

Reprint from

RECENT ADVANCES
IN DOPING ANALYSIS
(6)

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

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D. GOUDREAULT, C. AYOTTE:
The Identification of Fenozolone Urinary Metabolites
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D. Goudreault and C. Ayotte

The identification of fenozolone urinary metabolites

INRS-Santé, Montréal, Québec

Fenozolone (2-ethylamino-5-phenyl-4-oxo-2-oxazolidine, Ordinator®) is a psychostimulant drug used to overcome fatigue. Although its utilization is banned by the I .O.C., its metabolism still remains unknown and the only method reported in the literature to detect its abuse is non-specific. Gielsdorf and al. reported in 1982 the detection of fenozolone, pemoline and thozalinone in human urine by the formation of a common hydrolysis product : 5-phenyl-2,4-oxazolidinedione ¹. Fenozolone has a chemical structure very similar to pemoline : N-ethyl tautomer of pemoline . The difficulties to analyse fenozolone by GC/MS as for pemoline seem to be related to its poor extraction ^{1,2} and GC chromatographic properties^{1,2,3}. However in 1993, Ayotte reported the detection of pemoline by GC/MS as its trimethylsilyl (tms) derivative ⁴. We are now reporting the GC/MS detection of fenozolone and its main metabolite pemoline by GC/MS as their tms derivatives after oral administration of Ordinator® 10 mg to two female volunteers. The parent compound and its metabolite were extracted from urine sample on a Sep Pak C₁₈ cartridge (same extraction procedure as for anabolic agents ⁵). Fenozolone and pemoline are excreted in urine mainly in the free form, only less than 10% of pemoline is conjugated to glucuronic acid. The presence of fenozolone can be confirmed up to 48 hres by GC/MS in SIM mode analysis and pemoline for more than three days in the full scan mode. As determined by the analysis of blank urine samples spiked with fenozolone and pemoline, the limits of detection in the SIM mode (ions m/z 348, 333, 178, 163(fenozolone) and 392, 377, 178, 163 (pemoline)) are 5ng/mL and 3,3ng/mL respectively and in the full scan mode 33.3ng/mL for both of them.

Finally, if pemoline is detected in urine sample, the presence or absence of fenozolone should be verified in order to identify correctly the substance which was abused.

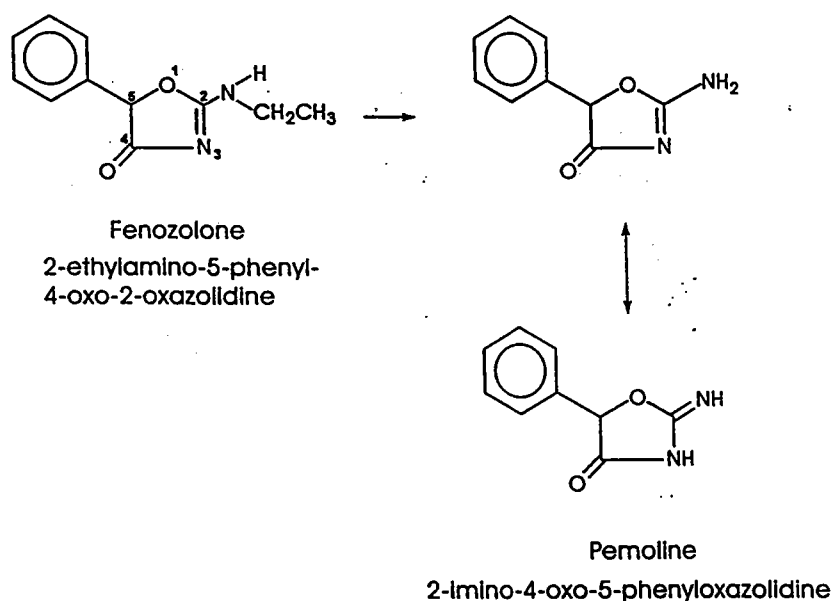
¹Gielsdorf W., J. Clin. Chem., Clin. Biochem, 20, 65 (1982)

²Vermeulen N.P.E., De Roode D. and Breimer, D.D., J. Chromatogr., 137, 333 (1977)

³Libeer J.C. and Schepens P., J. Pharmaceut. Sc., 67, 419 (1978)

⁴Ayotte C., Proceedings of the 11th Cologne Workshop on Dope Analysis, March 1993, eds. M. Donike, H. Geyer, A. Gotzmann, U. Mareck-Engelke and S. Rauth, 111 (1994)

⁵Ayotte C., Goudreault D. and Charlebois A., J. Chromatogr. B Biomed. Applic., 687, 3 (1996)



Fenzolone: Ordinator[®] 10mg from Synthelabo, France

Use: fatigue, psychostimulant and antidepressant activities.

Metabolism: unknown

Analysis: GC/MS analysis of acid hydrolysis product (5-phenyl-2,4-oxazolidinedione), common to pemoline.

Pemoline:

Metabolism: . mainly unchanged, detectable in urine up to 48 hres maximum 2-4h.

- . 4% as 5-phenyloxazolidine-2,4-dione, small part as conjugated pemoline and malic acid.

Analysis: .GC and GC/MS analysis of the acid hydrolytic product
 .recently the GC/MS analysis of pemoline as N,O-tri tms derivative.

Preparation of the samples for GC/MS analysis

1) Extraction of free, glucuronide and sulfate, drugs.

A) free fraction

- 1.5mL urine + 2mL acetate buffer 0.2M pH 5.2)
- Pass through Sep-pak C₁₈ cartridge, elute with MeOH.
- L/L extraction (triple) diethyl ether pH 9.
- Derivatization 50 µL MSTFA:TMSI:Ethanethiol (50:1:1) 70°C/30 min.

B) Glucuronic fraction

- Aqueous phase (free fraction) + 2mL acetate buffer 0.2M pH 5.2 + 400µL HCL 4M (pH ~6.5-7)
- Pass through Sep-pak C₁₈ cartridge, elute with MeOH.
- Enzymatic hydrolysis: 2000 units of β-glucuronidase from E. Coli. Type IX-A, 1h/ 50°C
- L/L extraction (triple) diethyl ether pH 9.
- Derivatization 50 µL MSTFA:TMSI:Ethanethiol (50:1:1) 70°C/30 min.

C) Sulfate Fraction

- Aqueous phase (glucuronic fraction) + 2mL acetate buffer 0.2M pH 5.2 + 400µL HCL 4M (pH ~6.5-7)
- Pass through Sep-pak C₁₈ cartridge, elute with MeOH.
- 1mL ethyl acetate + 2µL H₂SO₄ 4M, 40°C/1h
- Add 4mL ethyl acetate + 1mL of sodium bicarbonate 5% (w/v)
- Wash organic phase with water saturated with NaCl.
- Derivatization 50 µL MSTFA:TMSI:Ethanethiol (50:1:1) 70°C/30 min.

2) Screening procedure: free and glucuronic fractions are extracted together (see B)

3) Confirmation procedure: 3mL urine are used, and only the free drugs are extracted (see A)

Instrumental details

GC/MS analyses in the scan and sim modes are carried out with Hewlett Packard HP5890 gas chromatograph with direct coupling with HP-MSD 5970.

The injections are carried out in the splitless mode (1 μ L) and the separation is achieved on HP-5 capillary columns (5% phenyl methylsilicone phase, 25m x 0.25mm, film thickness:0.33 μ m).

Chromatographic parameters

carrier gas: He

injector port: 270°C

transfer line: 310°C

injection mode: splitless 30 sec

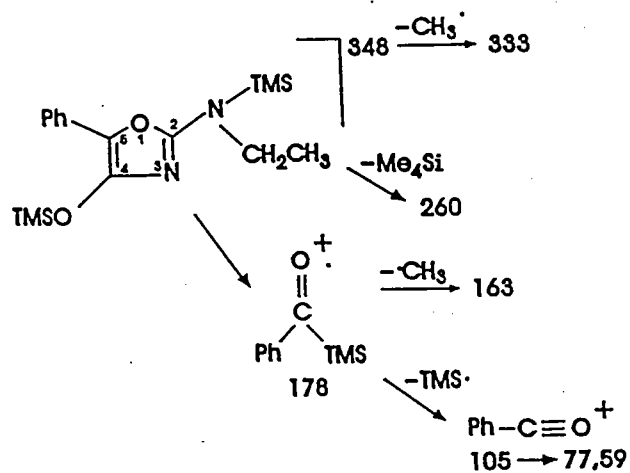
initial temperature of the oven: 100°C (1 min.)

initial rate: 20°C/min.

first temperature: 220°C

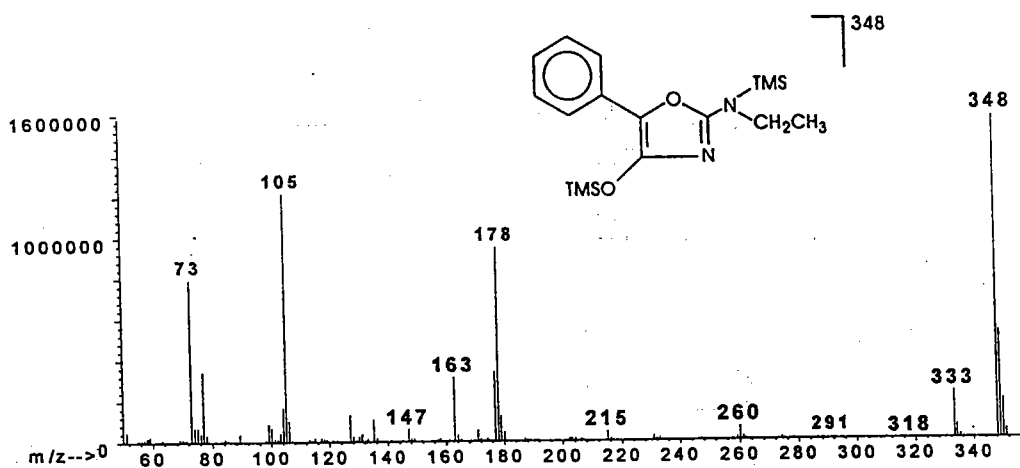
final rate: 4.4°C/min.

final temperature : 320°C (5.27 min.)

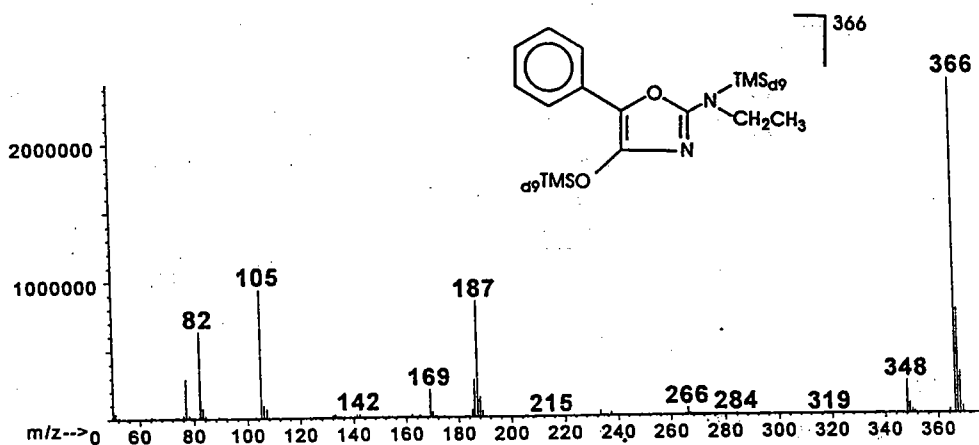


Proposed fragmentation pathways for fenozolone N,O-bis tms derivative.

A)

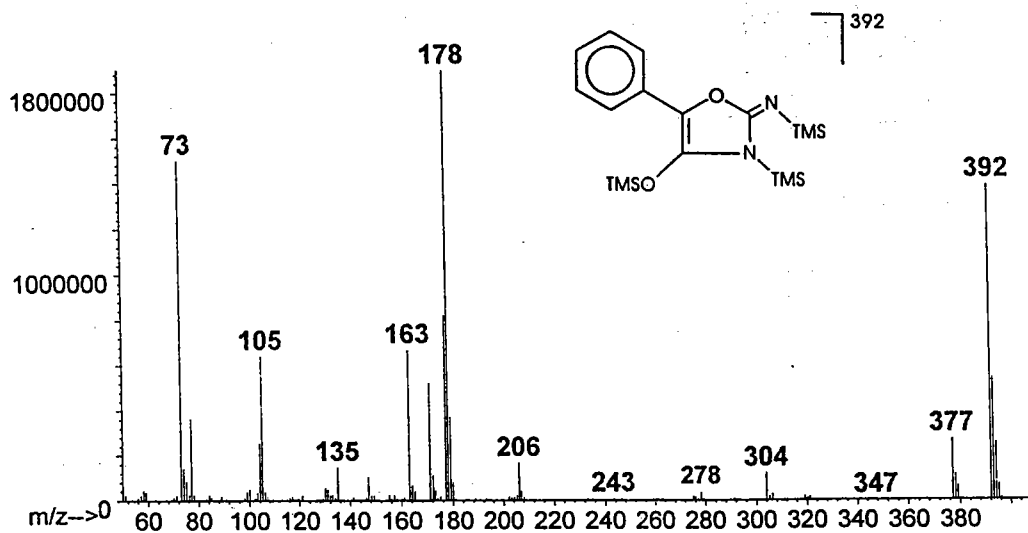


B)

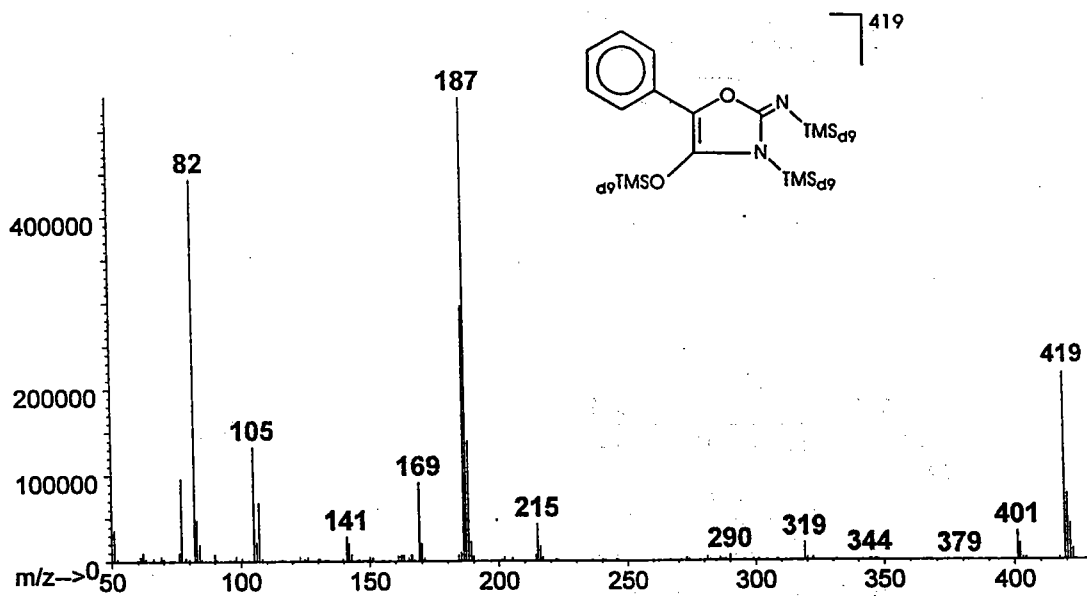


Mass spectra of fenozolone as A) N,O-bis tms and B) N,O-bis d_9 -tms derivatives (authentic standard).

A)



B)

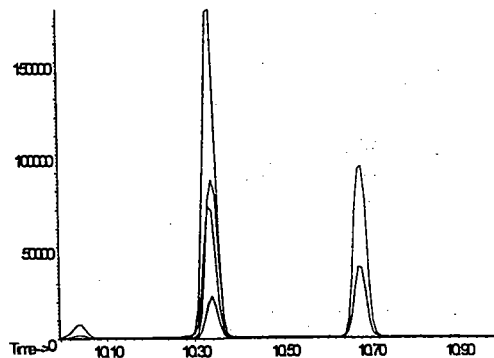
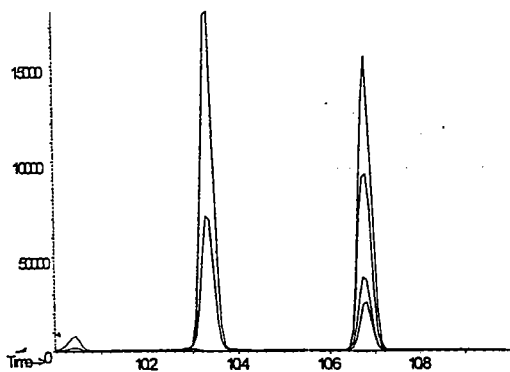


Mass spectra of pemoline as A) N,O-tri tms and B) N,O-tri d₉-tms derivatives (authentic standard).

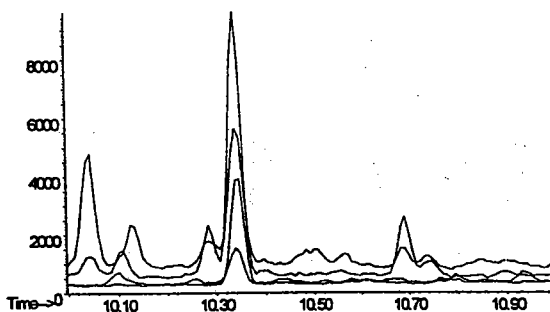
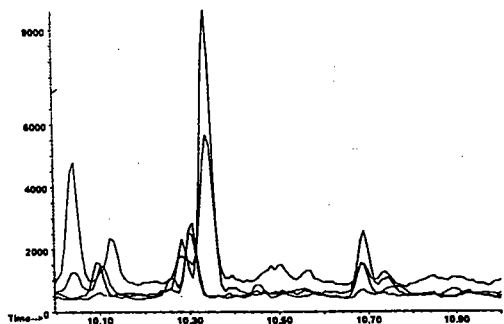
FENOZLONE
m/z 163, 178, 333, 348

PEMOLINE
m/z 163, 178, 377, 392

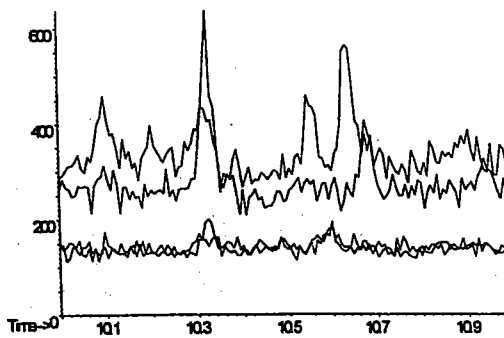
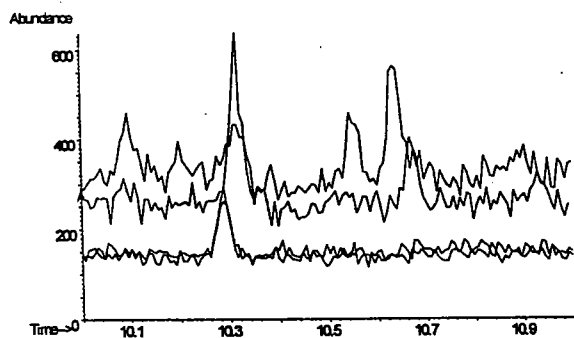
Free fraction



Glucuronic fraction

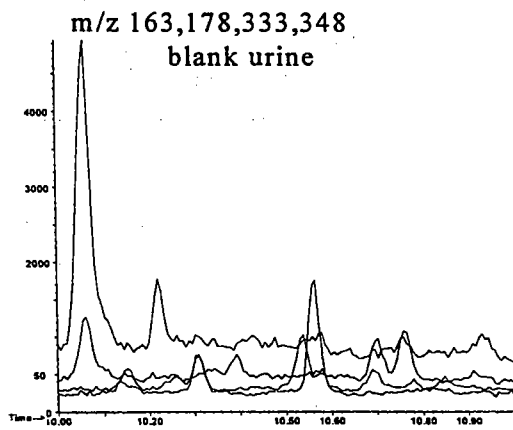


Sulfate fraction

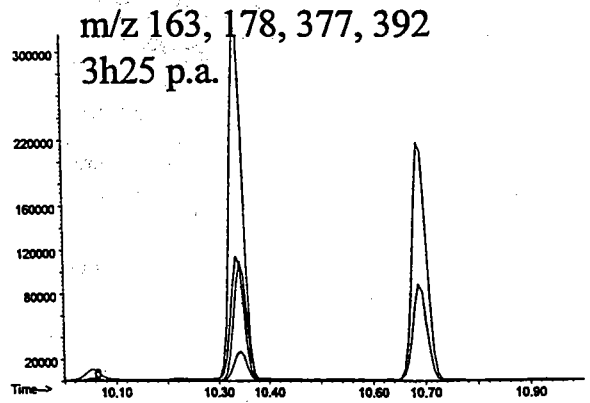
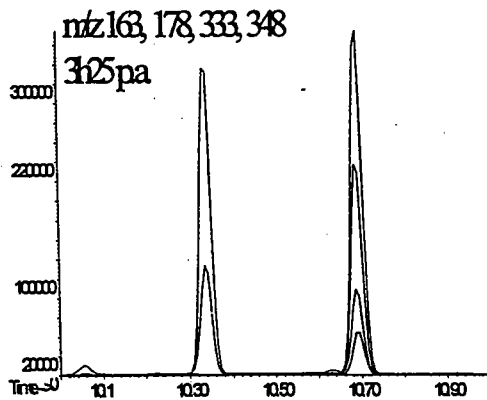
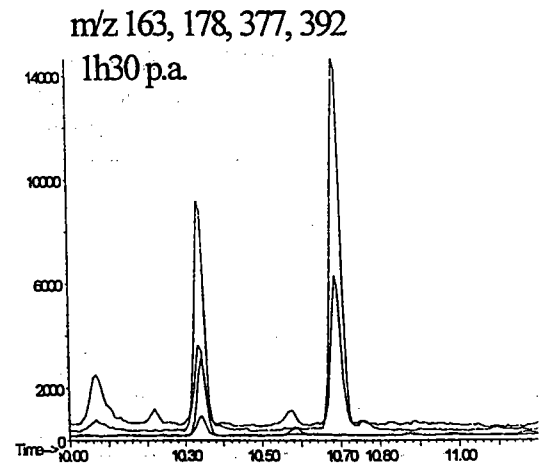
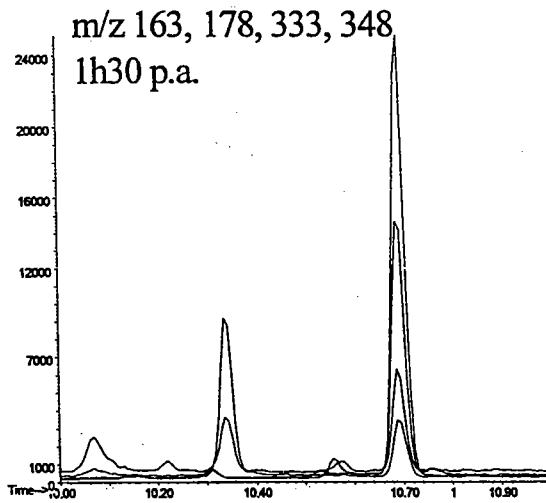
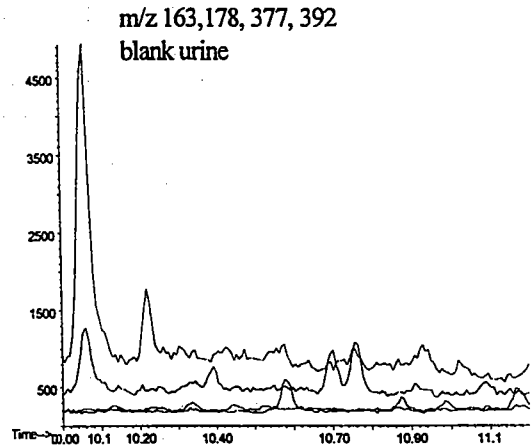


GC/MS (SIM mode) analysis of fenzolone reference urine.
(prepared according to full procedure).

FENOZOLONE

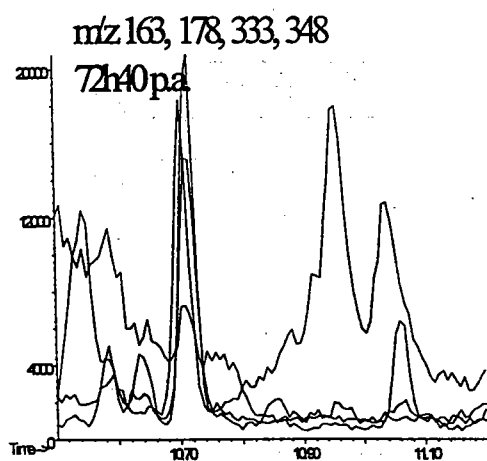
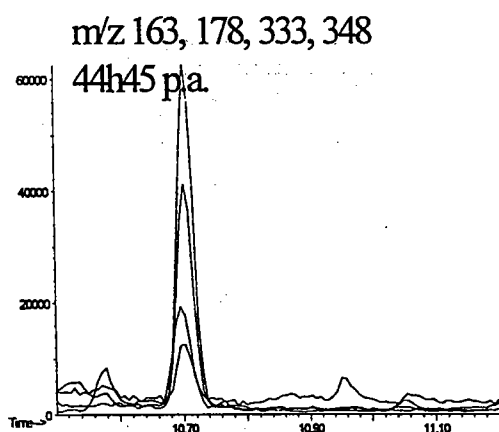
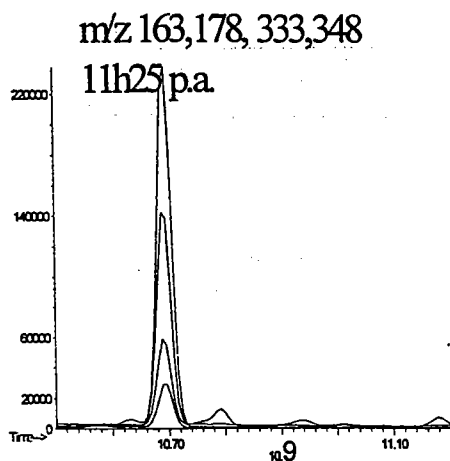


PEMOLINE

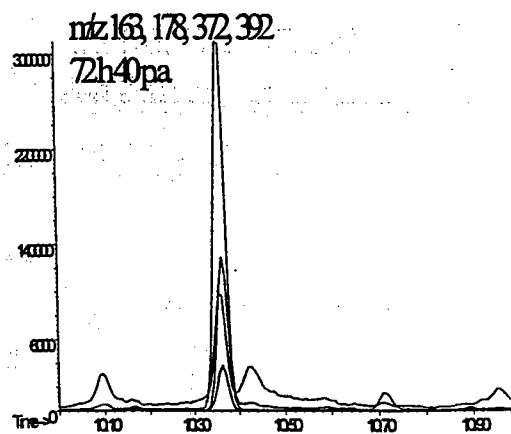
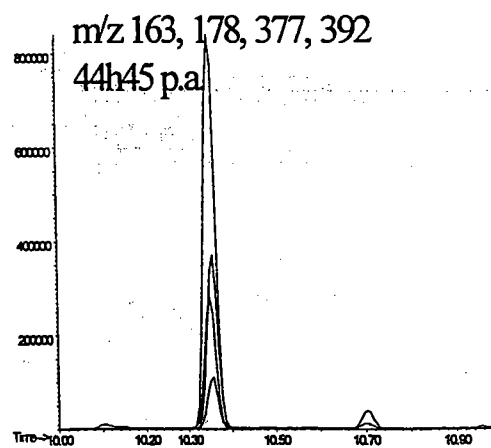
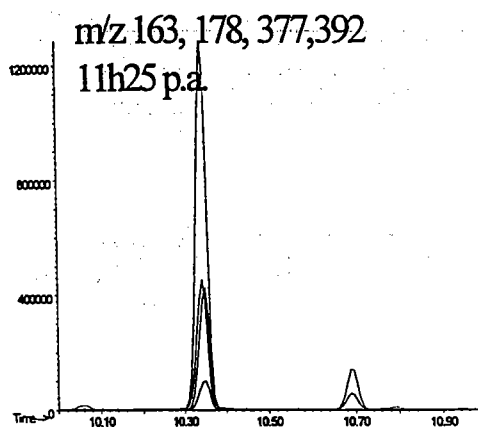


GC/MS (SIM mode) analysis of fenozolone reference urine samples prepared according to screening procedure (only FF; pH 9).

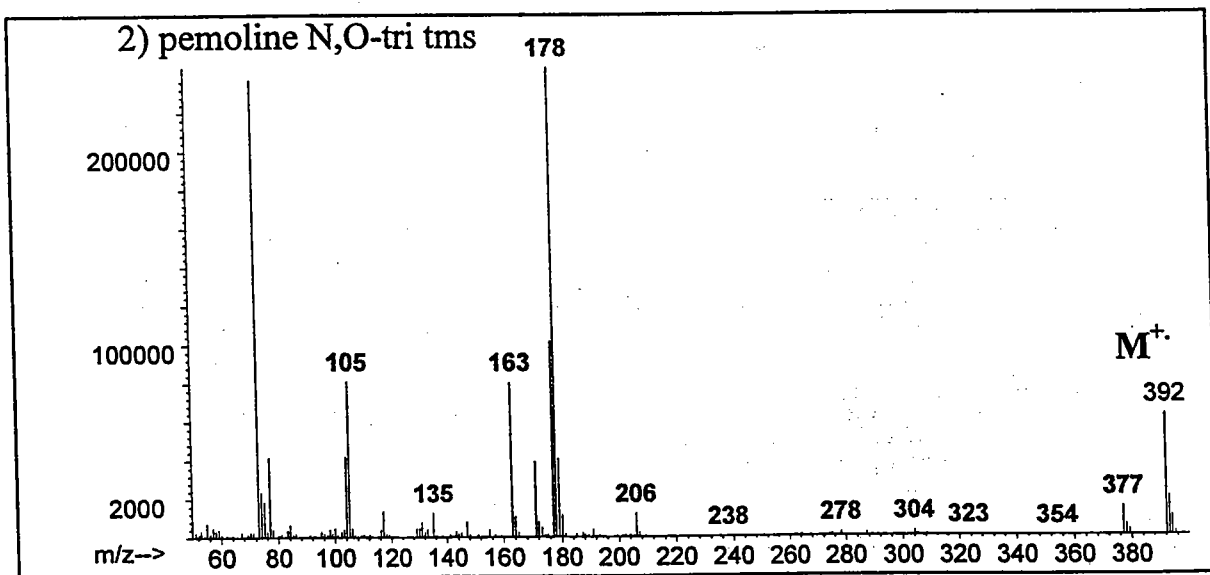
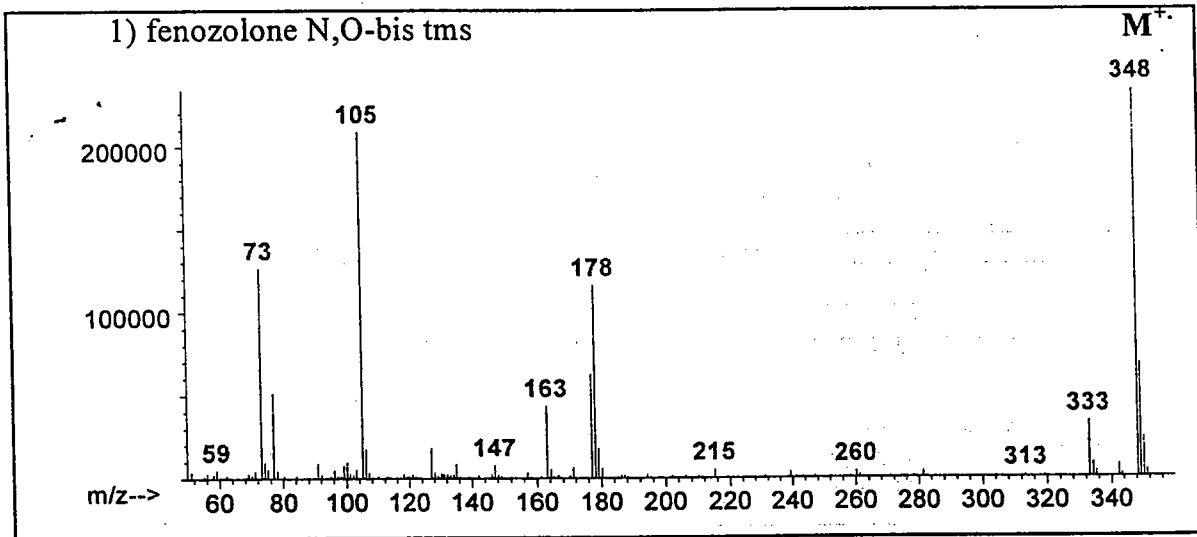
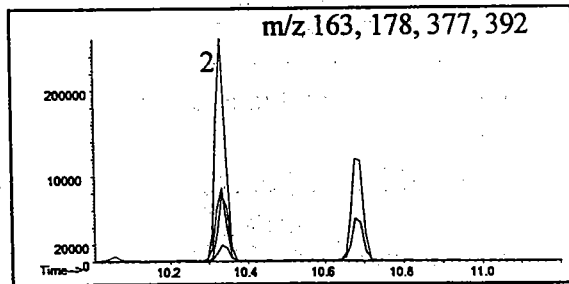
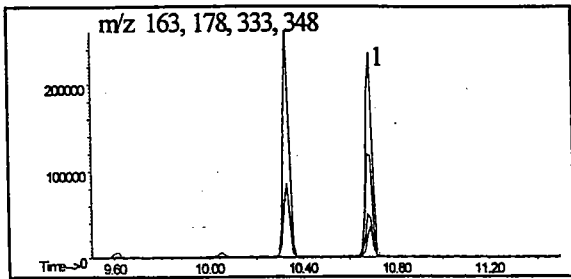
FENOZOLONE



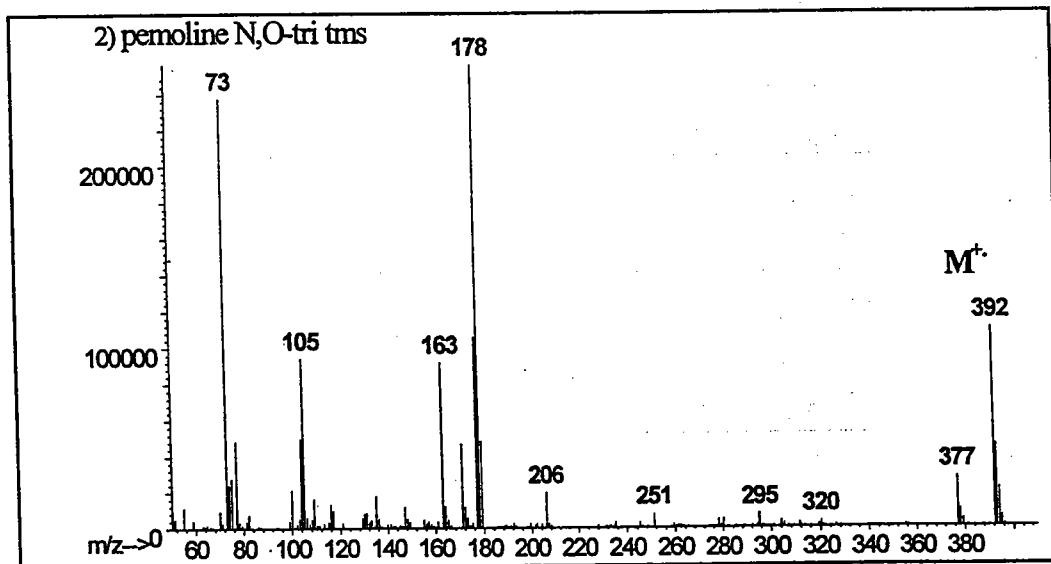
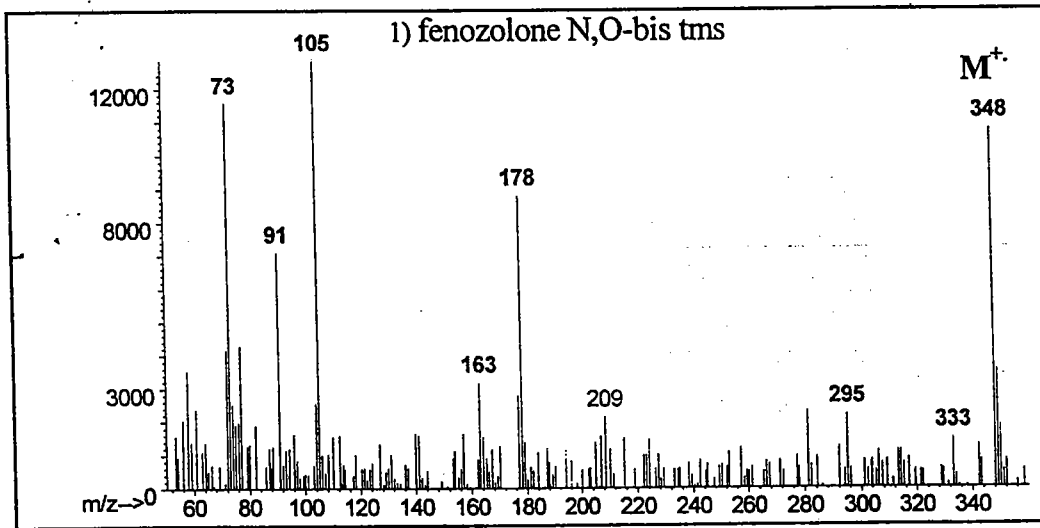
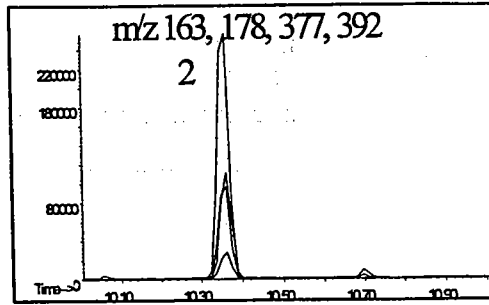
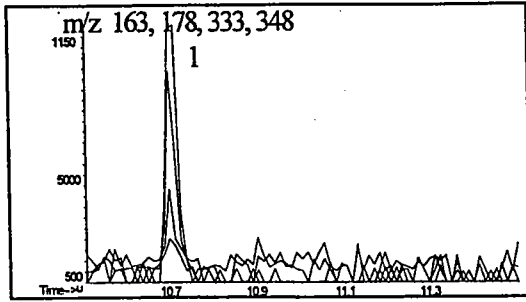
PEMOLINE



GC/MS (SIM mode) analysis of fenozolone reference urine samples prepared according to screening procedure (only FF, pH 9).



Mass spectra (full scan mode) of fenozolone reference urine sample 3h25 p.a. (prepared according to screening procedure (only FF, pH 9))



Mass spectra of fenozolone reference urine sample 44h45 p.a. (prepared according to confirmation procedure (only FF, pH9)

Ion ratios for the confirmation of fenozolone N,O-bis tms

Sample	105 (%)	163 (%)	178 (%)	333 (%)	348 (%)	Remarks
fenozolone std ¹	93.2 ±2.0	20.3± 0.5	63.3±0.9	13.1 (0)	100	
st3-7 25h30 ²	87.7 (5.9) ⁴	20.2 (0.5)	60.7 (4.1)	14.1 (7.6)	100	confirmed and full mass spectra
st3-11 44h45	86.2 (7.5)	19.3 (4.9)	61.8 (2.4)	13.7 (4.6)	100	confirmed and full mass spectra
st3-15 68h45	84.4 (9.4)	20.1 (10.0)	21.3 (18)	18 (37.4)	100	not confirmed
st3-16 72h40	90.3 (3.1)	20.8 (2.5)	82.4 (30.2)	14.2 (8.4)	100	not confirmed
st4-7 21h15 ³	94.2 (1.1)	19.1 (5.9)	60.6 (4.3)	13.1(0)	100	confirmed and full mass spectra
st4-9 30h00	96.1 (3.1)	19.4 (4.4)	60.7 (8.9)	14.9 (13.7)	100	confirmed
st4-11 36h50	91.0 (2.4)	20.8 (2.5)	64.2 (1.4)	13.9 (6.1)	100	confirmed
st4-12 45h00	86.1 (7.6)	19.9 (2)	63.4 (0.2)	13.9 (6.1)	100	confirmed
st4-13 51h20	86.6 (7.1)	18.8 (7.4)	65.8 (3.9)	13.7(4.6)	100	confirmed

¹ Authentic standard of fenozolone were analysed along with the reference urines. (two aliquots of 1 and 2 ng/µL injected, spiked in blank urine and prepared as described in confirmation procedure (FF, pH 9)

² Urine samples from first volunteer (#st3) who received 10mg Ordinator®. (prepared according to confirmation procedure (FF, pH 9)

³ Urine samples from second volunteer (#st4) who received 10mg Ordinator®. (Prepared according to confirmation procedure (FF, pH 9)

⁴ Variation (%)