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Analysis of Etozolin and Piretanide in Human Urine by Gas Chromatography/Mass Spectrometry

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Summary

Liquid-liquid extraction technique has used for treatment of urine samples in the analysis of etozolin and piretanide. The excretion kinetics of etozolin and piretanide have been studied by gas chromatography/mass spectrometry after administration of single theurapeutic dose by volunteers. A quantity of 65% piretanide and 5% of etozolin as metabolite have been determined in collected urine samples up to the 48th hour. Their mass spectra as well the spectrum of etozolin standard have been presented and discussed.

Introduction

The diuretics have different pharmacological properties and are classified in groups as carbonic anhydrase inhibitors, loop diuretics, thiazide type, potassium-sparing and uricosuric agents. Of all the groups, the loop diuretics are the most potent ones. Forth and Henschler (1) have established that furosemide, bumetanide and ethacrynic acid as diuretics increasing the urine flow by a factor of 30 compared with a normal flow. The same substances have been excreted unchanged to a high extent.
This paper investigates the excretion kinetic of etozolin and piretanide after their administration.

Experimental

The studies have been performed with urine samples from two volunteers after oral administration of a single therapeutic dose of 200 mg etozolin (Elkapin mite, Gödecke tablet, first volunteer) and 6 mg piretanide (Arelix, Cassella-Riedel tablet, 2nd volunteer). The urine samples have been collected up to the 48th hour. The quantitative determination of the excreted drugs in urine has been measured by gas chromatography/mass spectrometry (GC/MS) with selected ions monitoring (SIM) mode after liquid-liquid extraction (LLE) and methylation.

The experiments were performed using 3 ml of urine sample with ethacrynic acid as internal standard with concentration 10.4μg/ml. The suitable pH value for extraction of the substances was chosen after series of experiments with piretanide standard and urine samples of etozolin. The pH value of 5 was achieved using KH_2PO_4, pH 7 using KH_2PO_4/Na_2HPO_4, pH 9 and 11 using 5M NaOH. The next chemical treatment of the urine samples follows the routine procedure of our laboratory for screening of diuretics (Fig. 1).

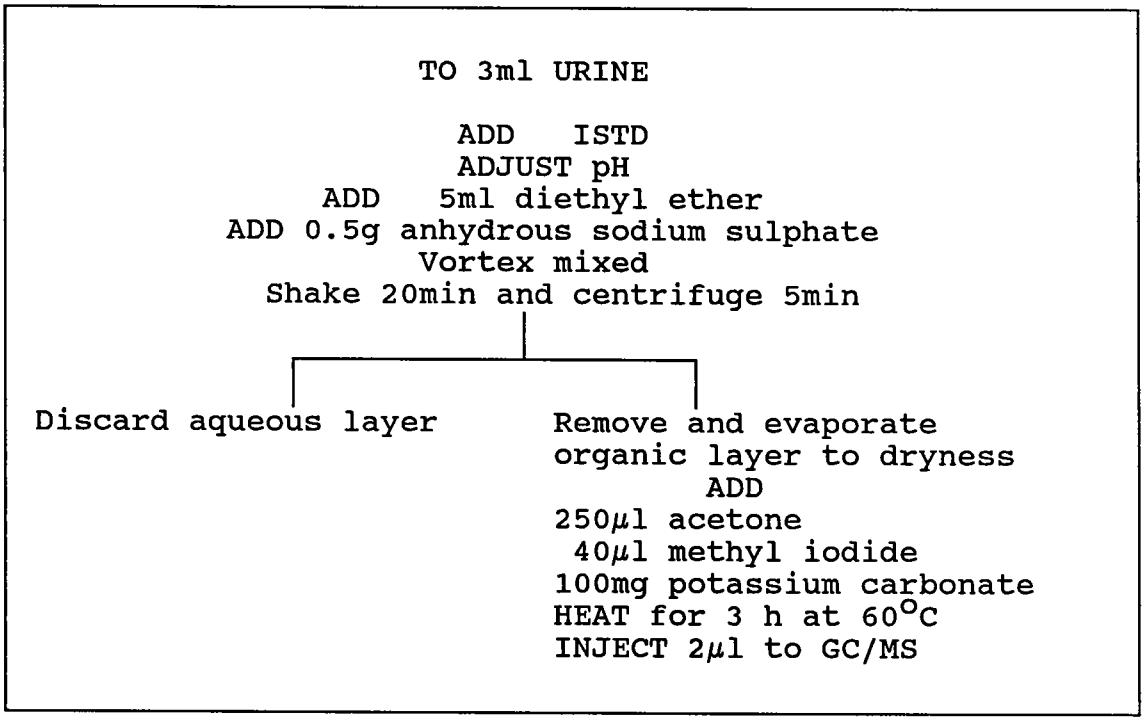

Fig.1: Extraction and derivatization of diuretics

A Hewlett Packard GC/MS 5995-direct system was used and the GC/MS conditions are presented on Fig.2. The mass spectrometer was operated in the electron impact (EI) mode.

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ANALYTICAL INSTRUMENT: HP 5995C GC/MS System

CHROMATOGRAPHIC CONDITIONS:
COLUMN : Fused silica capillary, cross linked
         5% phenylmethyl silicone
         length - 18, film thickness - 0.32μm,
         internal diameter - 0.2mm;
INJECTOR TEMPERATURE       : 280°C;
TRANSFER LINE TEMPERATURE  : 280°C;
MASS ANALYSER TEMPERATURE  : 220°C;
ION SOURCE TEMPERATURE     : 250°C;
INJECTION MODE             : Splitless;
CARRIER GAS                : helium at 0.93ml/min;
TEMPERATURE PROGRAM        : initial - 190°C
                             rate    -  30°C/min
                             final   - 300°C
                             total time - 12min;
MASS SPECTROMETER: SCAN mode, range m/z 35-450
                   SIM mode.
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Fig.2: Gas chromatographic/mass spectrometric conditions for analysis of diuretics

Results and discussions

Dr.Park et al. (4) have established that LLE method could be applied for the analysis and confirmation of diuretic agents with suitable pH value. Having in mind the published results we have used LLE. At the beginning we studied the influence of the pH value on the percent extraction of the investigated substances by standard solution of piretanide with concentration 60μg/ml and urine samples of etozolin. The obtained results pointed out that pH 5 was suitable for extraction of piretanide and etozolin metabolite.

Excretion study etozolin

Table 1 shows the obtained results of the excretion kinetic of etozolin after administration of a single therapeutic dose (200 mg). The urine flow is increased from the second to the seventh hour and has a maximum between the 3rd and 4th hour after administration of the drug. The diuretic effect of etozolin is lower than other loop diuretics.

The etozolin is an ethyl ester of [3-Methyl-4-oxo-5-(piperidinyl)-2-thiazolidinylidene]acetic acid. Gladigou (2) and Hodenburg (3) discuss the acid named ozotolin as a main metabolite of etozolin. We could not find it in the collected urine samples (at pH 5 and 1). We have not detected the unchanged etozolin in the investigated samples which agrees with the conclusion of these authors.

In all studied samples we have found only one metabolite of etozolin. Its relative retention time (RRT) is 1.41 while RRT of etozolin standard is 1.08 (using GC conditions). Table 1 shows excretion kinetic of this metabolite in collected urine samples. In all urine samples studied, approximately 5% of the administrated dose of etozolin was detected as metabolite. The results show that a positive screening till the 48th hour after administration of etozolin is possible. The excretion curve is shown on Fig.3.

Table 1: Excretion of etozolin metabolite after administration of a single oral dose (200 mg)

Time h	Urine volume ml	Urine flow ml/min	Etozolin metabolite µg/ml	Total excreted Etozolin Met. mg
1^{25}	50	0.6	2.15	0.11
2^{30}	120	1.8	12.98	1.56
3_{40}	80	2.7	28.10	2.25
4^{55}	290	2.9	12.65	3.67
7	160	0.8	8.43	1.35
12	30	0.1	6.94	0.21
24	120	0.2	0.47	0.06
36	40	0.1	0.21	0.01
46	100	0.2	0.47	0.05
48	40	0.3	(12.50ng/ml)	(0.51µg)

Excretion study piretanide

Table 2 shows the obtained results for the excretion kinetic of piretanide after administration of a single therapeutic dose (6 mg). The urine flow is increased from the first to the third hour and has a maximum of urine flow about the second hour after administration of the drug. The diuretic effect is strong and comparable with those of furosemide and bumetanide (5).

The piretanide is a 3-(Aminosulfonyl)-4-phenoxy-5-(1-pyrrolidinyl) benzoic acid. We have discovered unchanged piretanide in the collected urine samples. Its total excretion quantity is 65% of the administered dose. The concentration of the piretanide is rather low (about 5 ng/ml) after the 20th hour from administration. The excretion curve is presented on Fig.4. The methylated piretanide is detected with RRT=2.16 using GC condition given on table 2.

Table 2: Excretion study of piretanide after administration of a single oral dose (6 mg)

Time h	Urine volume ml	Urine flow ml/min	Piretanide unchanged µg/ml	Total Pireta-nide unchanged µg	Excreted Piretanide %
1	600	10.0	1.80	1077.20	17.95
2	700	11.7	2.63	1842.90	70.72
3	425	7.1	1.43	607.40	10.12
7	225	0.8	1.62	364.70	6.08
11	50	0.2	0.11	5.71	0.10
			ng/ml		
14^{45}	40	0.2	60.9	2.44	0.04
19^{45}	150	0.5	15.1	2.27	0.04
26^{30}	150	0.4	2.7	0.40	0.01
30^{30}	175	0.7	2.8	0.50	0.01
36	200	0.6	2.8	0.56	0.01
48	100	0.2	1.6	0.18	-

Gas chromatographic / mass spectrometric confirmation

The mass spectrum of the etozolin (pill) is shown on Fig.5 (A). Its molecular ion (m/z 284) is clearly visible. Two other intensive ions are obvious. The predominant one is m/z 84 formed by the piperidinyl radical. The ion of m/z 211 is obtained by elimination of a carboxyethyl group from the molecular ion M^+-73 (M^+-$OOCCH_2CH_3$). Loss of the HS produces the ion of m/z 251 (M^+-33).

The mass spectrum of detected etozolin metabolite from the collected urine samples given on Fig.5 (B) shows an intensive molecular ion of m/z 298 and the other three ions of m/z 82, m/z 169 and m/z 241 (M^+ - O=C-N-CH_3, M^+-57). The ion of m/z 269 is the fragment after loss of ethyl group (M^+-C_2H_5). The elimination of the carboxyethyl group forms an ion of m/z 225 with a low intensity. The ion of m/z 169 is formed from M^+-73-57 (M^+-$OOCCH_2CH_3$,-O=C-N-CH_3).

The mass spectrum of thrimethyl piretanide is shown on Fig.5 (C). The molecular ion of m/z 404 is clearly visible. The basic ion is m/z 295 formed by elimination of sulphonamide cation fragmented from the molecular ion (M^+-SO_2-N(CH_3)$_2$). In the spectrum there is a small ion of m/z 373, formed by M^+-31 (M^+-OCH_3). The other ones are very small as well.

Literature

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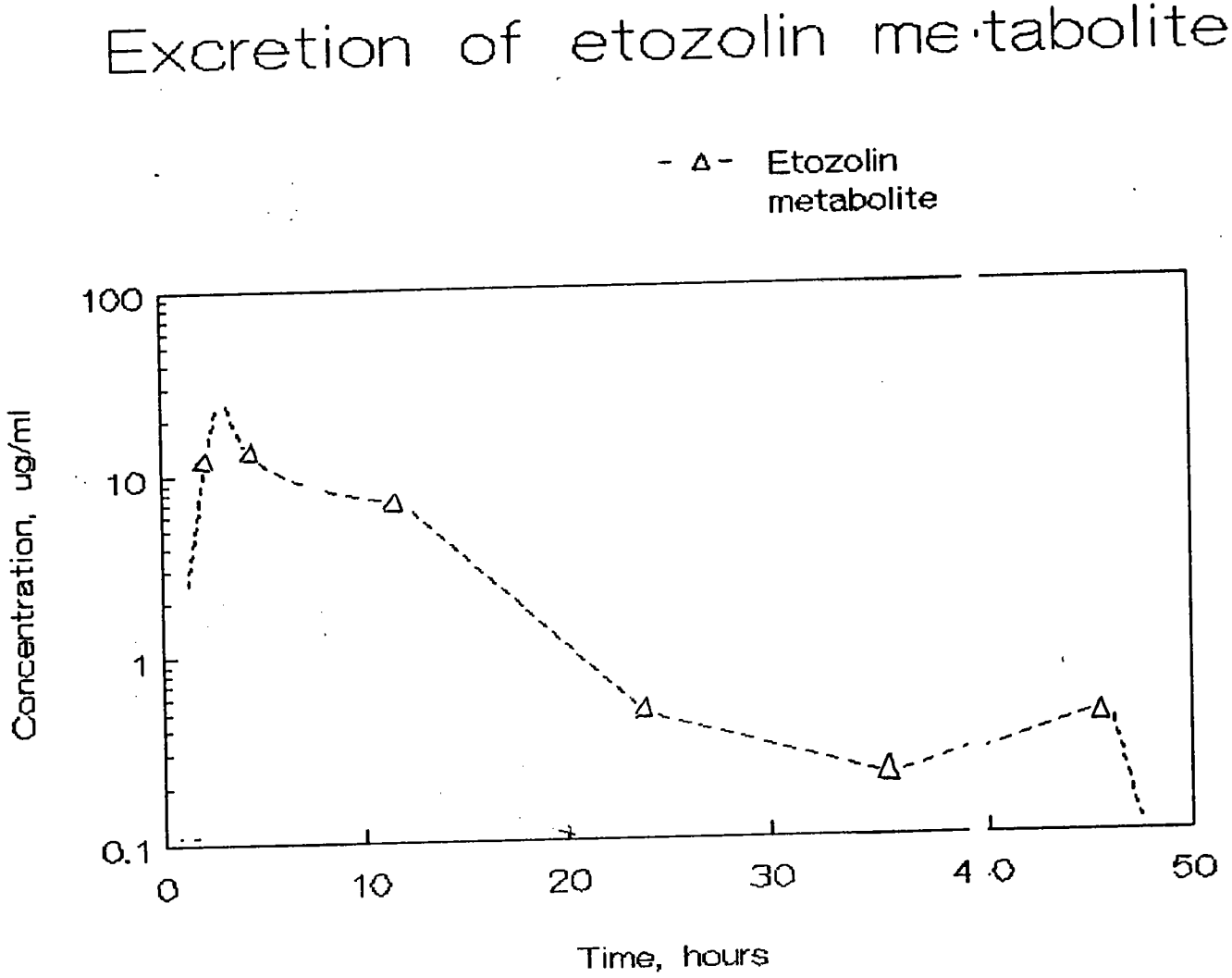

Excretion of etozolin metabolite
- Δ - Etozolin metabolite
Concentration, ug/ml
100
10
1
0.1
0
10
20
30
40
50
Time, hours

Fig.3.

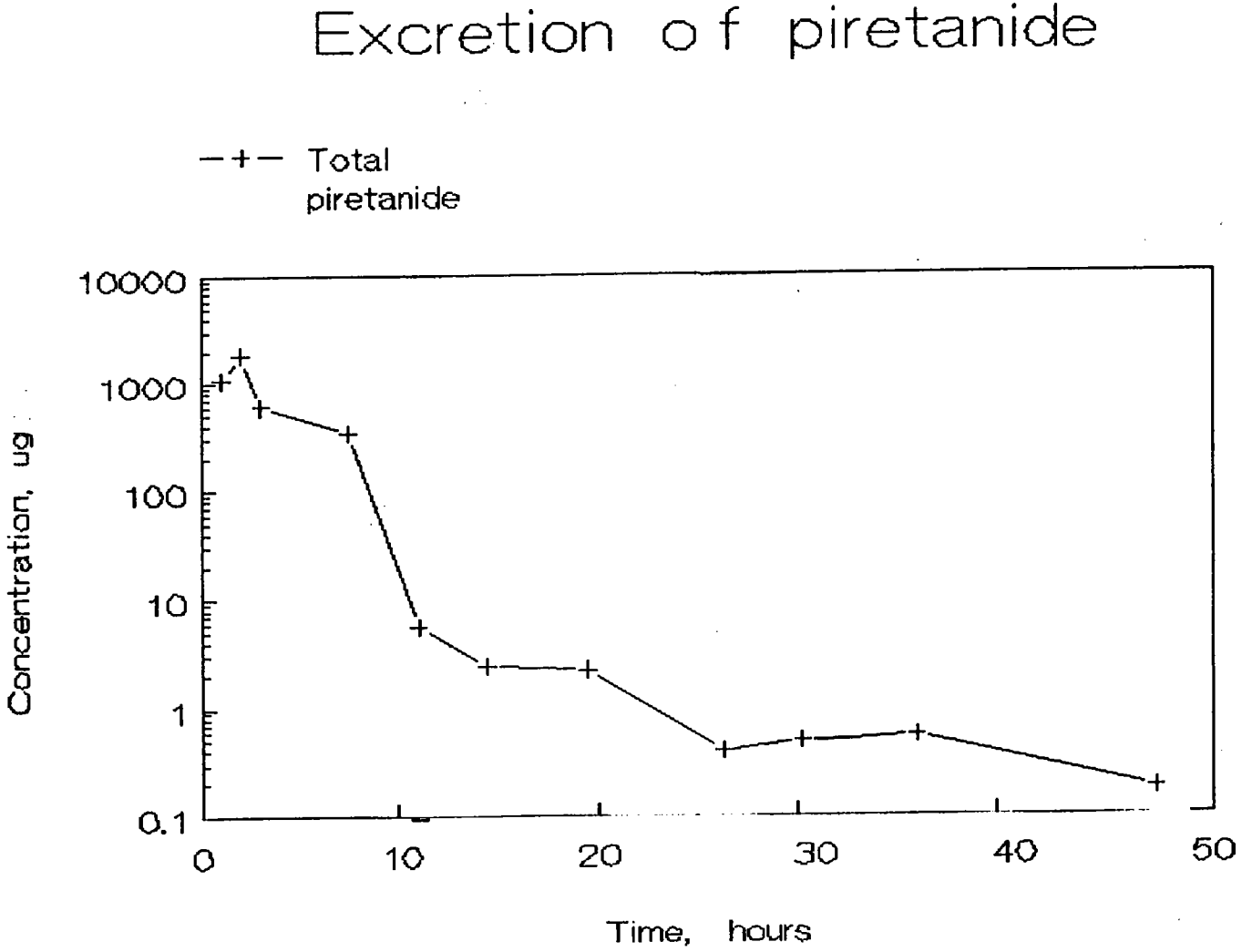
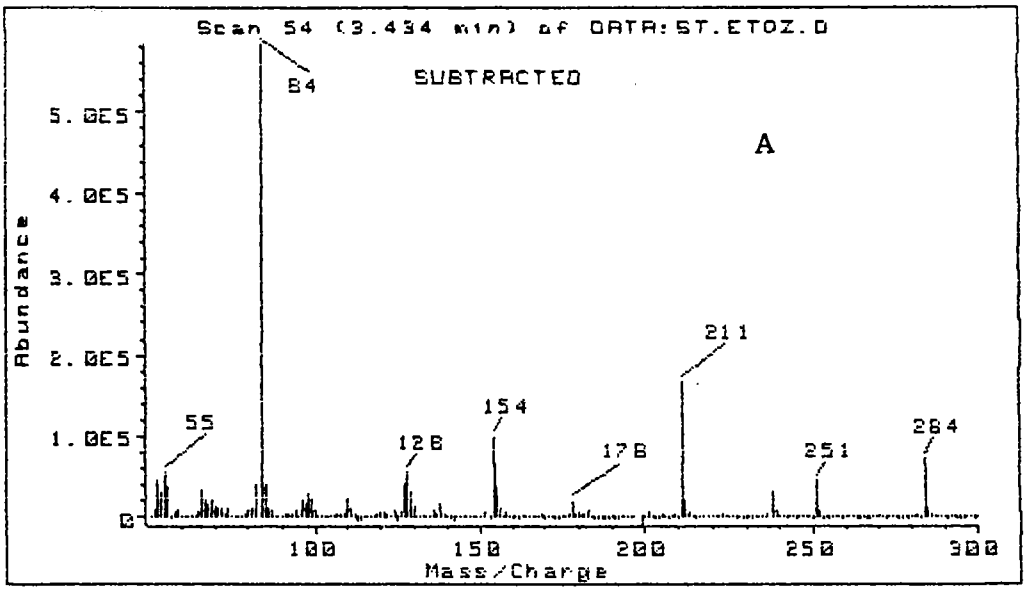
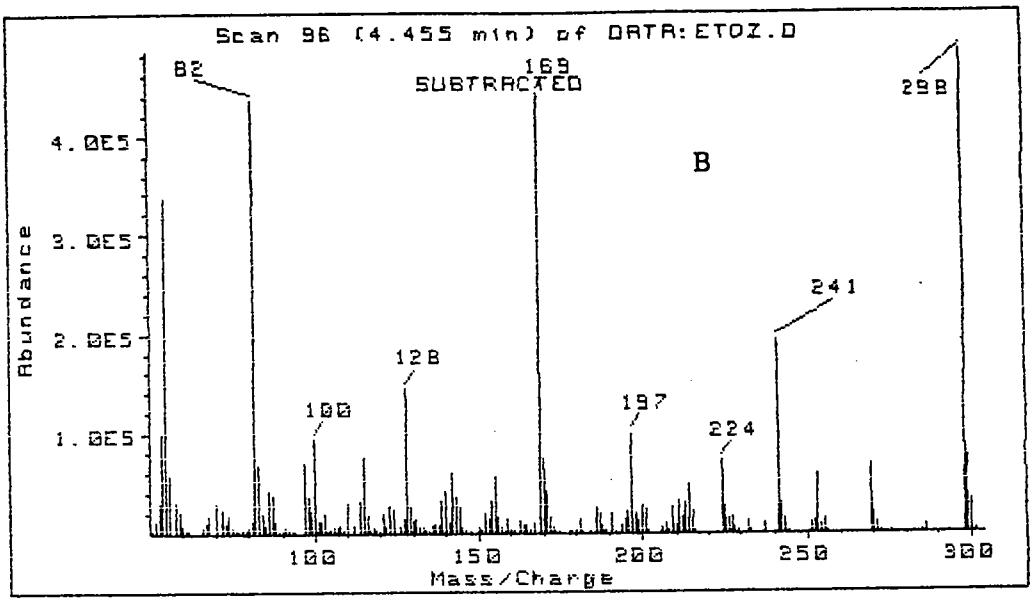

Excretion of piretanide
Total piretanide
10000
1000
100
10
1
0.1
Concentration, ug
0
10
20
30
40
50
Time, hours

Fig. 4.





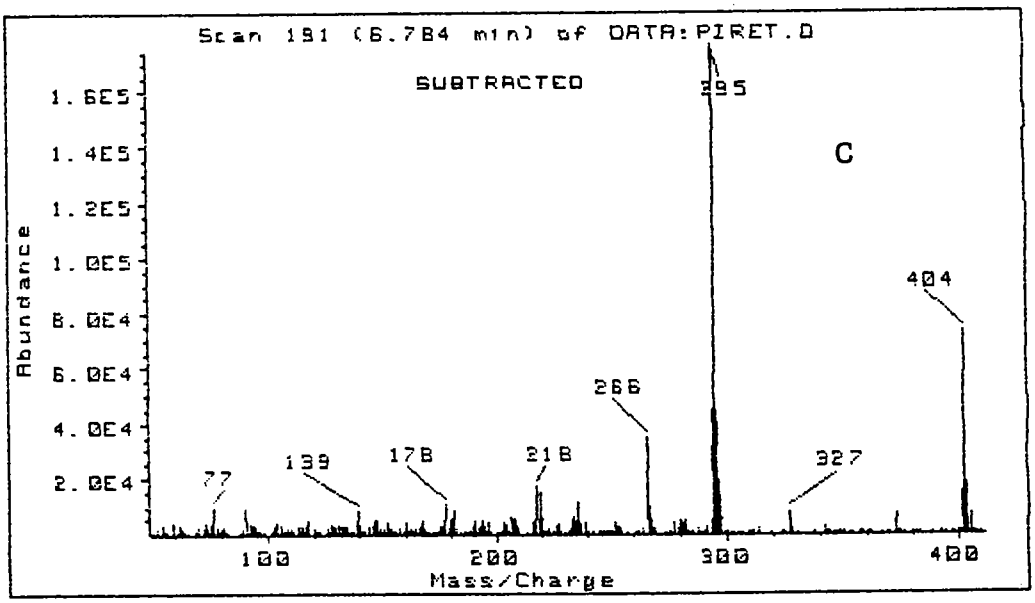


Fig.5 Mass spectrum of : A. Unchanged Etozolin

B. Metabolite of Etozolin

C. Three methyl derivative of Piretanide