

Reprint from

RECENT ADVANCES
IN DOPING ANALYSIS
(6)

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

Sport und Buch Strauß, Köln, 1999

V.A. SEMENOV, S.L. BOLOTOV, V.F. SIZOI:
New Stimulant of Russia – Carphedon
In: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck-Engelke (eds.) Recent advances in
doping analysis (6). Sport und Buch Strauß, Köln, (1999) 337-348

Semenov V.A., Bolotov S.L., Sizoi V.F.

New stimulant of Russia - CARPHEDONE

Moscow Antidoping Centre. 107005 Moscow, Elizavetinskii pr, 10, ADC.

Abstract

The metabolism of carphedone [(2-Oxo-4-phenylpyrrolidine)acetamide] and dynamics of the metabolites excretion have been investigated. After administration of single dose of the drug the carphedone itself and two metabolites in urine were recognised. It was found that the hydroxylation takes place during metabolism. Hydroxy group is added as to the phenyl radical and heterocyclic ring. During derivatization the dehydration of the molecule is occurred forming corresponding acetonitriles.

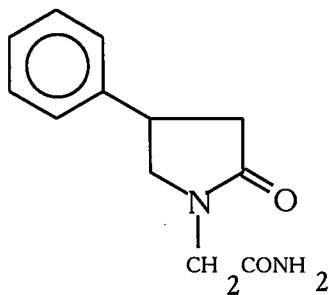
The mass spectra of the carphedone and metabolites are discussed.

Introduction

Carphedone (phenotropil) (I) was developed in Russia at the beginning of 1990's. It is phenyl derivative of nootropil. Pharmacological study showed the increasing of the physical activity and resistance to the cold, besides adaptogenic effect after carphedone administration.

At the end of 1997 carphedone was included into the list of the banned substances by the IOC Medical Commission. The aim of this work is the investigation of the carphedone metabolism, excretion and detection ability by existing screening procedures. For this purposes Hewlett Packard instruments 5890 with NPD, HPLC 1090, MSD 5971 and 5972 were used.

Results and discussion



I

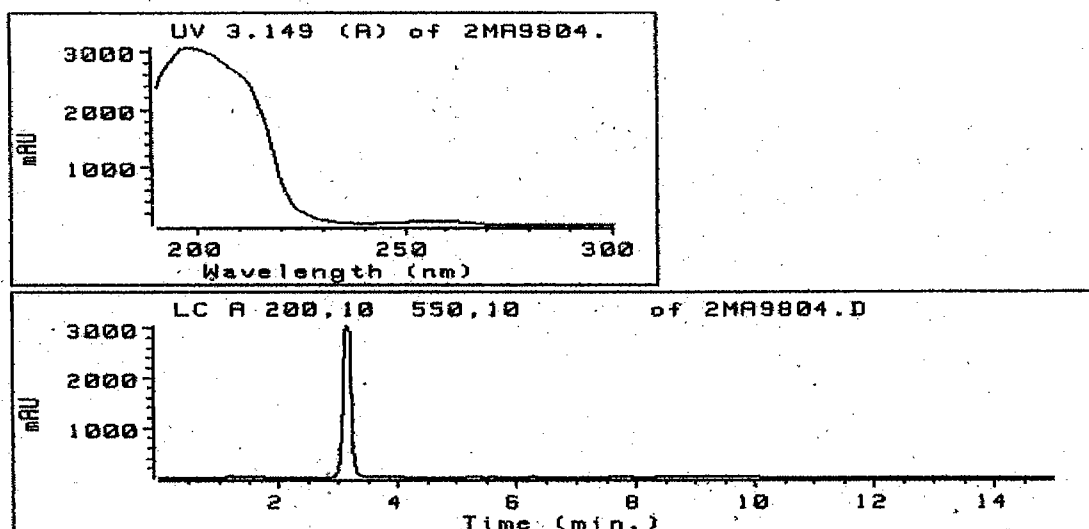


Fig.1 Chromatogram of the carphedone standard.

Then the electron impact mass spectrum of the native carphedone was obtained. It is shown on the Fig.2 with the fragmentation scheme. The ions with the m/z 201, 91, 77 and 44, has the structure correspondingly $M^+ - NH_3$, $PhCH_2^+$, Ph^+ и $CONH_2^+$. It have to be mentioned that the main fragmentation paths remain the same for the N-trifluoroacetyl (Fig.3), N-trimethylsilyl (Fig.4), N-tert-butyl-dimethylsilyl (Fig. 5) derivatives obtained. Mass spectra of the all studied derivatives (except only silyl derivatives) are characterised by the quit intensive molecular ion. Ion at m/z 201 is produced due to the amide cleavage with simultaneous hydrogen migration. It is specific only for nonderivatized carphedone and its trifluoroacetate. The silyl derivatives are characterised by the cleavage of CH_2-CO bond in side chain resulting in ions at m/z 175 and 174. Ions at m/z 104, 117 and 145 are the result of

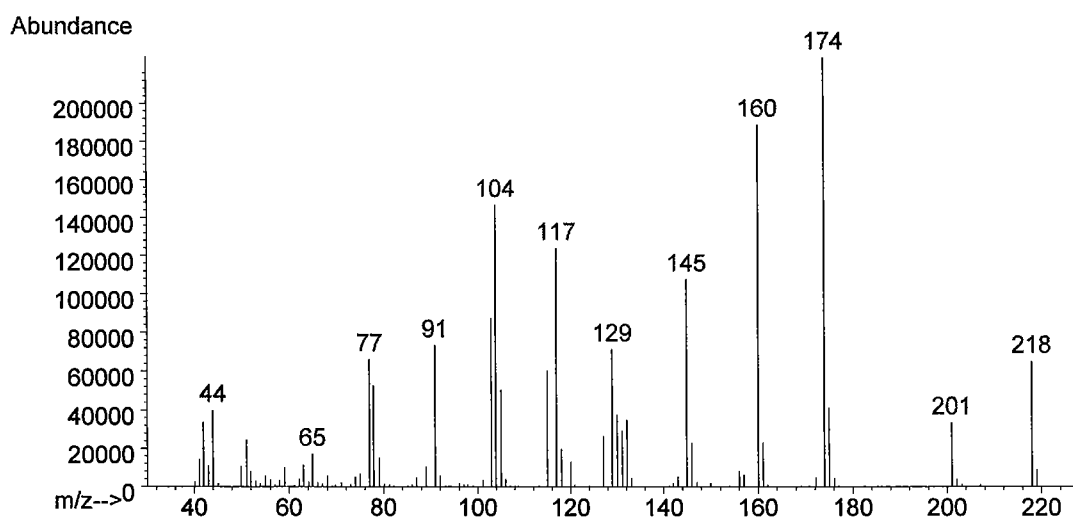
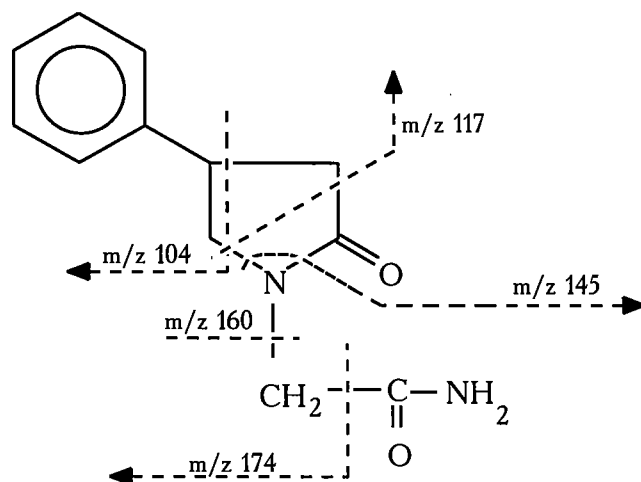


Fig.2 Mass spectrum of carphedone.

break of the bonds in pyrrolidine cycle.

Trimethylsilylation of carphedone gives as N-trimethylsilyl derivative (Fig.4) and dehydrated derivative with the mass spectrum shown on the Fig.6. It was found that derivatisation by the enol-MSTFA especially increases its content. This process is quite known in so called "wet" organic chemistry; - acylamides easily give corresponding nitriles with the presence of water accepting reagents.

Metabolism of carphedone was studied after oral administration of the single dose (50 mg) by the volunteer. In conditions of procedure I a very intensive peak with the mass-spectrum of the native carfedone could be seen (Fig. 2). Any additional metabolites were not found in this procedure. For the correct identification in screening procedure I carfedone presents a certain difficulties. Carfedone usually is administrated in a high doses and gives a big overload peak

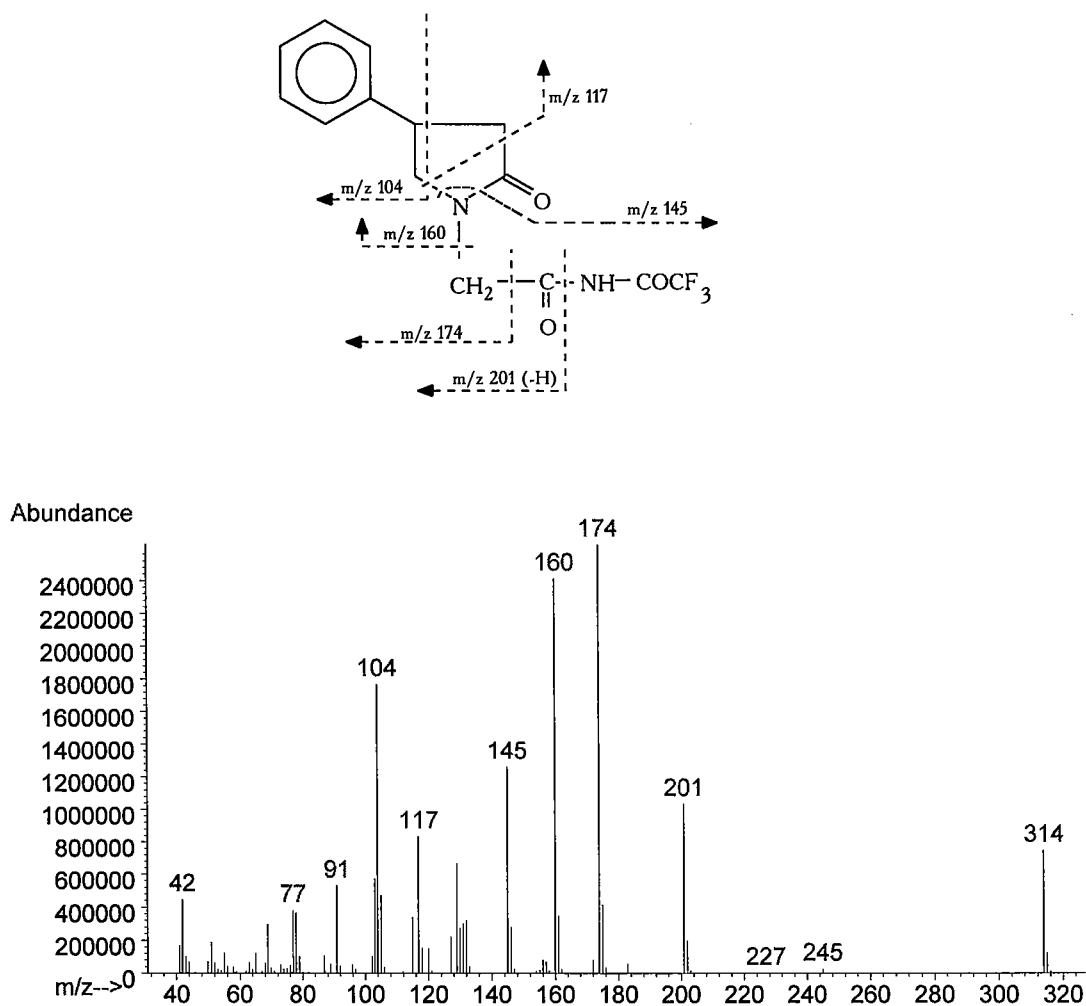


Fig.3 Mass spectrum of N-trifluoroacetyl-carphedone ($M^+ = 314$)

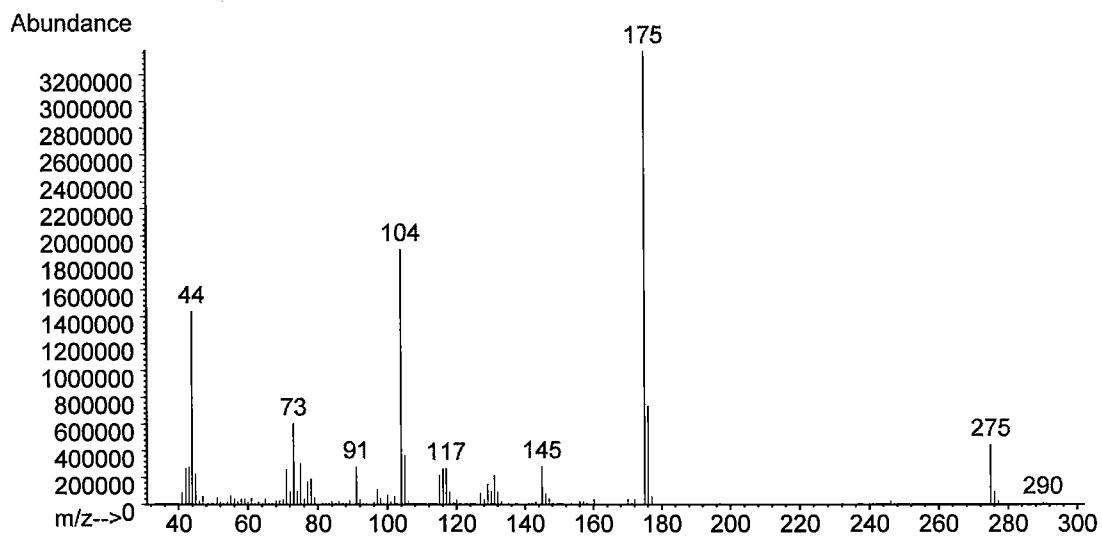
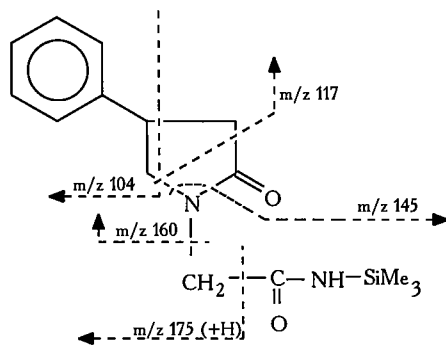


Fig. 4 Mass spectrum of N-trimethylsilyl-carphedone ($M^+ = 290$)

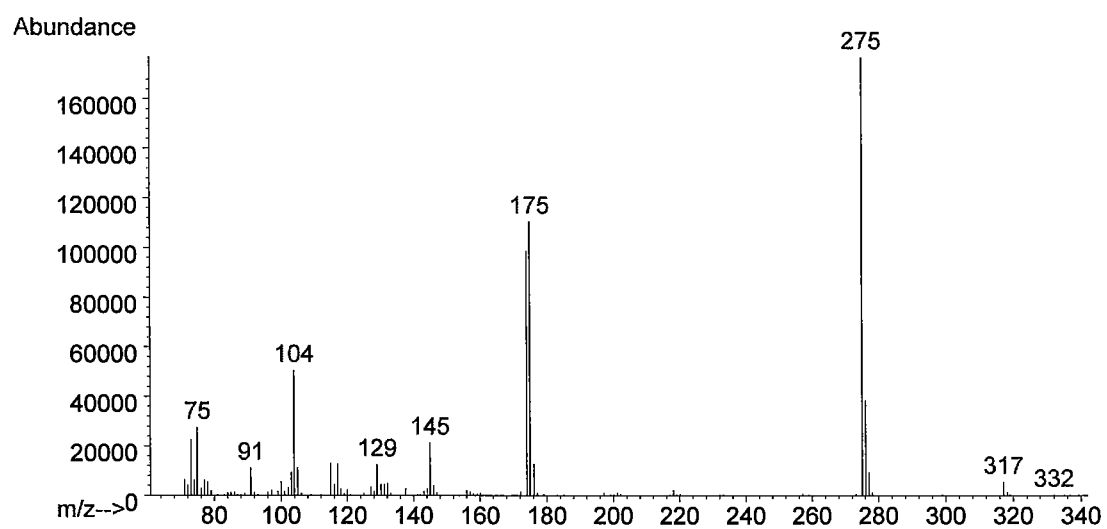
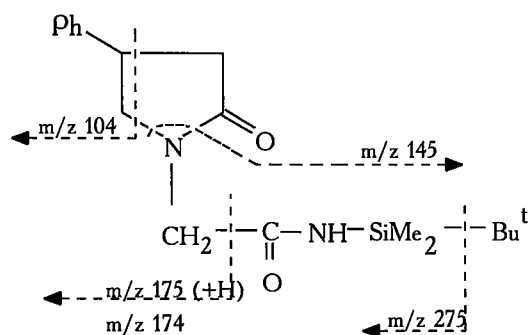


Fig.5 Mass spectrum of N-tert-butyl-4-phenyl-1-(dimethylsilyl)carphedone ($M^+ = 332$)

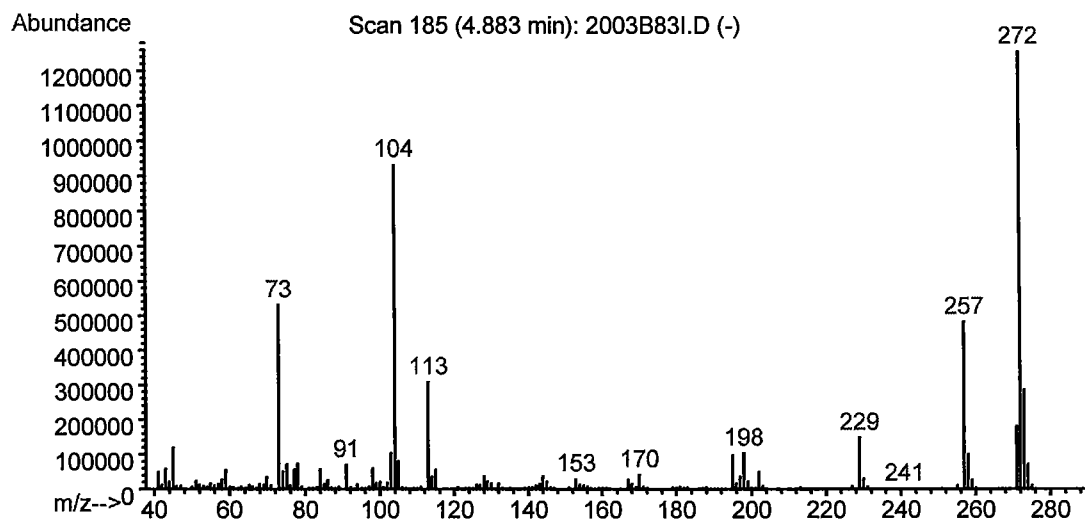
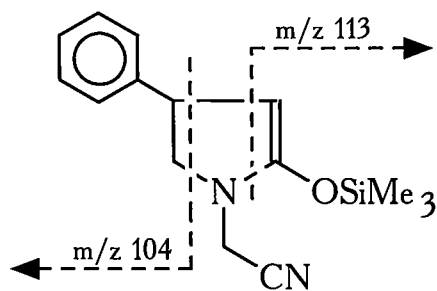


Fig.6 Mass spectrum of 2-oxo-4-phenylpyrrolidineacetonitrile, enol-O-TMS ($M^+ = 272$)

with the sharp essentially shifted from the regular relative retention (Fig. 7). But from the other hand this specific shape itself could help in carfedone determination by the operator.

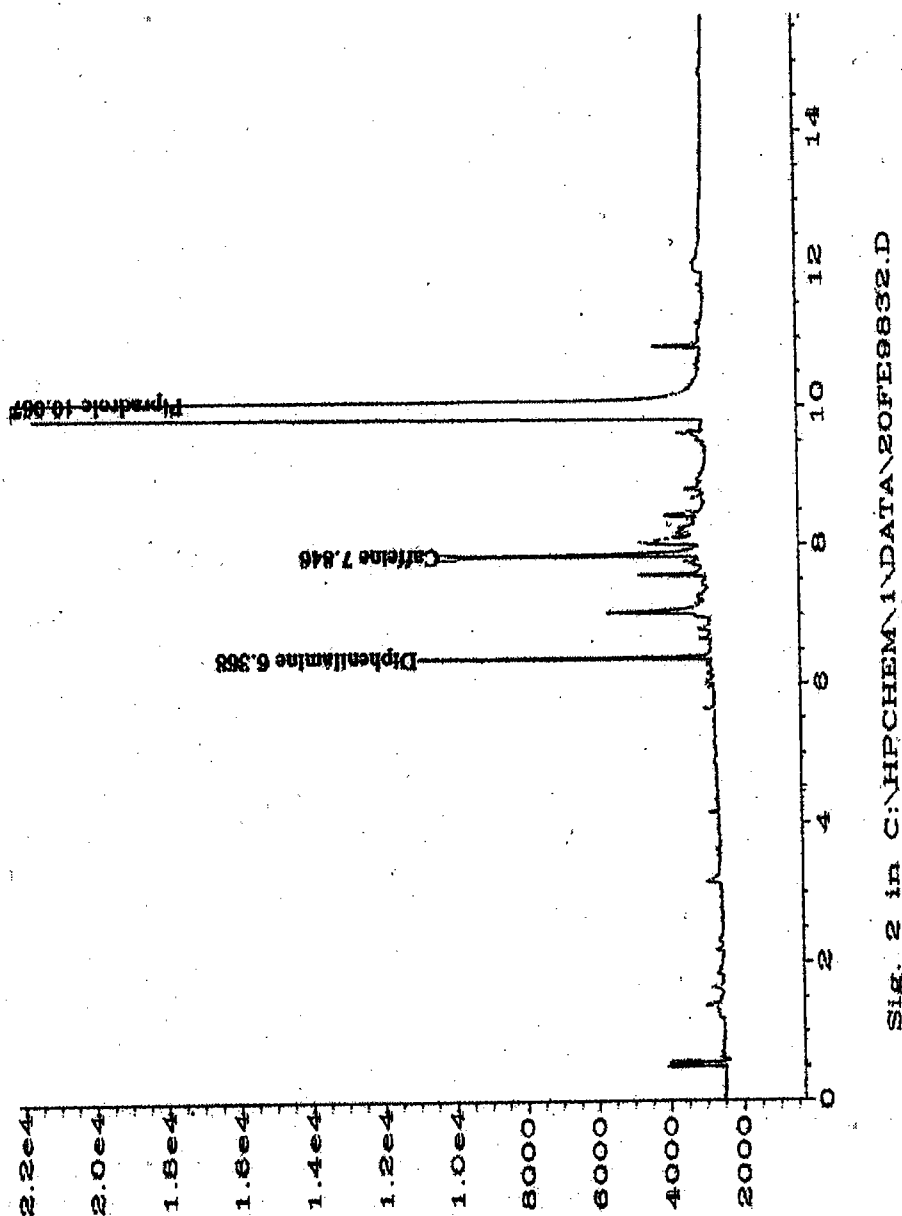


Fig.7 Screening procedure I.

After following trimethylsilylation of the dry residue of the same sample by the MSTFA with the presence of the ammonium iodide the mass spectrum corresponding to the listed on the Fig.6 was obtained. So dehydration to the corresponding nitrile takes place for the urine sample as far as for carfedone standard as the result of this derivatisation type.

Then the urine sample was prepared following procedure IV (enzyme hydrolysis and

trimethylsilylation). In this case besides intensive carphedone peak (Fig. 8) several weak signals of the hydroxylated metabolites were observed. Mass spectra of the biggest two are shown on Fig.9 and Fig.10.

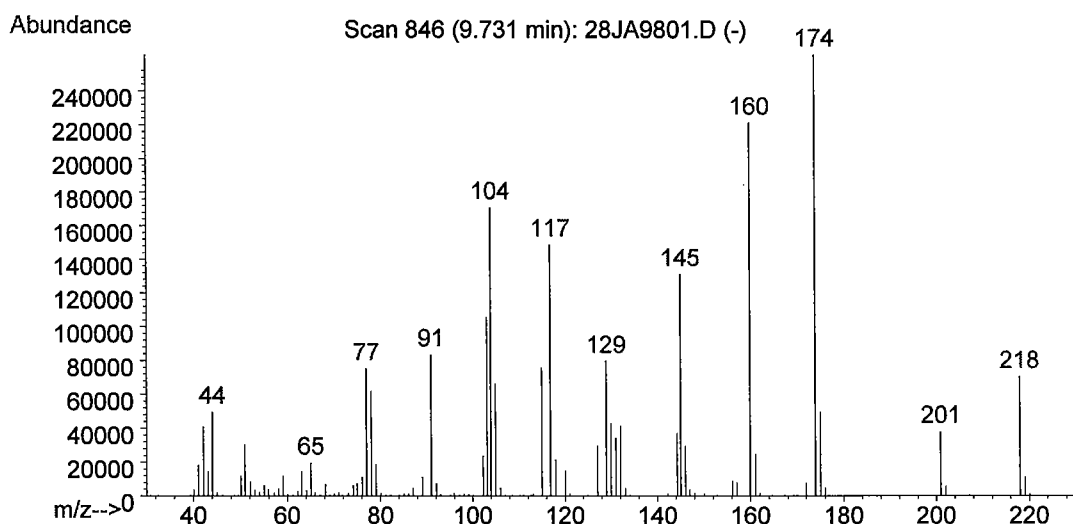
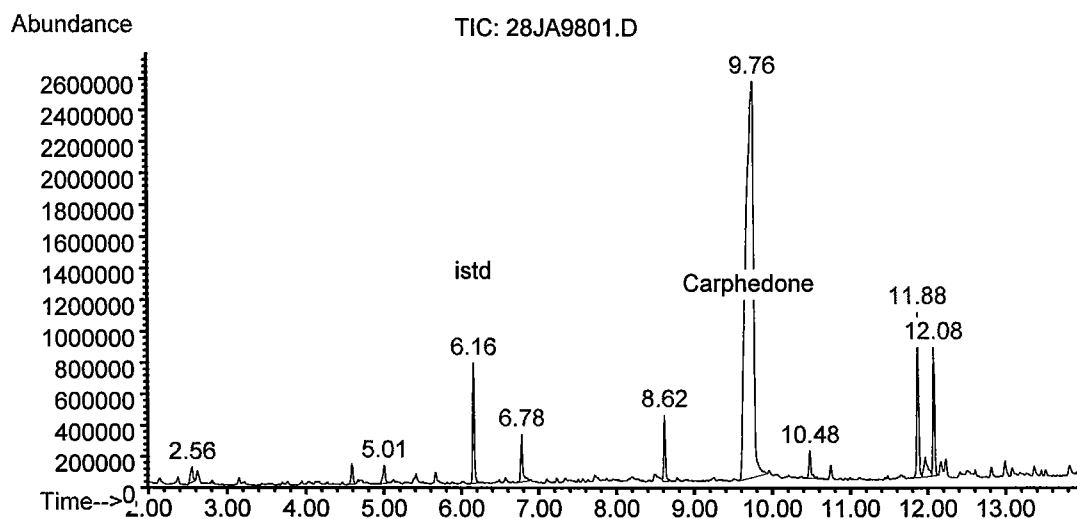


Fig.8 Total Ion Current and mass spectrum of compound at RT 9.76 min

Metabolite **II** is the hydroxylated carphedone in position three of pyrrolidine cycle. Metabolite **III** contains hydroxy group in aromatic ring. This conclusion is based on the presence of the ion m/z 202 $[\text{Me}_3\text{SiO}-\text{CH}=\text{CH}-\text{OSiMe}_3]^+$ containing C2 and C3 atoms of the pyrrolidine ring and the ion m/z 104 $[\text{PhCH}=\text{CH}_2]^+$ belonged to the nonsubstituted carphedone.

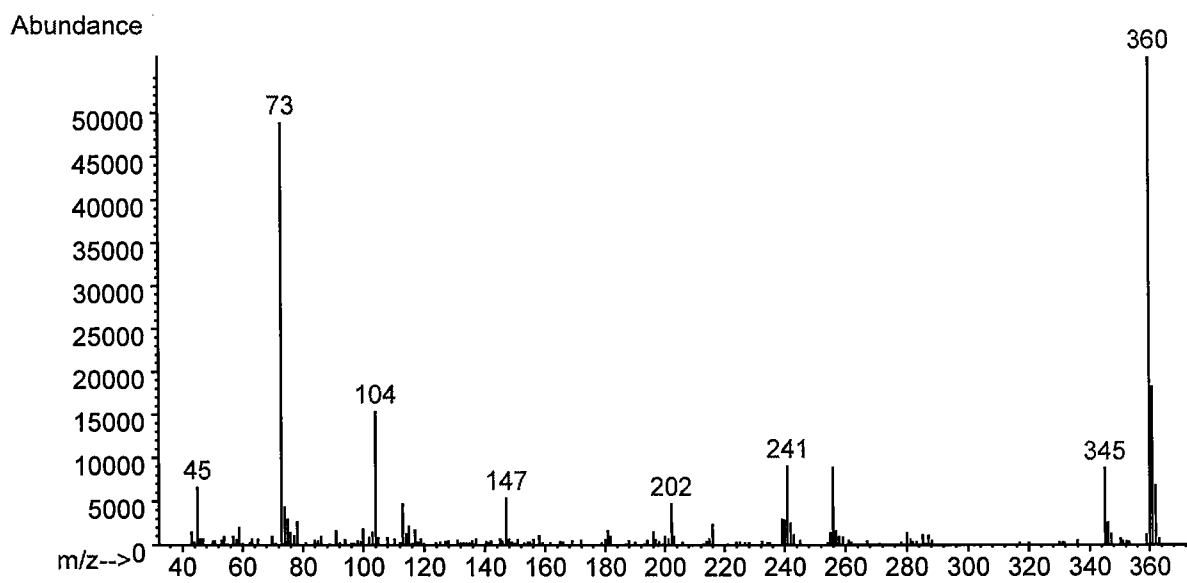
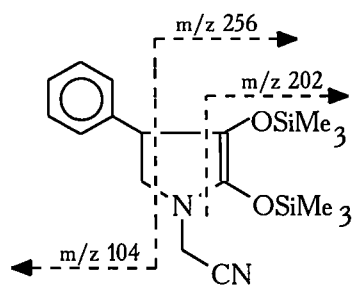


Fig.9 Mass spectrum of dehydrated 3-hydroxycarphedone (II), enol-bis-TMS ($M^+ = 360$)

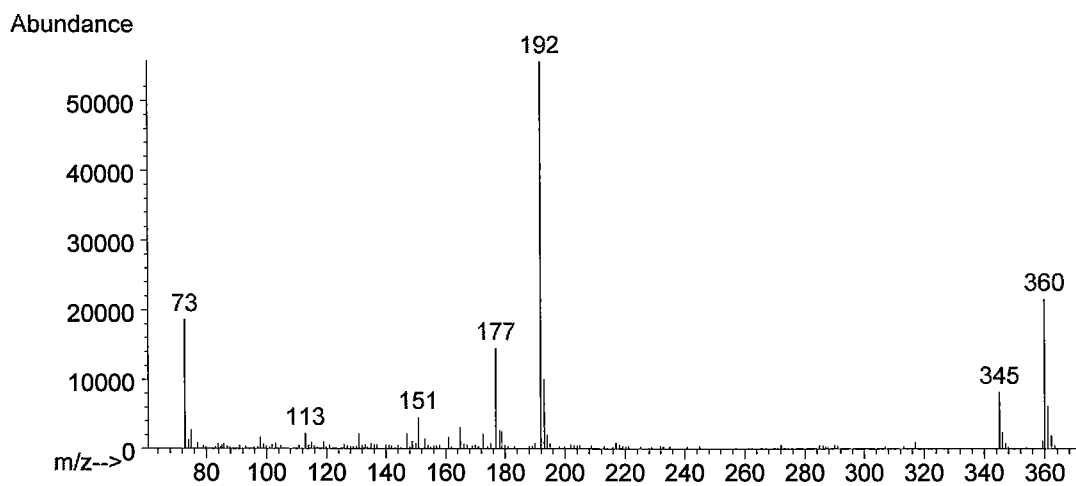
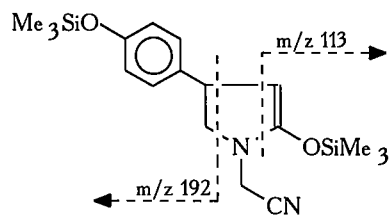
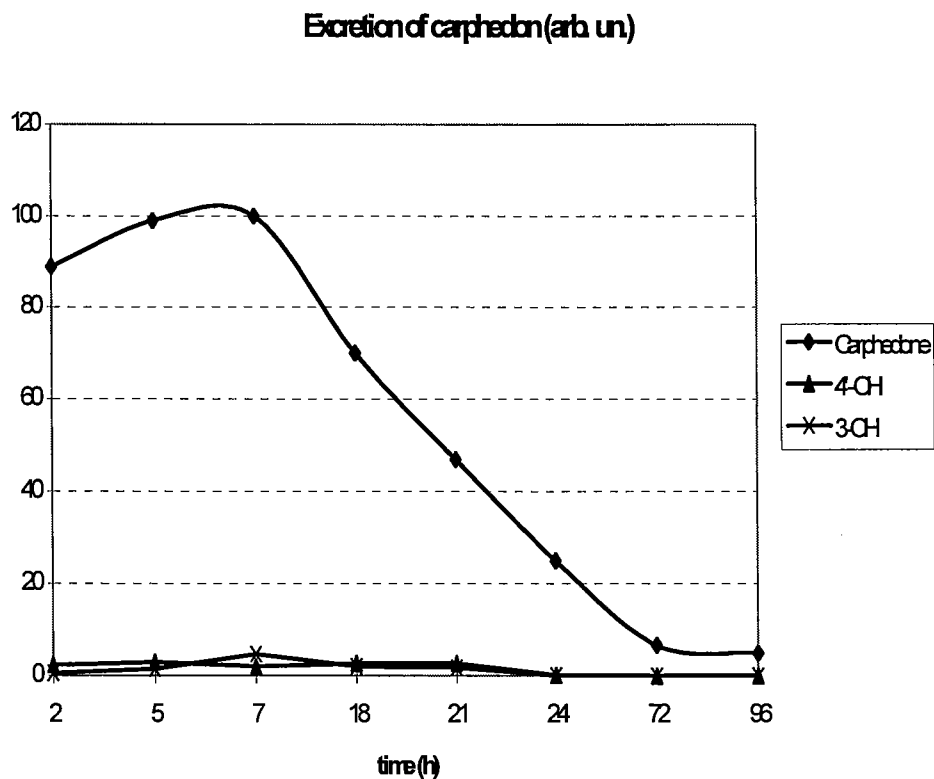


Fig.10 Mass spectrum of dehydrated 4'-hydroxycarphedone (III), enol-bis-TMS ($M^+ = 360$)

Mass spectrum of metabolite **III** has the base ion peak at m/z 192 supposed structure 4- $\text{Me}_3\text{SiOC}_6\text{H}_4\text{NH}=\text{CH}_2^+$ and has not ion peak with m/z 104.

Excretion of carphedone and its hydroxylated metabolites is shown on Fig. 11.



It is seen that carphedone reliably could be detected at 96 hours after administration.

So carphedone itself is detected and confirmed in procedure I and IV. Besides it has several weak metabolites are detected after hydrolysis in procedure IV.