail samples (No.1 – No. 8) and of deuterated internal standards.

Table 3.11-1. Quantitation of endogenos steroids in nail

No.	Name	Nail Sample 01, 8.32 mg				
		Peak area	I.Std area	Ratio	Total (ng)	C (pg/mg)
1	d3-NEA	44736032	44736032	1.0000		
2	AND	809637	44736032	0.0181	0.0181	2.18
3	ETIO					
4	DOP					
5	DHEA	*256902*	239070953	0.0011	0.0060	0.72
6	epiAND	*734927*	239070953	0.0031	0.0038	0.46
7	epiTEST	*2010500*	239070953	0.0084	0.0208	2.50
8	d3-TEST	239070953	239070953	1.0000		
9	TEST	8497842	239070953	0.0355	0.0411	4.94

No.	Name	Nail Sample 02, 15.10 mg				
		Peak area	I.Std area	Ratio	Total (ng)	C (pg/mg)
1	d3-NEA	20535570	20535570	1.0000		
2	AND	619765	20535570	0.0302	0.0302	2.00
3	ETIO					
4	DOP					
5	DHEA					
6	epiAND	*1178585*	254627378	0.0046	0.0057	0.38
7	epiTEST					
8	d3-TEST	254627378	254627378	1.0000		
9	TEST	18032104	254627378	0.0708	0.0819	5.42

No.	Name	Nail Sample 03, 1.84 mg				
		Peak area	I.Std area	Ratio	Total (ng)	C (pg/mg)
1	d3-NEA	50711124	50711124	1.0000		
2	AND	*349115*	50711124	0.0069	0.0069	3.75
3	ETIO					
4	DOP					
5	DHEA	210808	226908419	0.0009	0.0049	2.66
6	epiAND	*455244*	226908419	0.0020	0.0025	1.36
7	epiTEST	*158496*	226908419	0.0007	0.0017	0.92
8	d3-TEST	226908419	226908419	1.0000		
9	TEST	2421613	226908419	0.0107	0.0124	6.74

Note: Peak area = area of endogenous steroid peak in mass chromatogram, I.Std area = area of internal standard peak, d3-NEA = Norepiandrosterone-d_3, d3-Test = Testosterone-d_3, Total (ng) = total weight in ng of endogenous steroid present, C = concentration of endogenous steroids in pg/mg of nail. Numerical values underlined in **Table 3.11-1** were calculated manually.

Table 3.11-2. Quantitation of endogenos steroids in nail						
No.	Name	Nail Sample 04, 4.24 mg				
		Peak area	I.Std area	Ratio	Total (ng)	C (pg/mg)
1	d3-NEA	19319589	19319589	1.0000		
2	AND	371599	19319589	0.0192	0.0192	4.53
3	ETIO					
4	DOP					
5	DHEA	*905713*	327652941	0.0028	0.0153	3.61
6	epiAND	*697974*	327652941	0.0021	0.0026	0.61
7	epiTEST	13590560	327652941	0.0415	0.1026	24.20
8	d3-TEST	327652941	327652941	1.0000		
9	TEST	45995392	327652941	0.1404	0.1624	38.30

No.	Name	Nail Sample 05, 35.91 mg				
		Peak area	I.Std area	Ratio	Total (ng)	C (pg/mg)
1	d3-NEA	52861302	52861302	1.0000		
2	AND	1619555	52861302	0.0306	0.0306	0.85
3	ETIO					
4	DOP					
5	DHEA	*3510129*	284930111	0.0123	0.0673	1.87
6	epiAND	677282	284930111	0.0024	0.0030	0.08
7	epiTEST	1131874	284930111	0.0040	0.0099	0.28
8	d3-TEST	284930111	284930111	1.0000		
9	TEST	*4592514*	284930111	0.0161	0.0181	0.50

No.	Name	Nail Sample 06, 38.52 mg				
		Peak area	I.Std area	Ratio	Total (ng)	C (pg/mg)
1	d3-NEA	35625802	35625802	1.0000		
2	AND	*1005906*	35625802	0.0282	0.0282	0.73
3	ETIO					
4	DOP					
5	DHEA	*5039407*	301798807	0.0167	0.9140	23.73
6	epiAND	*341196*	301798807	0.0011	0.0014	0.04
7	epiTEST	*1471868*	301798807	0.0049	0.0121	0.31
8	d3-TEST	301798807	301798807	1.0000		
9	TEST	102621502	301798807	0.3400	0.3933	10.21

Note: Peak area = area of endogenous steroid peak in mass chromatogram, I.Std area = area of internal standard peak, d3-NEA = Norepiandrosterone-d_3, d3-Test = Testosterone-d_3, Total (ng) = total weight in ng of endogenous steroid present, C = concentration of endogenous steroids in pg/mg of nail. Numerical values underlined in **Table 3.11-2** were calculated manually.

Table 3.11-3. Quantitation of endogenos steroids in nail

No.	Name	Nail Sample 07, 20.09 mg				
		Peak area	I.Std area	Ratio	Total (ng)	C (pg/mg)
1	d3-NEA	340443	340443	1.0000		
2	AND	*31279*	340443	0.0919	0.0919	4.57
3	ETIO					
4	DOP					
5	DHEA	*24297*	842317	0.0288	0.1576	7.84
6	epiAND	*26036*	842317	0.0309	0.0381	1.90
7	epiTEST	*42870*	842317	0.0509	0.1258	6.26
8	d3-TEST	842317	842317	1.0000		
9	TEST	*12717*	842317	0.0151	0.0175	0.87

No.	Name	Nail Sample 08, 75.28 mg				
		Peak area	I.Std area	Ratio	Total (ng)	C (pg/mg)
1	d3-NEA	35390471	35390471	1.0000		
2	AND	*264097*	35390471	0.0075	0.0075	0.10
3	ETIO					
4	DOP					
5	DHEA	14129134	279165398	0.0506	0.2768	3.68
6	epiAND	*2513360*	279165398	0.0090	0.0110	0.15
7	epiTEST	*5129319*	279165398	0.0184	0.0455	0.60
8	d3-TEST	279165398	279165398	1.0000		
9	TEST	*6023063*	279165398	0.0216	0.0250	0.33

Note: Peak area = area of endogenous steroid peak in mass chromatogram, I.Std area = area of internal standard peak, d3-NEA = Norepiandrosterone-d_3, d3-Test = Testosterone-d_3, Total (ng) = total weight in ng of endogenous steroid present, C = concentration of endogenous steroids in pg/mg of nail. Numerical values underlined in **Table 3.11-3** were calculated manually.

3.3.3.6. Blood (plasma) samples

The concentrations of steroids in plasma samples from two healthy persons were measured along with the concentrations of steroids in the fingernails of steroid abusers for the purposes of method verification and comparison between the different matrices. The concentrations of endogenous steroids in plasma were higher than in nail [**Table 3.12**].

Table 3.12. Quantitation of endogenous steroids in plasma.

No.	Name	010304sim07 (plasma)				
		Peak area	I.Std area	Ratio	Total (ng)	C (ng/mL)
1	d3-NEA	271210	271210	1.0000		
2	AND	1460760	271210	5.3861	17.8299	17.8299
3	ETIO	566059	271210	2.0872	8.4567	8.4567
4	DOP					
5	DHEA	89098	2276386	0.0391	0.3823	0.3823
6	epiAND					
7	epiTEST	698526	2276386	0.3069	0.9530	0.9530
8	d3-TEST	2276386	2276386	1.0000		
9	TEST	591373	2276386	0.2598	0.6783	0.6783

No.	Name	010304sim06 (plasma)				
		Peak area	I.Std area	Ratio	Total (ng)	C (ng/mL)
1	d3-NEA	2117346	2117346	1.0000		
2	AND	*34344613*	2117346	16.2206	53.7210	53.7210
3	ETIO	*8077801*	2117346	3.8151	15.4576	15.4576
4	DOP					
5	DHEA	226985176	27269223	8.3239	81.3811	81.3811
6	epiAND					
7	epiTEST	13380377	27269223	0.4907	1.5238	1.5238
8	d3-TEST	27269223	27269223	1.0000		
9	TEST	90837415	27269223	3.3311	8.6972	8.6972

Note: Peak area = area of endogenous steroid peak in mass chromatogram, I.Std area = area of internal standard peak, d3-NEA = Norepiandrosterone-d_3, d3-Test = Testosterone-d_3, Total (ng) = total weight in ng of endogenous steroid present, C = concentration of endogenous steroids in ng/mL of plasma. Numerical values underlined in **Table 3.12** were calculated manually.

3.3.3.7. Urine samples

As for the plasma samples, the concentrations of steroids in urine collected from the same two healthy persons were measured along with the concentrations of steroids in fingernail from steroid abusers for the sake of comparison **[Table 3.13]**. In each case, the concentrations of endogenous steroids in the urine samples were high compared with those in the nail samples.

However, the following considerations are necessary with respect to the experimental procedure used and its effect on steroid concentrations. That is, the steroid analytes were extracted from the urine samples with an SPE method, and subsequently the steroids were re-extracted after enzyme hydrolysis. Therefore, the recovery of each steroid became lower. However, the measured concentrations of the steroids are accurate because deuterated internal standard samples were added at the same time, which would compensate for losses.

Table 3.13. Quantitation of endogenos steroids in urine.

No.	Name	061103sim5				
		Peak area	I.Std area	Ratio	Total (ng)	C (ng/mL)
1	d3-NEA	***24354***	***24354***	1.0000		
2	AND	***99576***	***24354***	4.0887	4.4240	0.8848
3	ETIO	***142112***	***24354***	5.8353	4.3491	0.8698
4	DOP					
5	DHEA	2307512	7794203	0.2961	0.0710	0.0142
6	epiAND	**664817**	**7794203**	**0.0853**	0.1098	0.0220
7	epiTEST	212030	7794203	0.0272	0.0370	0.0074
8	d3-TEST	7794203	7794203	1.0000		
9	TEST	659309	7794203	0.0846	0.0681	0.0136

No.	Name	061103sim2				
		Peak area	I.Std area	Ratio	Total (ng)	C (ng/mL)
1	d3-NEA	***1342659***	***1342659***	1.0000		
2	AND	***1228466***	***1342659***	0.9150	1.7002	0.3400
3	ETIO	***1529742***	***1342659***	1.1393	1.7969	0.3594
4	DOP					
5	DHEA	18756211	189755389	0.0988	0.0516	0.0103
6	epiAND	**4886584**	**189755389**	**0.0258**	0.0492	0.0098
7	epiTEST	7175217	189755389	0.0378	0.0438	0.0088
8	d3-TEST	189755389	189755389	1.0000		
9	TEST	12277462	189755389	0.0647	0.0548	0.0110

Note: Peak area = area of endogenous steroid peak in mass chromatogram, I.Std area = area of internal standard peak, d3-NEA = Norepiandrosterone-d_3, d3-Test = Testosterone-d_3, Total (ng) = total weight in ng of endogenous steroid present, C = concentration of endogenous steroids in ng/mL of urine. Numerical values underlined in **Table 3.13** were calculated manually.

3.3.4. Results obtained

The endogenous anabolic steroids androsterone (1.5–12.8 pg/mg), etiocholanolone, dehydroepiandrosterone (1.4–25.3 pg/mg), epiandrosterone (0.7–14.3 pg/mg), epitestosterone (0.6–11.0 pg/mg), and testosterone (0.6–25.0 pg/mg) were identified and quantified in fingernail samples. The concentration of each steroid in the fingernail clippings was extremely low compared with the concentrations in urine and blood **(plasma)** as shown in parentheses. In general, higher concentrations of testosterone were associated with higher concentrations of androsterone, dehydroepiandrosterone, epiandrosterone and epitestosterone. In particular, the concentrations of endogenous anabolic steroids were extremely high in the fingernail clippings of the anabolic steroid abusers who also showed high concentrations of testosterone in urine.

The presence of exogenous **(synthetic)** anabolic steroids was investigated using gas chromatography - mass spectrometric analysis of fingernail clippings from five anabolic steroid abusers. As a result of these qualitative analyses, it was found that testosterone isocaproate and testosterone enanthate were difficult to detect. Although the analytical results provided some evidence for the presence of anabolic steroids in the samples from steroid users, including testosterone and testosterone esters at low concentrations, it has not yet been possible to confirm this due to interference from other endogenous substances. Further work is necessary to clean up the initial extracts and to resolve the interference.

As a result of this study, a fundamental analytical method was obtained for anabolic steroids in fingernail clippings by GC-MS along with initial analytical data. However, more work is needed in future on the analysis of nail samples from a larger population of doping steroid abusers to overcome the problems identified here.

3.4. Conclusions

Qualitative and quantitative analyses of anabolic steroids in nail samples were successfully carried out by GC-MS using the cryogenic grinding method for sample preparation. Trimethylsilyl (**TMS**) derivatives of doping anabolic steroids were used for sample preparation for GC-MS analysis. An amended method was found to work well for the preparation of the TMS derivatives compared with methods described in published articles. This overcame the problem of dissolving the catalyst, ammonium iodide in the silanising reagent, MSTFA and was good for trimethylsilylation of doping steroids having enolic, ketonic and sterically hindered hydroxy groups because of complete mixing of the reagents. As a result, endogenous anabolic androgenic steroids (androsterone, etiocholanolone, dehydroepitestosterone, epiandrosterone, epitestosterone, and testosterone) were identified and determined by GC-MS in the selected ion monitoring mode.

Exogenous (**synthetic**) anabolic steroids from nail samples of anabolic steroid users could not be detected though testosterone isocaproate and testosterone enanthate were tentatively identified. A peak appeared in the nail extracts at the same retention time as that of a reference standard of testosterone isocaproate. It was not possible to confirm its presence due to the co-elution of an interfering component, although the mass spectrum of the peak corresponded to the mass spectrum of the reference standard. A similar result was obtained with respect to the identification of testosterone enanthate. If high-resolution GC-MS, GC-MS-MS or GC-MS^n analysis were used along with deuterated internal standards (deuterated testosterone esters), the identification of these dubious peaks might be able to be confirmed.

In general, endogenous steroid concentrations in nail were low, in the pg/mg range. However, it was noted that the concentrations of endogenous steroids in two nail samples

from steroid abusers were much higher than those in other samples. The testosterone/epitestosterone ratio was high in these two samples compared with the other samples in this study. The reason for this is not known, as concentrations of endogenous steroids are expected to decrease when exogenous anabolic steroids are administered, the basis of the current urine test for anabolic steroid abuse.

Qualitative and quantitative analyses of androgenic steroids in the urine and plasma samples of healthy volunteers were carried out at the same time. As a result, in general, elevated concentrations of testosterone in nail were positively associated with high concentrations in plasma and urine.

Nail remains a potential, but still to be confirmed, alternative biological specimen to hair for the detection of past exposure to doping steroids. It will be necessary to analyse nail samples from a much larger population and collect a statistically-significant set of data for elucidation of these unresolved problems. Moreover, it will be necessary to analyse conjugated steroids in the fingernail as well as free steroids. Further methodological developments will be needed to improve the clean-up of nail extracts to reduce the effects of interferences on the analysis of steroids.

4. Analysis of Opium Alkaloids using Pyrolysis GC-MS and Matrix Assisted Laser Desorption Ionisation - Time of Flight - Mass Spectrometry.

This study was conducted in **the Identification Research Laboratory, National Research Institute of Police Science,** Tokyo, Japan, during a period of study at an external institution approved by **the Faculty of Science, University of Glasgow.**

A. Analyses of Opium Alkaloids using Pyrolysis GC-MS

4.1. Introduction

Gas chromatography-mass spectrometry (**GC-MS**) has been the most commonly used method for the analysis of opium alkaloids. When opium alkaloids in fingernail clippings are analysed, the analytical procedure necessarily involves GC-MS after extraction of the alkaloids from the fingernail samples. However, it is possible that street heroin residues in fingernail samples can be directly analysed by pyrolysis GC-MS (**Py-GC-MS**). Moreover, the analytical procedure for street heroin in nail would be simplified, and there is the simultaneous possibility of being able to apply the method to the analysis of seized street heroin.

Published articles on the analysis of opium alkaloids by Py-GC-MS are extremely few in number [183, 184, 185, 186, 187] though many other analytical methods have been used, as described in the section on the analysis of street heroin in **Chapter 2.**

The most important reason is that thermal analysis instruments are not yet widespread in the field of forensic science. In the references of many Py-GC-MS papers concerning opium alkaloids, few report that the temperature of the thermal degradation is

correctly controlled. In this study, Py-GC-MS of opium was achieved by using both the Curie-point pyrolyser and the double shot pyrolyser, as well as a normal gas chromatographic instrument that allowed the temperature of thermal degradation to be set to the optimum value.

There were two purposes in this Py-GC-MS study. The first was to prove whether it is possible to analyse opium alkaloids in nail directly using Py-GC-MS without extracting the drug from the fingernail.

The second was to reduce the weight of the nail sample needed for the analyses of opium alkaloids by thermal analysis. In the work carried out, fingernail samples containing opium alkaloids were not available, and spiked samples were prepared by adding opium alkaloids to powdered fingernail clippings instead.

As a result, it was found that opium alkaloids could be analysed by Py-GC-MS. There was no thermal degradation of the alkaloids if they were converted to TMS derivatives and excellent Py-GC-MS analysis could be performed.

4.2. Methods and Materials - Samples

1) Turkish opium

2) Unknown opium

The opium samples were obtained from stock of the Department of Forensic Medicine and Science, University of Glasgow. The opium was dissolved with methanol and a working solution with a concentration of 1.0 μg/mL of opium was prepared.

3) Nail clippings

Nail clipping samples were obtained from healthy volunteers who were non-opiate users.

4.3. Methods and Materials - Instruments

The following instruments were used:

1) **HP6890 GC + HP5973N MSD (HP, USA);**

2) **HP6890 GC + HP5973N MSD with Double-shot pyrolyser, model PY-2020D, (Frontier Lab, Japan);**

3) **Shimadzu GC-MS QP-5000 (Shimadzu, Japan)** with Curie-point pyrolyser **(Japan Analytical Industry Co., Japan).**

4.4. Pyrolysis GC-MS without pyrolyser (injection port of gas chromatograph)

4.4.1. Principle

The analyte is decomposed by heating in the injection port of gas chromatograph **(GC).**

4.4.2. Gas chromatography - mass spectrometric (GC-MS) analysis

Two (2) μL of a methanol solution of the opium (2 μg) was injected into the gas chromatograph - mass spectrometer **(GC-MS).**

4.5. Pyrolysis GC-MS with pyrolyser (double shot furnace)

4.5.1. Principle

Figure 4.1 shows the main body of the double-shot pyrolyser. **Figure 4.2** shows the state to set up the pyrolyser and the controller of the double-shot pyrolyser in the **Hewlett Packard GC.**

As shown in **Figure 4.3**, the crucible, made of stainless steel, is set up in the upper part of the furnace and kept at a constant temperature. A sample of about 0.1 mg in weight is put in this crucible. The crucible falls into the furnace and the sample is decomposed by heating. The generated gas is introduced into the GC for six (6) sec and

analysed. The injection port of gas chromatograph (**GC**) is modified to meet the pyrolyser, but the split ratio (10:1) of the GC is the same as in conventional gas chromatography.

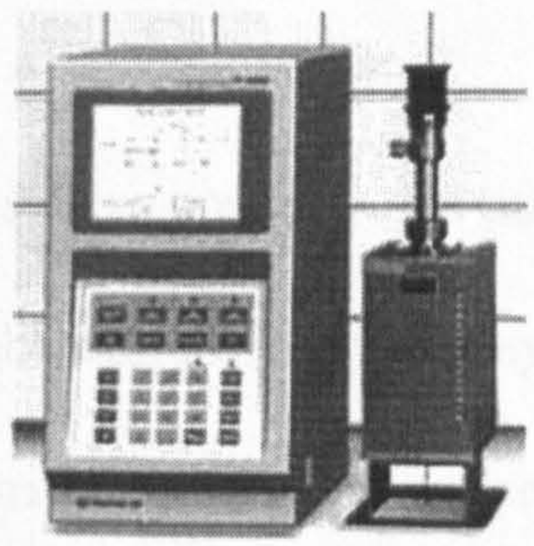


Figure 4.1. Double-shot pyrolyser, model PY-2020D

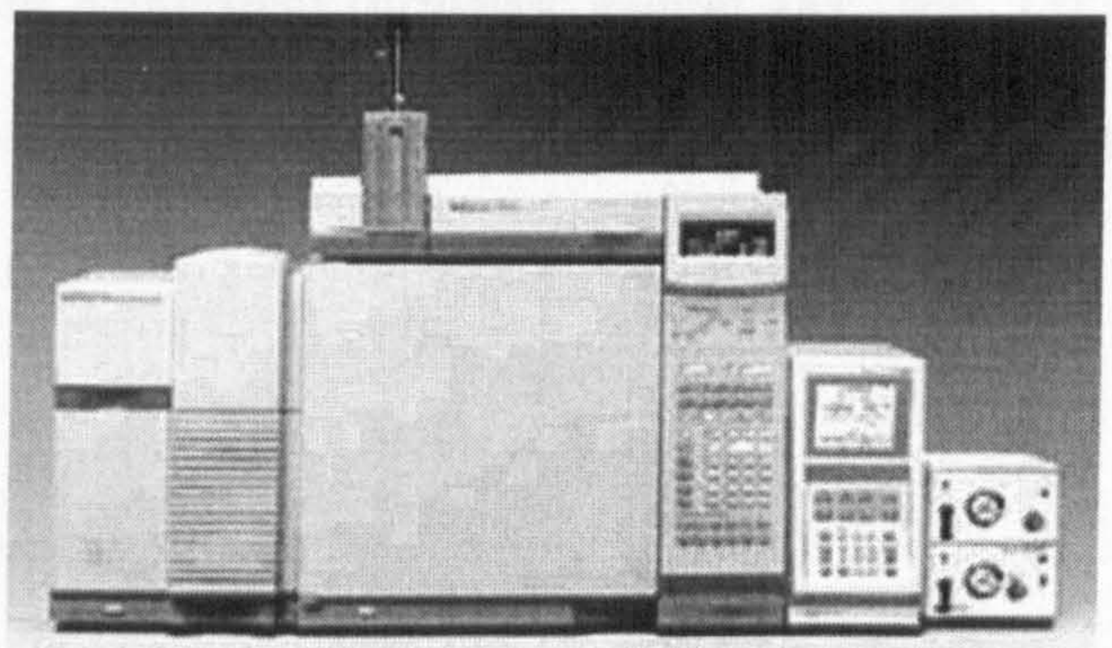


Figure 4.2. HP GC-MS fitted with Frontier Lab's pyrolyser
(model: Thermal analysis system 2020)

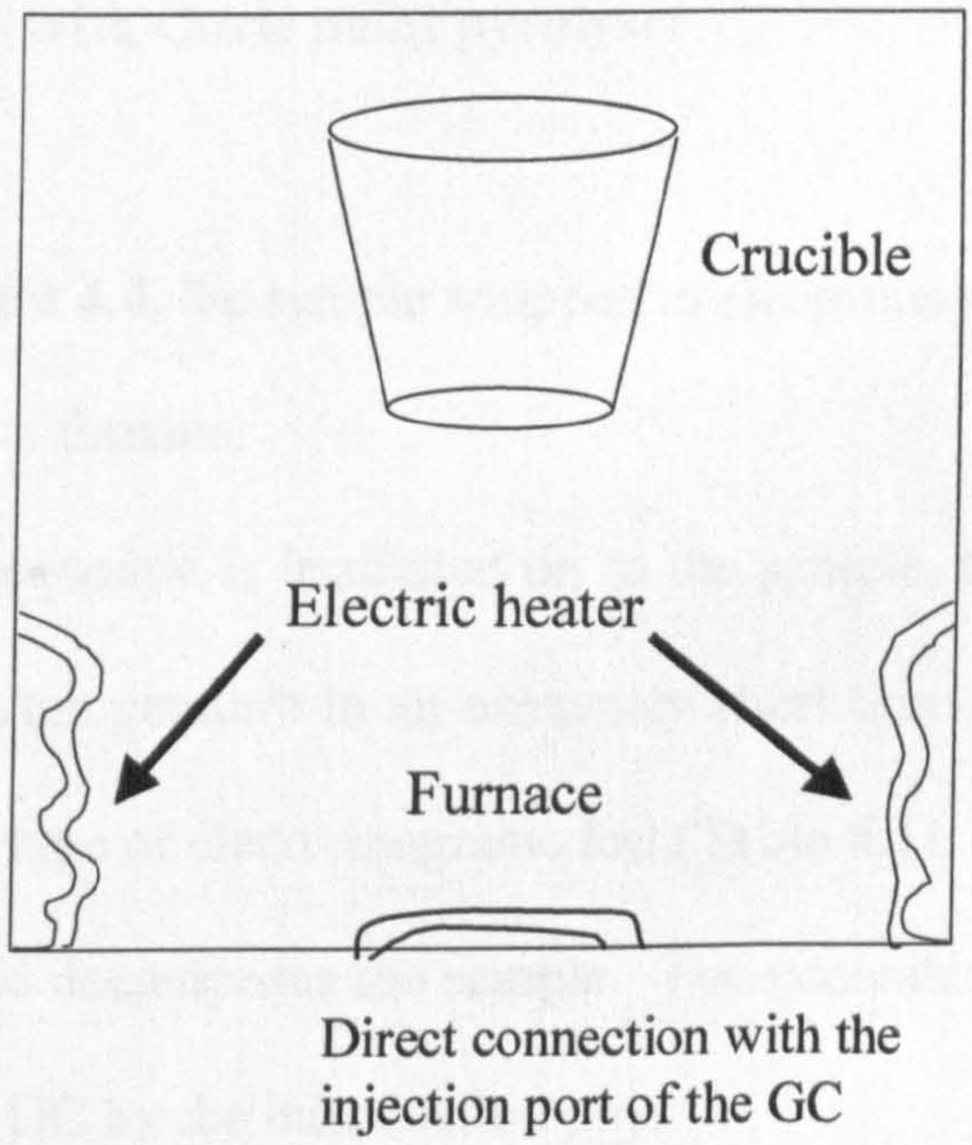


Figure 4.3. Outline of the double shot pyrolyser

4.5.2. Sample preparation for pyrolysis GC-MS

300 µL of a solution of ethylmorphine hydrochloride in methanol (1.01 µg/µL) and 300 µL of a solution of morphine in methanol (1.06 µg/µL) were added to 15.3 mg of powdered fingernail clippings in a micro-test tube.

The nail-methanol mixtures were concentrated and dried using a rotary evaporator (**Buchi**, Germany). The sample therefore contained ethylmorphine (20 µg) and morphine (20 µg) respectively per milligram of fingernail powder.

The nail powder was obtained by pulverising nail clippings with a cryogenic milling device (**Japan Analytical Industry, model JFC-300**, Tokyo, Japan), for 5 min under liquid nitrogen, after the samples had been decontaminated by the method described in **Chapter 2**.

4.5.3. Pyrolysis GC-MS analyses

0.3–2.0 mg of powdered nail samples was analysed by Py-GC-MS.

4.6. Pyrolysis GC-MS with Curie point pyrolyser

4.6.1. Principle

As shown in **Figure 4.4**, the sample wrapped in electromagnetic foil is set up in the high frequency induction furnace.

When the high frequency is irradiated on to the sample, the electromagnetic foil reaches a constant high temperature in an extremely short time (sec). The temperature reached depends on the type of electromagnetic foil (**Table 4.1**).

The heat generated decomposes the sample. The generated gas is introduced into the injection port of the GC by the introduction pipe.

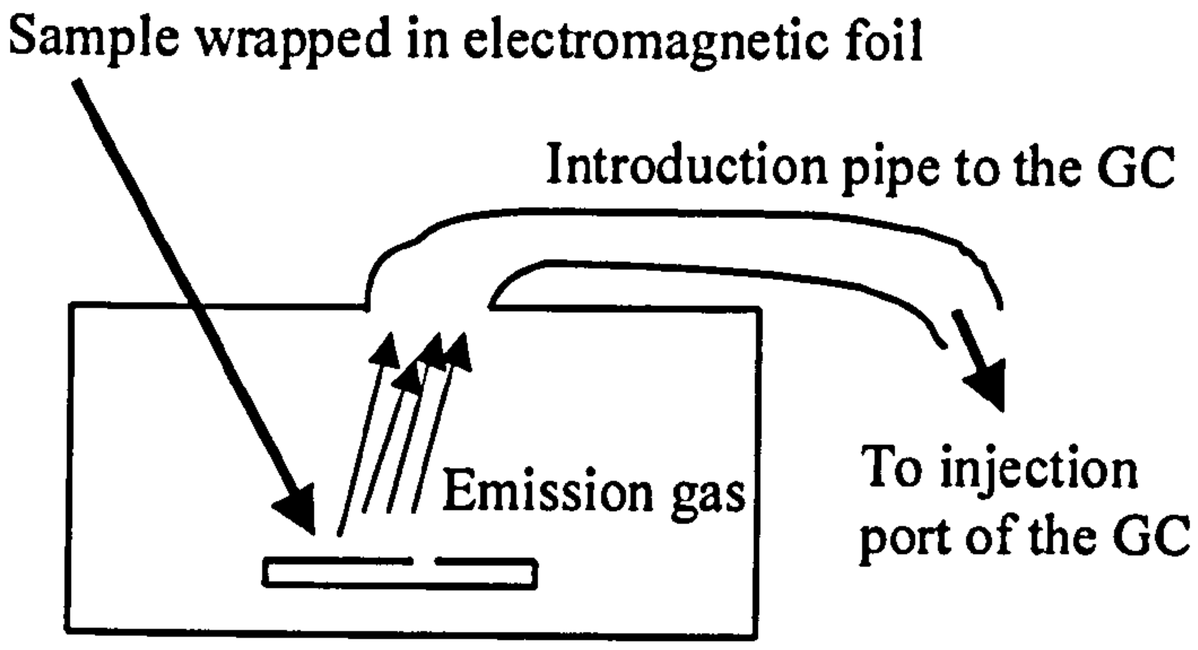


Figure 4.4. Outline of the Curie point pyrolyser

Table 4.1. Curie point temperatures of pyrolysis probes

Curie point temperature (°C)	Composition
1040	Co
920	Co, Fe
764	Fe
740	Fe, Other
670	Fe, Other
650	Stainless
590	Co, Ni
500	Co, Ni
485	Ni, Other
445	Ni, Fe, Other
386	Ni, Fe, Other
358	Ni, Other
280	Ni, Cu
255	Ni, Cu, Other
235	Ni, Cu, Other
220	Ni, Cu, Other

The introduction pipe is kept at a constant high temperature, and prevents the emission gas being adsorbed.

4.6.2. Sample preparation for pyrolysis GC-MS

The samples used were the same sample as for Curie point Py-GC-MS.

4.7. Results and Discussion

4.7.1. Pyrolysis GC-MS without pyrolyser (Thermal degradation at injection port of the GC)

(a) Free (base) opium alkaloids

Figure 4.5 shows total ion chromatogram (**TIC**) of the GC-MS analysis of 2 μg of the methanol extracts of Turkish opium.

Codeine, morphine, and thebaine were determined as the main morphine alkaloids present as shown in **Table 4.2**. The quality parameter in **Table 4.2** shows degree (%) of agreement obtained between the sample spectrum and the library spectrum in a computer search of the mass spectral database from **Hewlett Packard Co.**

Similarly, **Figure 4.6** shows the TIC from GC-MS of 2 μg of the methanol extracts of the unknown opium. Codeine, morphine, and thebaine were also determined to be the main morphine alkaloids present, as shown in **Table 4.3.**

(b) Trimethylsilylated opium alkaloids.

To examine the thermal stability of the morphine alkaloids during GC, the TMS derivatives were analysed using the same GC-MS conditions (**Figure 4.7**).

Trimethylsilylated opium was prepared by reacting opium with N,O-bis (trimethylsilyl)trifluoroacetamide (**BSTFA**) containing 1% of dimethylchlorosilane (**DMCS**) at 60 °C for 30 min. As shown in **Table 4.4,** the pyrolysis compounds were detected with a higher degree of certainty compared with **Table 4.1.**

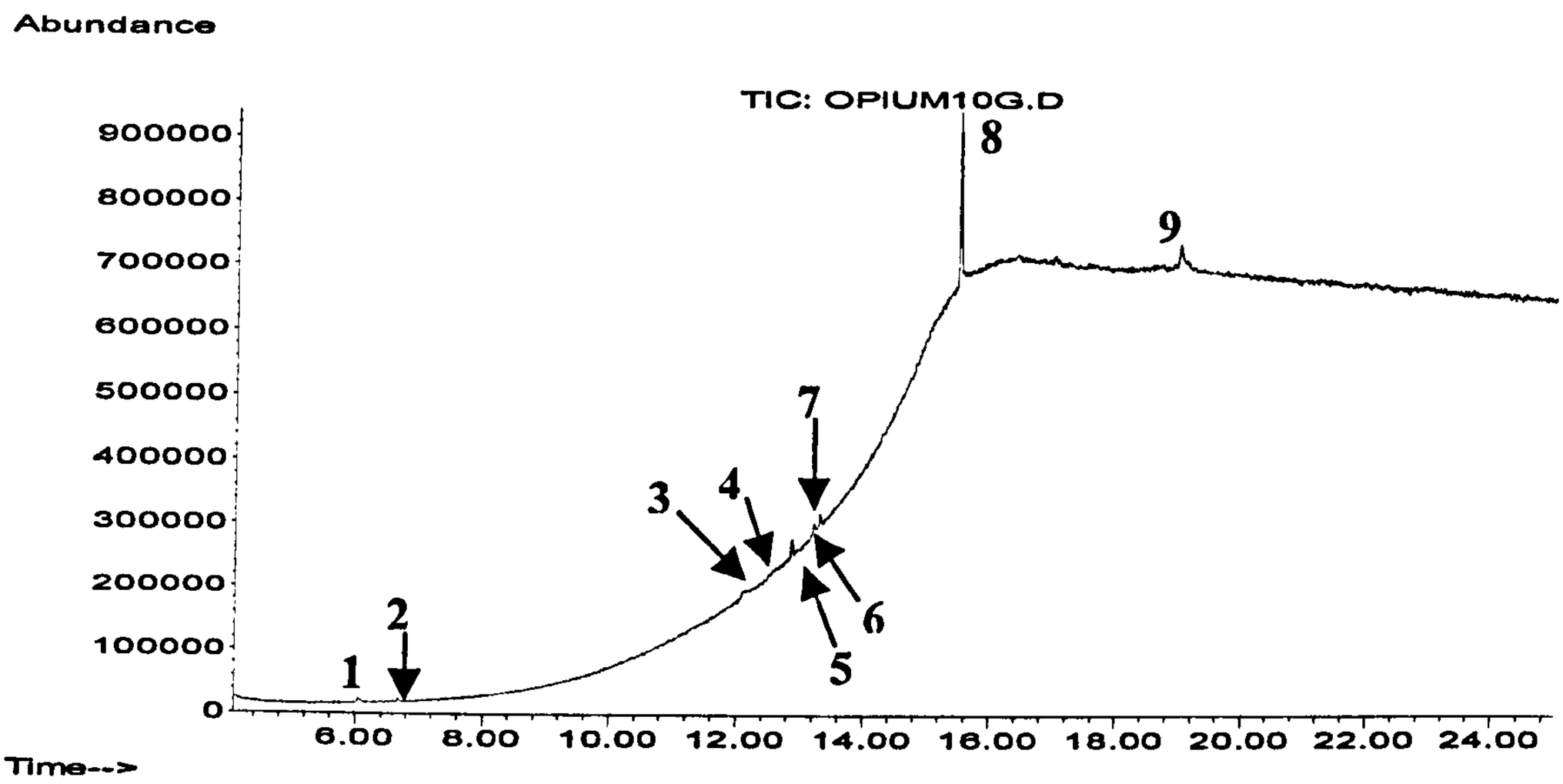


Figure 4.5. Total ion chromatogram (**TIC**) of Turkish opium (2 μg)

Table 4.2. Detected opiates and retention times (Turkish opium)

Peak No.	Rt (min)	Compound	Ions used for identification	Quality (%)
1	6.041	Meconin	165, ***194***, 147	52
2	6.673	Hydrocotarnine	220, ***221***, 178	76
3	12.109	Codeine	***299***, 162, 229	55
4	12.541	Morphine	***285***, 162, 215	0
5	12.882	Thebaol	***203***, 175	89
6	13.223	Thebaine	***311***, 255, 296	70
7	13.323	Thebaine	***311***, 255, 296	78
8	15.520	Papaverine	324, 338, ***339***	99
9	19.020	Noscapine	220, 205	64

Notes: Rt = Retention time, Quality (%) = Match (% fit) of mass spectrum between sample and library spectrum. ***Bold, italic and underline*** shows molecular ion (*m/z*).

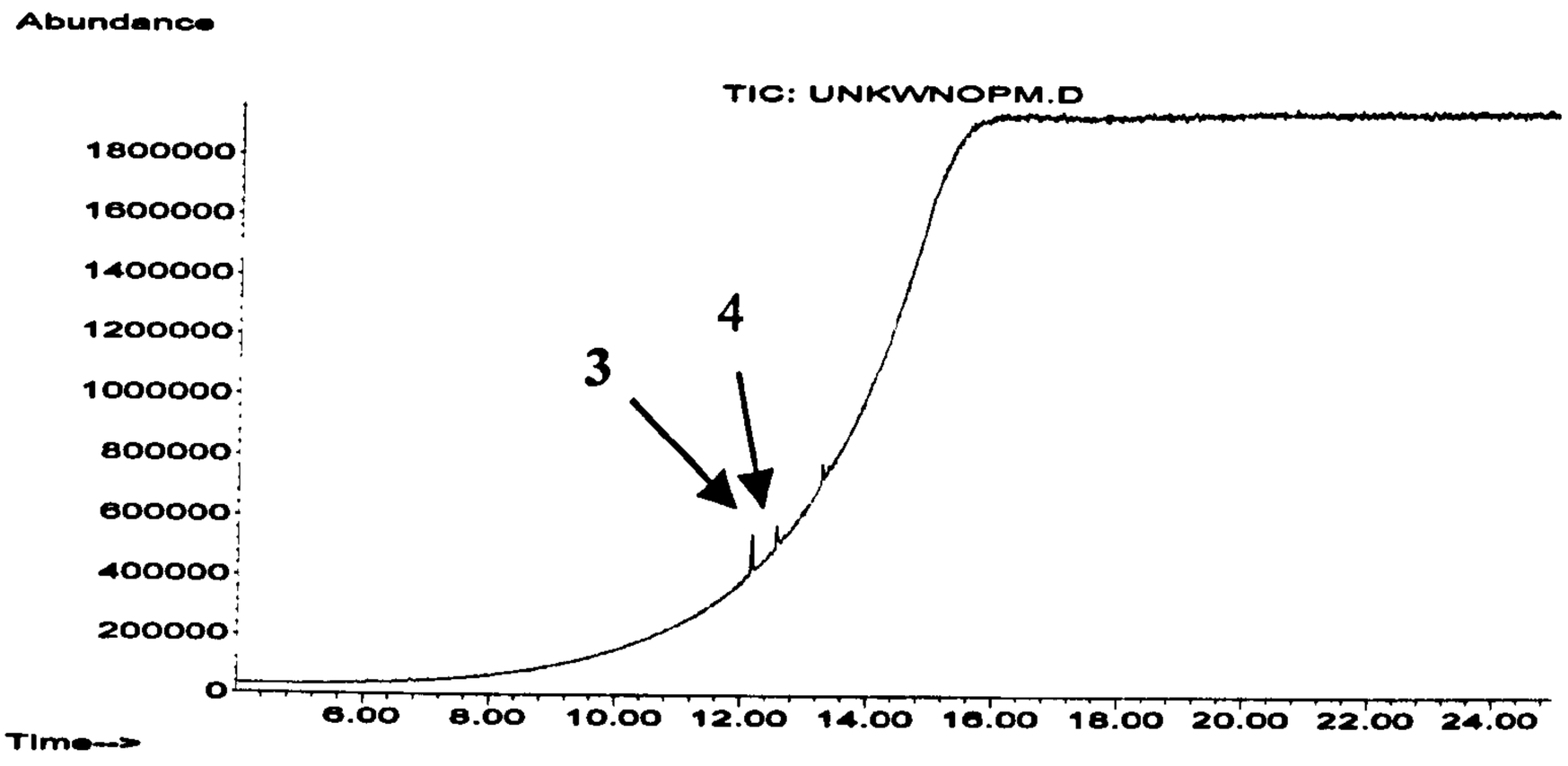


Figure 4.6. TIC of GC-MS (Unknown opium, 2 μg)

Table 4.3. Detected opiates and retention times (Unknown opium)

Peak No.	Rt (min)	Compound	Ions used for identification	Quality (%)
1	6.089	Meconin	165, ***194***, 147	38
2	6.756	Hydrocotarnine	220, ***221***, 178	64
3	12.177	Codeine	***299***, 162, 229	98
4	12.578	Morphine	***285***, 162, 215	94
5	13.290	Thebaine	***311***, 255, 296	86
6	13.401	Thebaine	***311***, 255, 296	50
7	15.607	Papaverine	324, 338, ***339***	25
8	19.178	Noscapine	220, 205	10

Notes: Rt = Retention time, Quality (%) = Match (% fit) of mass spectrum between sample and library spectrum. ***Bold, italic and underline*** shows molecular ion (*m/z*).

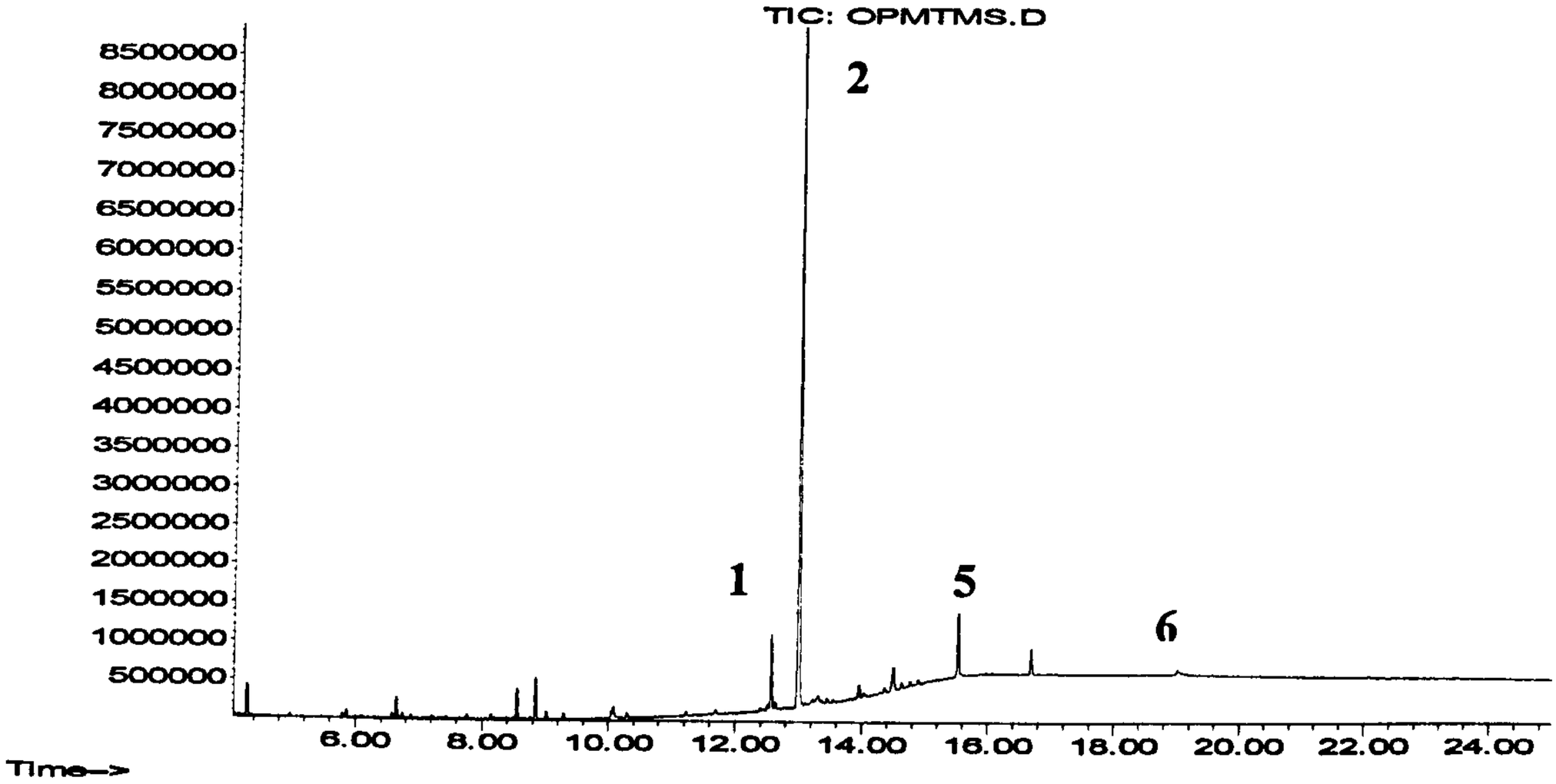


Figure 4.7. TIC of GC-MS (TMS-Turkish opium)

Table 4.4. Retention time and matching rate of TMS-Turkish opium (10 μg of TMS-Turkish opium)

Peak No.	Rt (min)	Compound	Ions used for identification	Quality (%)
1	12.571	Codeine-TMS	***371***, 234, 313	98
2	13.002	Morphine-di-TMS	***429***, 41, 401	99
3	13.223	Thebaine	***311***, 255, 296	83
4	13.323	Thebaine	***311***, 255, 296	99
5	15.529	Papaverine	324, 338, ***339***	99
6	19.020	Noscapine	220, 205	72

Notes: Rt = Retention time, Quality (%) = Match (% fit) of mass spectrum between sample and library spectrum. ***Bold, italic and underline*** shows molecular ion *(m/z)*.

4.7.2. Pyrolysis GC-MS with double shot pyrolyser

The thermal stability of opium free bases and their derivatives, including acetyl and trimethylsilyl derivatives, were examined.

4.7.2.1. GC-MS analyses of free opium alkaloids

A quantity of opium weighing about 1.0 mg was analysed by Py-GC-MS as shown in **Figures 4.8** and **4.9** and **Tables 4.5** and **4.6**. The pyrolysis temperature was 350 °C.

This temperature was set as an optimum temperature of pyrolysis following trials of a range of temperatures. The pyrolysis temperature in the injection port of the GC was also set at 280 °C as an optimum temperature. Therefore, it can be confirmed that the sample was decomposed in pyrolysis GC-MS analysis if a pyrolyser was used.

3,5-dimethoxy-4,5-epoxyphenanthrene was detected as a product of thermal degradation from both the Turkish and the unknown opium. Also, Neopine (**ß-codeine**) was detected as an interesting compound from Turkish opium.

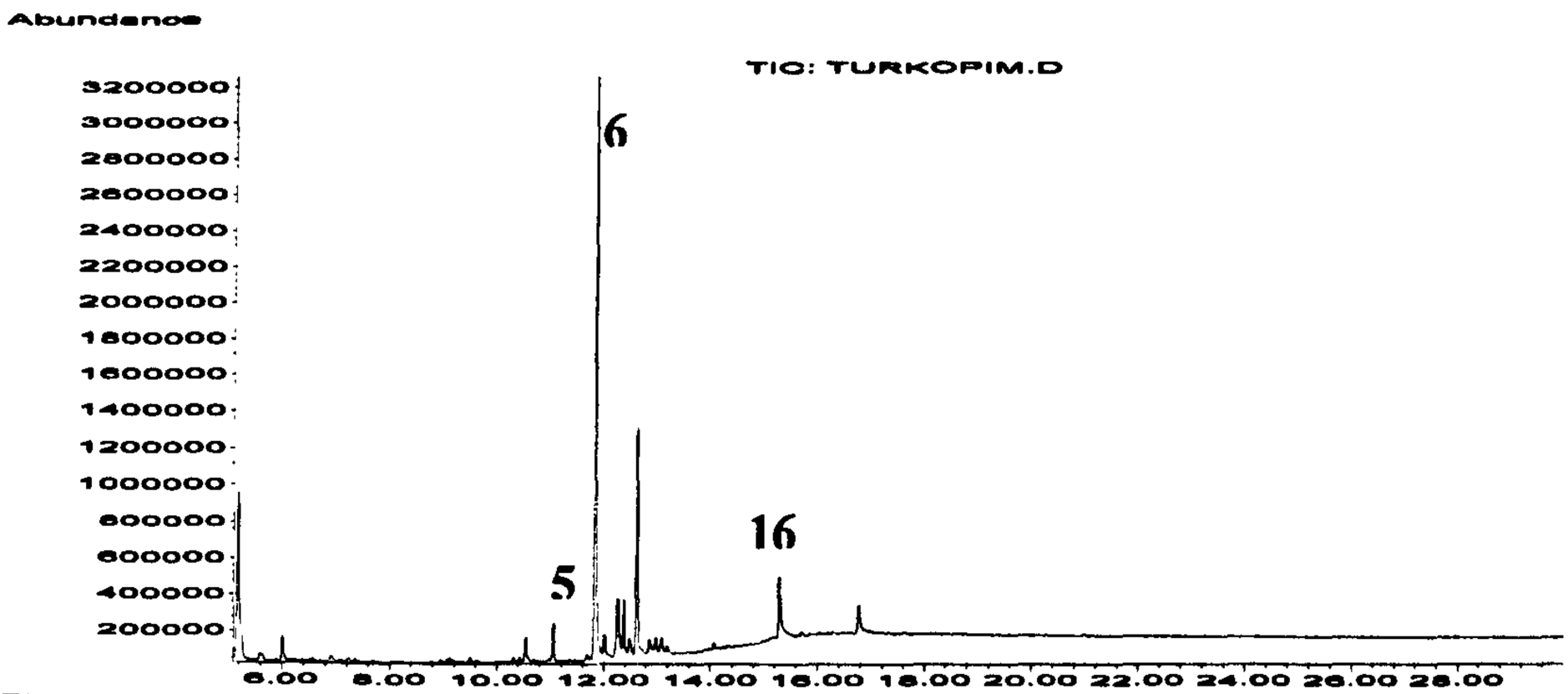


Figure 4.8. TIC of Py-GC-MS (about 1 mg Turkish opium)

Table 4.5. Detected opiates and retention time by Py-GC-MS (Turkish opium)

Peak No.	Rt (min)	Compound	Ions used for identification	Quality (%)
1	5.170	Meconin	165, ***194***, 147	99
2	5.593	?		
3	6.007	Hydrocotarnine	220, ***221***, 178	97
4	10.544	?		
5	11.052	3,6-DiMeO-4,5-epoxyphenanthrene	***252***	98
6	11.833	Codeine	***299***, 162, 229	99
7	12.012	Neopine (ß-Codeine)	***299***, 254, 285	94
8	12.247	Morphine	***285***, 162, 215	99
9	12.360	MW 313?		
10	12.473	MW 313?		
11	12.624	Thebaol	***203***, 175	99
12	12.859	MW 317?		
13	12.981	Thebaine	***311***, 255, 296	89
14	13.085	Thebaine	***311***, 255, 296	96
15	13.188	MW 313?		
16	15.287	Papaverine	324, 338, ***339***	99
17	18.431	Noscapine	220, 205	

Notes: Rt = Retention time, Quality (%) = Match (% fit) of mass spectrum between sample and library spectrum. ***Bold, italic and underline*** shows molecular ion (*m/z*).

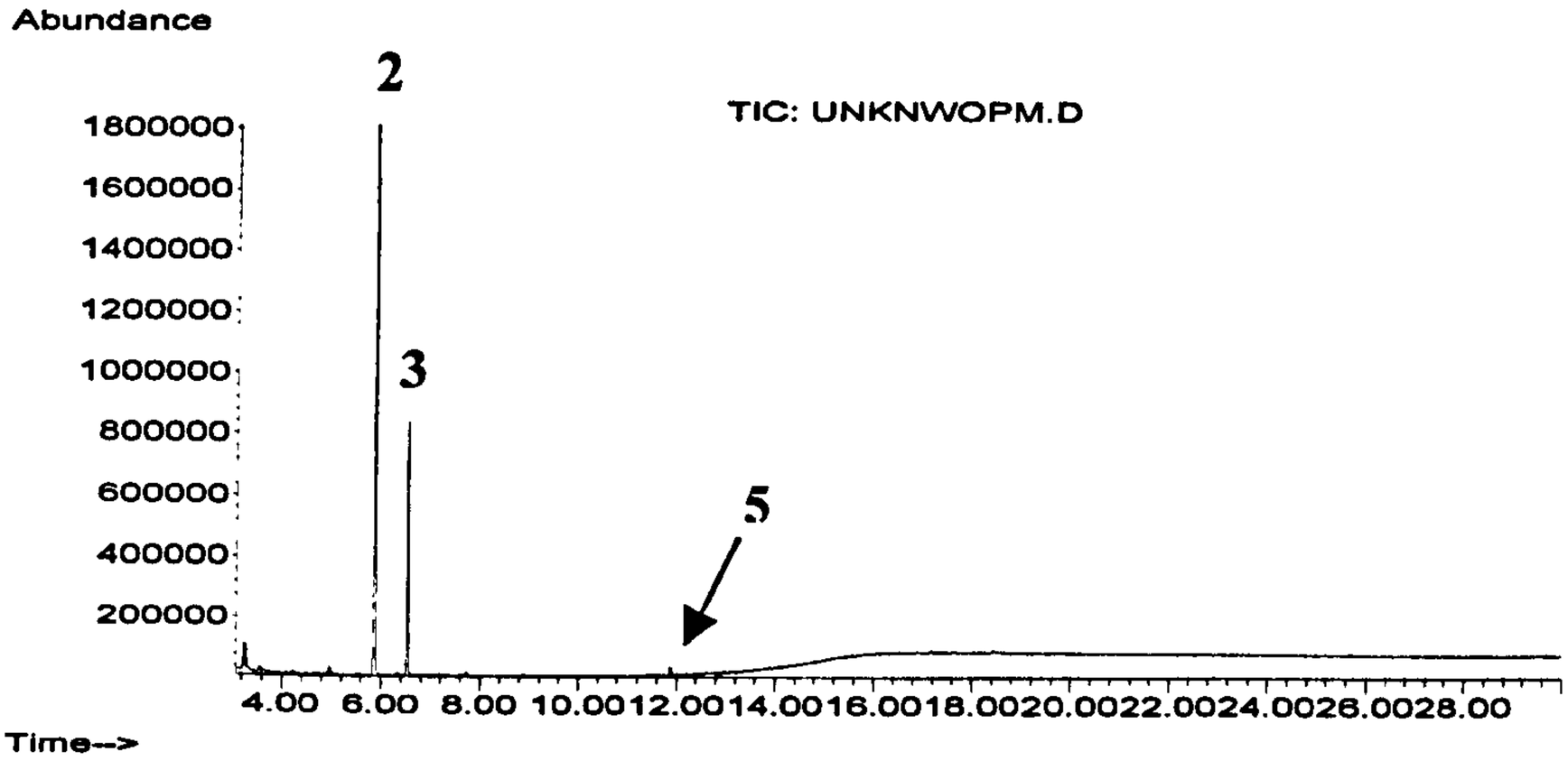


Figure 4.9. TIC of Py-GC-MS (Unknown opium)

Table 4.6. Detected opiates and retention time by Py-GC-MS (Unknown opium)

Peak No.	Rt (min)	Compound	Ions used for identification	Quality (%)
1	3.264	4-Hydroxy-benzeneethanol	107, ***138***	90
2	5.871	Meconin	165, ***194***, 147	
3	6.530	Hydrocotarnine	220, ***221***, 178	95
4	11.122	3,6-DiMeO-4,5-epoxyphenanthrene	***252***	86
5	11.875	Codeine	***299***, 162, 229	98
6	12.280	Morphine	***285***, 162, 215	99
7	12.657	Thebaol	***203***, 175	7
8	13.005	Thebaine	***311***, 255, 296	9
9	13.108	Thebaine	***311***, 255, 296	5
10	15.282	Papaverine	324, 338, ***339***	49
11	18.426	Noscapine	220, 205	42

Notes: Rt = Retention time, Quality (%) = Match (% fit) of mass spectrum between sample and library spectrum. ***Bold, italic and underline*** shows molecular ion (*m/z*).

4.7.2.2. Acetylated opium alkaloids in nail

Samples were prepared in which ground fingernail was treated with the acetylated derivative of opium, to simulate fingernail samples containing street heroin. There were two aims. The first was to examine the thermal stability of the acetyl derivative. The second was to establish the amount of fingernail that is required for analysis.

The acetylation of opium was carried out with opium and acetic anhydride using pyridine as catalyst at 60 °C for 30 min. The acetylated opium was dissolved with ethyl acetate and mixed with decontaminated pulverised nail clippings using a rotary evaporator. As a result, pulverised nail contained about 500 ng of acetylated opium per milligram was obtained.

Figure 4.10 shows the TIC from the pyrolysis GC-MS of 0.3 mg of the blank nail sample. The peak at a retention time of about 7.7 min is dibutyl phthalate (**DBP**, $C_{16}H_{22}O_4$ = 278.35).

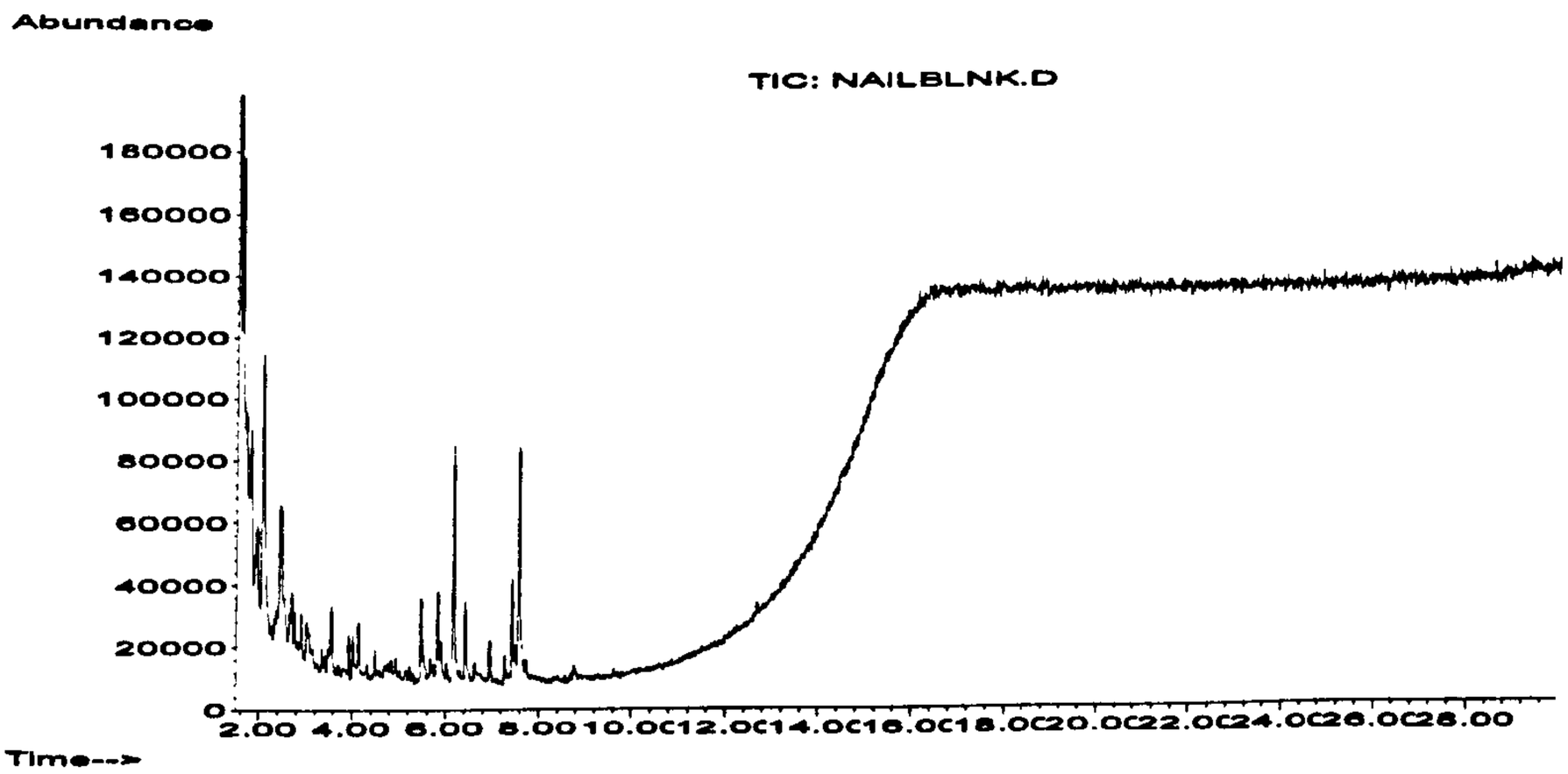


Figure 4.10. TIC of Py-GC-MS (0.3 mg blank nail)

Figure 4.11 shows the TIC from Py-GC-MS of a nail sample containing 500 ng of the acetylated unknown opium. **Tables 4.7** and **4.8** show retention times (min) and

library search fit factors (%) of mass spectra of the detected compounds of the unknown and the acetylated Turkish opium, respectively. In **Tables 4.7** and **4.8**, the thermal decomposition compound, 3,6-dimethoxy-4,5-epoxy-phenanthrene was detected.

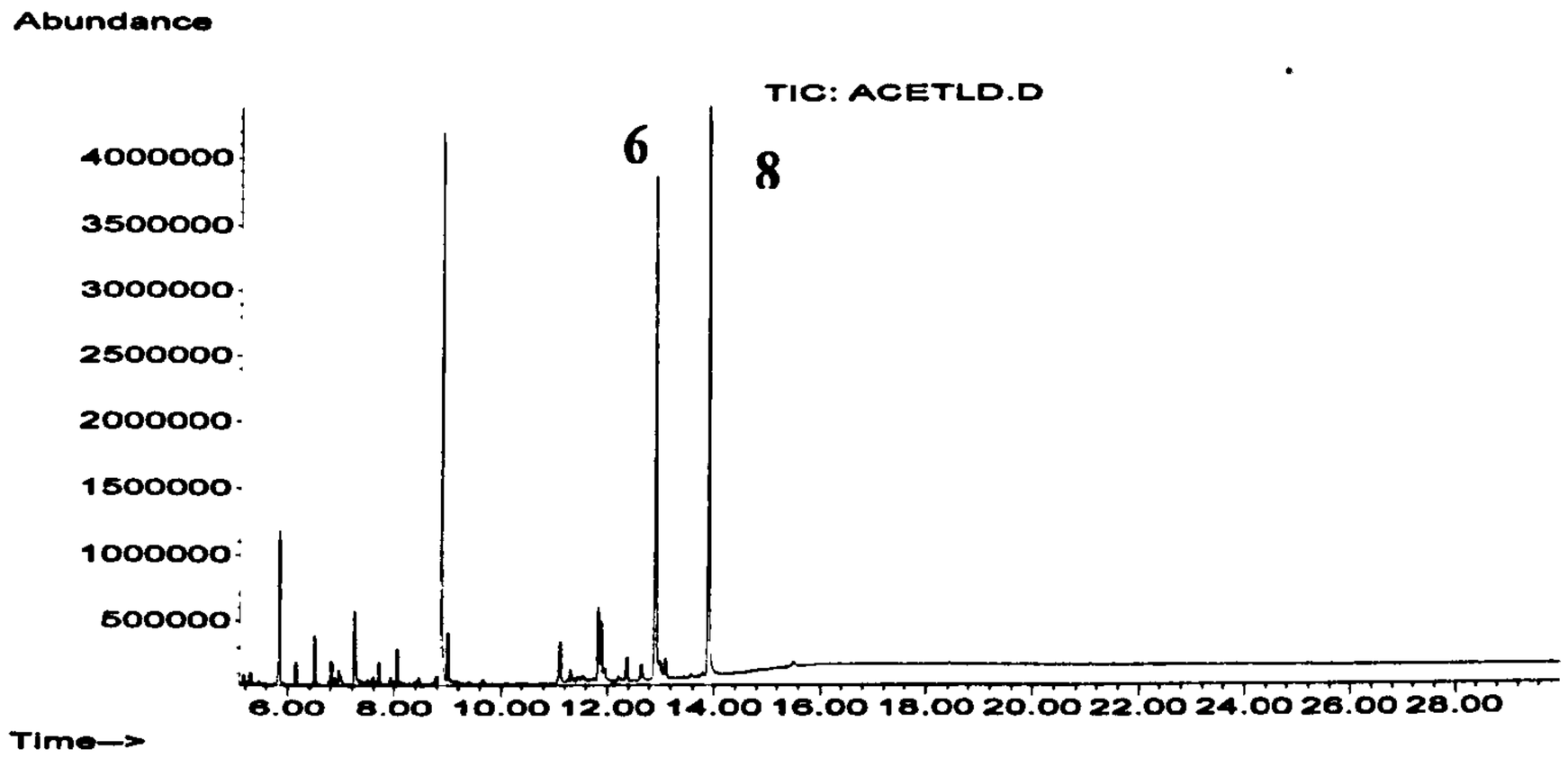


Figure 4.11. TIC of Py-GC-MS (500 ng of acetylated unknown opium, 0.2 mg nail)

Table 4.7. Retention time and library search fit factor of acetylated unknown opium

Peak No.	Rt (min)	Compound	Ions used for identification	Quality (%)
1	5.838	6,7-DiMeO-isobenzofuranone	***180***	98
2	6.148	4,5-DiMeO-isobenzofuranone-1,3-dione	***208***	97
3	6.506	Hydrocotarnine	220, ***221***, 178	96
4	11.099	3,6-DiMeO-4,5-epoxyphenanthrene	***252***	95
5	12.633	Thebaol	***203***, 175	89
6	12.906	6-Acetylcodeine	***341***, 282, 229	99
7	13.094	Thebaine	***311***, 255, 296	94
8	13.885	Heroin	327, ***369***, 308	99

9	15.268	Papaverine	324, 338, ***339***	10
10	18.412	Noscapine	220, 205	38

Quality (%) = Match (% fit) of mass spectrum between sample and library spectrum.

Bold, italic and underline shows molecular ion (*m/z*).

Table 4.8. Retention time and library search fit factor of acetylated Turkish opium

Peak No.	Rt (min)	Compound	Ions used for identification	Quality (%)
1	10.986	3,6-DiMeO-4,5-epoxyphenanthrene	***252***	95
2	12.586	Thebaol	***203***, 175	99
3	12.868	6-Acetylcodeine	***341***, 282, 229	99
4	12.953	6-Monoacetylmorphine	287, 340, ***399***	95
5	13.066	Acetylthebaol	***296***	98
6	13.885	Heroin	327, ***369***, 308	99
7	15.278	Papaverine	324, 338, ***339***	99
8	18.421	Noscapine	220, 205	53

Notes: Rt = Retention time, Quality (%) = Match (% fit) of mass spectrum between sample and library spectrum. ***Bold, italic and underline*** shows molecular ion (*m/z*).

4.7.2.3. Reproducibility and thermal stability of opium alkaloids

Sample preparation:

Nail samples were prepared containing 20 μg/mg and 20 ng/mg each of ethylmorphine-HCl and morphine respectively using a rotary evaporator.

The nail samples (1–2 mg) were used for quantitative analysis by Py-GC-MS and GC-MS as TMS derivatives and underivatised samples.

Ethylmorphine ($C_{19}H_{23}NO_3$ = 313.40), morphine ($C_{17}H_{19}NO_3$ = 285.34) and their TMS derivatives were analysed by quantitative analysis and thermal stability as shown in **Figure 4.12.**

4.7.2.4. *Quantitative analysis*

(a) Reproducibility of free morphine alkaloids

Tables 4.9 and **4.10** show analytical data for TMS derivatives of ethylmorphine **(I.Std)** and morphine.

Nail samples (containing 20.0 μg/mg each of morphine and ethylmorphine-HCl) were added directly to the Py-GC-MS sample holder, and the Py-GC-MS was carried out. Reproducibility of the peak area ratio of morphine to ethylmorphine was poor as shown in **Table 4.9.**

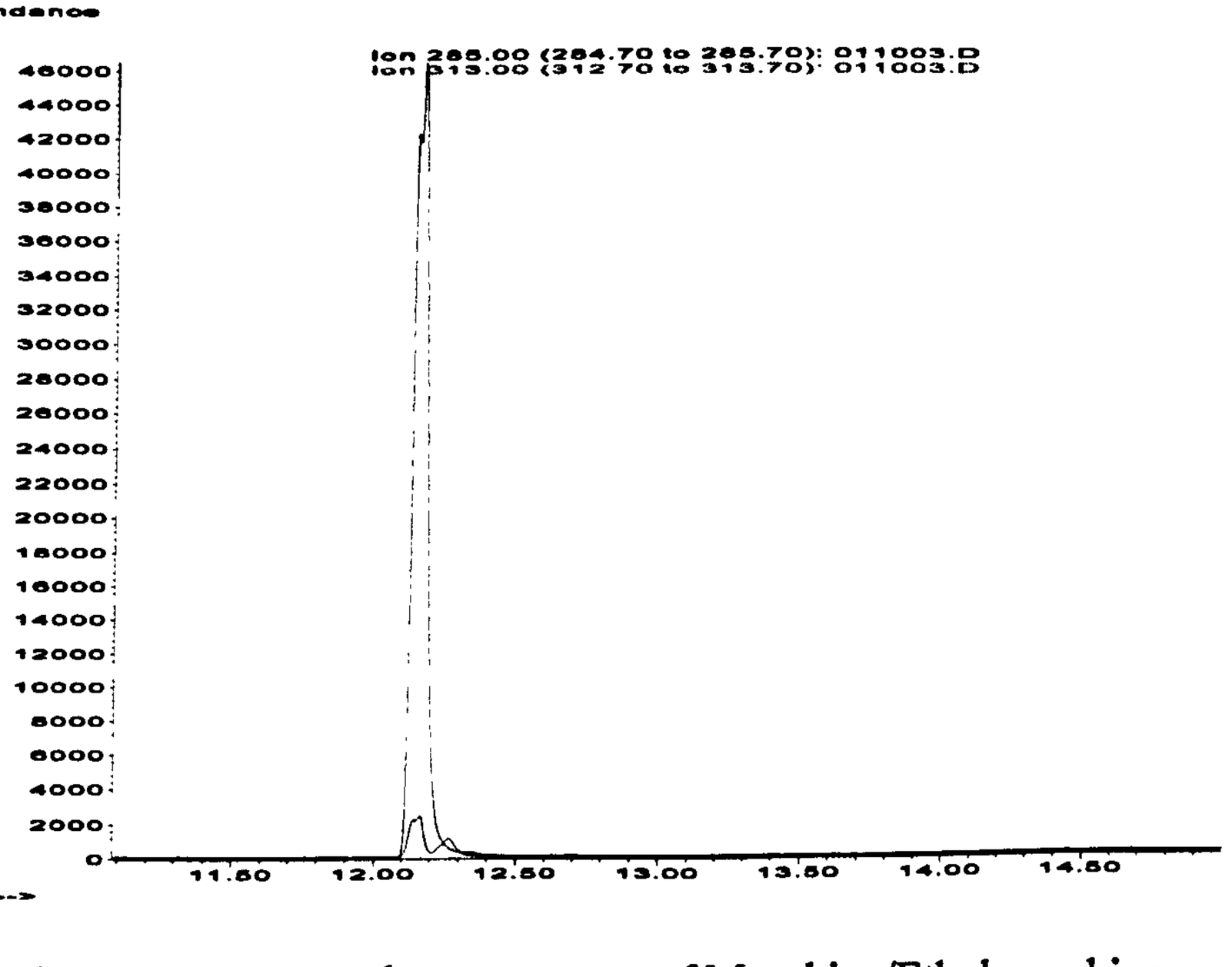


Figure 4.12. Mass chromatograms of Morphine/Ethylmorphine (20 μg each), nail 1.0 mg

Table 4.9. Quantitative analysis of morphine in nail by Py-GC-MS

File Name Py-GC-MS	Nail Wt. (mg)	Intensity *m/z* 313	Intensity *m/z* 285	Peak Area Ratio (%)	Et-Morp. Rt (min)	Morphine Rt (min)
011001.d	1.2	0	0	0.00	0.000	0.000
011002.d	1.7	46411	192900	24.06	12.163	12.265
011003.d	1.0	43067	1027429	4.19	12.169	12.270
011004.d	1.2	52071	415290	12.54	12.169	12.270

Notes: Rt = Retention time, Et-Morp. = Ethylmorphine.

(b) Reproducibility of trimethylsilylated morphine alkaloids

10 μL of BSTFA containing 1% of DMCS and 20 μL of dimethylformamide was added to the nail and heated for 30 min at 60 °C.

One (1) μL of trimethylsilylated sample was injected into the HP GC-MS. The intensity of mass chromatogram was high and reproducibility was good, as shown in **Table 4.10.** The reproducibility of data was 98.6% (n=4).

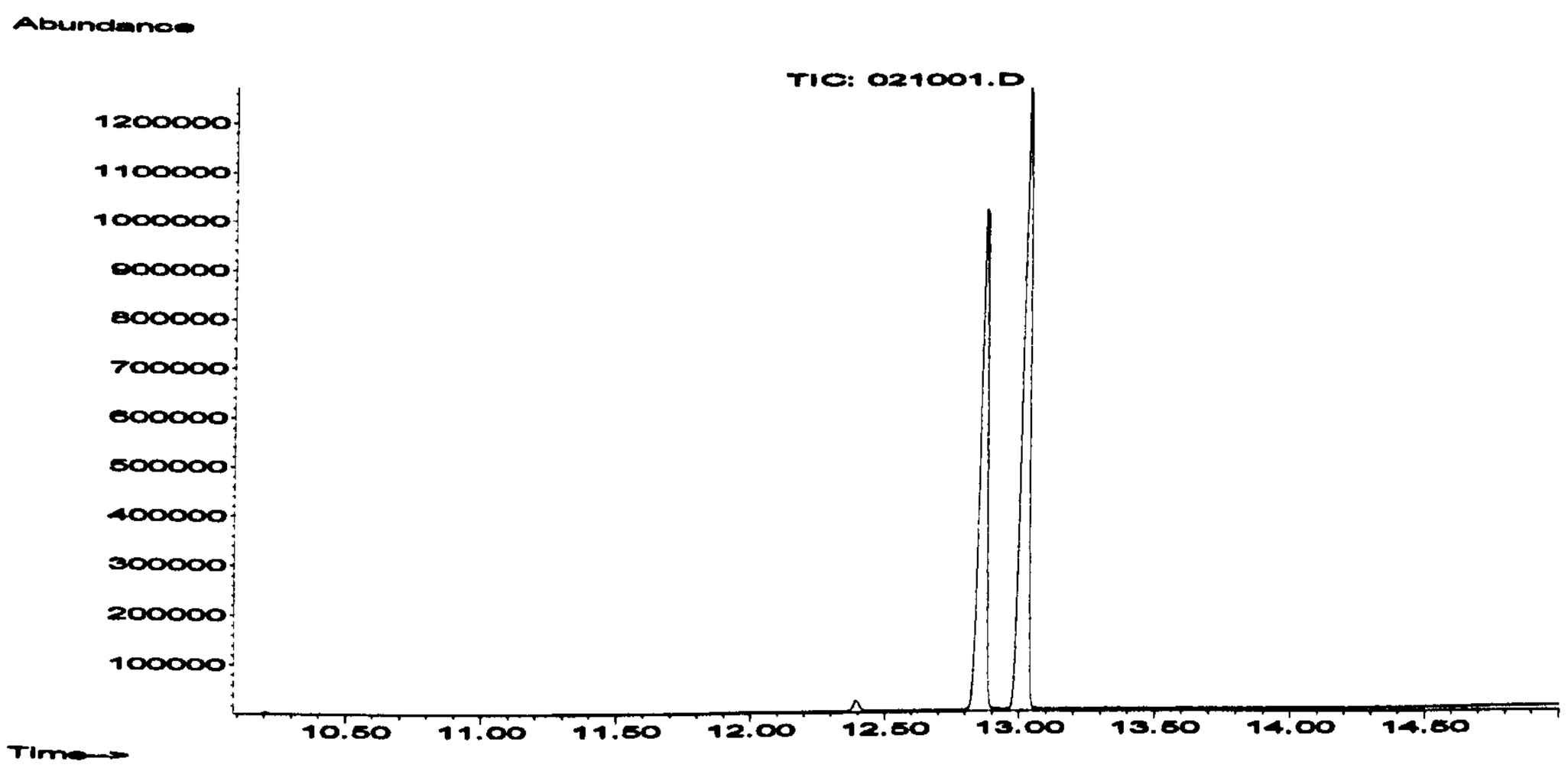


Figure 4.13. MC of Ethylmorphine-TMS/Morphine-di-TMS (1.47 μg)

Table 4.10. Quantitative analysis of TMS derivatives

File Name GC-MS	Nail Wt. (mg)	Intensity *m/z* 429	Intensity *m/z* 385	Peak Area Ratio (%)	Et-Morp. Rt (min)	Morphine Rt (min)
021001.d	2.2	13863392	13741395	100.89	12.867	13.024
021002.d	1.7	14490133	14334135	101.09	12.836	12.998
021003.d	1.8	17779749	18279387	97.27	12.867	13.029
021004.d	1.4	16936573	17815775	95.07	12.868	13.020

Notes: Rt = Retention time, Et-Morp. = Ethylmorphine.

(c) Reproducibility of trimethylsilylated morphine alkaloids by Py-GC-MS

2 μL of the same sample solution was injected into the Py-GC-MS sample holder. As shown in **Table 4.11**, reproducibility of the peak area ratio was poor compared with conventional GC-MS.

Table 4.11. Quantitative analysis of TMS derivative

File Name Py-GC-MS	Nail Wt. (mg)	Intensity *m/z* 429	Intensity *m/z* 385	Peak Area Ratio (%)	Et-Morp. Rt (min)	Morphine Rt (min)
011010.d	2.2	3262	2971	109.79	12.639	12.809
011012.d	1.7	7024	6174	113.77	12.648	12.813
011013.d	1.8	28290	17082	165.61	12.643	12.809
011014.d	1.4	57264	41906	136.65	12.643	12.809

Notes: Rt = Retention time, Et-Morp. = Ethylmorphine.

(d) Reproducibility of low concentrations of trimethylsilylated morphine alkaloids by GC-MS

10 μL of BSTFA containing 1% of DMCS and 20 μL of dimethylformamide were added to nail samples (containing 20.0 ng/mg each of morphine and ethylmorphine-HCl) and heated for 30 min at 60 °C. One (1) μL of trimethylsilylated sample was injected into the HP GC-MS.

There was reproducibility in some degree though the intensities of the mass chromatogram were low, as shown in **Table 4.12.**

(e) Reproducibility of morphine alkaloids by Curie point Py-GC-MS

There was no reproducibility of morphine alkaloids regarding Curie point Py-GC-

MS at the optimum pyrolysis temperature. Pyrolysis temperature and temperature of the introduction pipe were 341 °C and 260 °C, respectively. If a newer model (directly connected to the injection port of the GC instrument) had been used for the Py-GC-MS, the reproducibility might have been better.

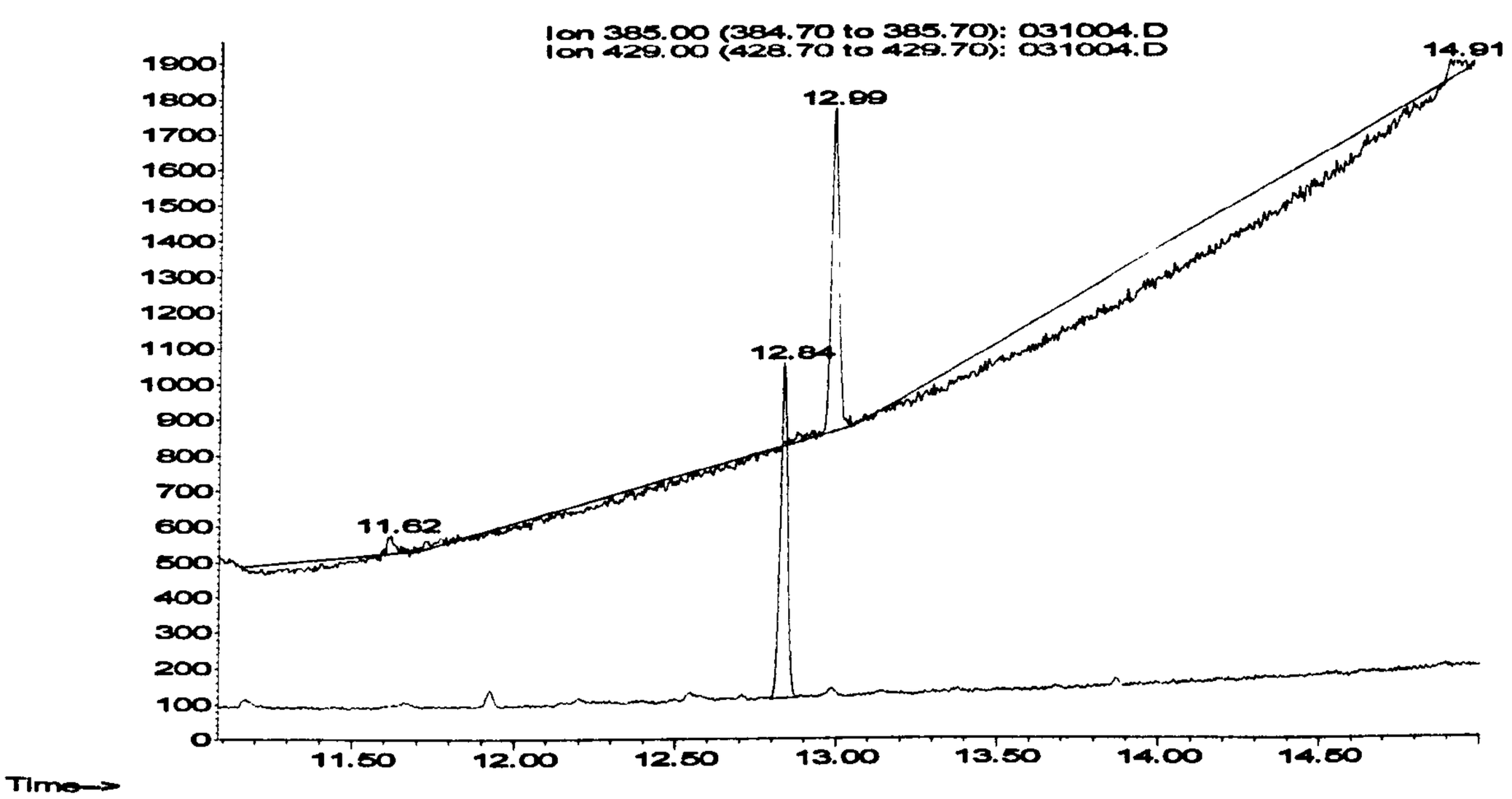


Figure 4.14. MC of Morphine-di-TMS/Ethylmorphine-TMS (each 1.33 ng)

Table 4.12. Quantitative analysis of TMS derivatives

File Name GC-MS	Nail Wt. (mg)	Intensity *m/z* 429	Intensity *m/z* 385	Peak AreaEt- Ratio (%)	Morp. Rt (min)	Morphine Rt (min)
031001.d	2.1	194509	229320	84.82	12.841	12.994
031002.d	2.0	10508	11853	88.65	12.828	12.985
031003.d	2.2	17075	14335	119.11	12.836	12.989
031004.d	2.0	15445	14524	106.34	12.836	12.989

Notes: Rt = Retention time, Et-Morp. = Ethylmorphine.

4.8. Conclusions

1) Py-GC-MS analyses that assumed the availability of a minimum amount of nail sample were carried out. The pyrolysis GC-MS was performed for the analysis of illicit opioids in nail clippings using GC-MS combined with several types of pyrolyser. The optimum temperature for pyrolysis GC-MS of opium using the **double shot pyrolyser (Frontier Lab Co.**, Japan) was 350 °C. and reproducible data was obtained.

2) Good analytical data were not obtained with the **Curie point pyrolysis GC-MS** instrument because of adsorption and thermal degradation of analytes in the interface pipe leading to the injection port of the gas chromatograph. Morphine was decomposed by pyrolysis as shown **Tables 4.1–4.4** and **Figures 4.5–4.6**, and **Figures 4.8–4.9**. However, TMS derivatives of the morphine alkaloids were not decomposed [**Figure 4.7**].

3) Minor components of opium such as **Meconin, Hydrocotarnine, Neopine, Thebaine** and **Thebaol** were detected in the opium. These compounds could not be detected in nail clippings of heroin abusers.

4) The reproducibility of the extraction rate of morphine was 98.6% (n=4), when the TMS derivative was used (**Table 4.10**). However, the reproducibility of the extraction rate of morphine was poor when the TMS derivative of low concentrations of morphine was used (**Figure 4.14** and **Table 4.12**). There was no reproducibility of the extraction rate of morphine when no derivative of morphine was used.

B. Analysis of Morphine Alkaloids using Matrix Assisted Laser Desorption Ionisation-Time of Flight-Mass Spectrometry

4.9. Introduction

The analysis of street heroin is equivalent to the analysis of opium alkaloids. However, the analysis of street heroin in urine and blood becomes complex because of the presence of metabolites and impurities besides the opium alkaloids themselves. In addition, the amount of the fingernail sample available becomes a problem in opium alkaloid analysis in the fingernail.

The mass spectrometry is the most excellent method of identifying ultra trace amounts of chemical compounds. After opium alkaloids are extracted from fingernail clippings, GC-MS analysis is carried out to identify the morphine alkaloids which are present.

The smallest amount of fingernail clipping is available for the GC-MS analysis and extraction of the analyte is necessary. The sensitivity of detection and the resolution of the mass spectrometer coupled with laser excitation are high. Moreover, the analyte does not need to be separated as in GC analysis.

In this study, it was intended to assess the possibility of analysing trace amounts of opium alkaloids in small samples of fingernail collected directly from the finger with a TOF-MS instrument. The results given below showed that an extremely small quantity of morphine alkaloids could be easily identified with high sensitivity by MALDI-TOF-MS without prior separation of impurities.

4.10. Methods and Materials

4.10.1. Samples

Morphine, ethylmorphine hydrochloride (**I.Std**), methanol extracts of opium (**Turkish**), methanol extracts of opium (**unknown origin**), and cocaine were used as test samples.

The MALDI matrix was α-cyano-4-hydroxycinnamic acid (**CHCH**, $C_{10}H_7NO_3$ = 189.17)

4.10.2. Instrumentation

The Matrix Assisted Laser Desorption Ionisation-Time of Flight-Mass Spectrometry (**MALDI-TOF-MS**) was a **Voyager System model 4181** from **Perkin Elmer Biosystems** (USA).

4.10.3. Principle of MALDI-TOF-MS

As shown in **Figure 4.15,** when a sample and matrix mixture was irradiated with a nitrogen laser emitting light at 337 nm, ions are generated. Mass spectrometry is carried out by introducing the ions into a mass spectrometer of the time of flight (**TOF**) type.

2,5-dihydroxybenzoic acid ($C_7H_6O_4$ = 154.13), 2,6-dihydroxyacetophenone ($C_8H_8O_3$ = 152.15) and **CHCH**, etc. are typically used as the matrices.

Compounds with high polarity, which promote the excitation of ions, are suitable as a matrix. The TOF-MS has extremely high resolution and is chiefly used in fields related to biochemistry, such as peptides and proteins having high molecular weights.

In this research, an assessment was made of whether the TOF-MS can also be applied to opium alkaloids with low molecular weights.

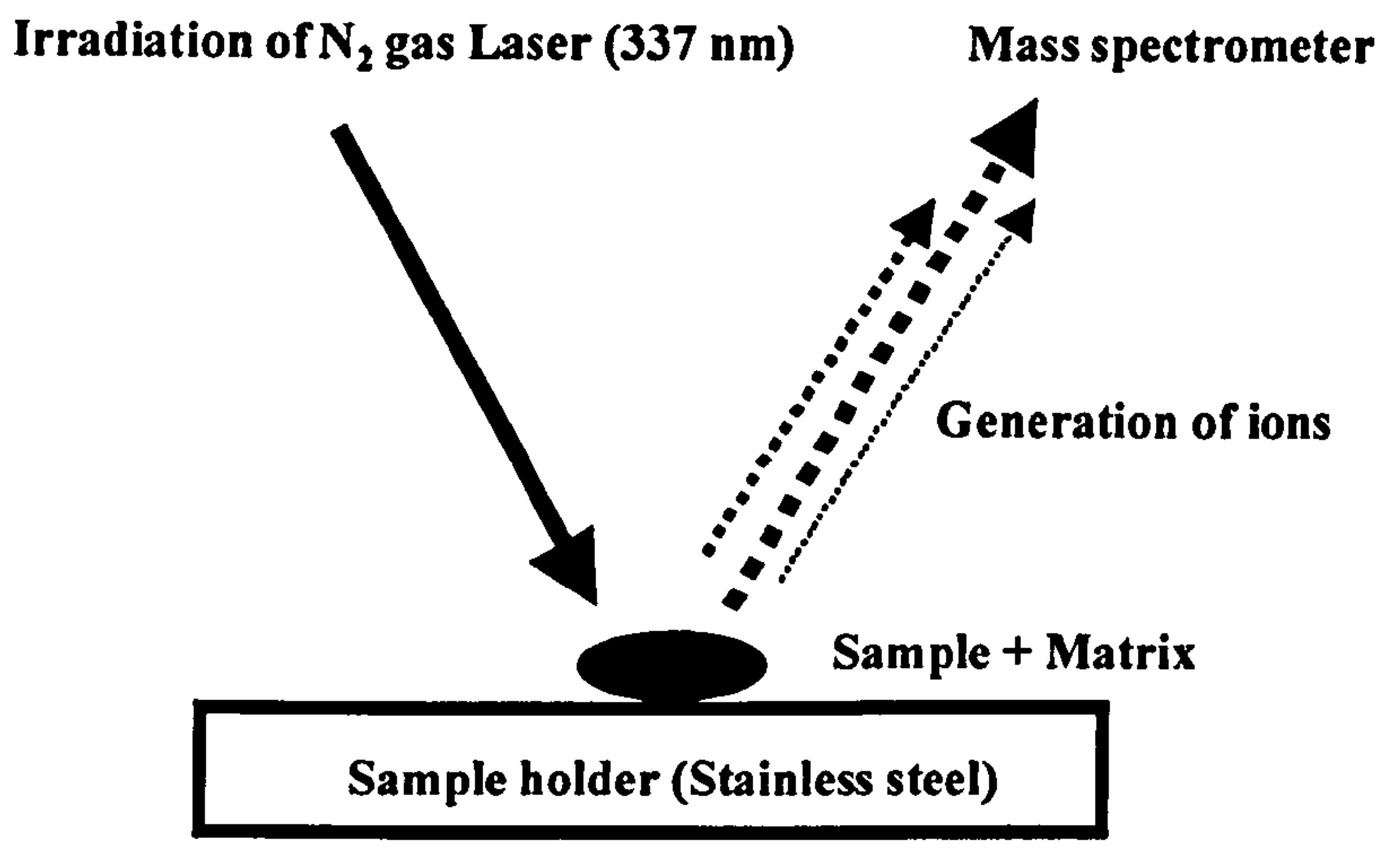


Figure 4.15. Principle of MALDI-TOF-MS

4.10.4. Sample preparation

A trace amount of the sample was collected in the stainless steel sample holder, and the sample was mixed with **CHCH** as the matrix. **CHCH** is generally used for the analysis of peptides and glycopeptides.

4.11. Results and Discussion

4.11.1. Morphine

As shown in **Figure 4.16**, the $(M+1)^+$ ion of morphine ($C_{17}H_{19}NO_3$ = 285.34), *m/z* 286 was identified as the base peak ion. Other ions such as *m/z* 294, 379 were background ions (see **Figure 4.21**).

4.11.2 Ethylmorphine

Similarly, the $(M+1)^+$ ion of ethylmorphine ($C_{19}H_{23}NO_3$ = 313.40), *m/z* 314 was recognised as base peak ion as shown in **Figure 4.17**. Other ions such as *m/z* 212, 294, 379 were background ions respectively (see **Figure 4.21**).

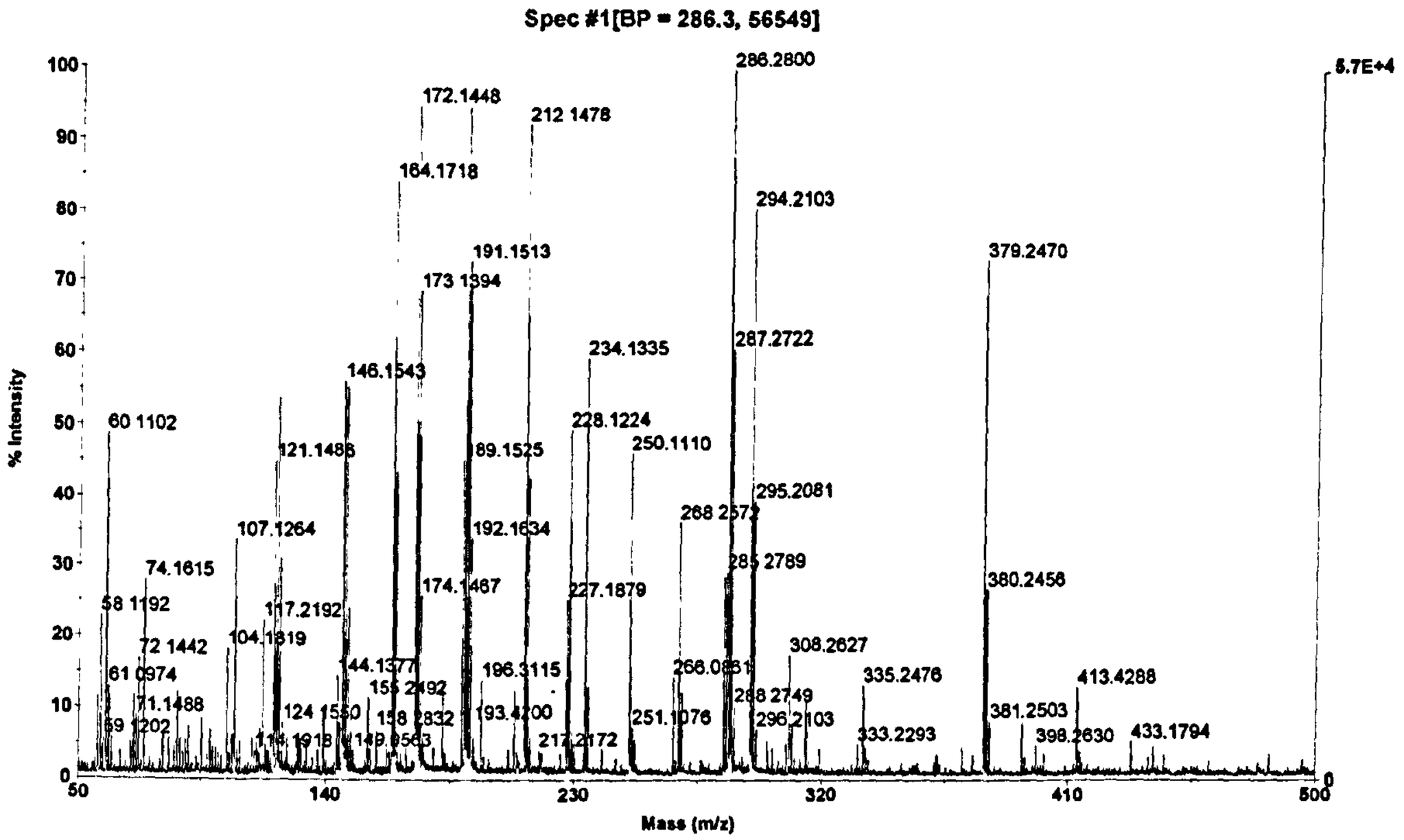


Figure 4.16. MALDI mass spectrum of Morphine

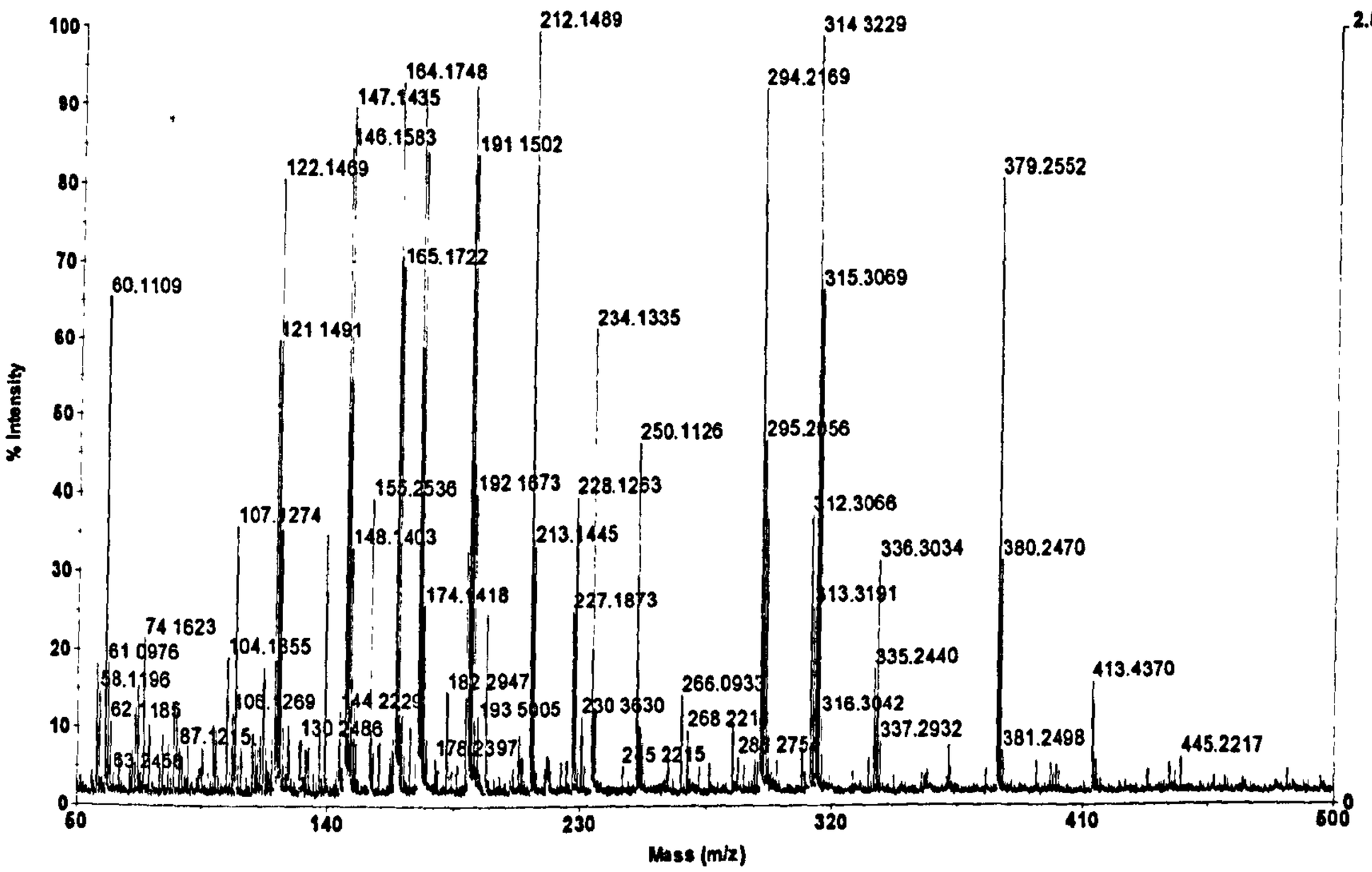


Figure 4.17. MALDI mass spectrum of Ethylmorphine

4.11.3. Opium extracts (Turkish)

Figure 4.18 shows the mass spectrum of Turkish opium extracted with methanol. The ions at *m/z* 286, 300, 340, and 414 are $(M+1)^+$ ions of morphine, codeine ($C_{18}H_{21}NO_3$ = 299.36), papaverine ($C_{20}H_{21}NO_4$ = 339.38), and noscapine ($C_{22}H_{23}NO_7$ = 413.43), respectively.

In addition, ions at *m/z* 220, 221 and 222 were recognised as the $(M-1)^+$, M^+, and $(M+1)^+$ ions of hydrocotarnine ($C_{12}H_{15}NO_3$ = 221.31) respectively. The M^+ ion of thebaol ($C_{16}H_{14}O_3$ = 254.29) was also identified at *m/z* 254. A fragment ion could be detected at *m/z* 253 that is the $(M+1)^+$ ion of 3,6-dimethoxy-4,5-epoxyphenanthrene ($C_{16}H_{12}O_3$ = 252.27).

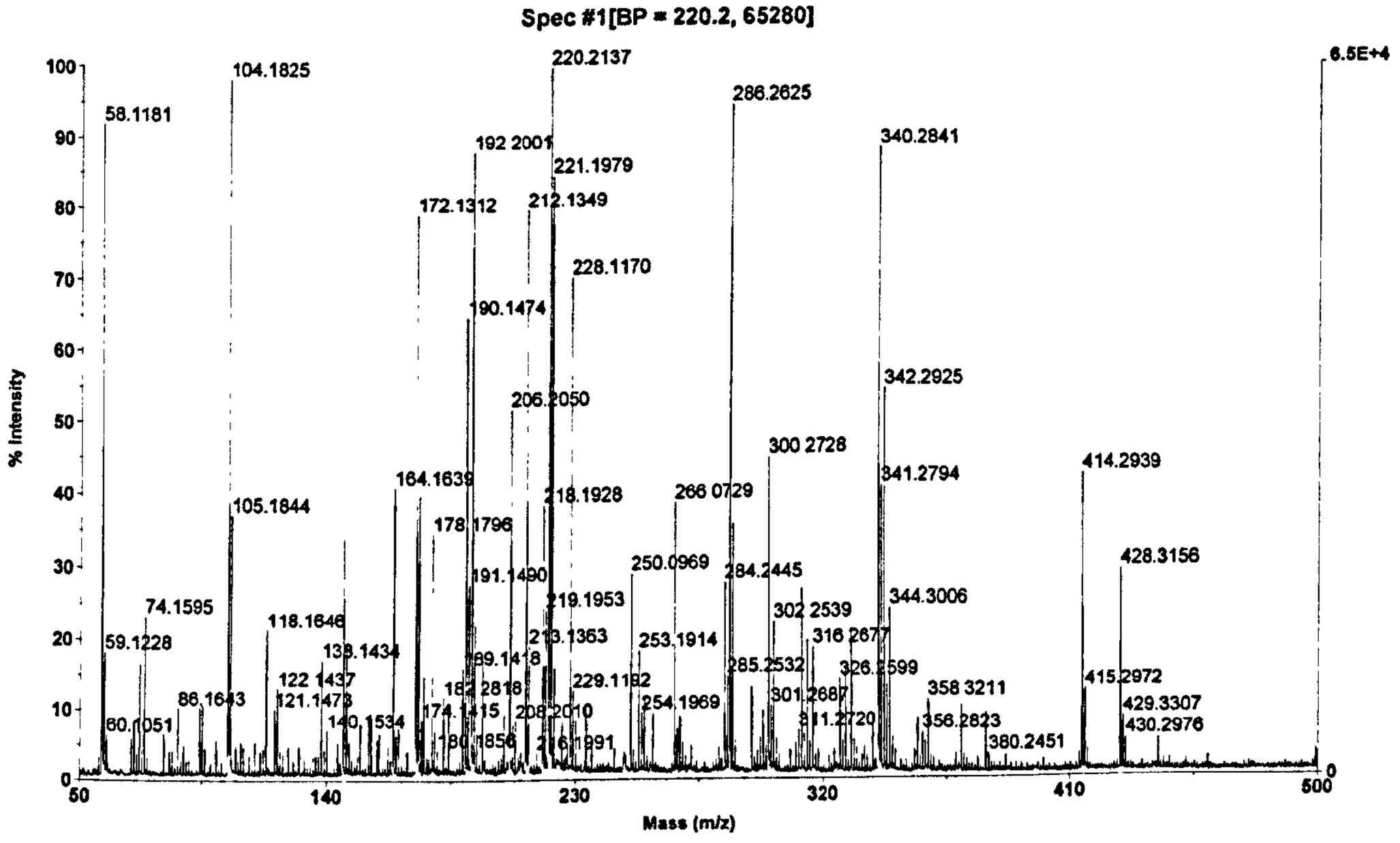


Figure 4.18. MALDI MS of extract from Turkish opium

4.11.4. Extracts of opium of unknown origin

Figure 4.19 similarly shows the mass spectrum of an extract with methanol of opium of unknown country. The ions at *m/z* 286, 300, 340, 414, 312, and 255 are $(M+1)^+$ ions of morphine, codeine, papaverine, noscapine, thebaine ($C_{19}H_{21}NO_3$ = 311.37) and

thebaol, respectively.

In addition, ions at *m/z* 220, 221, 222 were recognised as the $(M-1)^+$, M^+, and $(M+1)^+$ ions of hydrocotarnine ($C_{12}H_{15}NO_3$ = 221.31), respectively. The M^+ ion of thebaol ($C_{16}H_{14}O_3$ = 254.29), *m/z* 254 was also recognised. The ion at *m/z* 253 is the $(M+1)^+$ ion of 3,6-dimethoxy-4,5-epoxyphenanthrene ($C_{16}H_{12}O_3$ = 252.27).

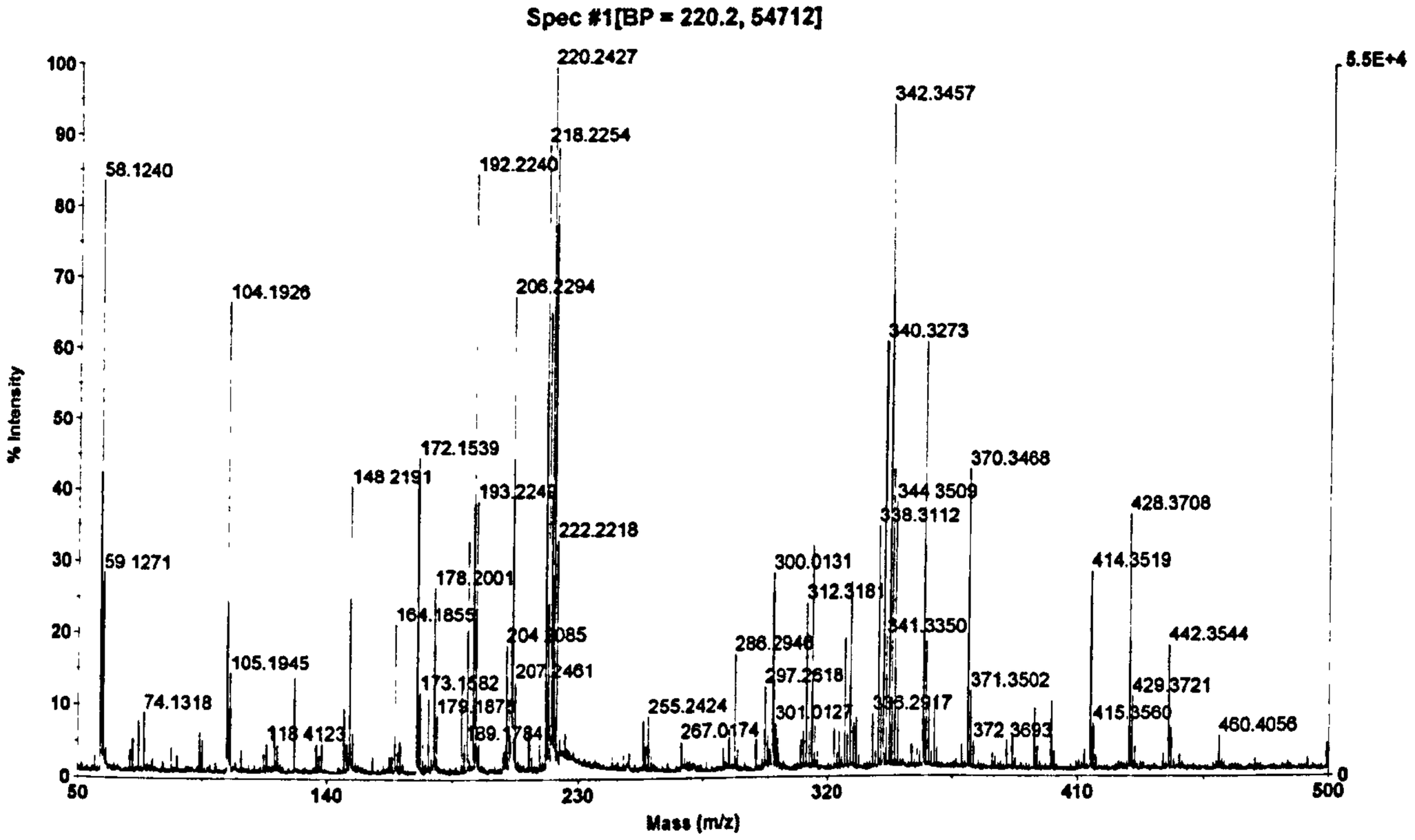


Figure 4.19. MALDI mass spectrum of opium of unknown origin

4.11.5. Cocaine

Figure 4.20 shows the MALDI mass spectrum of cocaine. The $(M+1)^+$ ion of cocaine ($C_{17}H_{21}NO_4$ = 303.36) is at *m/z* 304.

4.11.6. α-cyano-4-hydroxycinnamic acid

The $(M+1)^+$ ion of α-cyano-4-hydroxycinnamic acid ($C_{10}H_7NO_3$ = 189.17) was detected at *m/z* 190 as shown in **Figure 4.21.**

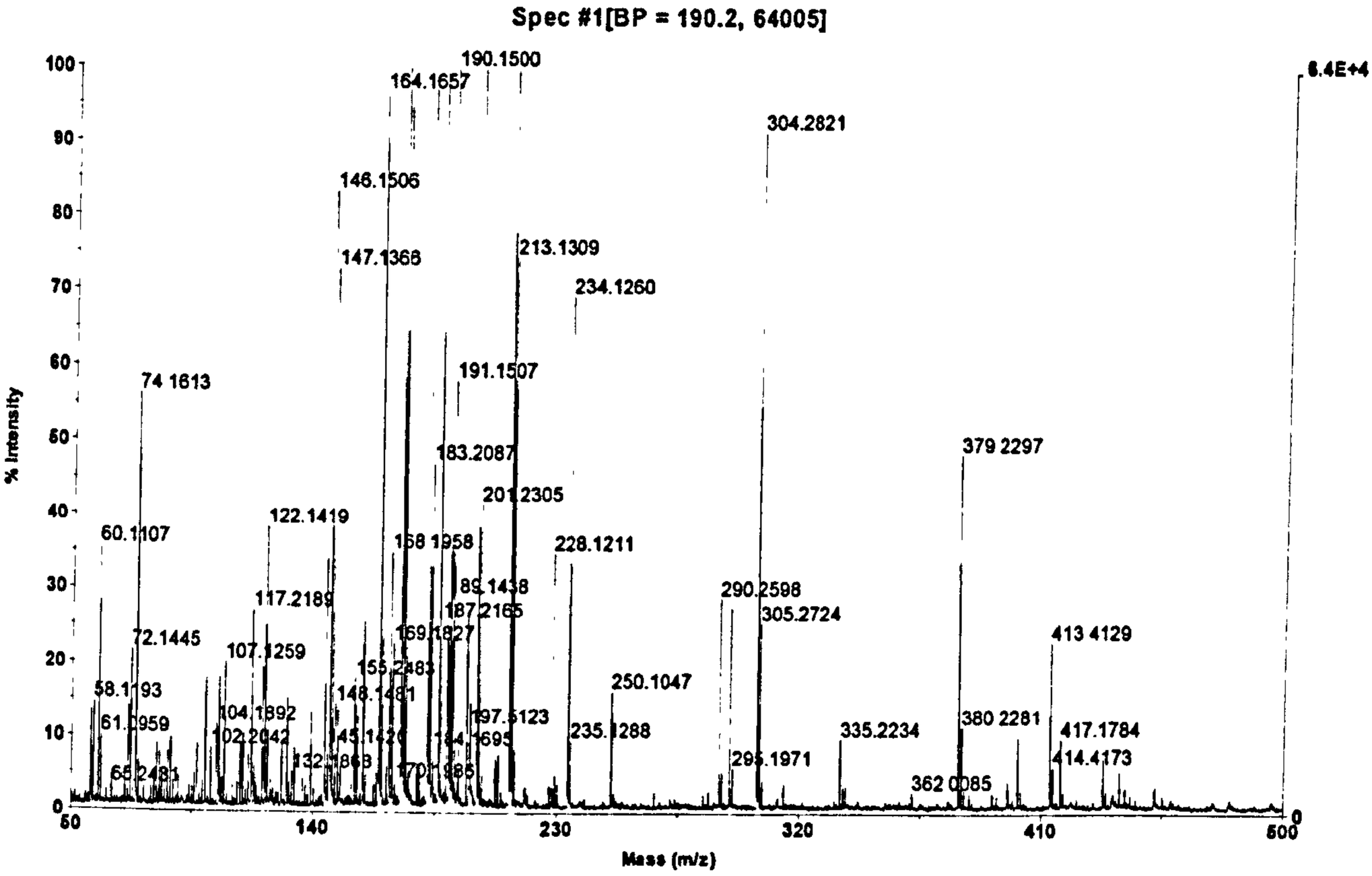


Figure 4.20. MALDI mass spectrum of Cocaine

Figure 4.21. MALDI mass spectrum of α-cyano-4-hydroxycinnamic acid

4.12. Conclusions

1) MALDI-TOF-MS was evaluated as an analytical method that assumed the availability of a minimum amount of nail sample without prior extraction of the target analytes. The analytical data for opium alkaloids by MALDI-TOF-MS indicate that the technique was able to identify unextracted opium alkaloids. The opium alkaloids were easily measured, though the analytical conditions of the MALDI-TOF-MS were in the measurement mode used for polymers (peptide, etc.) and there were interfering peaks derived from the matrix compound.

2) If MALDI-TOF-MS was applied to analysis of illicit drug in the fingernail sample, there is a possibility to be able to analyse without extracting the drug in the ultra trace amount of the fingernail.

5. General Conclusions

The aims of the work carried out for this thesis were outlined at the beginning of this report and were (a) to develop and assess improved methods of analysis for nail samples when used as an alternative matrix in forensic toxicology, (b) to evaluate this improved methodology for the analysis of nail specimens from controlled drug users and from doping steroid users and (c) to investigate instrumental methods of analysis which might be suitable for direct analysis of target analytes in very small samples of nail. All of these objectives were successfully achieved during the project.

Nail has been shown to be an effective and useful sample in forensic toxicology and it is clear that nail analysis should be given more attention in future. A relatively convenient method of extracting illicit drugs from the difficult nail matrix based on cryogenic anabolic steroid users, respectively. It was found that this extraction method had the advantage grinding was developed and was applied to nail samples from cannabis, heroin and that analytes could be extracted directly without hydrolysis compared with previously used alkaline hydrolysis procedures. This method therefore overcomes the potential criticism of nail analysis based on alkaline hydrolysis, that the range of detectable analytes is unacceptably low, by extending the accessible range of analytes to include all of those which are now routinely analysed in hair. The results obtained indicate that analytes of a wide range of polarities are readily extracted from the finely ground nail powder.

As a result, cannabinoids and opium alkaloids including heroin could be identified and determined in fingernails of cannabis and heroin abusers without chemical degradation of the target analytes, despite the fact that the analytes are present at low (ppb) concentrations. In particular, heroin could easily be detected without chemical degradation whereas it is difficult to identify heroin in conventional biological samples

such as blood or urine. Therefore, for these specimens, 6-monoacetylmorphine (6-MAM) is used as a biological marker for heroin use. The cryogenic grinding method was also successfully used for detection and identification of 6-acetylcodeine, papaverine and noscapine together with heroin in nail clippings. This study is the first to report detection of some of the impurities of street heroin in biological samples and suggests that they might be used in the near future as biological markers to prove that street heroin had been administered.

Nail was shown to be useful as a forensic toxicology sample in a number of different contexts. In addition to abuse of street drugs, the cryogenic grinding method was applied to nail samples from doping steroid users. This approach might in future provide a viable alternative to hair and have a significant influence on fair play in sporting events.

Endogenous anabolic steroids could be identified and determined in nail. The concentrations were found to be extremely low compared with those in urine and blood. However, exogenous anabolic steroids could only be tentatively identified and determined due to the presence of interfering endogenous compounds. Nevertheless, it seems probable that exogenous steroids are present in nail and can be extracted and analysed as for the drugs of abuse. The cryogenic grinding method is particularly valuable in this respect because many doping steroids are esters of testosterone and its analogues and would therefore be hydrolysed if the nail specimens were processed using an alkaline hydrolysis procedure.

Finally, looking towards the future practical application of nail analysis, especially as an alternative to hair, an assessment was made of instrumental methods of analysis which might permit direct analysis of analytes in the nail matrix and which might pave the way for analysis of very small nail samples (microgram amounts). The value of this

would be to allow nails samples to be collected at different locations on the nail and not just from the tip, as it might take several weeks for drugs to reach the latter location. Hair analysis currently requires a period of 4 weeks for hair to grow until drugs in circulation and entrapped in the hair matrix emerge from the scalp. This forms the basis of the current protocol for date rape drug testing in hair. The techniques investigated (pyrolysis GC-MS and MALDI-TOF-MS) potentially might provide the basis of direct nail analysis, using microgram amounts of sample. However, the sensitivity of these instruments at present seems to be too low.

Future Work

The immediate need is to establish nail as a true and effective alternative for hair in forensic toxicology. This will require the collection and analysis of nail samples from a large number of drug abusers to establish concentration ranges and distribution patterns. Controlled dosing studies will be needed to establish the pharmacokinetics of the distribution of drugs and other substances into the nail matrix, their binding and elimination, if any, and the effects of cosmetic treatment on the analytes within the matrix.

In addition, the use of deuterated internal standards of exogenous (**synthetic**) anabolic steroids is desirable for the analysis of exogenous steroids in nail clippings. Improved analytical methods based on **GC-MS^n** or **LC-MS^n** might overcome the interference that was found in this project, which used GC-MS only. It is anticipated that a method can be established which can allow the analysis of nail clippings without extracting the drug and in trace amounts of nail samples. When this is achieved, nail will become a very useful sample with a promising future. When **MALDI-TOF-MS,** a high sensitivity analytical method, was experimentally applied to opium alkaloids, excellent

analytical data were obtained. However, problems remain to be solved in this technique with respect to the introduction of nail samples and subsequent quantitation of the analytes.

The final conclusion and observation which might be made are that the subject of drug analysis in nail is still in its infancy, at the stage of potential rapid development into which hair analysis entered more than ten years ago. The work described in this thesis has provided one route by which future progress might be made.

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Appendix

Table 3.8 is a database of doping steroids collected throughout research of this project. More than 600 kinds of the steroids collected consist of anabolic androgenic steroids, and its relative metabolites, estrogens, and progestagens.

Notes:

* **Bold** = steroids of stock (stored)

** **Bold and underline** = purchased steroids

*** **Bold and double underline** = synthesised steroids by author

**** ***Bold, italic and underine*** = deuterated steroids. "***-D***" is put on the deuterated steroids behind the compound name.

* Vol 1 - Vol 4 = Recent advances in doping analysis, Sports & Buch Strauss, **W. Schanzer** et al.

* Name of the number shows the same steroid name.

Table 3.8. Database of doping steroids (Androgen, Estrogen, Progestagen)

No.	Compound Name	Mol. Form.	MW	Mass Spectrum
1	acetylthio-19-nortestosterone, 7-	C20H28SO3	348.51	
2	algestone acetophenide	C29H36O4	448.61	-----
3	allylestrenol (17α-Allyl-estr-4-en-17ß-ol)	C21H32O	300.48	241, 55, 79, 91, 67, free-
4	altrenogest (17α-Allyl-trenbolone)	C21H26O2	310.44	
5	androisoxazole (17ß-OH-17α-Me-androsta[3,2-c]isoxazole)	C21H31NO2	329.48	
6	androst-13-en-3α-ol, 7α,17,17-trimethyl, 5ß-	C21H34O	302.50	
7	androst-16-en, 5α-	C19H30	258.44	243, 67, 79, ***258***, free-
8	androst-16-en-3α-ol, 5α-	C19H30O	274.45	***274***, 94, 148, 259, 79, free-
9	androst-16-en-3-one, 5α-	C19H28O	274.44	272, 257, 94, 79, 149, free-
10	androst-1-en-17ß-ol-3-one, 5α- (4-Dihydro-boldenone)	C19H28O2	288.43	246, ***288***, 79, 109, free-
11	androst-1-en-17ß-ol-3-one, 5ß-	C19H28O2	288.43	122, ***288***, 270, 109, free-
12	androst-1-en-3α-ol-17-one, 5ß-	C19H28O2	288.43	
13	androst-1-ene-3,17-dione, 5α-	C19H26O2	286.42	415, ***430***, 73, 416, di-
14	androst-1-ene-3,17-dione, 5ß-	C19H26O2	286.42	122, 244, ***286***, 109, free-
15	androst-1-ene-3α,17ß-diol, 5ß-	C19H30O2	290.45	
16	androst-1-ene-3α,6ß,16ß,17ß-tetrol-4-chloro-17α-methyl, 5ß-	C20H31ClO4	370.92	218, 219, 73, 231, ***658***, tetra-
17	androst-1-ene-3α,6ß,17ß-triol-16-one-4-chloro-17α-methyl, 5ß-	C20H29ClO4	368.90	***656***, 73, 658, 657, 244, tetra-
18	androst-1-ene-6ß-ol-3,17-dione, 5ß-	C19H26O3	302.42	
19	androst-4-en-17ß-ol-3-one-6α-Me-17a-(1-propynyl) (Dimethisterone)	C23H32O2	340.51	
20	androst-4-en-3α-ol-17-one-4-chloro	C19H27ClO2	322.87	451, ***466***, 468, di-
21	androst-4-ene-11ß-ol-3,17-dione	C19H26O3	302.42	***302***, 163, 123, 77, 91, free-
22	***androst-4-ene-11ß-ol-3,17-dione-D7***	***C19D7H19O3***	309.36	
23	androst-4-ene-19-ol-3,17-dione	C19H26O3	302.42	272, ***302***, 273, 79, 81, 77, free-
24	androst-4-ene-3,11,17-trione (Adrenosterone)	C19H24O3	300.40	122, ***300***, 91, 79, 105, free-
25	**androst-4-ene-3,16-dione**	**C19H26O2**	286.42	415, ***430***, 73, 416, di-
26	**androst-4-ene-3,17-dione**	C19H26O2	286.42	***434***, 143, 142, 434, di-

39	**androst-5-en-3ß-ol-17-one, 3ß-acetate**	C21H30O3	330.47	270, 255, 271, 269, free-
40	androst-5-en-3ß-ol-17-one, 3ß-enanthate (DHEA enanthate)	C26H40O3	400.61	-----
41	***androst-5-en-3β-ol-17-one-D2* (Dehydroepiandrosterone-D2)**	***C19D2H26O2***	290.43	
42	androst-5-ene-3,17-dione	C19H26O2	286.42	***286***, 177, 271, free-
43	androst-5-ene-3α,17α-diol	C19H30O2	290.45	
44	androst-5-ene-3α,17ß-diol	C19H30O2	290.45	
45	androst-5-ene-3ß,16α,17ß-triol	C19H30O3	306.45	
46	***androst-5-ene-3β,16α,17β-triol-D5***	***C19D5H25O3***	311.45	
47	androst-5-ene-3ß,16α,18-triol-17-one	C19H28O4	320.43	288, 255, 159, 270, free-
48	**androst-5-ene-3ß,16α-diol-17-one (16α-OH-DHEA)**	**C19H28O3**	304.43	***304***, 213, 286, 271, 231, free-
49	**androst-5-ene-3ß,17α-diol**	**C19H30O2**	290.45	254, 305, 215, 343, 249, di-
50	androst-5-ene-3ß,17α-diol, diacetate	C23H34O4	374.53	314, 239, 254, 315, free-
51	**androst-5-ene-3ß,17ß-diol**	**C19H30O2**	290.45	239, 305, 254, 344, 329, 215, di-
52	androst-5-ene-3ß,17ß-diol, 17-acetate	C21H32O3	332.49	
53	androst-5-ene-3ß,17ß-diol, 17ß-benzoate	C26H34O3	394.56	105, 77, 161, ***394***, free-
54	androst-5-ene-3ß,17ß-diol, 3-acetate	C21H32O3	332.49	272, 43, 121, 91, 105, 107, free-
55	androst-5-ene-3ß,17ß-diol, 3ß-Ac-17ß-benzoate	C28H36O4	436.60	105, 376, 77, 121, 133, free-
56	**androst-5-ene-3ß,17ß-diol, diacetate**	C23H34O4	374.53	314, 43, 315, 146, 121, free-
57	**androst-5-ene-3ß,17ß-diol, dipropionate**	C25H38O4	402.58	57, 328, 121, 133, 146, free-
58	**androst-5-ene-3ß,17ß-diol-16-one**	**C19H28O3**	304.43	***304***, 91, 79, 105, 286, 213, free-
59	androst-5-ene-3ß-ol-7,17-dione, 3ß-Ac (7-oxo-DHEA)	C21H28O4	344.46	284, 285, 256, 151, free-
60	androsta-1,4-dien-17ß-ol-3-one-4-chloro-17α-methyl	C20H27ClO2	334.87	
61	androsta-1,4-diene-3,11,17-trione (Δ1-Adrenosterone)	C19H22O3	298.40	***298***, 160, 91, 121, 254, free-
62	androsta-1,4-diene-3,17-dione (Boldione)	C19H24O2	284.40	122, ***284***, 159, 121, 91, free-
63	androsta-1,4-diene-3,17-dione, 1-Me- (Atamestane)	C20H26O2	298.43	
64	androsta-1,4-diene-6ß,12x,17ß-triol-3-one-4-chloro-17α-methyl	C20H27ClO4	334.89	
65	androsta-1,4-diene-6ß,16ß,17ß-triol-3-one-4-chloro-17α-methyl	C20H27ClO4	334.89	
66	androsta-1,4-diene-6ß,17ß-diol-3-one	C19H26O3	302.42	
67	androsta-1,4-diene-6ß,17ß-diol-3-one-4-chloro	C19H25ClO3	336.86	
68	androsta-1,4-diene-6ß,17ß-diol-3-one-4-chloro-17α-methyl	C20H27ClO3	350.89	143, 315, 73, 243, di-

69	androsta-1,4-diene-6ß-ol-3,17-dione	C19H24O3	300.40	
70	androsta-4,13-dien-11ß-ol-3-one-9α-fluoro-18-nor-17,17-diMe	C20H27FO2	318.43	
71	**androstan, 5α-**	**C19H32**	260.46	245, ***260***, 95, 203, free-
72	androstan, 5ß- (Etiocholane)	C19H32	260.46	245, 67, 95, 81, 203, ***260***, free-
73	**androstan-17-one, 5α-**	**C19H30O**	274.45	***274***, 67, 230, 241, 259, free-
74	androstan-17-one, 5ß-	C19H30O	274.45	***274***, 55, 67, 81, 108, free-
75	**androstan-17ß-ol, 5α-**	**C19H32O**	276.47	217, ***276***, 261, 67, 81, free-
76	androstan-17ß-ol-3-one-1α,17α-dimethyl-, 5α-	C21H34O2	318.50	43, 41, 81, 55, 67, 69, 79, free-
77	**androstan-3α-ol, 5α-**	**C19H32O**	276.47	243, 67, 95, 81, 258, free-
78	androstan-3α-ol-17-one, 3-benzoate, 5α-	C26H34O3	394.56	
79	androstan-3α-ol-17-one, 3-propionate, 5α-	C22H34O3	346.51	
80	**androstan-3α-ol-17-one, 5α- (Androsterone)**	**C19H30O2**	290.45	419, ***434***, 73, 329, 169, di-
81	**androstan-3α-ol-17-one, 5ß- (Etiocholanolone)**	**C19H30O2**	290.45	419, ***434***, 329, 73, 169, di-
82	androstan-3α-ol-17-one-4-chloro, 5α-	C19H29ClO2	324.89	453, ***468***, 470, di-
83	androstan-3α-ol-17-one-4-chloro, 5ß-	C19H29ClO2	324.89	
84	***androstan-3α-ol-17-one-D2, 5α- (Androsterone-D2)***	***C19D2H28O2***	292.45	
85	***androstan-3α-ol-17-one-D2, 5β- (Etiocholanolone-D2)***	***C19D2H28O2***	292.45	
86	***androstan-3α-ol-17-one-D4, 5α- (Androsterone-D4)***	***C19D4H26O2***	294.45	423, ***438***, 73, 333, di-
87	***androstan-3α-ol-17-one-D4, 5β- (3α-Etiocholanolone-D4)***	***C19D4H26O2***	294.45	423, ***438***, 73, 333, di-
88	**androstan-3-one, 5α-**	**C19H30O**	274.45	202, 203, ***274***, 259, 187, free-
89	androstan-3ß-ol, 3ß-acetate, 5α-	C21H34O2	318.50	243, 258, 258, 67, 244, free-
90	androstan-3ß-ol, 5α-	C19H32O	276.47	243, 67, 81, 95, 93, ***276***, free-
91	androstan-3ß-ol-16-one, 5α-	C19H30O2	290.45	***290***, 93, 67, 79, 257, free-
92	**androstan-3ß-ol-17-one, 5α- (Epiandrosterone)**	**C19H30O2**	290.45	419, ***434***, 73, 329, 239, di-
93	androstan-3ß-ol-17-one, 5ß- (Epietiocholanolone)	C19H30O2	290.45	***290***, 67, 79, 244, 93, 81, free-
94	androstan-3ß-ol-17-one-4chloro, 5ß-	C19H29ClO2	324.89	
95	androstan-3ß-ol-17-one-4x-chloro, 5α-	C19H29ClO2	324.89	
96	***androstan-D1, 5α- (16-d1 form)***	***C19DH31***	261.46	
97	androstane-3,11,17-trione, 5α-	C19H26O3	302.43	***302***, 124, 41, 55, free-
98	**androstane-3,17-dione, 5α-**	C19H28O2	288.43	73, 275, ***432***, 417, 290, di-

99	androstane-3,17-dione, 5ß-	C19H28O2	288.43	275, 73, 417, 290, di-
100	androstane-3,17-dione-4-chloro, 5ß-	C19H27ClO2	322.88	
101	androstane-3α,16x-diol-17-one-4x-chloro, 5x-	C19H29ClO3	340.89	
102	androstane-3α,17α-diol, 5α-	C19H32O2	292.47	241, 129, 75, 331, 256, di-
103	androstane-3α,17α-diol, 5ß-	C19H32O2	292.47	215, 67, 256, 274, free-
104	androstane-3α,17α-diol, diacetate, 5α-	C23H36O4	376.54	256, 241, 316, 215, free-
105	androstane-3α,17α-diol, diacetate, 5ß-	C23H36O4	376.54	256, 241, 257, 215, free-
106	androstane-3α,17α-diol-7a,17ß-dimethyl, 5α-	C21H36O2	320.52	
107	androstane-3α,17α-diol-7α,17ß-dimethyl, 5ß-	C21H36O2	320.52	
108	androstane-3α,17α-diol-7ß,17ß-dimethyl, 5ß-	C21H36O2	320.52	
109	**androstane-3α,17ß-diol, 5α-**	**C19H32O2**	292.47	75, 241, 129, 257, 215, di-
110	**androstane-3α,17ß-diol, 5ß-**	**C19H32O2**	292.47	256, 241, 73, 129, 75, 215, di-
111	androstane-3α,17ß-diol, diacetate, 5α-	C23H36O4	376.54	316, 241, 256, 262, free-
112	androstane-3α,17ß-diol, diacetate, 5ß-	C23H36O4	376.54	316, 256, 241, 215, 301, free-
113	androstane-3α,17ß-diol-7α,17α-dimethyl, 5α-	C21H36O2	320.52	
114	androstane-3α,17ß-diol-7α,17α-dimethyl, 5ß-	C21H36O2	320.52	
115	androstane-3α,17ß-diol-7ß,17α-dimethyl, 5α-	C21H36O2	320.52	
116	androstane-3α,17ß-diol-7ß,17α-dimethyl, 5ß-	C21H36O2	320.52	
117	***androstane-3α,17β-diol-D2, 5α- (16,16-d2 form)***	***C19D2H30O2***	294.45	
118	***androstane-3α,17β-diol-D3, 5α- (16,16,17-d3 form)***	***C19D3H29O2***	295.45	
119	androstane-3α-ol-11,17-dione, 5α- (Androsterone-11-one)	C19H28O3	304.43	***304***, 79, 67, 105, free-
120	**androstane-3α-ol-11,17-dione, 5ß- (Etiocholanolone-11-one)**	**C19H28O3**	304.43	***304***, 232, 191, 286, 150, free-
121	androstane-3ß,11ß-diol-17-one, 5α-	C19H30O3	306.45	***306***, 273, 79, 93, free-
122	androstane-3ß,17α-diol, 5α-	C19H32O2	292.47	241, 129, 73, 75, 421, di-
123	androstane-3ß,17α-diol, 5ß-	C19H32O2	292.47	256, 241, 346, 331, 129, di-
124	androstane-3ß,17α-diol, diacetate, 5α-	C23H36O4	376.54	316, 241, 256, 148, free-
125	androstane-3ß,17α-diol, diacetate, 5ß-	C23H36O4	376.54	256, 241, 316, 215, free-
126	androstane-3ß,17α-diol-4x-chloro, 5x-	C19H31ClO2	326.91	
127	**androstane-3ß,17ß-diol, 5α- (Drostandiol)**	**C19H32O2**	292.47	129, 73, 75, 241, 421, di-
128	androstane-3ß,17ß-diol, 5ß-	C19H32O2	292.47	256, 241, 129, 346, di-
129	androstane-3ß,17ß-diol, diacetate, 5α-	C23H36O4	376.54	256, 301, 241, 67, 316, free-

130	androstane-3ß,17ß-diol, diacetate, 5ß-	C23H36O4	376.54	316, 241, 256, 301, free-
131	androstane-3ß,17ß-diol-7α,17α-dimethyl, 5α- (Tetrahydrobolasterone)	C21H36O2	320.51	
132	***androstane-3ß,17ß-diol-D2, 5α- (16,16-d2 form)***	***C19D2H30O2***	294.45	
133	***androstane-3ß,17ß-diol-D3, 5α- (16,16,17-d3 form)***	***C19D3H29O2***	295.45	
134	androstane-3ß-ol-11,17-dione, 5α- (Epiandrosterone-11-one)	C19H28O3	304.43	***304***, 79, 67, 91, free-
135	**androstane-3ß-ol-7,17-dione, 5α-**	**C19H28O3**	304.41	257, ***304***, 79, 67, free-
136	beclomethasone (9α-Cl-16ß-Me-Prednisolone)	C22H29ClO5	408.92	121, 223, 147, 91, 135, free-
137	beclomethasone dipropionate	C28H37ClO7	521.05	57, 295, 331, 121, 277, free-
138	**betamethasone (9α-F-16ß-Me-Prednisolone)**	**C22H29FO5**	392.47	122, 121, 41, 91, 123, 55, free-
139	betamethasone-17α-valerate	C27H37FO6	476.59	60, 121, 41, 56, 85, free-
140	**bolandiol (Estr-4-ene-3ß,17ß-diol)**	C18H28O2	276.42	75, ***420***, 74, 73, 240, di-
141	bolandiol dipropionate	C24H36O4	388.55	
142	**bolasterone (7α-Me-17α-Me-Testo.)**	C21H32O2	316.48	298, 124, 175, 135, ***316***, free-
143	bolazine (2α-Me-5α-DHT azine)	C40H64N2O2	604.97	
144	bolazine capronate	C46H74N2O4	719.11	
145	boldenone,α- (Epiboldenone)	C19H26O2	286.42	
146	**boldenone, ß- (1,2-Dehydro-Testo.)**	**C19H26O2**	286.42	206, ***430***, 325, 415, di-
147	**boldenone-17ß-acetate**	C21H28O3	328.46	122, 147, ***328***, free-
148	**boldenone-17ß-benzoate**	C26H30O3	390.53	105, 122, 77, 147, 91, free-
149	boldenone-17ß-undecylenate	C30H44O3	452.68	122, 147, 55, 121, 123, 41, free-
150	bolenol (19-Nor-17α-pregn-5-en-17ß-ol)	C20H30O2	302.46	
151	bolmantalate (Nortestosterone-1-adamantanecarboxylate)	C29H40O4	452.64	
152	**calusterone (7ß-Me-17α-Me-Testo.)**	C21H32O2	316.48	***316***, 124, 259, free-
153	**canrenone (17α-(2-Carboxyethyl)-6-ene-Testo.) lactone)**	C22H28O3	340.46	267, 107, 91, 136, ***340***, free-
154	chlormadinone (6-Cl-Pregna-4,6-diene-17α-ol-3,20-dione)	C21H27ClO3	362.89	***578***, 473, tri-
155	chlormadinone acetate	C23H29ClO4	404.93	175, 43, 234, 344, 91, 115, free-
156	**clobetasol-17α-propionate**	C25H32ClFO5	466.00	331, 71 (Clobetasone)
157	clomegestone (6-Cl-17-OH-16α-Me-Pregna-4,6-diene-3,20-dione)	C22H29ClO3	376.93	
158	**clostebol (4-Cl-Testo.)**	C19H27ClO2	322.87	73, 129, 358, 268, ***466***, di-
159	clostebol diol	C19H29ClO2	324.89	

160	**clostebol-17ß-acetate**	C21H29ClO3	364.90	73, ***436***, mono-
161	clostebol-17ß-caproate	C27H41ClO3	463.09	
162	clostebol-17ß-propionate	C22H31ClO3	378.93	
163	cyano-19-nortestosterone, 7-	C19H25NO2	299.41	
164	cyclopenten-1-yloxy-5ß-androst-1-en-3α-ol, 1-, (17ß-)	C24H36O2	356.55	
165	cyclopenten-1-yloxy-5ß-androst-1-en-3-one, 1-, (17ß-)	C24H34O2	354.54	
166	cyclopentyloxy-estra-1,3,5(10)-triene-16α,17ß-diol, 3- (Quinestradiol)	C23H32O3	356.51	
167	cyproterone (6-Cl-1α,2α-diH-17-ol-3'H-Cyclopropa[1,2]pregna-4,6-diene-3,20-dione)	C22H27ClO3	374.91	
168	cyproterone acetate	C24H29ClO4	406.94	
169	**danazol (17α-Ethynyl-17ß-OH-4-androsta[2,3-d]isoxazole)**	**C22H27NO2**	337.46	***337***, 146, 91, 132, 147, 173, free-
170	dehydro-17α-methyltestosterone, 9(11)-	C20H28O2	300.44	-----
171	dehydrocorticosterone, 11-	C21H28O4	344.45	313, 285, 91, 121, 41, 122, free-
172	dehydroestradiol, 9(11)-	C18H22O2	270.37	***270***, 211, 160, 147, 158, 157, free-
173	delmadinone acetate (Δ1-Chlormadinone acetate)	C23H27ClO4	402.92	
174	dexamethasone (9α-F-16α-Me-Prednisolone)	C22H29FO5	392.48	121, 122, 315, 223, 43, 91, free-
175	**dihydrotestosterone, 5α- (5α-Androstan-17ß-ol-3-one)(5α-DHT)**	**C19H30O2**	290.45	73, ***434***, 143, 142, di-
176	dihydrotestosterone, 5ß- (5ß-Androstan-17ß-ol-3-one)(5ß-DHT)	C19H30O2	290.45	***290***, 220, 247, free-
177	**dihydrotestosterone-17ß-benzoate**	C26H34O3	394.56	-----
178	dihydrotestosterone-17ß-bromoacetate	C21H32BrO3	412.39	-----
179	**dihydrotestosterone-17ß-enanthate**	C26H42O3	402.62	-----
180	dihydrotestosterone-17ß-propionate	C22H34O3	346.51	
181	dihydrotestosterone-17ß-valelate	C24H38O3	374.57	
182	***dihydrotestosterone-D2, 5α- (5α-DHT-D2)***	***C19D2H28O2***	292.45	
183	***dihydrotestosterone-D3, 5α- (5α-DHT-D3)***	***C19D3H27O2***	293.45	73, 143, ***437***, 75, di-
184	***dihydrotestosterone-D3, 5ß- (5ß-DHT-D3)***	***C19D3H27O2***	293.45	
185	**dromostanolone (2α-Me-DHT)**	C20H32O2	304.47	***448***, 73, 141, 405, 157, di-
186	**dromostanolone acetate**	C22H34O3	346.51	55, 94, 95, 93, 79, 67, 149, free-
187	**dromostanolone propionate**	C23H36O3	360.54	286, 149, 271, 94, free-
188	drospirenone	C24H30O3	318.50	
189	dydrogesterone (10α-Isopregnenone)	C21H28O2	312.45	---

190	enestebol (4-OH-Methandrostenolone)	C20H28O3	316.44	
191	epiandrosterone-3ß-acetate	C21H32O3	332.49	
192	epiclostebol (4-Cl-17α-Epitestosterone)	C19H27ClO2	322.87	
193	epiestriol, 16- (Estra-1,3,5-triene-3,16ß,17ß-triol)	C18H24O3	288.39	***288***, 289, 213, 160, free-
194	epifluoxymesterone (9α-F-11ß-OH-17ß-Me-Testo.)	C20H29FO3	336.45	
195	epimethandrostenolone, 17α-	C20H28O2	300.44	206, ***444***, 339, 73, di-
196	epimethandrostenolone-6ß,16ß-diol	C20H28O4	332.43	
197	epimethenolone	C20H30O2	302.46	
198	epimethyltestosterone, 17ß-	C20H30O2	302.46	
199	epioxandrolone, 17α-	C19H30O3	306.45	
200	**epitestosterone, 17α-**	**C19H28O2**	288.43	73, ***432***, 433, 417, di-
201	***epitestosterone-D3, 17α- (16,16,17-d3 form)***	***C19D3H25O2***	291.43	***435***, 73, 420, di-
202	epitiostanol (2α,3α-Epithio-5α-androstan-17ß-ol)	C19H30OS	306.51	272, 91, 81, 93, 105, 55, free-
203	estr-1-en-3α-ol-17-one, 5α- (1,2-deH-19-Norandrosterone)	C18H26O2	274.41	403, ***418***, 404, 419, 313, di-
204	estr-1-en-3α-ol-17-one, 5ß- (1,2-deH-19-Noretiocholanolone)	C18H26O2	274.41	403, ***418***, 404, 419, 313, di-
205	estr-4-en-17α-ethynyl-17ß-ol (Lynestrenol)	C20H28O	284.44	91, 79, 67, 77, 201, free-
206	estr-4-en-17α-ol-3-one (19-nor-epi-Testo.)	C18H26O2	274.41	***274***, 256, 91, 79, 232, free-
207	estr-4-en-17α-vinyl-17ß-ol-3-one (Norvinisterone)	C20H28O2	300.44	
208	estr-4-en-3α-ol-17-one-4-chloro	C18H25ClO2	308.85	
209	**estr-4-ene-3,17-dione**	C18H24O2	272.39	***272***, 110, 91, 41, 79, 186, free-
210	estr-4-ene-3,17-dione-4-chloro	C18H23ClO2	306.84	
211	***estr-4-ene-3,17-dione-D4***	***C18D4H20O2***	276.39	
212	estr-4-ene-3ß,17ß-diol	C18H28O2	276.42	-----
213	estr-4-ene-6ß-ol-3,17-dione (6ß-OH-Norandrostenedione)	C18H24O3	288.39	***504***, 73, 505, 147, tri-
214	estr-5(10)-en-3α-ol,17-one	C18H26O2	274.41	403, ***418***, 404, 419, di-
215	estr-5(10)-en-3ß-ol-17-one	C18H26O2	274.41	
216	estr-5(10)-ene-3,17-dione	C18H24O2	272.39	***272***, 91, 216, 244, free-
217	estr-5(10)-ene-3α,17α-diol	C18H28O2	276.42	
218	estr-5(10)-ene-3α,17ß-diol	C18H28O2	276.42	
219	estr-5(10)-ene-3ß,17α-diol	C18H28O2	276.42	

220	estr-5(10)-ene-3ß,17ß-diol	C18H28O2	276.42	258, 91, 79, 240, free-
221	estra-1,3,5(10)-triene-17α-ethynyl-11ß-MeO-3,17ß-diol (Moxestrol)	C21H26O3	326.44	
222	estra-1,3,5(10)-triene-17ß-MeO-3-propoxy (Promestriene)	C22H32O2	328.50	
223	estra-1,3,5(10)-triene-2-MeO-3,16α,17ß-triol (2-MeO-estriol)	C19H26O4	318.42	***318***, 319, 189, 137, 176, 231, free-
224	estra-1,3,5(10)-triene-2-MeO-3,16α-diol-17ß-one (2-MeO-estrone)	C19H24O3	300.40	***300***, 301, 176, 137, 215, 150, free-
225	estra-1,3,5(10)-triene-2-MeO-3,17ß-diol (2-MeO-estradiol)	C19H26O3	302.42	***302***, 137, 303, 176, 189, 175, free-
226	estra-1,3,5(10)-triene-3,15α,16α,17ß-tetrol	C18H24O4	304.39	
227	estra-1,3,5(10)-triene-3,15α,16α-triol-17-one	C18H22O4	302.37	
228	estra-1,3,5(10)-triene-3,16α,17ß,18-tetrol	C18H24O4	304.39	
229	estra-1,3,5(10)-triene-3,16α,18-triol-17-one	C18H22O4	302.37	
230	estra-1,3,5(10)-triene-3-MeO-16-Me-16ß,17ß-diol (Mytatrienediol)	C20H28O3	316.44	
231	estra-4,9-dien-17α-methyl-17ß-propionyl-3-one (Promegestone)	C22H30O2	326.48	
232	estradiol, 17α-	C18H24O2	272.39	***272***, 160, 172, 146, 213, free-
233	**estradiol, 17ß- (Estra-1,3,5(10)-triene-3,17ß-diol)**	**C18H24O2**	272.39	***272***, 213, 160, 172, free-
234	estradiol-16-one	C18H22O3	286.37	***286***, 213,172, 214, 287, free-
235	estradiol-17ß-cipionate	C26H36O3	396.57	***272***, 160, 146, 213, 172, free-
236	estradiol-17ß-enantate	C25H36O3	384.56	
237	estradiol-17ß-hexahydrobenzoate	C25H34O3	382.55	
238	estradiol-17ß-phenylpropionate	C27H32O3	404.67	
239	estradiol-17ß-undecylate	C29H44O3	440.67	57, 55, 159, ***440***, 133, 146, free-
240	estradiol-17ß-valerate	C23H32O3	356.51	
241	estradiol-3,17-dipropionate	C24H32O4	384.52	328, 329, 57, 172, ***384***, free-
242	**estradiol-3-benzoate**	C25H28O3	376.50	105, 77, ***376***, 106, 377, free-
243	**estradiol-3-methyl ether, 17ß-**	**C19H26O2**	286.42	***286***, 186, 173, 160, 227, free-
244	***estradiol-D3, 17β-* (16,16,17-d3 form)**	***C18D3H21O2***	275.39	***419***, 339, di-
245	estran-17ß-ol-3-one, 5α- (Nordihydrotestosterone, 19-)	C18H28O2	276.42	41, 55, 67, 81, 79, 68, 217, free-
246	estran-3α-ol-17-one, 3α-acetate, 5α- (19-Norandrosterone-Ac)	C20H30O3	318.46	258, 91, 230, 201, ***318***, free-
247	estran-3α-ol-17-one, 3α-acetate, 5ß- (19-Noretiocholanolone-Ac)	C20H30O3	318.46	258, 93, 187, 91, 214, 201, free-
248	**estran-3α-ol-17-one, 5α- (19-Norandrosterone)**	C18H28O2	276.42	405, ***420***, 315, 73, di-
249	**estran-3α-ol-17-one, 5ß- (19-Noretiocholanolone)**	C18H28O2	276.42	***276***, 91, 201, 202, 199, free-

250	estran-3α-ol-17-one-4x-chloro, 5α-	C18H27ClO2	310.86	
251	estran-3α-ol-17-one-4x-chloro, 5ß-	C18H27ClO2	310.86	
252	***estran-3α-ol-17-one-D3, 5α-* (19-Norandrosterone-D3)**	***C18D3H25O2***	279.42	
253	***estran-3α-ol-17-one-D3, 5ß-* (19-Noretiocholanolone-D3)**	***C18D3H25O2***	279.42	
254	estran-3ß-ol-17-one, 5α- (Norepiandrosterone)	C18H28O2	276.42	
255	estran-3ß-ol-17-one, 5ß- (Norepietiocholanolone)	C18H28O2	276.42	***276***, 258, 91, 201, 187, free-
256	***estran-3β-ol-17-one-D3, 5a-* (Norepiandrosterone-3α,4,5α-D3)**	***C18D3H25O2***	279.36	***279***, 79, 69, 55, 204, free-
257	estrane-3,17-dione, 5α-	C18H26O2	274.41	***274***, 230, 215, 218, 256, free-
258	estrane-3α,16-diol-17-one, 5α- (16-OH-19-Norandrosterone)	C18H28O3	292.42	
259	estrane-3α,16x-diol-17-one-4x-chloro, 5x-	C18H27ClO3	326.86	
260	estrane-3α,17ß-diol, 5ß-	C18H30O2	278.44	203, 260, 201, 91, 216, 242, free-
261	estrane-3α,17ß-diol-17α-ethyl, 5α-,	C20H34O2	306.44	
262	estrane-3α,17ß-diol-17α-ethyl, 5ß-	C20H34O2	306.44	
263	estrane-3α,17ß-diol-17α-ethynyl, 5α- (Tetrahydronorethindrone, 3α,5α-)	C20H30O2	302.46	
264	estrane-3α,17ß-diol-17α-ethynyl, 5ß- (Tetrahydronorethisterone, 3α,5ß-)	C20H30O2	302.46	
265	estrane-3ß,17α-diol, 5α-	C18H30O2	274.41	260, 201, 91, 216, free-
266	estrane-3ß,17α-diol-4x-chloro, 5x-	C18H29ClO2	312.88	
267	**estriol (Estra-1,3,5(10)-triene-3,16α,17ß-triol)**	**C18H24O3**	288.39	***288***, 160, 146, 213, free-
268	**estrone (Estra-1,3,5(10)-trien-3-ol-17-one)**	**C18H22O2**	270.37	73, 155, ***414***, 399, di-
269	ethinylestradiol 3-cyclopentyl ether, 17α- (Quinestrol)	C25H32O2	364.50	213, 160, 133, ***364***, 41, 159, free-
270	ethinylestradiol, 17α-	C20H24O2	296.41	213, ***296***, 160, 133, free-
271	ethisterone (17α-Ethinyl-Testo.)	C21H28O2	312.45	124, ***312***, 91, 79, 121, 229, free-
272	ethylestrenol (17α-Ethyl-estr-4-en-17ß-ol)	C20H32O	288.46	216, 201, 241, 91, ***288***, free-
273	ethynodiol (Estr-4-ene-17α-ethynyl-3ß,17ß-diol)	C20H28O2	300.44	
274	ethynodiol diacetate	C24H32O4	384.52	43, 324, 91, 199, 79, 325, free-
275	etonogestrel (3-Keto-desogestrel)	C22H28O2	324.47	
276	**fludrocortisone**	**C21H29FO5**	380.46	43, 321, 225, 41, 283, 55, free-
277	flumethasone	C22H28F2O5	410.46	
278	**fluoxymesterone (9α-F-11ß-OH-17α-Me-Testo.)**	C20H29FO3	336.45	73, ***552***, 462, 407, 319, tri-
279	fluoxymesterone-6ß,11ß-diol	C20H29FO4	352.45	

280	flurogestone acetate	C23H31FO5	406.49	
281	formebolone (2-Formyl-11α-OH-methandrostenolone)	C21H28O4	344.45	-----
282	furazabol (17ß-OH-17α-Me-5α-Androsta[2,3-c]-furazan	C20H30N2O2	330.47	143, 209, ..***402***, mono-
283	fusidic acid	C31H48O6	516.73	95, 220, 177, 43, 123, 123, free-
284	gestodene	C21H26O2	310.44	
285	gestonorone caproate (17α-ol-19-norpregn-4-ene-3,20-dione hexanoate)	C26H38O4	414.60	273, 99, 255, 71, 274, 371, free-
286	gestrinone (13ß-Et-17α-Ethynyl-17ß-OH-4,9,11-Gonatrien-3-one)	C21H24O2	308.42	
287	halometasone (2-Cl-6α,9-diF-11α,17,21-triol-16α-Me-Pregna-1,4-diene-3,20-dione)	C22H27ClF2O5	444.91	
288	hydroxy-17α-methyltestosterone, 11α-	C20H30O3	318.46	
289	hydroxy-17α-methyltestosterone, 4-	C20H30O3	318.46	
290	hydroxy-17α-methyltestosterone, 6ß-	C20H30O3	318.46	143, 73, 283, 405, di-
291	hydroxy-4-Cl-testosterone, 6-	C19H27ClO3	338.87	73, 519, 503, ***554***, tri-
292	hydroxyandrosterone, 11ß-	C19H30O3	306.45	***522***, 73, 168, 417, tri-
293	hydroxyandrosterone, 6ß-	C19H30O3	306.45	73, ***522***, 327, 417, 507, tri-
294	***hydroxyandrosterone-D4, 11ß- (2,2,4,4-d4 form)***	***C19D4H26O3***	310.45	***526***, 73, 168, 421, 256, tri-
295	***hydroxyandrosterone-D7, 11ß- (2,2,4,6,6,16,16-d7 form)***	***C19D7H23O3***	313.45	
296	hydroxyboldenone, 6ß-	C19H26O3	302.42	73, 209, ***446***, di-
297	hydroxyepiandrosterone, 6ß-	C19H30O3	306.45	73, ***522***, 507, 75, 417, 327, tri-
298	hydroxyepistanozolol, 16ß-	C21H32N2O2	344.50	
299	hydroxyepistanozolol, 3'-	C21H32N2O2	344.50	
300	hydroxyestradiol, 15α-	C18H24O3	288.39	***288***, 83, 85, 160, free-
301	hydroxyestradiol, 2-	C18H24O3	288.39	***288***, 160, 213, 107, free-
302	hydroxyestradiol, 4-	C18H24O3	288.39	
303	hydroxyestradiol, 6α-	C18H24O3	288.39	270, 158, 157, 144, 211, free-
304	hydroxyestradiol, 6ß-	C18H24O3	288.39	
305	hydroxyestriol, 2-	C18H24O4	304.39	
306	hydroxyestriol, 5α-	C18H24O4	304.39	
307	hydroxyestrone, 16α-	C18H22O3	286.37	213, ***286***, 172, 91, free-
308	hydroxyestrone, 16ß-	C18H22O3	286.37	
309	hydroxyestrone, 2-	C18H22O3	286.37	***286***, 287, 201, 188, 97, free-
310	hydroxyestrone, 4-	C18H22O3	286.37	***286***, 287, 162, 219, free-

311	hydroxyestrone, 6α-	C18H22O3	286.37	
312	hydroxyestrone, 6ß-	C18H22O3	286.37	
313	hydroxyestrone-3,16α-diacetate, 16α-	C22H26O5	370.45	328, 214, 329, 172, ***370***, free-
314	hydroxyethyl-5α-estrane-3α,17ß-diol, 17α-(2-)	C20H34O3	322.49	
315	**hydroxyetiocholanolone, 11ß-**	**C19H30O3**	306.45	***306***, 67, 79, 97, free-
316	hydroxyetiocholanolone, 16α-	C19H30O3	306.45	-----
317	hydroxyetiocholanolone, 6ß-	C19H30O3	306.45	73, 147, ***522***, 327, 377, tri-
318	***hydroxyetiocholanolone-D7, 11ß-* (2,2,4,6,6,16,16-d7 form)**	***C19D7H23O3***	313.45	
319	**hydroxyfluoxymesterone, 6ß-**	C20H29FO4	352.45	143, 73, 75, 144, 129, di-
320	hydroxyfurazabol, 16ß-	C20H30N2O3	346.47	218, 231, 117, 147, di-
321	hydroxymethandriol, 16-	C20H32O3	320.47	
322	hydroxymethandrostenolone, 16-	C20H28O3	316.44	117, 218, 249, 368, 278, tri-
323	**hydroxymethandrostenolone, 6ß-**	C20H28O3	316.44	517, 518, 73, 229, 294, tri-
324	hydroxymethyl-11a-OH-methandrostenolone, 2x-	C21H30O3	330.47	
325	hydroxymethylene-17α-Me-5α-androstan-17ß-ol-3-one, 2x-	C21H34O3	334.50	
326	hydroxymethylene-17α-Me-5α-androstane-16x,17ß-diol-3-one, 2x-	C21H34O4	350.50	
327	hydroxymethylene-17α-Me-5α-androstane-3x,17ß-diol, 2x-	C21H36O3	336.52	
328	hydroxymethylene-17α-Me-5α-androstane-3x,6x,17ß-triol, 2x-	C21H36O4	352.52	
329	hydroxymethylene-17α-Me-5α-androstane-6x,17ß-diol-3-one, 2x-	C21H34O4	350.50	
330	hydroxymethyl-ethisterone, 2-	C22H30O3	342.48	
331	hydroxyoxandrolone, 16x-	C20H30O4	334.46	
332	**hydroxypregnenolone, 21-**	**C21H32O3**	332.49	301, 255, ***332***, 55, 41, 91, free-
333	hydroxyprogesterone, 16α-	C21H30O3	330.47	43, 231, 100, 124, 91, 79, 41, free-
334	hydroxyprogesterone-17α-acetate	C23H32O4	372.51	43, 269, 287, 270, 229, 330, free-
335	hydroxyprogesterone-17α-caproate	C27H40O4	428.62	
336	hydroxyprogesterone-3-cyclopentyl enol ether, 17α- (Pentagestrone)	C26H38O3	398.58	
337	**hydroxystanozolol, 16ß-**	C21H32N2O2	344.50	218, ***560***, 217, 231, tri-
338	**hydroxystanozolol, 3'-**	C21H32N2O2	344.50	143, 545, ***560***, 254, tri-
339	hydroxystanozolol, 4α-	C21H32N2O2	344.50	
340	hydroxystanozolol, 4ß-	C21H32N2O2	344.50	143, ***560***, 73, 254, 545, tri-

341	***hydroxystanozolol-D3, 16ß-***	***C21D3H29N2O2***	347.50	
342	***hydroxystanozolol-D3, 3-***	***C21D3H29N2O2***	347.50	
343	***hydroxystanozolol-D3, 4ß-***	***C21D3H29N2O2***	347.50	
344	hydroxytestosterone, 11ß-	C19H28O3	304.43	***304***, 163, 91, 124, 79, free-
345	hydroxytestosterone, 14α-	C19H28O3	304.43	271, 286, 91, 124,79, free-
346	hydroxytestosterone, 15α-	C19H28O3	304.43	
347	hydroxytestosterone, 15ß-	C19H28O3	304.43	
348	hydroxytestosterone, 16α-	C19H28O3	304.43	***304***, 124, 91, 79, 262, free-
349	hydroxytestosterone, 17ß-benzoate, 19-	C26H32O4	408.54	
350	hydroxytestosterone, 19-	C19H28O3	304.43	274, ***304***, 275, 273, 91, 110, free-
351	hydroxytestosterone, 2α-	C19H28O3	304.43	260, ***304***, 261, 91, free-
352	hydroxytestosterone, 2ß-	C19H28O3	304.43	
353	hydroxytestosterone, 6α-	C19H28O3	304.43	***304***, 79, 245, 81, 275, free-
354	hydroxytestosterone, 6ß-	C19H28O3	304.43	433, 392, ***448***, 73, di-
355	isoflupredone (9-Fluoropredonisolone)	C21H27FO5	378.44	
356	**ketotestosterone, 11-**	C19H26O3	302.42	122, ***302***, 91, 79, 181, free-
357	ketotestosterone, 16-	C19H26O3	302.42	
358	mebolazine (2α-Me-5α-Me-DHT azine)	C42H68N2O2	633.02	
359	medrogestone (Dimethyl-6-deH-progesterone, 6,17α-)	C23H32O2	340.51	***340***, 43, 297, 109, 189, 175, free-
360	**medroxyprogesterone (6α-Me-17α-OH-Pregn-4-ene-3,20-dione)**	C22H32O3	344.50	***560***, 545, tri-
361	medroxyprogesterone-17α-acetate	C24H34O4	386.54	283, 91, 137, 145, 105, 93, free-
362	***medroxyprogesterone-D3***	***C22D3H32O3***	347.49	
363	megestrol	C22H30O3	342.48	
364	megestrol acetate	C24H32O4	384.52	281, 43, 282, 187, 91, 107, free-
365	***megestrol-D3***	***C22D3H27O3***	345.48	
366	melengestrol (6-Me-16-methylene-17α-OH-6-ene-Progesterone)	C23H30O3	354.49	
367	melengestrol acetate	C23H30O3	354.49	
368	***melengestrol-D3***	***C23D3H27O3***	357.49	
369	mepitiostane	C25H40O2S	404.66	
370	mesabolone (17ß-[(1-MeO-cyclohexyl)oxy]-5α-androst-1-en-3-one)	C26H40O3	400.61	

371	**mestanolone (17α-Me-5α-DHT)**	**C20H32O2**	304.48	73, 143, ***448***, 216, 358, di-
372	**mesterolone (1α-Me-5α-DHT)**	**C20H32O2**	304.47	141, 73, 157, 433, ***448***, di-
373	mesterolone-17ß-acetate	C22H34O3	346.51	
374	**mestranol (17α-Ethynyl-estradiol-3-Me-ether)**	**C21H26O2**	310.44	227, ***310***, 174, 147, 228, free-
375	**methandriol (17α-Me-androst-5-ene-3ß,17ß-diol)**	**C20H32O2**	304.47	253, 213, ***304***, 145, free-
376	**methandriol-diacetate**	C24H36O4	388.55	-----
377	**methandriol-dipropionate**	C26H40O4	416.51	57, 342, 253, 268, 43, 145, free-
378	**methandrostenolone (17α-Me-androsta-1,4-dien-17ß-ol-3-one)**	**C20H28O2**	300.43	73, 206, ***444***, 143, 339, di-
379	methandrostenolone-6ß,12-diol	C20H28O4	332.43	
380	methandrostenolone-6ß,16α-diol	C20H28O4	332.43	
381	methandrostenolone-6ß,16ß-diol	C20H28O4	332.43	
382	**methenolone (1-Me-1,2-dehydro-5α-DHT)**	C20H30O2	302.46	73, ***446***, 208, 193, 129, di-
383	**methenolone-17ß-acetate**	**C22H32O3**	344.50	123, 136, 135, 91, 79, 93, free-
384	**methenolone-17ß-enanthate**	C27H42O3	414.63	195, 208, ..***486***, mono-
385	methyl-19-nor-pregna-4,9-diene-3,20-dione, 17α- (Demegestone)	C21H28O2	312.46	
386	methyl-5α-androst-1-en-16x-ol-17-one, 2-	C20H30O2	302.46	
387	methyl-5α-androst-1-en-3α-ol-17-one, 2-	C20H30O2	302.46	
388	methyl-5α-androst-1-en-3α-ol-17-one, 2x-	C20H30O2	302.46	
389	methyl-5α-androst-1-ene-16α,16ß-diol-3,17-dione, 2-	C20H28O4	332.44	
390	methyl-5α-androst-1-ene-16α-ol-3,17-dione, 1-	C20H28O3	316.44	
391	methyl-5α-androst-1-ene-16x,17ß-diol-3-one, 2-	C20H30O3	318.46	
392	methyl-5α-androst-1-ene-18-ol-3,17-dione, 1-	C20H28O3	316.44	
393	methyl-5α-androst-1-ene-3α,16α-diol-17-one, 1-	C20H30O3	318.46	
394	methyl-5α-androst-1-ene-3α,17ß-diol, 1-	C20H32O2	304.48	
395	methyl-5α-androst-1-ene-3x,16x-diol-17-one, 2-	C20H30O3	318.46	
396	methyl-5α-androst-1-ene-6ß-ol-3,17-dione, 1-	C20H28O3	316.44	
397	methyl-5α-androstan-17ß-ol, 17a-	C20H34O	290.49	
398	methyl-5α-androstan-17ß-ol-3-one, 17ß-acetate, 1-	C22H34O3	346.51	
399	methyl-5α-androstan-17ß-ol-3-one, 17ß-enanthate, 1-	C27H44O3	416.65	
400	methyl-5α-androstan-2-oxa-17α-ol-3-one, 17ß-	C20H30O3	318.46	

401	methyl-5α-androstan-3α-ol-17-one, 1α-	C20H32O2	304.45	73, 433, ***448***, 75, 343, di-
402	methyl-5α-androstan-3α-ol-17-one, 2α-	C20H32O2	304.45	
403	methyl-5α-androstan-3α-ol-17-one, 2x-	C20H32O2	304.45	
404	methyl-5α-androstane-18-ol-3,17-dione, 1α-	C20H30O3	318.46	
405	methyl-5α-androstane-3,17-dione, 1α-	C20H30O2	302.46	73, 290, 275, 75, di-
406	methyl-5α-androstane-3α,16α-diol-17-one, 1α-	C20H32O3	320.49	521, 73, ***536***, 147, tri-
407	methyl-5α-androstane-3α,17α-diol, 17α-	C20H34O2	306.45	
408	methyl-5α-androstane-3α,17α-diol, 17ß-	C20H34O2	306.45	
409	***methyl-5α-androstane-3α,17β-diol-D3, 17α-*** **(17α-Cd3 form)**	***C20D3H31O2***	309.45	274, 364, 146, di-; CI
410	methyl-5α-androstane-3α,17ß-diol, 17α-	C20H34O2	306.45	271, 361, 143, di-; CI
411	methyl-5α-androstane-3α,17ß-diol, 1α-	C20H34O2	306.45	73, 75, 145, 129, di-
412	methyl-5α-androstane-3α,17ß-diol, 2α-	C20H34O2	306.45	
413	methyl-5α-androstane-3α,17ß-diol-2ß-carboxylic acid, 17α-	C20H32O4	336.43	
414	methyl-5α-androstane-3α,18-diol-17-one, 1α-	C20H30O3	318.46	
415	methyl-5α-androstane-3α,6ß,18-triol-17-one, 1α-	C20H30O4	334.46	
416	**methyl-5α-androstane-3ß,17ß-diol, 17α-**	**C20H34O2**	306.49	
417	methyl-5α-androstane-3ß,18-diol-17-one, 1α-	C20H30O3	318.46	
418	methyl-5ß-androst-1-en-17α-ol-3-one, 17ß-	C20H30O2	302.46	
419	methyl-5ß-androst-1-en-17ß-ol-3-one, 17α-	C20H30O2	302.46	
420	methyl-5ß-androst-1-ene-16,17ß-diol-3-one, 17α-	C20H30O3	318.46	218, 117, 231, 147
421	methyl-5ß-androst-1-ene-3α,17α-diol, 17ß-	C20H32O2	304.48	358, 216, 143, di-
422	methyl-5ß-androst-1-ene-3α,17ß-diol, 17α-	C20H32O2	304.48	
423	methyl-5ß-androst-1-ene-6,16,17ß-triol-3-one, 17α-	C20H28O4	332.44	218, 117, 579
424	methyl-5ß-androst-1-ene-6ß,17ß-diol-3-one, 17α-	C20H30O3	318.46	180, 143, 73, 173, ..***534***, tri-
425	methyl-5ß-androstane-3α,17α-diol, 17α-	C20H34O2	306.46	
426	methyl-5ß-androstane-3α,17α-diol, 17ß-	C20H34O2	306.45	
427	methyl-5ß-androstane-3α,17ß-diol, 17α-	C20H34O2	306.46	274, 364, 146, di-; CI
428	***methyl-5ß-androstane-3α,17β-diol-D3, 17α-*** **(17α-Cd3 form)**	***C20D3H31O2***	309.46	
429	methyl-5ß-androstane-3ß,16α,17ß-triol, 17α-	C20H34O3	322.49	218, 231, 219, ***538***, tri-
430	methyl-5ß-androstane-3ß,17ß-diol, 17α-	C20H34O2	306.45	
431	methyl-androst-4-en-17ß-ol-(3,2c)-pyrazol, 17α-	C21H30N2O	326.48	

432	methyl-androst-4-ene-11α,17ß-diol-3-one, 17α-	C20H28O3	316.44	
433	methyl-androst-5-en-17ß-ol-3-one, 17α-	C20H30O2	302.46	-----
434	***methylboldenone-D3* (17α-Me-androsta-1,4-diene-17ß-ol-3-one-D3)**	***C20D3H25O2***	305.34	
435	methyl-clostebol	C20H29ClO2	336.90	
436	methylene-17α-OH-6-ene-progesterone, 16-	C22H28O3	340.46	
437	methylene-5α-androstan-3α-ol-17-one, 1-	C20H30O2	302.46	***446***, 431, di-
438	methylene-5α-androstane-2ß-ol-3,17-dione, 1-	C20H28O3	316.44	
439	methylene-5α-androstane-3α,6ß-diol-17-one, 1-	C20H30O3	318.46	
440	methyl-estra-4,9-dien-17ß-(2-OH-propionyl)-3-one, 17α- (Trimegestone)	C22H30O3	450.66	
441	methyl-nandrolone, 7α- (Trestolone; 7α-Me-nor-Testo.)	C19H28O2	288.43	***288***, 91, 73, 289, free-
442	methyl-prednisolone	C22H30O5	374.48	136, 135, 91, 121,free-
443	methyl-progesterone, 16α-	C22H32O2	328.50	
444	**methyl-testosterone, 17α-**	**C20H30O2**	302.46	73, ***446***, 301, 75, 447, di-
445	methyl-testosterone-3-cyclopentyl enol ether, 17α-	C25H38O2	370.58	
446	***methyltestosterone-D3, 17α-* (20,20,20-d3 form)**	***C20D3H27O2***	305.46	
447	metribolone (Methyltrienolone, Methyltrenbolone)	C19H24O2	284.40	
448	**mibolerone (7α,17α-diMe-Nor-Testo.)**	C20H30O2	302.46	431, ***446***, 301, 341, 356, di-
449	mifepristone	C29H35NO2	429.61	
450	***momethasone furoate***	**C27H30Cl2O6**	521.44	-----
451	**nandrolone (19-Nor-Testo.)**	**C18H26O2**	274.41	***274***, 110, 91, 41, 79, free-
452	**nandrolone-17ß-benzoate**	C25H30O3	378.52	
453	nandrolone-17ß-caproate	C24H36O3	372.55	
454	nandrolone-17ß-cyclohexanecarboxylate	C25H36O3	384.56	
455	nandrolone-17ß-cyclohexylpropionate	C27H40O3	412.62	
456	nandrolone-17ß-cyclopentylpropionate	C26H38O3	398.59	
457	**nandrolone-17ß-decanoate**	C28H44O3	428.66	274, 155, 110, 256, 147, 91, free-
458	nandrolone-17ß-dodecanoate	C30H48O3	456.71	-----
459	nandrolone-17ß-furylpropionate	C25H32O4	396.53	
460	nandrolone-17ß-hexyloxyphenylpropionate	C33H46O4	506.73	
461	nandrolone-17ß-hydrogen succinate	C24H30O5	398.50	

462	nandrolone-17ß-phenylpropionate	C27H34O3	406.57	91, 257, 105, 79, 147, 107, free-
463	**nandrolone-17ß-propionate**	C21H30O3	330.47	-----
464	nandrolone-17ß-undecanoate	C29H46O3	442.69	
465	***nandrolone-D3, 17ß-* (16,16,17-d3 form)**	***C18D3H23O2***	277.41	
466	nor-17,17-dimethyl-2-oxa-5α-androst-13-en-3-one, 18-	C20H28O2	300.44	
467	nor-17,17-dimethyl-5α-androst-13-en-3α-ol, 18-	C20H32O	288.48	
468	nor-17,17-dimethyl-5ß-androsta-1,13-dien-3α-ol, 18-	C20H30O	286.46	253, 216, 201, ***358***, mono-
469	nor-17,17-dimethyl-androsta-1,4,13-trien-3-one, 18-	C20H26O	266.43	
470	nor-17α-pregna-4,9-dien-17α-ol-3-one-21-nitrite, 19-	C20H25NO2	311.43	
471	nor-5α-pregnane-3α,17ß-diol, 19-	C20H34O2	306.49	
472	nor-5α-pregnane-3ß,16α,17ß-triol, 19-	C20H34O3	322.49	
473	nor-5α-pregnane-3ß,16ß,17ß-triol, 19-	C20H34O3	322.49	
474	nor-5α-pregnane-3ß,17ß,20R-triol, 19-	C20H34O3	322.49	
475	nor-5α-pregnane-3ß,17ß,20S-triol, 19-	C20H34O3	322.49	
476	nor-5α-pregnane-3ß,17ß-diol, 19-	C20H34O2	306.49	
477	nor-5ß-pregnane-3α,17ß-diol, 19-	C20H34O2	306.49	
478	nor-5ß-pregnane-3ß,17ß-diol, 19-	C20H34O2	306.49	
479	norbolethone (13ß-Et-17ß-OH-18,19-dinor-17α-pregn-4-en-3-one)	C21H32O2	316.48	
480	norclostebol	C18H25ClO2	308.84	
481	norclostebol-17ß-acetate	C20H27ClO3	350.88	
482	**norethandrolone (17α-Et-19-Nor-Testo.)**	**C20H30O2**	302.46	***446***, 287, 356, di-
483	norethindrone (Norethisterone) (17α-Ethynyl-19-nor-Testo.)	C20H26O2	298.43	***298***, 231, 110, free-
484	norethindrone-17ß-acetate	C22H28O3	340.47	***340***, 325, 298, 231, 283, free-
485	norethindrone-17ß-enanthate	C27H38O3	410.60	
486	**norethynodrel (17α-Ethynyl-estr-5(10)-en-17-ol-3-one)**	**C20H26O2**	298.43	91, 215, 79, free-
487	norgesterone (19-Norpregna-5(10),20-dien-17α-vinyl-17ß-ol-3-one)	C20H28O2	369.50	
488	norgestimate (Dexnorgestrel)	C23H31NO3	362.50	
489	norgestomet (19-Norpregn-4-ene-11ß-Me-3,20-dioxo-17α-yl-Ac)	C23H32O4	372.50	
490	**norgestrel, levo- (17-Ethynyl-18-Me-19-nor-Testo.)**	C21H28O2	312.45	91, 79, 245, 110, ***312***, free-
491	norgestrienone (17α-Ethynyl-estra-4,9,11-trien-17ß-ol-3-one)	C20H22O2	294.39	

492	normegestrol-17ß-acetate	C23H30O4	370.49	
493	normethandrone (17α-Me-19-Nor-Testo.)	C19H28O2	288.43	***288***, 71, 55, 231, 91, 110, free-
494	norpregnanetriol (19-Nor-5ß-pregnane-3α,17α,20α-triol)	C20H34O3	322.49	
495	norprogesterone (19-Norpregn-4-ene-3,20-dione)	C20H28O2	300.44	43, 110, 91, 41, 79, ***300***, free-
496	norstanozolol, 17α-	C20H30N2O	314.47	
497	oxabolone (4-OH-19-Nor-Testo.)	C18H26O3	290.41	419, 73, 329, 303, ***434***, di-
498	oxabolone cypionate	C26H38O4	414.59	
499	***oxandrolone*** **(2-Oxa-17α-Me-5α-DHT)**	C19H30O3	306.45	273, 363, 213, 161, 227, mono-
500	***oxymesterone*** **(4-OH-17α-Me-Testo.)**	C20H30O3	318.46	***534***, 143, tri-
501	**oxymetholone (2-Hydroxymethylene-17α-Me-DHT)**	**C21H32O3**	332.49	***548***, 73, 490, 281, 405, tri-
502	***pregn-16-ene-3ß-ol-20-one-D3, 5α- (3α,11,11-d3 form)***	***C21D3H29O2***	319.49	
503	pregn-4-en-17α-ol-20-one-6α-methyl (Anagestone)	C21H30O2	314.47	
504	pregn-4-en-17ß-ol-11-methylene-13ß-ethyl-18,19-dinor-20-yne (Desogestrel)	C22H30O	310.48	
505	pregn-4-ene-11ß,17,18,21-tetrol-3,20-dione (18-OH-cortisol)	C21H30O6	378.47	
506	pregn-4-ene-11ß,17,21-triol-3,20-dione-18-al (18-Oxacortisol)	C22H30O6	390.48	
507	**pregn-4-ene-11ß,17α,21-triol-3,20-dione (Hydrocortisone)**	**C21H30O5**	362.47	123, 163, 124, 41, 91, free-
508	pregn-4-ene-11ß,18,21-triol-3,20-dione (18OH-B)	C21H30O5	362.47	
509	**pregn-4-ene-11ß,21-diol-3,20-dione (Corticosterone)**	**C21H30O4**	346.47	315, 269, 55, 41, 91, 316, free-
510	pregn-4-ene-11ß,21-diol-3,20-dione-18-al (Aldosterone)	C21H28O5	360.46	329, 283, 255, 311, 91, 314, free-
511	**pregn-4-ene-14α,17α-propylidenedioxy-3,20-dione (Proligestone)**	**C24H34O4**	386.54	-----
512	pregn-4-ene-17α,20ß,21-triol-3,11-dione	C21H30O5	362.47	-----
513	pregn-4-ene-17α,21-diol-3,11,20-trione (Cortisone)	C21H28O5	360.46	122, 121, 330, 123, 300, free-
514	pregn-4-ene-17α,21-diol-3,11,20-trione, 21-Ac	C23H30O6	402.49	43, 122, 91, 121, 301, 342, free-
515	pregn-4-ene-17α,21-diol-3,20-dione (Cortexolone)	C21H30O4	346.47	316, 244, 124, 287, 286, free-
516	pregn-4-ene-17α-ol-3,20-dione (17α-OH-Progesterone)	C21H30O3	330.47	***546***, 531, tri-
517	pregn-4-ene-18,21-diol-3,20-dione	C21H30O4	346.47	
518	**pregn-4-ene-21-ol-3,20-dione (11-DOC)**	**C21H30O3**	330.47	299, 271, 253, 147, 300, free-
519	**pregn-4-ene-3,20-dione (Progesterone)**	**C21H30O2**	314.47	124, ***314***, 272, 229, 91, free-
520	***pregn-4-ene-3,20-dione-D3*** **(Progesterone-D3)**	***C21D3H27O2***	317.47	
521	pregn-4-ene-3ß,20α-diol	C21H34O2	318.50	

522	pregn-4-ene-3ß,20ß-diol	C21H34O2	318.50	
523	pregn-4-ene-6ß,11ß,17α,21-tetrol-3,20-dione (6ß-OH-Cortisol)	C21H30O6	378.47	318, 43, 55, 91, 44, 41, 69, free-
524	**pregn-5-en-3ß-ol**	**C21H34O**	302.50	***302***, 284, 303, 269, 287, free-
525	**pregn-5-en-3ß-ol-20-one (Pregnenolone)**	**C21H32O2**	316.48	45, 157, 73, 446, di-
526	***pregn-5-en-3ß-ol-20-one-D4 (Pregnenolone-D4)***	***C21D4H32O2***	320.48	
527	**pregn-5-ene-3ß,16α-diol-20-one**	**C21H32O3**	332.49	
528	**pregn-5-ene-3ß,17α,20α-triol (Pregnenetriol)**	**C21H34O3**	334.50	289, 271, 253, 290, 213, free-
529	**pregn-5-ene-3ß,17-diol-20-one (17-OH-Pregnenolone)**	**C21H32O3**	332.49	***332***, 271, 253, 289, 43, 213, free-
530	pregn-5-ene-3ß,20α-diol, diacetate	C25H38O4	402.58	
531	**pregn-5-ene-3ß,20s-diol (Pregnenediol)**	**C21H34O2**	318.50	***318***, 45, 189, 91, 105, 79, free-
532	pregna-1,4,6-triene-3,20-dione-6-chloro, (9ß,10α) (Trengestone)	C21H25ClO2	344.88	
533	pregna-1,4-diene-11ß-17α-21-triol-3,20-dione (Prednisolone)	C21H28O5	360.46	122, 121, 123, 147, 91, free-
534	pregna-1,4-diene-17α,21-diol-3,11,20-trione (Prednisone)	C21H26O5	358.44	121, 91, 147, 160, 159, free-
535	**pregnan-20-one, 5α-**	**C21H34O**	302.50	43, 84, 41, 55, 67, 217, 81, free-
536	**pregnan-3,20-dione, 5α-**	**C21H32O2**	316.47	43, 84, ***316***, 55, 81, 231, 41, free-
537	pregnan-3α-ol-20-one, 5α- (Tetrahydroprogesterone, 3α,5α-)	C21H34O2	318.50	43, 300, 41, 84, 81, 55, 95, free-
538	**pregnan-3α-ol-20-one, 5ß- (Pregnanolone)**	**C21H34O2**	318.50	43, 300, 41, 84, 81, 55, free-
539	***pregnan-3a-ol-20-one-D3, 5α- (5α-Pregnanolone-D3)***	***C21D3H31O2***	321.50	
540	pregnan-3ß-ol-20-one, 5α-	C21H34O2	318.50	43, 84, ***318***, 55, 215, 107, free-
541	pregnane-11ß,17α,21-triol-3,20-dione, 5ß- (5ß-DiH-cortisol)	C21H32O5	364.49	***364***, 287, 244, 304, 305, free-
542	pregnane-17α,21-diol-3,11,20-trione, 5ß- (5ß-DiH-cortisone)	C21H30O5	362.47	
543	pregnane-3α,11ß,17α,21-tetrol-20-one, 5α- (Allotetrahydrocortisol)	C21H34O5	366.50	-----
544	**pregnane-3α,11ß,17α,21-tetrol-20-one, 5ß- (Tetrahydrocortisol)**	**C21H34O5**	366.50	
545	pregnane-3α,11ß,21-triol-20-one, 5α- (αTHB)	C21H34O4	350.50	
546	**pregnane-3α,11ß,21-triol-20-one, 5ß- (THB)**	**C21H34O4**	350.50	
547	pregnane-3α,15ß,17-triol-20-one, 5ß- (15ß-OH-PDL)	C21H34O4	350.50	
548	pregnane-3α,17,20α,21-tetrol, 5ß- (HHS)	C21H36O4	352.52	
549	pregnane-3α,17,20α-triol, 3,20-diacetate, 5ß-	C25H40O5	420.60	
550	**pregnane-3α,17,21-triol-20-one, 5ß- (THS)**	**C21H34O4**	350.50	
551	pregnane-3α,17α,20α,21-tetrol-11-one, 5ß- (20a-Cortolone)	C21H34O5	366.50	287, 43, 55, 304, 305, 44, free-
552	**pregnane-3α,17α,20α-triol, 5ß- (Pregnanetriol)**	**C21H36O3**	336.50	255, 291, 273, 215, 256, free-

553	**pregnane-3α,17α,20α-triol-11-one, 5ß-**	**C21H34O4**	350.50	
554	pregnane-3α,17α,20ß,21-tetrol-11-one, 5ß- (20ß-Cortolone)	C21H34O5	366.50	
555	**pregnane-3α,17α,21-triol-11,20-dione, 5ß- (Tetrahydrocortisone)**	**C21H32O5**	364.48	-----
556	pregnane-3α,17α-diol-20-one, 5ß- (PDL)	C21H34O3	334.50	43, 255, 215, 229, ***334***, free-
557	**pregnane-3α,20α-diol, 5ß- (Pregnanediol)**	**C21H36O2**	320.52	216, 234, 81, 93, 302
558	pregnane-3α,20α-diol, diacetate, 5α-	C25H40O4	404.60	
559	***pregnane-3a,20a-diol-D4, 5α- (5α-Pregnanediol-D4)***	***C21D4H32O2***	324.52	
560	pregnane-3α,20s-diol, diacetate, 5ß-	C25H40O4	404.60	43, 284, 93, 269, 215, 107, free-
561	pregnane-3α,20ß-diol, diacetate, 5α-	C25H40O4	404.60	
562	pregnane-3α,20ß-diol, diacetate, 5ß-	C25H40O4	404.60	
563	**pregnane-3α,21-diol-11,20-dione, 5ß-**	**C21H32O4**	348.50	
564	pregnane-3α,21-diol-20-one, 5ß- (THDOC)	C21H34O3	334.50	
565	pregnane-3α,6α,11ß,21-tetrol-20-one, 5ß- (6αOH-THB)	C21H34O5	366.50	
566	pregnane-3α,6α,21-triol-11,20-dione, 5ß- (6αOH-THA)	C21H32O5	364.49	
567	**pregnane-3ß,17α,20α-triol, 5α-**	**C21H36O3**	336.52	
568	**pregnane-3ß,17α-diol-20-one, 5α-**	**C21H34O3**	334.51	***334***, 273, 43, 291, 255, 107, free-
569	pregnane-3ß,20α-diol, diacetate, 5ß-	C25H40O4	404.60	
570	pregnane-3ß,20ß-diol, diacetate, 5α-	C25H40O4	404.60	
571	pregnane-3ß,20ß-diol, diacetate, 5ß-	C25H40O4	404.60	
572	propethandrol (4-Et-17α-OH-17-propionyloxyl-estr-3-ene)	C23H36O3	360.54	
573	propylmesterolone (1α-Me-17α-propyl-5α-DHT)	C23H38O2	346.56	
574	quinbolone (17ß-(1-cyclopenten-1-yloxy)-androsta-1,4-dien-3-one)	C24H32O2	352.52	
575	roxibolone (2-Carboxy-11ß-OH-methandrostenolone)	C21H28O5	360.46	
576	silandrone (17ß-TMS-Testo.)	C22H36O2Si	360.62	
577	**stanozolol (17ß-OH-17α-Me-5α-Androst-2-eno[3,2-c]pyrazole)**	**C21H32N2O**	328.50	143, ***472***, 168, di-
578	stanozolol-4,16-diol	C21H32N2O3	360.50	
579	***stanozolol-D3* (20,20,20-d3 form)**	***C21D3H29N2O***	331.50	96, 94, ***331***, 95, 133, free-
580	stenbolone (2-Me-1,2-dehydro-5α-dihydro-Testo.)	C20H30O2	302.46	-----
581	stenbolone acetate	C22H32O3	344.50	
582	testolactone	C19H24O3	300.40	119, 121, 123, 91, .., free-

583	**testosterone (17ß-OH-Androst-4-en-3-one)**	**C19H28O2**	288.43	73, ***432***, 209, 417, 195, di-
584	**testosterone-17ß-acetate**	C21H30O3	330.47	387, ***402***, 247, 209, mono-
585	**testosterone-17ß-benzoate**	C26H32O3	392.54	449, ***464***, 209, mono-
586	testosterone-17ß-chloral hemiacetal (Cloxotestosterone)	C21H29Cl3O3	435.82	
587	testosterone-17ß-chloral hemiacetal acetate	C23H31Cl3O4	477.78	
588	testosterone-17ß-cyclohexylpropionate	C28H42O3	426.64	
589	**testosterone-17ß-cypionate**	C27H40O3	412.62	469, ***484***, 209, mono-
590	**testosterone-17ß-decanoate**	C29H46O3	442.69	***514***, 499, 209, mono-
591	**testosterone-17ß-enanthate**	C26H40O3	400.61	43, 124, 147, 113, 55, 41, free-
592	testosterone-17ß-furoate	C24H30O4	382.51	
593	testosterone-17ß-hexahydrobenzoate	C26H38O3	398.59	
594	testosterone-17ß-hexahydrobenzyl carbonate	C27H40O3	412.62	
595	testosterone-17ß-hexyloxyphenylpropionate	C34H46O4	518.74	
596	testosterone-17ß-isobutyrate	C23H34O3	358.53	
597	**testosterone-17ß-isocaproate**	C25H38O3	386.58	***458***, 443, 209, 247, mono-
598	testosterone-17ß-ketolaurate	C31H48O5	500.73	
599	testosterone-17ß-nicotinate	C25H31NO3	393.53	
600	**testosterone-17ß-phenylpropionate**	C28H36O3	420.60	***492***, 477, 247, 209, mono-
601	testosterone-17ß-phosphate	C19H29O5P	368.41	
602	**testosterone-17ß-propionate**	C22H32O3	344.50	401, ***416***, 209, 247, mono-
603	**testosterone-17ß-undecanoate**	C30H48O3	456.71	***528***, 209, 513, mono-
604	***testosterone-D2* (1,2-d2 form)**	***C19D2H26O2***	290.43	
605	***testosterone-D2* (16,16-d2 form)**	***C19D2H26O2***	290.43	
606	***testosterone-D3* (16,16,17-d3 form)**	***C19D3H25O2***	291.40	***435***, 73, 420, di-
607	tetrahydroaldosterone	C21H32O5	364.49	
608	tetrahydrogestrinone (THG)	C21H28O2	312.46	
609	tibolone (7α-Me,17α-Ethynyl-nor-Testo.)	C21H28O2	312.45	73, ***456***, 301, 182, 442, di-
610	tiomesterone (1α,7α-bis(acetylthio)-17α-Me-Testo.)	C24H36O4S2	450.66	43, 299, 121, 147, 298, 171, free-
611	trenbolone, 17α- (Epitrenbolone)	C18H22O2	270.37	***414***, 73, 283, 309, 193, di-

612	**trenbolone, 17ß- (17ß-OH-Estra-4,9,11-trien-3-one)**	**C18H22O2**	270.37	***414***, 73, 283, 309, 193, di-
613	**trenbolone-17ß-acetate**	**C20H24O3**	312.41	252, ***312***, 198, 237, 270, free-
614	trenbolone-17ß-cyclohexylmethylcarbonate	C26H34O4	410.56	
615	***trenbolone-D2, 17ß-***	***C18D2H20O2***	272.37	
616	triamcinolone	C21H27FO6	394.44	122, 121, 91, 270, 326, free

Table 3.8. (Continued) Database of doping steroids

No.	Cf., (Merck 12th ed, CAS No., Type etc.)	Literature
1		N. Kumar, J. Steroid Biochem., 71 (1999) 213-222
2	M0239; 24356-94-3; Cattle; Antiacne	E. Daeseleire, J. Chromatogr., A 674 (1994) 247-253
3	M0299; 432-60-0; Progestagen	D. Courtheyn, Anal. Chim. Acta, 473 (2002) 71-82
4	M0327; 850-52-2; Progestagen	
5	M0674; 360-66-7	
6	Bolasterone-M4	B.G. Wolthers, J. Chromatogr., B 843 (1999) 247-274
7	RI = 19.8	
8	1153-51-1	
9	18339-16-7	
10	65-06-5; RI = 25.7; 5b- = 10529-96-1	MH. Choi, Rapid Commun. MS., 13 (1999) 376-380
11	10529-96-1; Boldenone-M2; RI = 2451	Schanzer W, Biol. Mass Spectrom., 21 (1992) 3-16
12	Boldenone-M4	Schanzer W, Biol. Mass Spectrom., 21 (1992) 3-16
13	571-40-4; Boldenone-M7; RI = 26.0	Schanzer W, Biol. Mass Spectrom., 21 (1992) 3-16
14	21507-41-5; Boldenone-M6	Schanzer W, Biol. Mass Spectrom., 21 (1992) 3-16
15	Boldenone-M3	Schanzer W, Biol. Mass Spectrom., 21 (1992) 3-16
16		W. Schanzer, J. Steroid Biochem., 57 (1996) 363-376
17	Cl-Methandrostenolone-M4, RI = 2997	W. Schanzer, J. Steroid Biochem., 57 (1996) 363-376
18	Boldenone-M5	Schanzer W, Biol. Mass Spectrom., 21 (1992) 3-16
19	M3268; 79-64-1; Progetagen	
20	Clostebol-M1; CLOS-MET; RI = 2691	L.D. Bowers, J. Chromatogr., B 687 (1996) 69-78
21	382-44-5; RI = 28.4	W. Schanzer, Vol 4 (1996), p 199
22	2,2,4,6,6,16,16-d7	W. Schanzer, Vol 4 (1996), p 191
23	510-64-5	
24	M0174; 382-45-6; Nist#14350	
25	571-52-8; RI = 26.8	
26	M0678; 63-05-8; Androstenedione	D.H. Catlin, Steroids, 67 (2002) 559-564
27	Clostebol-M4	B. Bizec, Clin. Chem., 44 (1998) 973-984
28		J.F. Dorgan, Steroids, 67 (2002) 151-158
29	(98.4%)	D.H. Kerkhof, J. Chromatogr., A 954 (2002) 199-206
30	Clostebol-M7	B. Bizec, Clin. Chem., 44 (1998) 973-984
31	Fluoxymesterone-M2; RI = 2858	L.D. Bowers, J. Chromatogr., B 687 (1996) 69-78

32	1639-43-6; Androstenediol	VP. Uralets, J. Anal. Toxicol., 23 (1999) 357-366
33	566-48-3	
34		D.H. Catlin, Steroids, 67 (2002) 559-564
35	63-00-3; RI = 27.6 (free-)	J.F. Levesque, J. Chromatogr., B 780 (2002) 145-153
36	25824-80-0	
37	2283-82-1; RI = 24.7	
38	M7892; 53-43-0; DHEA, Prasterone	C.H.L Shackleton, Steroids, 62 (1997) 665-673
39	M0; 853-23-6; RI = 26.1	
40	23983-43-9	
41	16,16-d2	SJ. Gaskell, Label. Comp. & Radio., 17 (1980) 861-869
42	M0; 571-36-8; Androstenedione	
43		
44		
45		
46		
47	85922-89-0; RI = 27.1	
48	1232-73-1; 16a-Hydroxy-DHEA	
49	M0677; 1963-03-7; RI = 25.0 (free-)	L.D. Bowers, J. Chromatogr., B 687 (1996) 69-78
50	RI = 27.6	C.H.L. Shackleton, Steroids, 62 (1997) 665-673
51	M0677; 521-17-5; DHEA-M2	C.H.L. Shackleton, Steroids, 62 (1997) 665-673
52	M0677; 5937-72-4	
53	M0677; 1175-12-8	
54	M0677; 1639-43-6	
55	M0677; 5953-63-9	
56	M0677; 116262-99-8	C.H.L. Shackleton, Steroids, 62 (1997) 665-673
57	M0677; 2297-30-5; Bisexovis	
58	1159-66-6; RI = 26.3	
59	1449-61-2; RI = 28.4	I. Garret, Medical Hypotheses, 60 (2003) 391-397
60	4-Cl-Methandrostenolone, Chloroxomesterone	W. Schanzer, J. Steroid Biochem., 57 (1996) 363-376
61	7738-93-4; Nist# 126775	
62	897-06-3;Boldenone-M8; RI = 26.5	Schanzer W, Biol. Mass Spectrom., 21 (1992) 3-16
63	96301-34-7	
64	De-H-Cl-Me-Testo.-M3	B.G. Wolthers, J. Chromatogr., B 843 (1999) 247-274

65	De-H-Cl-Me-Testo.-M2	B.G. Wolthers, J. Chromatogr., B 843 (1999) 247-274
66	Boldenone-M9	Schanzer W, Biol. Mass Spectrom., 21 (1992) 3-16
67	6ß-OH-deH-Cl-Testo.	L.D. Bowers, J. Chromatogr., B 687 (1996) 69-78
68	De-H-4-Cl-Me-Testo.-M1	S. Rendic, J. Chromatogr., B 735 (1999) 73-83
69	Boldenone-M10	Schanzer W, Biol. Mass Spectrom., 21 (1992) 3-16
70	Fluoxymesterone-M5; RI = 2600	B.G. Wolthers, J. Chromatogr., B 843 (1999) 247-274
71	M0675; 438-22-2	K.R. Williams, University of Glasgow, Thesis (1999)
72	M3912; 438-23-3; RI = 19.8	
73	963-74-6; RI = 22.6	C. Ayotte, J. Chromatogr., B 687 (1996) 3-25
74	1912-61-4	V.P. Uralets, J. Anal. Toxicol., 24 (2000) 188-193
75	1225-43-0; RI = 22.7	
76	2881-21-2; Dimethylandrostanolone	
77	7657-50-3; RI = 22.6	
78	M0680	
79	M0680	
80	M0680; 53-41-8; Testo.-M	VP. Uralets, J. Anal. Toxicol., 23 (1999) 357-366
81	M0; 53-42-9; Testo.-M	VP. Uralets, J. Anal. Toxicol., 23 (1999) 357-366
82	4-Cl-Androsterone; Clostebol-M2	D. Thieme, Vol 4 (1996), pp 43-58
83	4-Cl-Etiocholanolone; Clostebol-M2	D. Thieme, Vol 4 (1996), pp 43-58
84	16,16-d2	W. Schanzer, Vol 4 (1996), p 199
85	16,16-d2	W. Schanzer, Vol 2 (1994), pp 93-112
86	2,2,4,4-d4; RI = 2501	W. Schanzer, Vol 2 (1994), pp 93- 112
87	2,2,4,4-d4; RI = 2519	W. Schanzer, Vol 2 (1994), pp 93- 112
88	1224-95-9; RI = 22.9	
89	1236-13-1; RI = 23.9	C.H.L. Shackleton, Steroids, 62 (1997) 665-673
90	1224-92-6; RI = 22.6	C.H.L. Shackleton, Steroids, 62 (1997) 665-673
91	571-51-7; RI = 25.0	
92	M3646; 481-29-8; DHEA-M1	B.G. Wolthers, J. Chromatogr., B 843 (1999) 247-274
93	571-31-3; RI = 24.7	C. Ayotte, J. Chromatogr., B 687 (1996) 3-25
94	Clostebol-M5	B. Bizec, Clin. Chem., 44 (1998) 973-984
95	Clostebol-M; Cattle	B.G. Wolthers, J. Chromatogr., B 843 (1999) 247-274
96		Schanzer, Vol 3 (1995), pp 201-213
97	1482-70-8	

98	846-46-8; Testo.-M; RI = 25.8	B.G. Wolthers, J. Chromatogr., B 843 (1999) 247-274
99	1229-12-5; RI = 25.5; Testo.-M	C. Ayotte, J. Chromatogr., B 687 (1996) 3-25
100		B. Bizec, Clin.Chem., 44 (1998) 973-984
101	Clostebol-M4	B.G. Wolthers, J. Chromatogr., B 843 (1999) 247-274
102	6165-21-5; RI = 24.2; 5a-DHT-M2	R. Aguilera, J. Chromatogr., B 727 (1999) 95-105
103	5856-10-0; RI = 24.7; 17a,5ßAD	B.G. Wolthers, J. Chromatogr., B 843 (1999) 247-274
104	RI = 26.9	C.H.L. Schackelton, Steroids, 62 (1997) 665-673
105	RI = 26.1	C.H.L. Schackelton, Steroids,62 (1997) 665-673
106	Bolasterone-M2	B.G. Wolthers, J. Chromatogr., B 843 (1999) 247-274
107	Bolasterone-M	B.G. Wolthers, J. Chromatogr., B 843 (1999) 247-274
108	Calusterone-M3	B.G. Wolthers, J. Chromatogr., B 843 (1999) 247-274
109	1852-53-5; 5a-DHT-M1; AD; 5aAD	L.D. Bowers, J. Chromatogr., B 687 (1996) 69-78
110	1851-23-6; Testo.-M; 5ßAD	C.H.L. Schackelton, Steroids, 62 (1997) 665-673
111	1247-66-1; RI = 27.2	C.H.L. Schackelton, Steroids, 62 (1997) 665-673
112	RI = 26.9	C.H.L. Schackelton, Steroids, 62 (1997) 665-673
113	Calusterone-M2	W. Schanzer, Vol 4 (1996), p 193
114	Calusterone-M1; Miboler.-M, Bolast.-M1	L.D. Bowers, J. Chromatogr., B 687 (1996) 69-78
115	Calusterone-M2	B.G. Wolthers, J. Chromatogr., B 843 (1999) 247-274
116	Bolasterone-M1; Calusterone-M1	B.G. Wolthers, J. Chromatogr., B 843 (1999) 247-274
117		Schanzer, Vol 3 (1995), pp 201-213
118		Schanzer, Vol 3 (1995), pp 201-213
119	1231-82-9; RI = 25.8	
120	739-27-5	
121	M0676; 514-17-0; RI = 27.2 (free)	
122	5856-11-1; RI = 25.7	R. Aguilera, J. Chromatogr., B 727 (1999) 95-105
123	19767-69-2; RI = 24.4	
124	909-07-9; RI = 27.4	C.H.L. Shackleton, Steroids, 62 (1997) 665-673
125	RI = 26.2	R. Aguilera, J. Chromatogr., B 727 (1999) 95-105
126		B. Bizec, Clin. Chem., 44 (1998) 973-984
127	571-20-0; Xenabol; WADA 2004 list	C. Jimenez, Anal. Chim. Acta, 460 (2002) 289-307
128	1852-53-5; Testo.-M; RI = 25.2	M. Ueki, Vol 3 (1995), p 245
129	RI = 27.1	C.H.L. Shackleton, Steroids, 62 (1997) 665-673
130	RI = 27.6	R. Aguilera, J. Chromatogr., B 727 (1999) 95-105

131		L.D. Bowers, J. Chromatogr., B 687 (1996) 69-78
132		Schanzer, Vol 3 (1995), pp 201-213
133		Schanzer, Vol 3 (1995), pp 201-213
134	7090-90-6; RI = 25.9	
135	49643-99-4; RI = 26.8	
136	M1047; 4419-39-0; Antiallergic	V. Cirimele, Forensic Sci. Int., 107 (2000) 381-388
137	M1047; 5534-09-8; Aerobec	R. Schilt, Analyst, 123 (1998) 2665-2670
138	M1226; 378-44-9	Y. Gaillard, Foren. Sci. Int., 107 (2000) 361-379
139	M1226; 2152-44-5	
140	M1352; 19793-20-5; Anabolic	
141	Norpropandrolate	
142	M1353; 1605-89-6; DiMe-Testo	R. G-Lumbreras, J. Chromatogr., B 754 (2001) 419-425
143	Metazine	
144		
145	ß-Boldenone-M	
146	M1354; 846-48-0	H.F. Brabander, J. Chromatogr., A 750 (1996) 105-114
147	M1354; 2363-59-9	
148	19041-66-8	
149	M1354; 13103-34-9; Equipoise	H.W. Hagedorn, Am. J. Vet. Res., 58(3), 224-227
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151	1491-81-2	
152	M1771; 17021-26-0; Methosarb	T. Kim, J. Chromatogr., B 687 (1996) 79-83
153	M1795; 976-71-6; Diuretic	R. G-Lumbreras, J. Chromatogr., B 754 (2001) 419-425
154	M2152	P. Marchand, J. Chromatogr., A 867 (2000) 219-233
155	M2152; 302-22-7; Progestagen	E. Daeseleire, J. Chromatogr., B 562 (1991) 673-679
156	M2423; 25122-46-7; Gluco; Anti-inflam.	
157		
158	M2475; 1093-58-9; RI = 2693	GP. Cartoni, J. Chromatogr., A 279 (1983) 515-522
159		A. Koole, J. Chromatogr., B 724 (1999) 41-51
160	M2475; 855-19-6; Steranabol	A. Koole, J. Chromatogr., B 724 (1999) 41-51
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163		N. Kumar, J. Steroid Biochem., 71 (1999) 213-222

164	Quinbolone-M2	B.G. Wolthers, J. Chromatogr., B 843 (1999) 247-274
165	Quinbolone-M1	B.G. Wolthers, J. Chromatogr., B 843 (1999) 247-274
166	M8238; 1169-79-5; Estrogen	
167		
168	Anti-androgen	
169	M2875; 17230-88-5	D. DeBoer, J. Anal. Toxicol., 16 (1992) 14-18
170	see M6018	
171	M2923; 72-23-1	
172	791-69-5 (9,11-dehydro)	
173	M2930; 13698-49-2	E. Daeseleire, J. Chromatogr., A 674 (1994) 247-253
174	M2986; 50-02-2; Antiinflammatory	E. Grippa, J. Chromatogr., B 2000 738 (2000) 17-25
175	M8950; 521-18-6; Stanolone	AT. Kicman, Clin. Chem., 41 (1995) 1617-1627
176	see M8950; 571-22-2	
177	M8950	
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179	M8950	S.B. Coutts, Clin. Chem., 43 (1997) 2091-2098
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182	16,16-d2	Schanzer, Vol 3 (1995), pp 201-213
183	16,16,17-d3	Schanzer, Vol 3 (1995), pp 201-213
184	16,16,17-d3	Schanzer, Vol 3 (1995), pp 201-213
185	M3504; 58-19-5; Antineoplastic	E. Harber, J. Chromatogr., B 755 (2001) 17-26
186	M3504	
187	M3504; 521-12-0; Masteron	W. Schanzer, J. Chromatogr., B 687 (1996) 93-108
188	M3510; 67392-87-4; Progestagen	
189	M3524; 152-62-5; Progestagen	S. T-Cartas, J. Liq. Chromatogr., 24 (2001) 1089-1103
190		GP. Cartoni, J. Chromatogr., A 279 (1983) 515-522
191	M3646	
192		B. Bizec, Clin. Chem., 44 (1998) 973-984
193	M3652; 547-81-9	
194	Fluoxymesterone-M4	B.G. Wolthers, J. Chromatogr., B 843 (1999) 247-274
195	Methandrostenolone-M3; RI = 2625	R. Massé, J Chromatogr., B 562 (1991) 323-340
196	Methandrostenolone-M	W. Schänzer, J. Steroid Biochem., 38(19914) 441-464

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199	Oxandrolone-M2; RI = 2666	B.G. Wolthers, J. Chromatogr., B 843 (1999) 247-274
200	M0; 481-30-1; RI = 2614	C.H.L Shackleton, Steroids, 62 (1997) 665-673
201	RI = 2597	W. Schanzer, Vol 2 (1994), pp 93- 112
202	M3662; 2363-58-8; Antineoplastic	
203		VP. Uralets, Vol 4 (1996), pp 35-41
204		
205	M5659; 52-76-6; Contraceptive	
206	4409-34-1; RI = 28.0	R. Draisci, J. Chromatogr., A 870 (2000) 511-522
207	M6814; 6795-60-4; Progestagen	
208	Norclostebol-M1	B.G. Wolthers, J. Chromatogr., B 843 (1999) 247-274
209	734-32-7; 19-Norandrostenedione	VP. Uralets, J. Anal. Toxicol., 23 (1999) 357-366
210		B. Bizec, Clin. Chem., 44 (1998) 973-984
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212	19-Norandrostenediol	VP. Uralets, J. Anal. Toxicol., 23 (1999) 357-366
213		J.F. Levesque, J. Chromatogr., B 780 (2002) 145-153
214	19-Nordehydroandrosterone	
215	19-Nordehydroepiandrosterone	VP. Uralets, J. Anal. Toxicol., 24 (2000) 188-193
216	3962-66-1; RI = 25.2	VP. Uralets, J. Anal. Toxicol., 24 (2000) 188-193
217		
218		
219		
220	25975-59-1 (5,6-dehydro); RI = 24.2	
221	M6372; 34816-55-2; Estrogen	
222	39219-28-8; Estrogen	
223	1236-72-7	
224	362-08-3	L. Dehennin, J. Steroid Biochem., 20 (1984) 465-471
225	362-07-2	L. Dehennin, J. Steroid Biochem., 20 (1984) 465-471
226	Estetrol	
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230	M6424; 5108-94-1; Estrogen	
231	M7969; 34184-77-5; Progestagen	
232	M3747; 57-91-0; RI = 25.8; ß-Estradiol-M	S. Hartmann, J. Chromatogr., B 704 (1997) 105-117
233	M3746; 50-28-2; Estrogen	Y. Gaillard, J. Chromatogr., B 735 (1999) 189-205
234	586-75-6; RI = 27.4	
235	313-06-4	
236	4956-37-0	
237	15140-27-9	
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239	3571-53-7	
240	979-32-8	
241	113-38-2	
242	M3746; 50-50-0; Pelanin	R. Schilt, Analyst, 123 (1998) 2665-2670
243	1035-77-4	
244		P. Marchand, J. Chromatogr., A 867 (2000) 219-233
245	1434-85-1	
246	RI = 25.9	J.C. Mathurin, J. Chromatogr., B 759 (2001) 267-275
247	RI = 25.3	J.C. Mathurin, J. Chromatogr., B 759 (2001) 267-275
248	1225-01-0; Nandrolone-M1; RI = 2438	J. Munoz-Guerra, J. Chromatogr., B 704 (1997) 129-141
249	33036-33-8; RI = 23.8; Nandrolone-M2	J. Munoz-Guerra, J. Chromatogr., B 704 (1997) 129-141
250	Norclostebol-M3	B.G. Wolthers, J. Chromatogr., B 843 (1999) 247-274
251	Norclostebol-M2	B.G. Wolthers, J. Chromatogr., B 843 (1999) 247-274
252	3ß,4ß,5ß-d3	
253	3ß,4ß,5ß-d3 (Radiant-Promochem)	L. Dehennin, J. Chromatogr., B 721 (1999) 301-307
254	Nandrolone-M	
255	RI = 24.2	G. Debruyckere, Anal. Chim. Acta, 275 (1993) 49-56
256	19-Norepiandrosterone-D3	
257	5696-58-2; RI = 24.0	
258		R. Masse, J. Chromatogr., B 339 (1985) 11-23
259	Norclostebol-M4	B.G. Wolthers, J. Chromatogr., B 843 (1999) 247-274
260	10002-97-8; RI = 24.0; Nandrolone-M	
261	Ethylestrenol-M1, Norethndrolone-M1	B.G. Wolthers, J. Chromatogr., B 843 (1999) 247-274
262	Ethylestrenol-M2, Norethandrolone-M2	J. Munoz-Guerra, J. Chromatogr., B 704 (1997) 129-141

263	see M3787; Progestagen	J.C. Mathurin, J. Chromatogr., B 759 (2001) 267-275
264		J.C. Mathurin, J. Chromatogr., B 759 (2001) 267-275
265	Nandrolone-M; RI = 24.1	
266	Clostebol-M; Cattle	
267	M3750; 50-27-1; Estradiol-M	S. Hartmann, J. Chromatogr., B 704 (1997) 105-117
268	M3751; 53-16-7; Estradiol-M	MH. Choi, Analyst, 125 (2000) 711-714
269	M8239; 152-43-2; Estrogen	
270	M3780; 57-63-6; Estrogen	H.F. Brabander, J. Chromatogr., A 750 (1996) 105-114
271	M3787; 434-03-7	S.A. Hewitt, Anal. Chim. Acta, 473 (2002) 99-109
272	M3851; 965-90-2; Maxibolein	
273	M3905; 1231-93-2; Contraceptive	
274	M3905; 297-76-7	
275	54048-10-1	
276	M4166; 127-31-1	S. Hartmann, J. Chromatogr., B 704 (1997) 105-117
277	M4173; 2135-17-3; Glucocorticoid	R. Draisci, J. Chromatogr., B 2001 (753) 217-223
278	M4223; 76-43-7; Halotestin	W. Schanzer, Steroids, 60 (1998) 353-366
279	Fluoxymesterone-M1	B.G. Wolthers, J. Chromatogr., B 843 (1999) 247-274
280	M4235; 337-03-1; Progestagen	D. Courtheyn, Anal. Chim. Acta, 473 (2002) 71-82
281	M4266; 2454-11-7	GP. Cartoni, J. High Resol. Chromatogr., 14 (1991) 838-842
282	M4319; 1239-29-8	T. Kim, J. Chromatogr., B 687 (1996) 79-83
283	M4340; 6990-06-3; Antibacterial	
284	M4421; 60282-87-3; Progestagen	
285	M4422; 1253-28-7; Progestagen	
286	M4423; 16320-04-0	
287	M4628;50629-82-8	
288	Formebolone-M	
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290	Formebolone-M2	S. Rendic, J. Chromatogr., B 735 (1999) 73-83
291	Clostebol-M8	B. Bizec, Clin. Chem., 44 (1998) 973-984
292	57-61-4	A. Heunerbein, J. Chromatogr., A 985 (2003) 375-386
293		J.F. Levesque, J. Chromatogr., B 780 (2002) 145-153
294		W. Schanzer, Vol 2 (1994), pp 93- 112
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296		S. Rendic, J. Chromatogr., B 735 (1999) 73-83
297		J.F. Levesque, J. Chromatogr., B 780 (2002) 145-153
298		W. Schanzer, J. Steroid Biochem., 57 (1996) 363-376
299	Stanozolol-M	M. Machnik, Vol 4 (1996), p 235
300	570-30-9	
301	362-05-0; RI = 28.2	L. Dehennin, J. Steroid Biochem., 20 (1984) 465-471
302		L. Dehennin, J. Steroid Biochem., 20 (1984) 465-471
303	1229-24-9	L. Dehennin, J. Steroid Biochem., 20 (1984) 465-471
304		L. Dehennin, J. Steroid Biochem., 20 (1984) 465-471
305		
306		
307	RI = 30.8	
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309	362-06-1; RI = 28.6; Estradiol-M	L. Dehennin, J. Steroid Biochem., 20 (1984) 465-471
310	3131-23-5; RI = 30.2	L. Dehennin, J. Steroid Biochem., 20 (1984) 465-471
311		
312		
313	1247-71-8; RI = 30.0	
314	Norethandrolone-M	
315	739-26-4; RI = 27.1; 11ß-OH-Androster.-M	A. Heunerbein, J. Chromatogr., A 985 (2003) 375-386
316		
317		J.F. Levesque, J. Chromatogr., B 780 (2002) 145-153
318		W. Schanzer, Vol 4 (1996), p 187
319		W. Schanzer, Steroids, 60 (1998) 353-366
320	Furazabol-M1	T. Kim, J. Chromatogr., B 687 (1996) 79-83
321	Methandriol-M1	C. Ayotte, J. Chromatogr., B 687 (1996) 3-25
322	Methandrostenolone-M	A.R. McKinney, J. Chromatogr., B 765 (2001) 71-79
323	Methandrostenolone-M2; Vol 4 pp. 242 & 273	L.D. Bowers, J. Chromatogr., B 687 (1996) 69-78
324	Formebolone-M1; RI = 3163	L.D. Bowers, J. Chromatogr., B 687 (1996) 69-78
325	Oxymetholone-M (Proposed)	V. Vladimirova, Vol 1 (1993) pp 101-120
326	Oxymetholone-M (Proposed)	V. Vladimirova, Vol 1 (1993) pp 101-120
327	Oxymetholone-M (Proposed)	V. Vladimirova, Vol 1 (1993) pp 101-120
328	Oxymetholone-M (Proposed)	W. Schanzer, Vol 1 (1993), p 99

329	Oxymetholone-M (Proposed)	V. Vladimirova, Vol 1 (1993) pp 101-120
330		D. DeBoer, Anal Toxicol., 16 (1992) 14-18
331	Oxandrolone-M1	B.G. Wolthers, J. Chromatogr., B 843 (1999) 247-274
332		
333	438-07-3	
334	M4886; 302-23-8	
335	M4886; 630-56-8; Hyproval; Progestagen	
336	M7251; 7001-56-1; Progestagen	
337	Stanozolol-M3; RI = 3334	V. Ferchaud, J. Chromatogr., B 695 (1997) 269-277
338	Stanozolol-M1; RI = 3218; Vol 4, p. 232	W. Schanzer, J. Chromatogr., B 687 (1996) 93-108
339	Stanozolol-M1; RI = 3238; Vol 1, p. 100	W. Schanzer, J. Chromatogr., B 687 (1996) 93-108
340	Stanozolol-M2; RI = 3218; Vol 4, p. 233	W. Schanzer, J. Chromatogr., B 687 (1996) 93-108
341		K.De Wasch, Anal. Chim. Acta, 473 (2002) 59-69
342		
343		K.De Wasch, Anal. Chim. Acta, 473 (2002) 59-69
344	M0; 1816-85-9; RI = 28.7 (free-)	R. G-Lumbreras, J. Chromatogr., B 754 (2001) 419-425
345	M0; 4075-20-1; RI = 28.2 (free-)	
346	M0; 2226-70-2	
347	M0	
348	M0; 63-01-4; RI = 28.3 (free)	
349	M0	
350	M0; 2126-37-6	
351	M0; 4075-14-3; RI = 27.2 (free)	
352	M0; 10390-14-4	
353	M0; 2944-87-8; RI = 27.8 (free)	
354	M0; 62-99-7	S. Rendic, J. Chromatogr., B 735 (1999) 73-83
355	M5190; 338-95-4; Anti-inflammatory	
356	M0; 564-35-2; RI = 27.7	R.G-Lumbreras, J. Chromatogr., B 754 (2001) 419-425
357	M0	
358	Dimethazine	
359	M5836; 977-79-7; Progestagen	A. Koole, J. Chromatogr., B 724 (1999) 41-51
360	M5838; 520-85-4	S. Hartmann, J. Chromatogr., B 704 (1997) 105-117
361	M5838; 71-58-9	D. Hooijerink, Analyst, 119 (1994) 2617-2622

362		P. Marchand, J. Chromatogr., A 867 (2000) 219-233
363	M5849; Progestagen	P. Marchand, J. Chromatogr., A 867 (2000) 219-233
364	M5849; 595-33-5; Progestagen	E. Daeseleire, J. Chromatogr., B 562 (1991) 673-679
365		P. Marchand, J. Chromatogr., A 867 (2000) 219-233
366	M5859; 5633-18-1; Progestagen	P. Marchand, J. Chromatogr., A 867 (2000) 219-233
367	M5859; 2919-66-6; Progestagen	I.G. Lange, J. Immu. & Imm., 24 (2003) 265-272
368		P. Marchand, J. Chromatogr., A 867 (2000) 219-233
369	M5904; 21362-69-6	
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371	M5973; 521-11-9; Androstalone	
372	M5974; 1424-00-6	R. Masse, J. Steroid Biochem., 42 (1992) 399-410
373	M5974	
374	M5976; 72-33-3; Conovid	
375	M6017; 521-10-8	W. Schanzer, Anal. Chim. Acta, 275 (1993) 23-48
376	M6017; 2061-86-1	
377	M6017; 3593-85-9; Probolin	
378	M6018; 72-63-9	R. Masse, J. Chromatogr., B 562 (1991) 323-340
379	Methandrostenolone-M	W. Schänzer, J. Steroid Biochem., 38(1991) 441-464
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