Reprint from

RECENT ADVANCES IN DOPING ANALYSIS (2)

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Sport und Buch Strauß, Köln, 1995

C. Ayotte, A. Charlebois, D. Goudreault, C. Lévesque:
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C. Ayotte, A. Charlebois, D. Goudreault, C. Lévesque.

Improvements of drug detection and analysis by appropriate preparation of the injection port of the gas chromatograph.

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In this paper, we will present several examples of drastic improvements of the quality of results obtained in GC/MS by reducing the "reactivity" of the injection chamber. The carrier gas (zero grade) is purified (O_2,H_2O) with a OMI-1 Indicating Purifier (Supelco, Canada) containing a resin of solid lithium compound. The glass liner and the glass wool are silanized overnight in a 5% DMDCS/toluene solution and rinsed three times with methanol. One of the most striking result was the obtention of the TMS-derivative of pemoline[1] described as impossible to detect through chromatography.

In the following pages, we are presenting selected examples taken from the GC/MS analysis of some TMS-ether, TMS-enol derivatives of anabolic androgenic steroids or their metabolites : oxandrolone,6β-hydroxymethandienone,6β-hydroxydehydrochlormethyltestosterone,stanozolol, T/E values and epi trenbolone. The improvements are observed with the detection levels, long term stability and high reproductibility.

The examples of propoxyphene, strychnine and codeine are also given.

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[1] C. Ayotte: Old dope agent, old method, 11th Köln Workshop in dope analysis, Köln (1993).

Procedure IV

GC/MS analyses in the SIM (selected ion monitoring) mode are carried out with Hewlett Packard HP 5890 gas chromatographs with direct coupling to HP-MSD 5970 quadrupolar filters. Systems are equipped with automatic samplers 7673 A and controlled by HP 59970 and Unix MS Chemstations. The injections are carried out in the splitless mode (1 μL) and the separation is achieved on HP-5 capillary columns.

Chromatographic parameters

carrier gas : He
injection port : 270°C
transfer line : 310°C
injection mode : splitless 30 sec.
initial temperature of the oven : 100°C (1 min)
initial rate : 16°C
first temperature : 220°C
final rate : 3.8°C
final temperature : 300°C (10 min)

PROCEDURE IV

SCREENING/CONFIRMATION
(Free and glucuronides fraction)

2 to 10 mL urine

↓

add

5 µg epietiocholanolone
500 ng 17α-methyl-5α-androstan-3β,17β-diol

↓

apply on Sep-Pak C18 cartridge
wash with 5 mL of water
wash with 3 mL of n-hexane
elute with 5 mL of methanol
evaporate methanol to dryness

↓

add

1 mL phosphate buffer 0.2M pH: 6.9
β-glucuronidase from E. Coli (type IX-A, 2000 units)

↓

37°C for 16 hours or 50°C for 1 hour

↓

add

100 mg solid buffer pH 9
5 mL diethyl ether

↓

shake mechanically 10 min. and centrifuge 5 min.
evaporate organic phase to dryness

↓

dissolve residue in 150 µL of methanol
transfer to vial containing 500 ng of 5α-androstan-17-one

↓

evaporate to dryness

↓

add

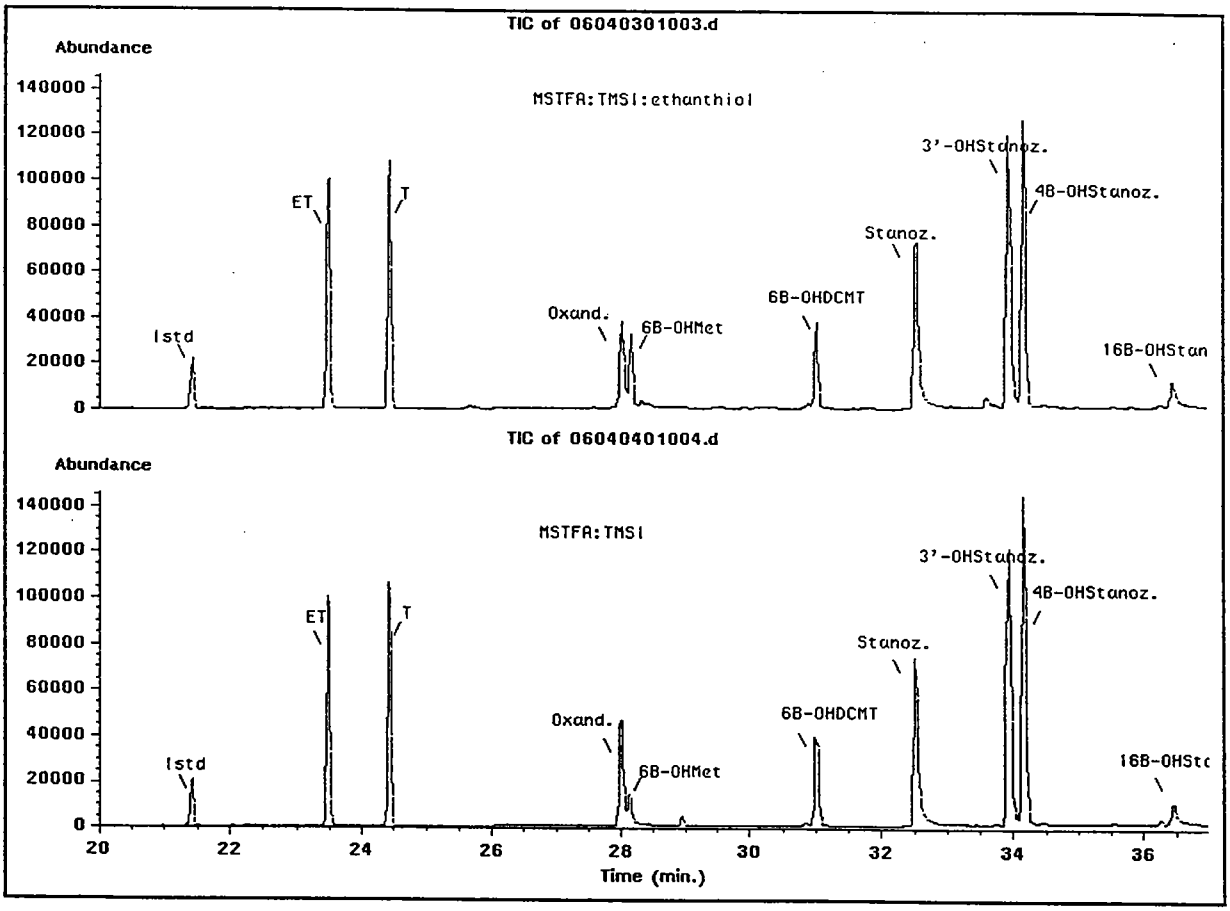
2µL ethanethiol
100 µL MSTFA
2 µL TMSI

↓

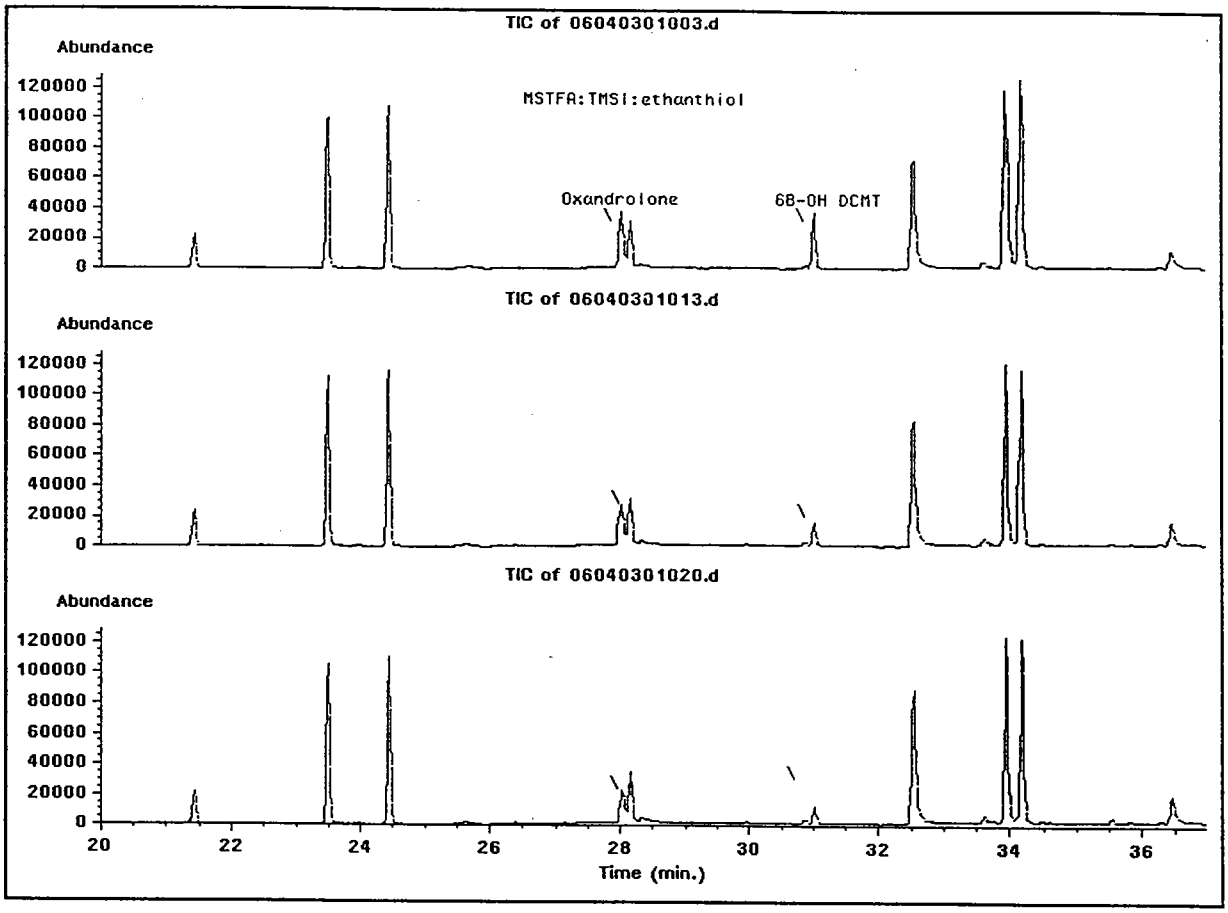
70°C for 30 min.

↓

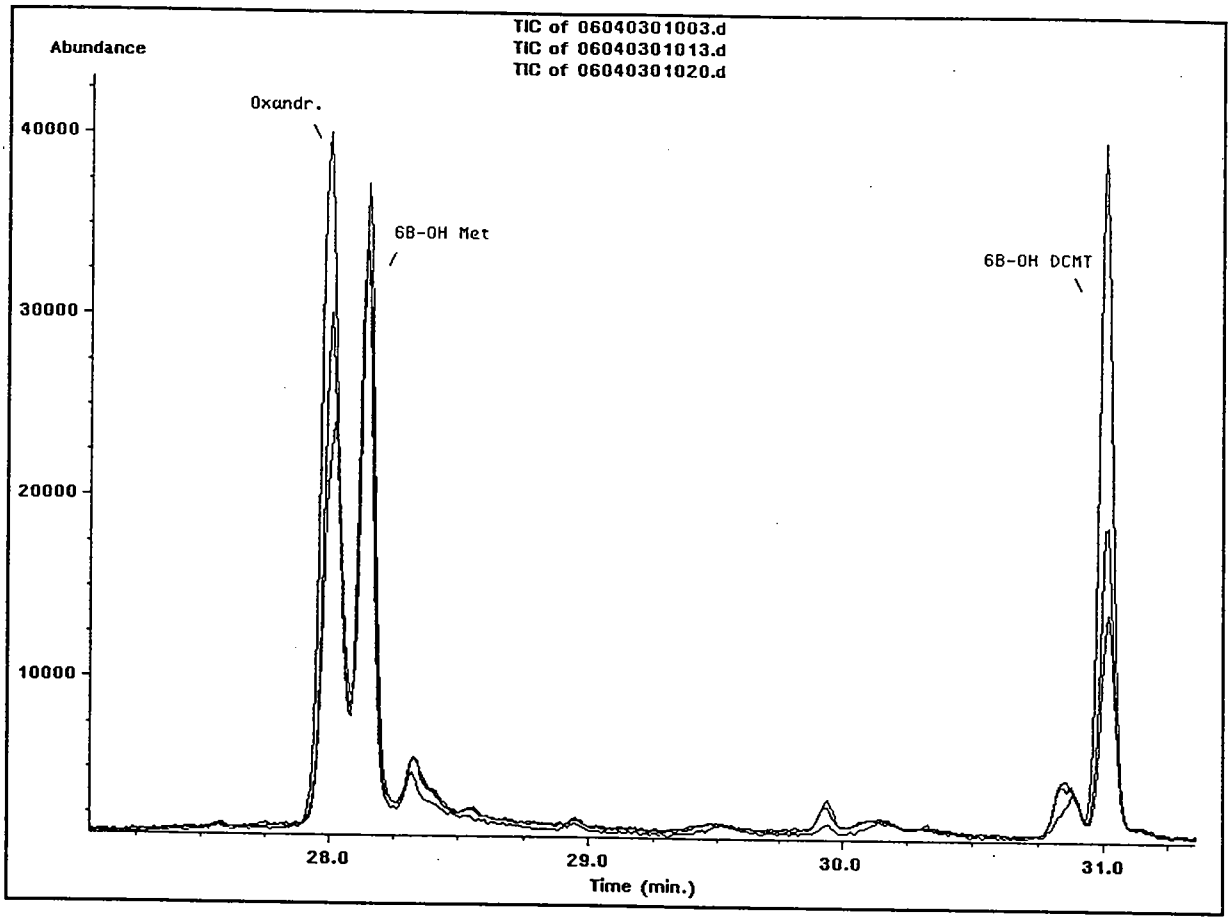
Inject into GC/MS and GC/FID



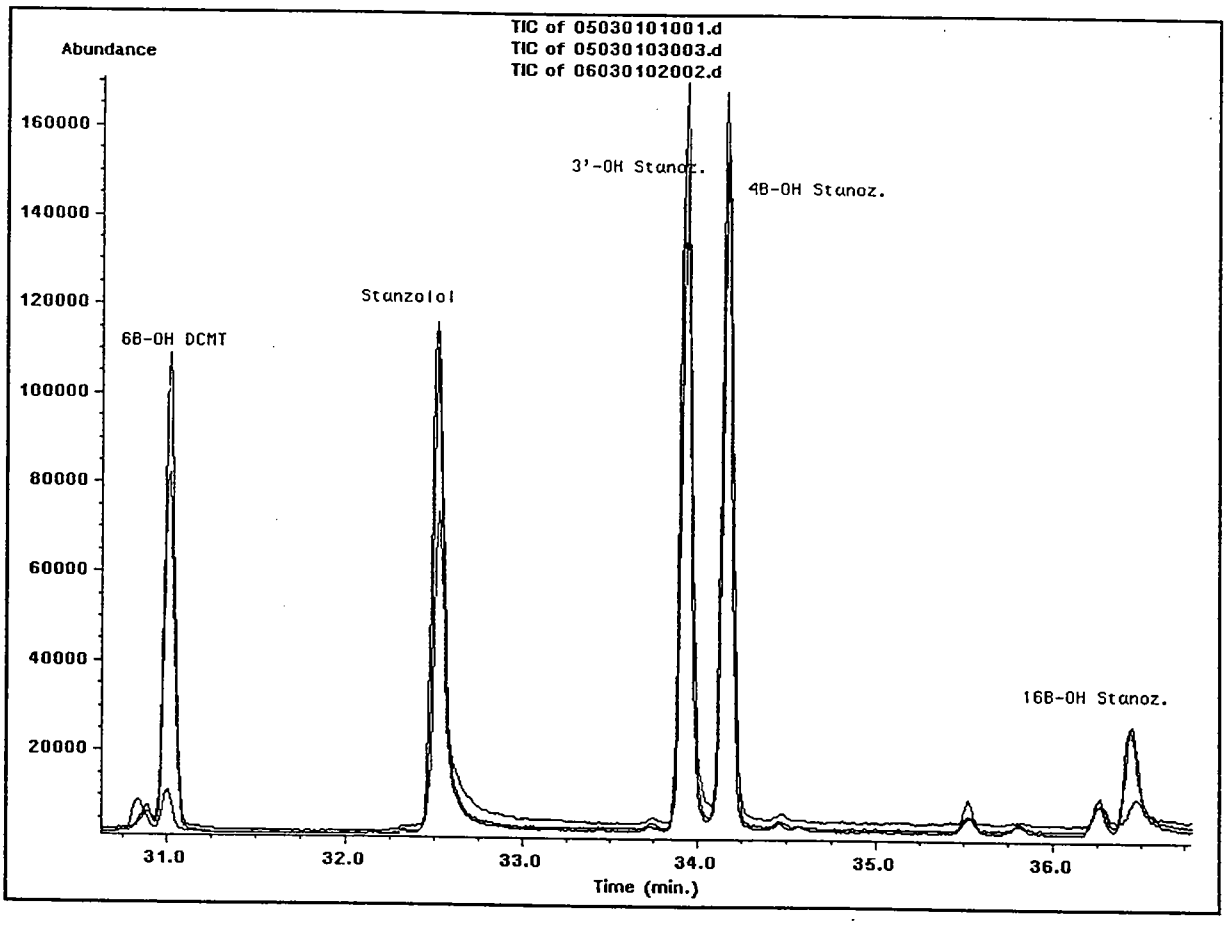
GC/MS (SIM mode) analysis of the TMS-ether, TMS-enol derivatives of a mixture of standards. Reactives used for the chemical derivatization; MSTFA:TMSI: ethanthiol (upper panel), MSTFA:TMSI (lower)



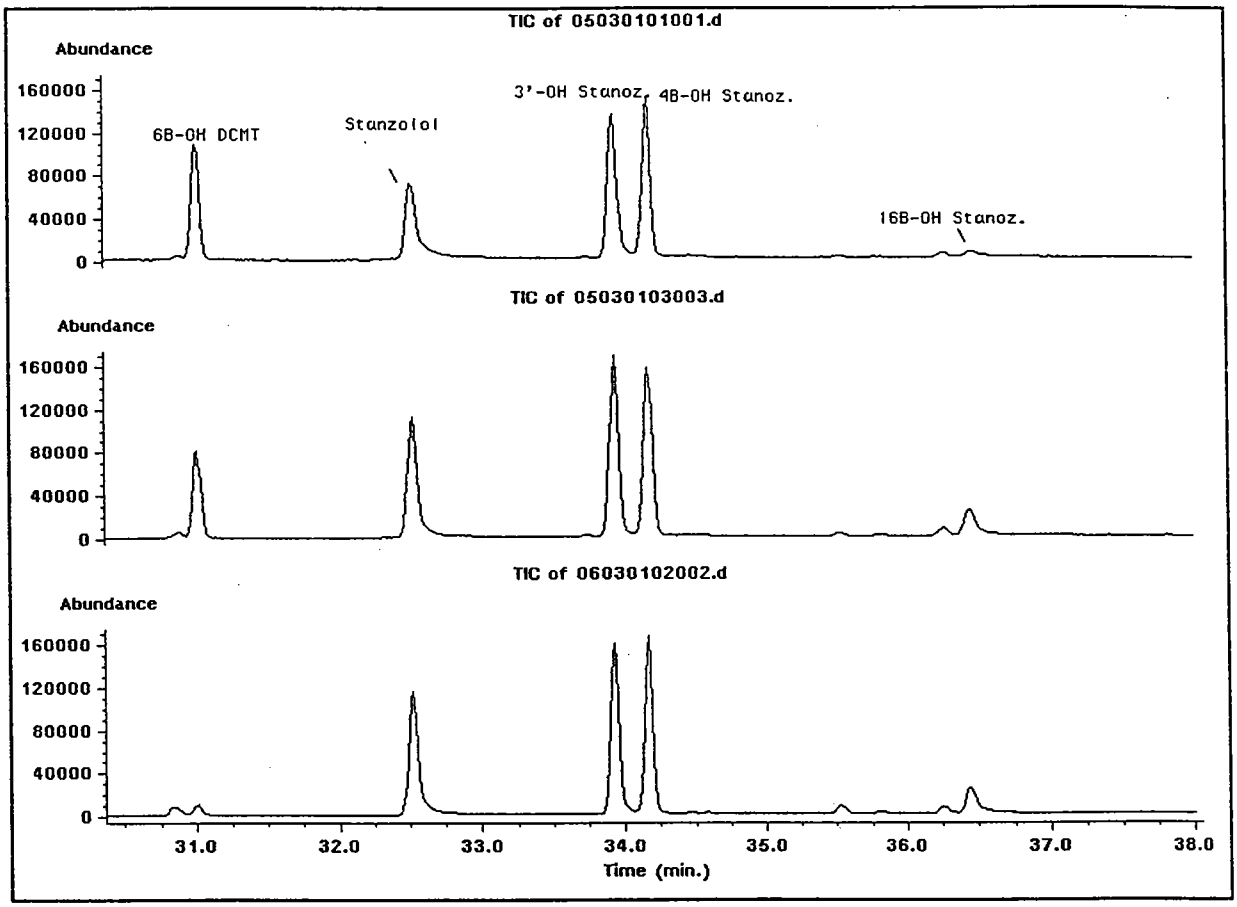
GC/MS (SIM mode) analysis of a mixture of standards injected over a 18 hours period on a silanized glass liner.



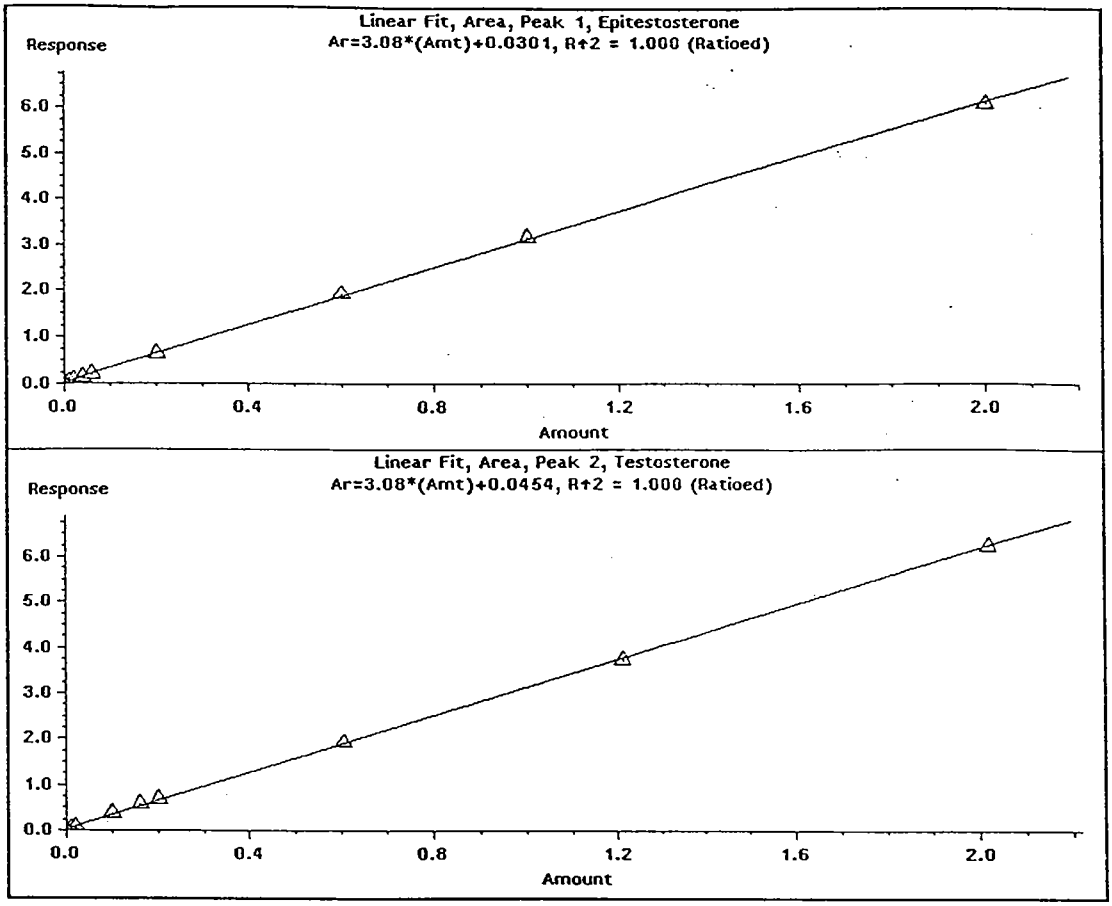
GC/MS (SIM mode) analysis of a mixture of standards injected over time (enlarged)



GC/MS (SIM mode) analysis of a mixture of standards (first, third and tenth injections)



GC/MS (SIM mode) analysis of a mixture of standards (first, third and tenth injections) (enlarged)



Calibration Table

[T]/[Et] Standards spikes/urine (Solutions #080394)

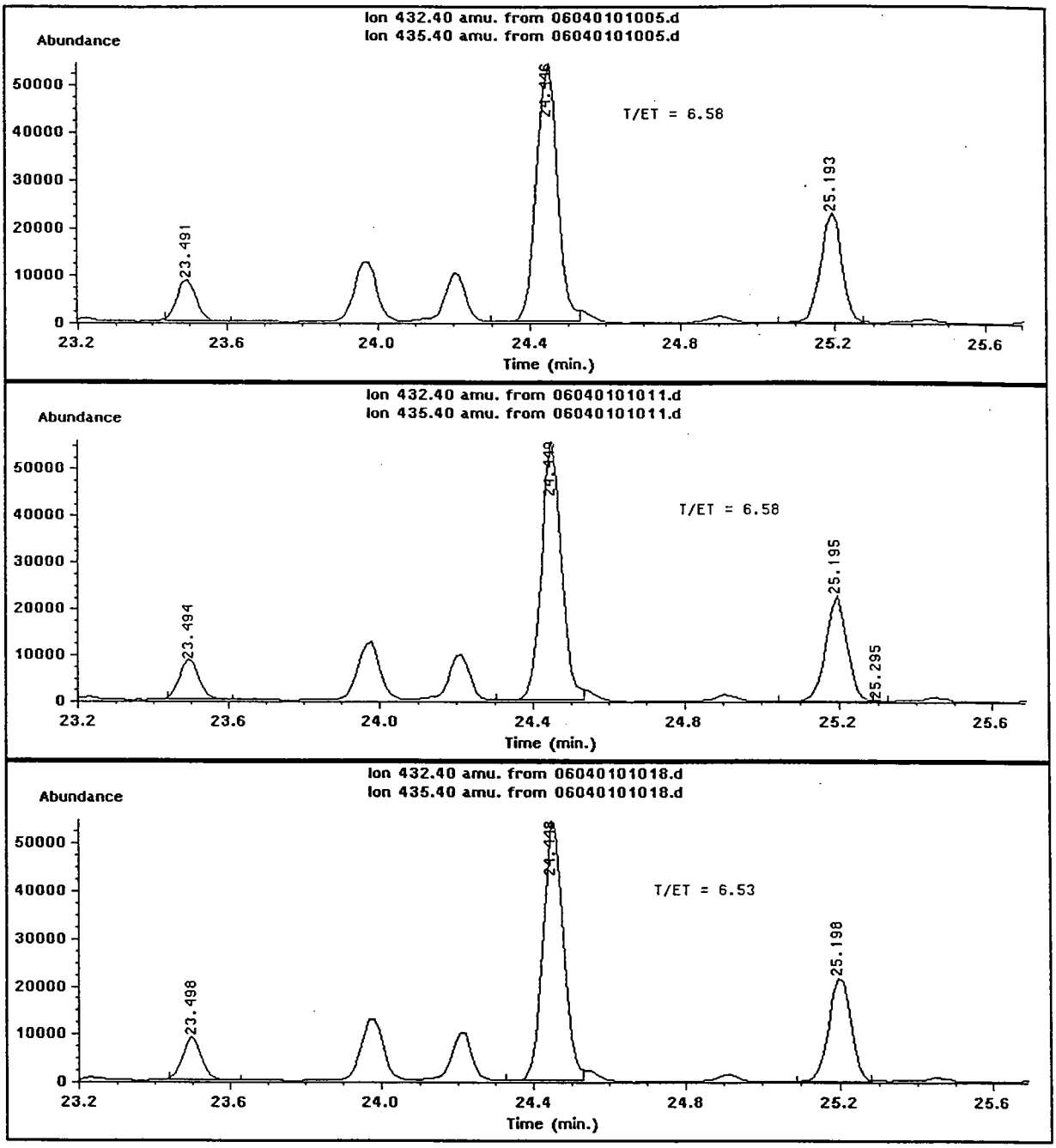
Calibration Table Last Updated: Thu Apr 07 14:07:04 1994

Reference Peak Window: 5.000 % of Retention Time
Non-reference Peak Window: 5.000 % of Retention Time

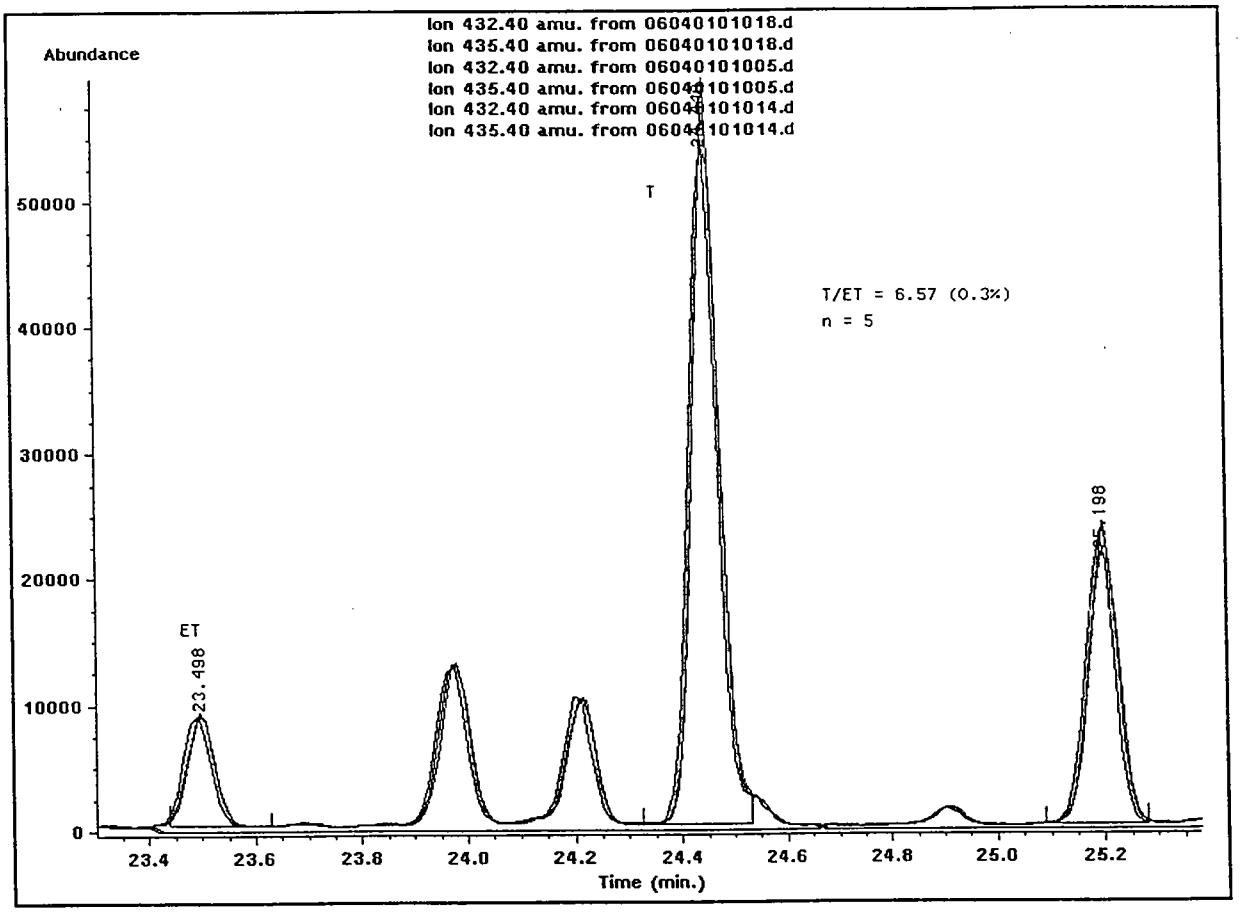
Default Sample Amount: 0
Uncalib. Peak Response Factor: 0
Default Multiplier: 1

Ret Time	Pk#	Signal Descr	Amt ng/ul	Lvl	Area	Pk-Type	Partial Name
23.465	1	432.40 amu	0.05007000	3	48448		Epitestostero
			0.1001000	1	92560		
			0.2003000	4	199001		
			0.3004000	2	251919		
			1.001000	7	867146		
			3.004000	6	2550640		
			5.007000	8	4514220		
			10.01000	5	5568990		
24.425	2	432.40 amu	0.05043000	1	66190		Testosterone
			0.1009000	2	106023		
			0.5043000	3	403990		
			1.009000	5	629639		
			0.8069000	4	736529		
			3.026000	6	2551990		
			6.052000	7	4944800		
			10.09000	8	8955800		
25.173	3	435.40 amu	5.000000	5	903818	+I	Istd 2
			5.000000	3	1068720		
			5.000000	1	1150270		
			5.000000	2	1274890		
			5.000000	4	1278840		
			5.000000	6	1315420		
			5.000000	7	1315760		
			5.000000	8	1429250		

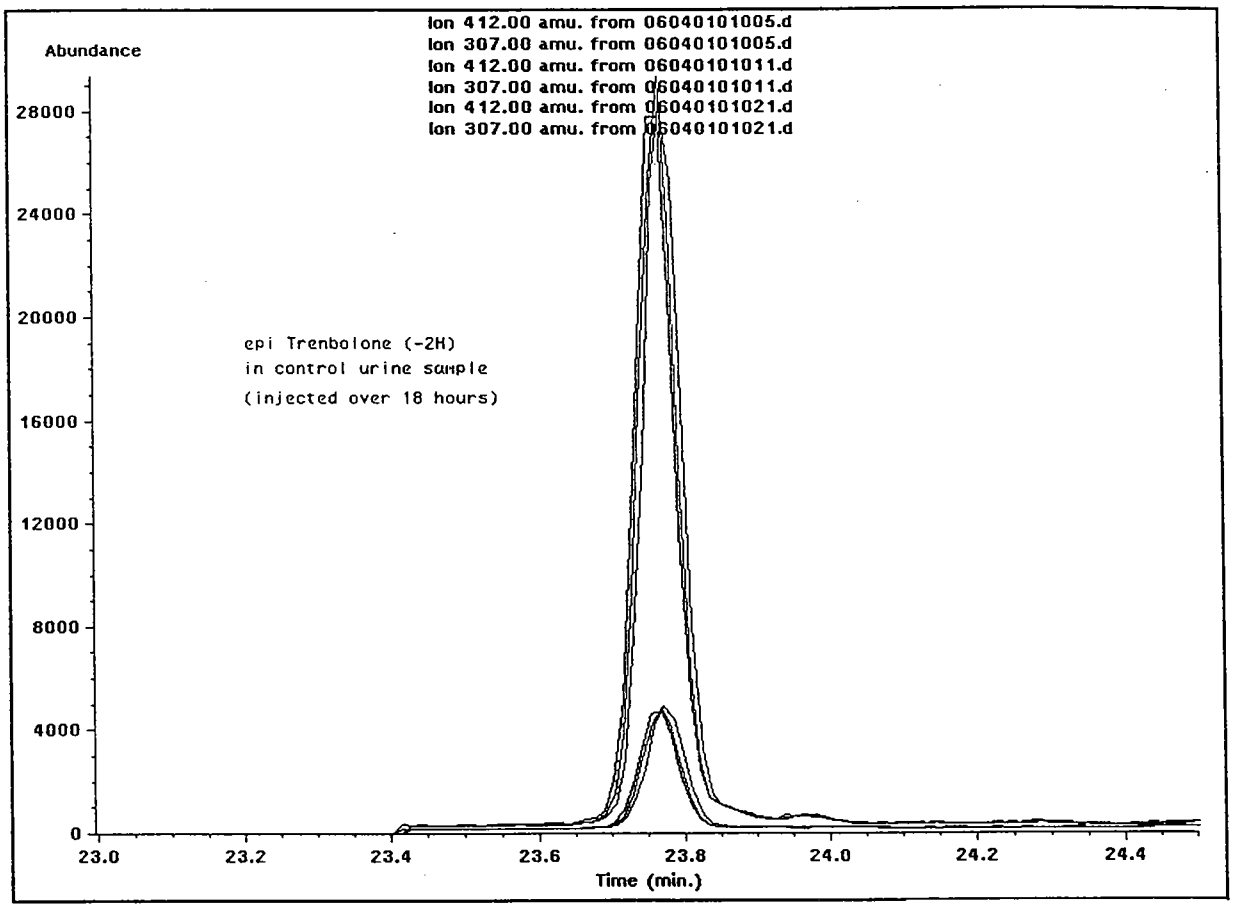
Calibration curves used for the determination of both testosterone and epitestosterone concentrations. 17a-methylandrostan-5a-3a,17-diol is used as internal standard. Analytical standards are spiked in urine samples and



Ion 432.4 and 435.4 amu from the GC/MS analysis of the control urine sample injected over a 18 hours period each 4 samples.

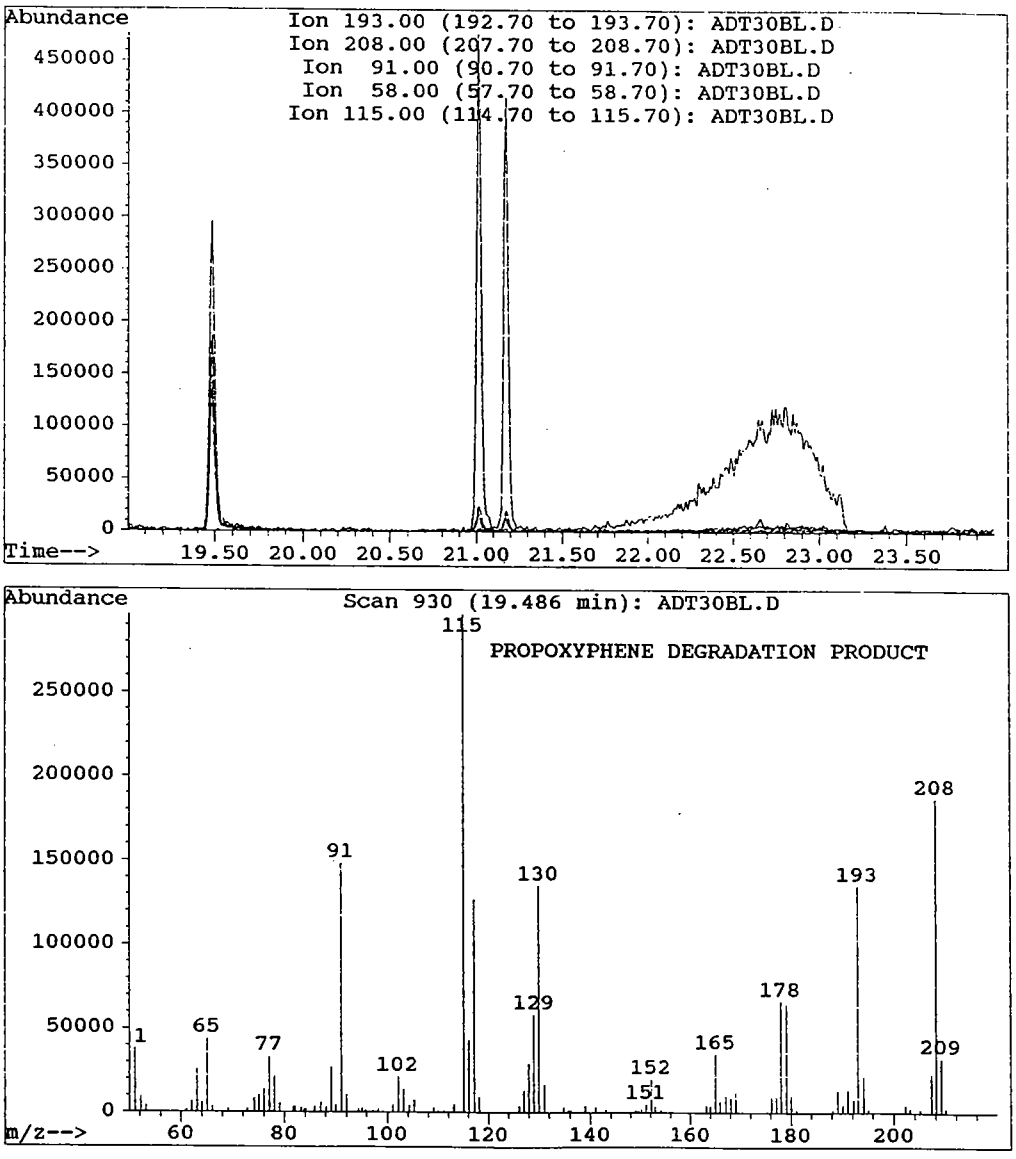


Superimposition of three ion chromatograms (432.4, 435.4 amu) obtained from the analysis of the control urine sample injected over a 18 hours period.



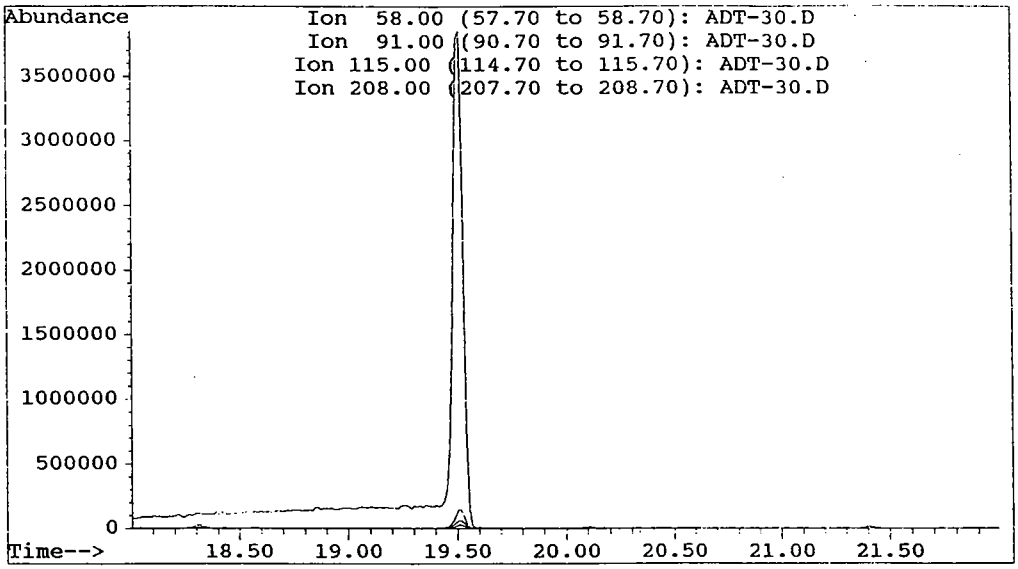
Superimposed ion chromatograms (ions 412.4 and 307.3) obtained from the analysis of the control urine sample over a 18 hours time period.

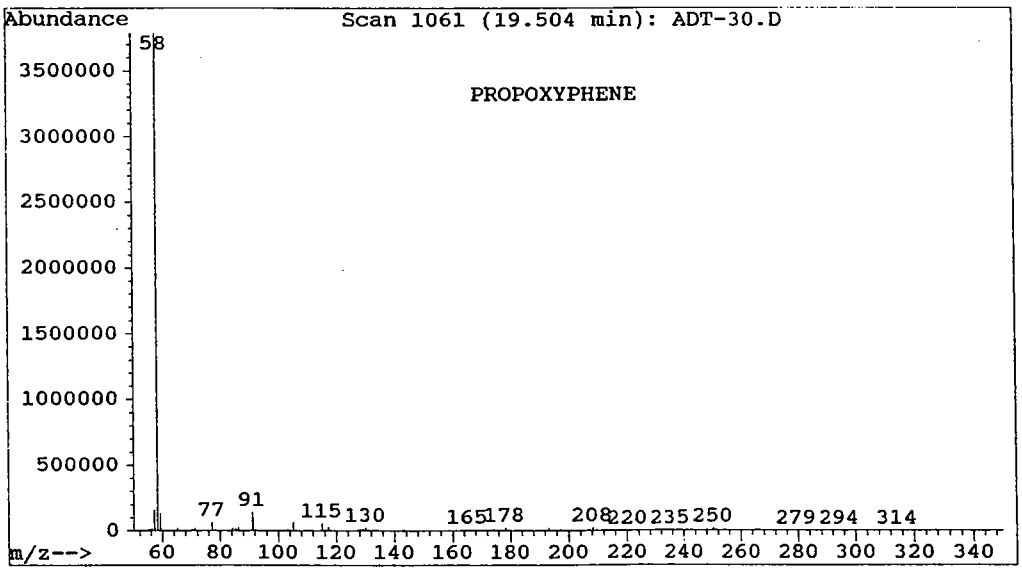
File : C:\HPCHEM\1\DATA\CAP92\ADT30BL.D
Operator : d.g.
Acquired : 15 Jan 92 3:20 pm using AcqMethod BLEUE.M
Instrument : MS_5971
Sample Name: adt-30 proc 1derive 20ul
Misc Info :
Vial Number: 1



GC/MS (full scan mode) analysis of a propoxyphene urine sample injected on an untreated glass liner

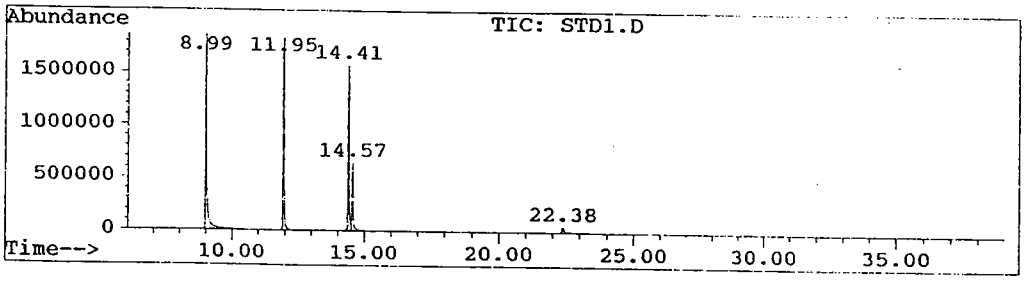
File : C:\HPCHEM\1\DATA\SMCC\ADT-30.D
Operator : C.L.
Acquired : 6 Mar 94 8:46 pm using AcqMethod STIM2
Instrument : 5971 - In
Sample Name: ADT-30 CAP 92 PR.I
Misc Info : PROPOXYPHENE
Vial Number: 8

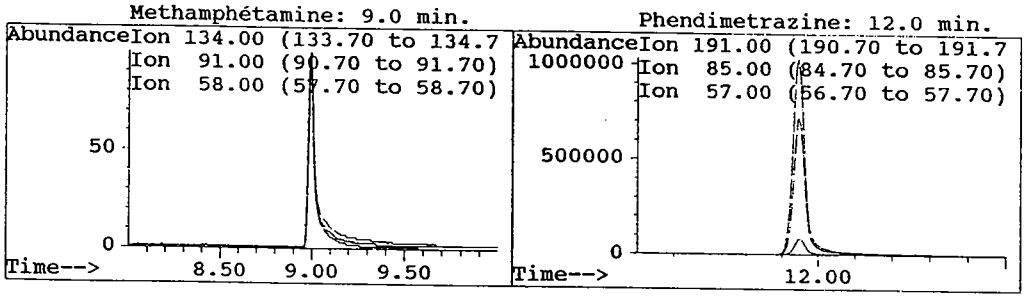


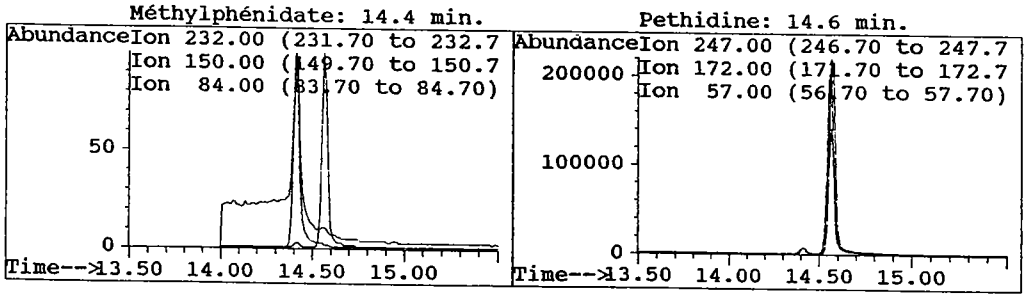


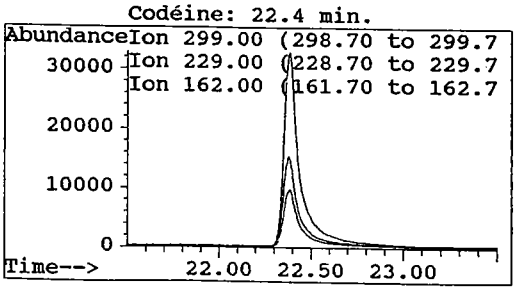
GC/MS (full scan mode) analysis of a propoxyphene urine sample injected on a silanized glass liner.

Fichier:	C:\HPCHEM\1\DATA\SMCC\STD1.D
Opérateur:	MANON
Date analysé:	6 Mar 94 12:34 pm
Instrument:	5971 - In
Méthode utilisée:	SIM.M
Échantillon:	STD DE PERFORMANCE
Information suppl:	WITHOUT SILANIZED GLASS WOOL
Vial no:	2







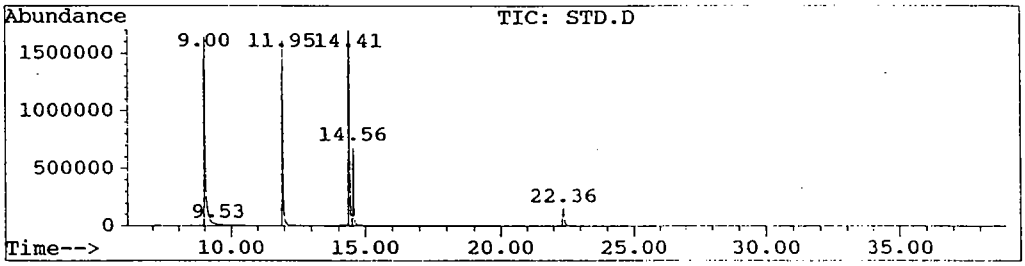


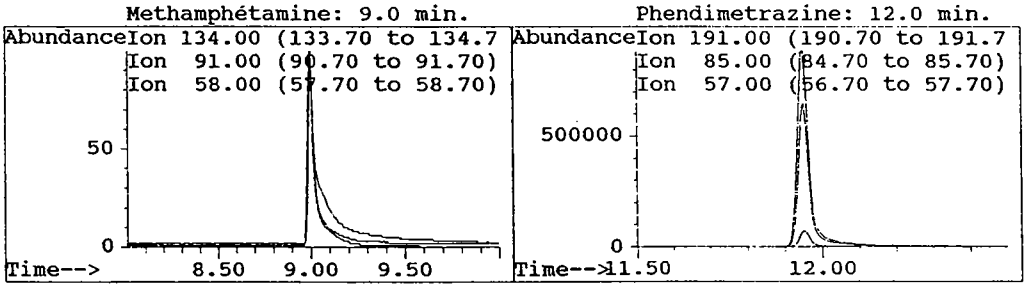
RÉSULTATS

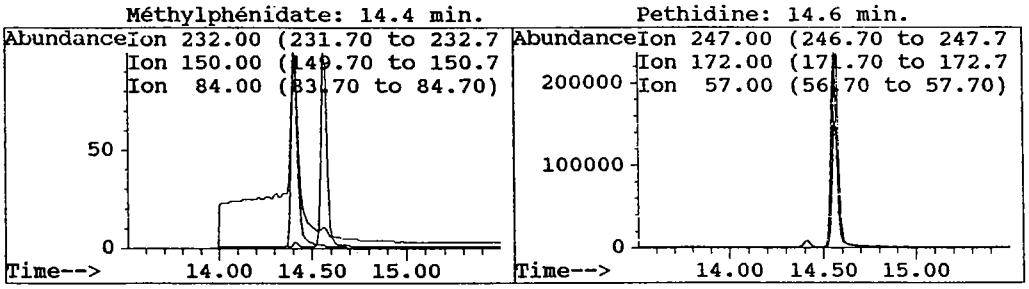
Produit	RT	Surface	Ratio
MA	8.99	57183377	3.07
PDM	11.95	43919861	2.36
MPH	14.41	38487621	2.07
Peth	14.57	18625288	
Codéine	22.38	3452812	185.38

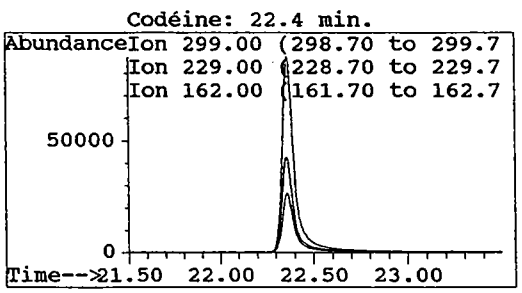
GC/MS (SIM mode) analysis of a mixture of standards injected on silanized glass liner.

Fichier: C:\HPCHEM\1\DATA\SMCC\STD.D
Opérateur: MANON
Date analysé: 6 Mar 94 10:48 am
Instrument: 5971 - In
Méthode utilisée: SIM.M
Échantillon: STD DE PERFORMANCE
Information suppl: SILANIZED GLASS WOOL
Vial no: 2







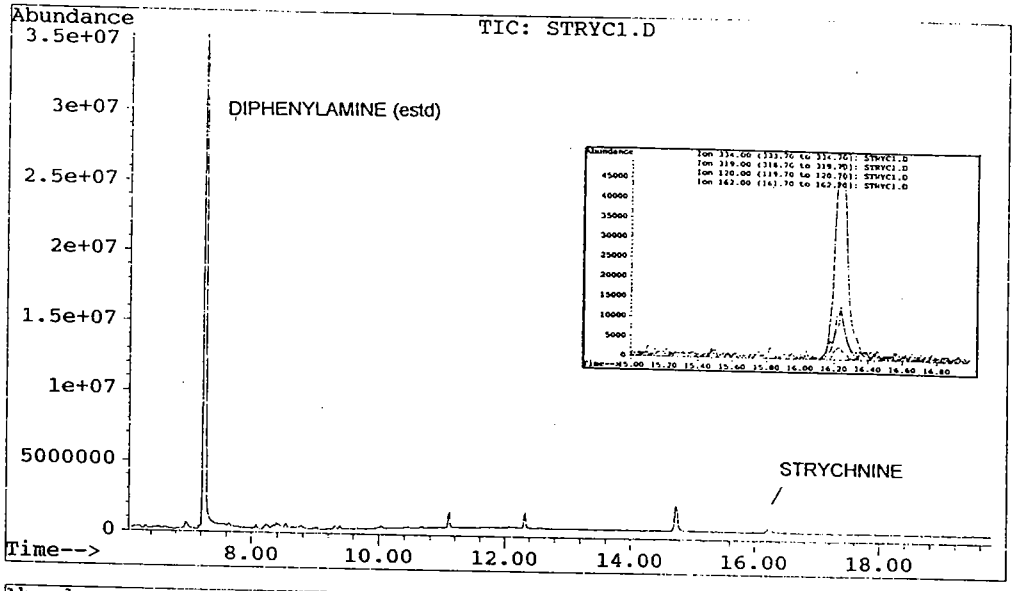


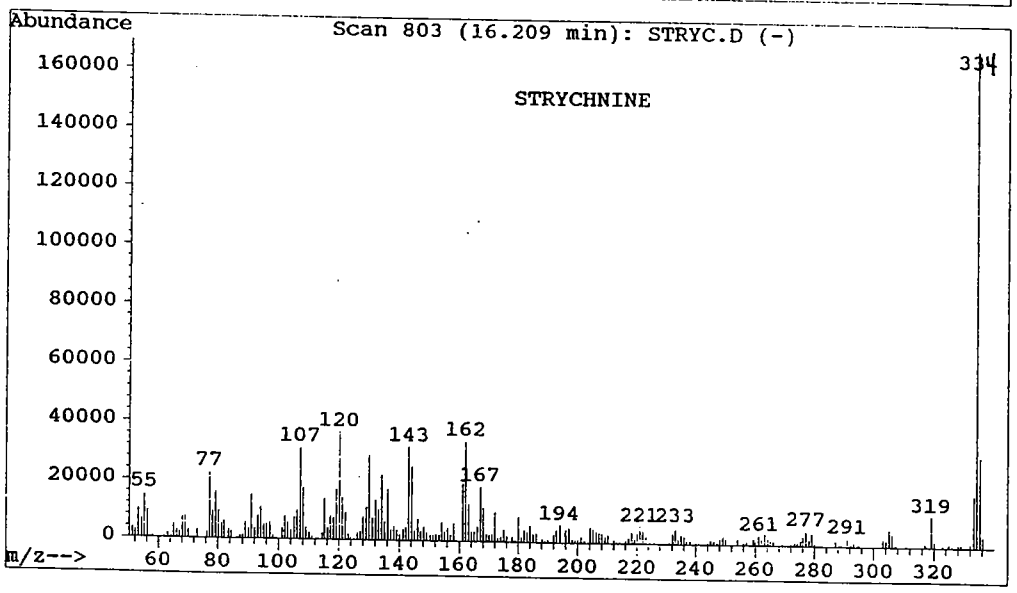
RÉSULTATS

Produit	RT	Surface	Ratio
MA	9.00	51597207	3.06
PDM	11.95	36445071	2.16
MPH	14.41	40323176	2.39
Peth	14.56	16871694	
Codéine	22.36	7242364	429.26

GC/MS (SIM mode) analysis of a mixture of standards injected on silanized glass wool and liner.

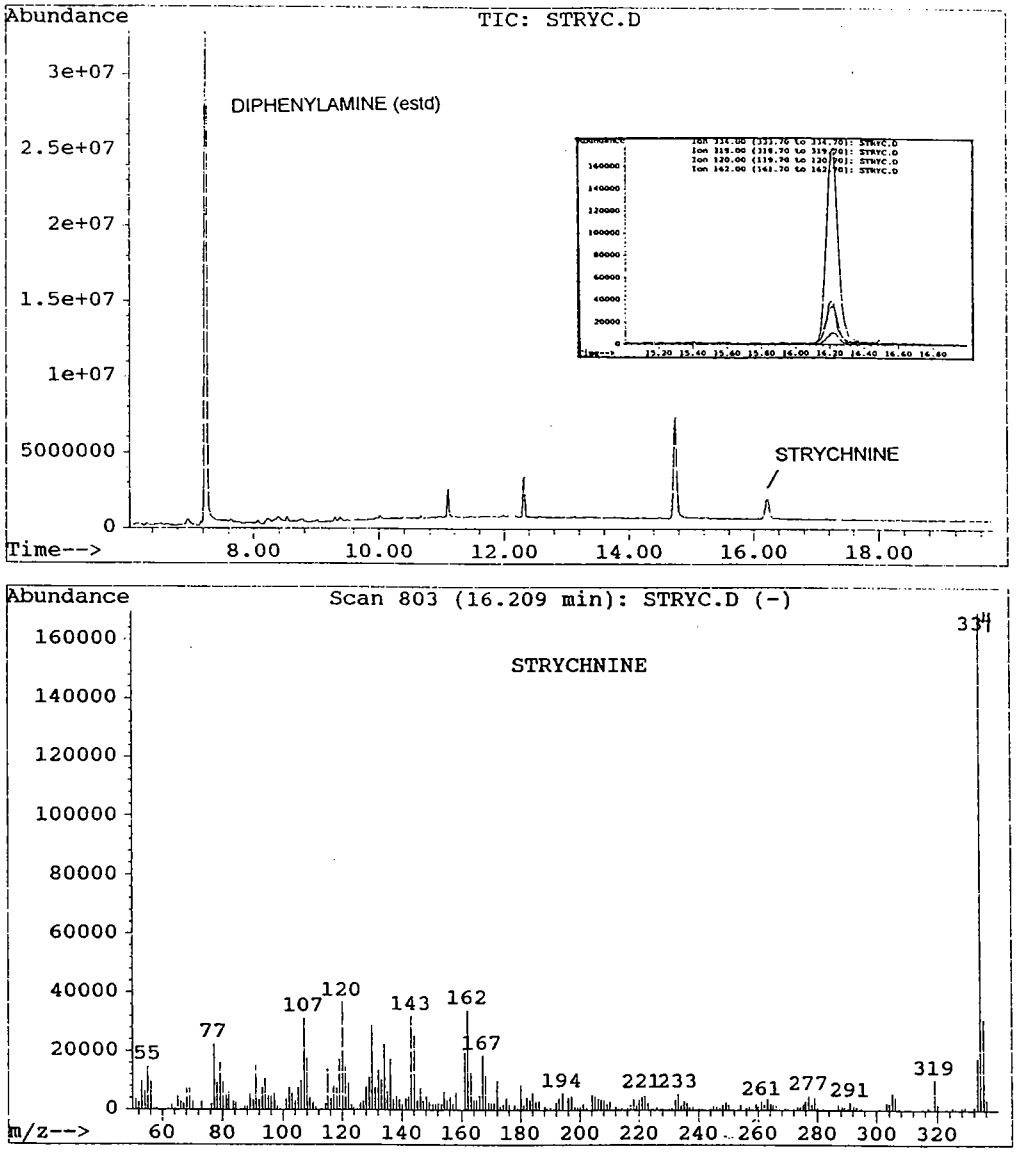
File : C:\HPCHEM\1\DATA\SMCC\STRYC1.D
Operator : MANON
Acquired : 6 Mar 94 12:04 pm using AcqMethod STRYC
Instrument : 5971 - In
Sample Name: STRYCHNINE 0.5UG/ML
Misc Info : WITHOUT SILANIZED GLASS WOOL
Vial Number: 1





GC/MS (full scan mode) analysis of strychnine authentic standard injected on silanized glass liner.

File : C:\HPCHEM\1\DATA\SMCC\STRYC.D
Operator : MANON
Acquired : 6 Mar 94 10:17 am using AcqMethod STRYC
Instrument : 5971 - In
Sample Name: STRYCHNINE 0.5UG/ML
Misc Info : LINER WITH SILANIZED GLASS WOOL
Vial Number: 1



GC/MS (full scan mode) analysis of a strychnine authentic standard injected on silanized glass wool and liner.