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Detection of Mesocarb in Urine by HPLC and GC-MSD

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Abstract

The purpose of this work is to develope a practically-used method for mesocarb detection in human urine. Acidic hydrolysis and acetylation were involved. A pyrolysis product of mesocarb was detected in hydrolyzed and unhydrolyzed urine samples by GC-MSD.
Coinciding in the results of HPLC and GC-MSD analysis the positive urine could be revealed with quite high creditability.
Whether the method could be used in doping control practice is still remaining questionable.

Introduction

Mesocarb is a psychostimulant which was recently written in the list of examples for the pharmacological class of stimulants by IOC [1]. The effect of this drug on central nervous system has been demonstrated by earlier researches [2]. The metabolism of mesocarb in rat was studied. And these researches indicated that the main metabolic pathway is hydroxylation of the parent compound, also detected in these work is the metabolite amphetamine [3]. Refering to its detection technique mass spectrometry combined with gas chromatograph or other chromatograph technique have been reported [4] and pyrolysis is the main difficulty many scientists faced in their studies. In most of cases neither the parent compound nor its metabolites could be detected as a whole molecule because of its thermal unstability unless some specific techniques were adopted.
To practise detection of mesocarb derivatization was performed in many researches; however, the pyrolysis still took place in a normal GC-MSD progress and only using GC-MSD is sometimes untrustworthy.
In our laboratory a procedure combining HPLC and GC-MSD analysis was attempted. By HPLC analysis two eluates were observed in the hydrolyzed and unhydrolyzed urine respectively.The spectra of these two eluates share totally same spectral profile but different retention time.
In the GC-MSD test we failed to find any clue which shows some correspondence to the eluates in HPLC test. The two GC eluates found in hydrolyzed urine and the unhydrolyzed

one showes that they possess the same mass spectra of the pyrolysis products. With acetylation an acetylated moiety was found but it was still a degraded compound.

Method procedure

Dosage

One tablet of mesocarb was taken orally by a volunteer. Urine was collected till 82 h. No obvious clinic sign was experienced.

Chemical treatment

5 ml of the collected urine was added by solid buffer to adjust pH to be about 9.3, after mixing and vortexing the sample was extracted with 4 ml of ethyl acetate. The extract was dried with a slow stream of nitrogen and redissolved in 300 μl of methanol and 10 μl was injected.

The methanol solution used in HPLC procedure was dried throughly, then mixed with 50 μl of acetone and 50 μl of trifluoroacetic anhydride (TFAA). Heated at 70 °C for 20 min it was dried again to drive off any remaining TFAA. Redissolved in 30 μl of acetate GC-MSD analysis was conducted.

To hydrolyze the sample 1 ml of 6N HCl and cysteine (100 mg) were added into 5 ml of urine and heated at 90 °C for 1 h. After clearance with 4 ml of ether the sample was extracted with 4 ml of ethyl acetate at pH 9.3, then the same procedure as above was applied.

Instrumentation and reagents

HP 1090M High Performance Liquid Chromatograph configurated with HP 9000-300 Computer, HP 9133 Disk Driver and PH Think Jet Printer HP 5890 Gas Chromatograph with HP-5 column and HP 5971 Mass selective detector equipted with Vectra QS/16S ChemStation

Mesocarb tablet was sent by Medical Commission of IOC

Basic solid buffer: sodium dicarbonate /potassium carbonate (3:2, w/w) the final pH from 9 to 9.5

Results and discussion

1. HPLC experiment

1.1 Mesocarb in different matrix

In test of mesocarb tablet using HPLC an eluate with Rt 13.9 min was observed. Its spectrum possesses 3 absorption maximums, they are 198 nm, 256 nm and 336 nm respectively (Fig.1).

In the unhydrolyzed positive urine an eluate with Rt 9.2 min was found, of which the spectral profile was totally same as that of mesocarb tablet (Fig.2).

After the urine was hydrolyzed an eluate with the same UV spectrum as first two appeared at 11.20 min (Fig.3).

According to the results of this test mesocarb could be considered to undergo a biotransformation during its excreting out even if they have same spectral profile.

1.2 Excretion of Mesocarb

The urine collected in different time period were treated as described above, then analyzed by HPLC. The eluate of unhydrolyzed urine was used as target compound and it could be detected in the urine samples of all time period till 59 h.

The intergrated area of the peaks were plotted against the excretion time (Fig.4). The excretion maximum could been seen at 6.5h.

Although the result has no quatitative meaning (we couldn't get the standard of the drug to have this test) the curve can forecast that in the time period of 4 to 59 h the mesocarb should be detected in urine. This excretion process is similar to that of M. Polgar's work finished in rat experiment.

2. GC-MSD experiment

To confirm the result of HPLC test GC-MSD analysis was conducted. Derivatized and underivatized procedure were involved in this experiment.

2.1 Extract analysis of GC-MSD

Extract gotten in the chemical treatment (extracted at pH 9.3) was directly subjected to the GC-MSD analysis. A pyrolysis product was scanned at Rt 7.3 min, the spectrum shown in Fig.5.

The characteristic ions are m/z 91, m/z 118, m/z 173 and m/z 203. Of them m/z 173 and m/z 203 are the sign of existing of sydnone imine but occasionally m/z 203 was not abundant enough to be recorded. This spectrum is identical to that of mesocarb tablet (Fig.6). In the urine sample of all time period, no matter when they were hydrolyzed or unhydrolyzed, we detected the peak bearing the same mass spectrum as above.

2.2 Derivatization

Because of the thermal unstability of mesocarb and its metabolite pyrolysis was irrepressible during a normal GC-MSD progress. To overcome this difficulty derivatization including methylation, silylation and acetylation were attempted. Unfortunately methylation and silylation were unsuccessful. Only by acetylation a derivatized eluate could be detected, but it still comes from a degraded moiety with 13.0 min of retention time. The pyrolysis scheme seems to be identical to that of the original mesocarb. The deduction of its spectrum demonstrated that the acetylated moiety possesses m/z 299, m/z 230, m/z 119 and m/z 91 as its characteristic ions (Fig. 7). Of them m/z 299 resulted from the adding of group COCF3 (m/z 203 + 96). Possibly the reaction site is the methine of the 5-membered ring. As comparison the spectra of mesocarb tablet and the blank urine treated with acetylation was made (fig.8 & Fig.9).

Although abundance, especially those of the higher mass fragments, were not too high the increase in the characteristic ions could make the detection more reliable.

2.3 Detection of Amphetamine

With acetylation in the urine of 23 h and 28 h amphetameine could be detected in trace amount (Fig.10). In the mass spectrum m/z 140 (base peak) is due to the cleavage of the whole moiety (m/z 231-91). Also m/z 91 and m/z 118 are the typical ions of amphetamine-TFA.

Compared with standard spectrum stored in the Libarary Data Base (NBS, HP 5988A) the common trait of their mass spectra could be announced.

3. Discussion

3.1 How to evaluate the different HPLC eluates in hydrolyzed and unhydrolyzed urine ?

As described above, in the hydrolyzed and unhydrolyzed urine, we found two elutes of which the spectra are indentical to each other and the retention time are different. Provided that in unhydrolyzed urine what we found is the parent mesocarb (Rt 9.2min) then the eluate from the hydrolyzed urine (Rt 11.2 min) may be a metabolite of this drug. If so these two spectra should have some difference of either wavelength of absorbtion maximum or the height ratio of the three absorbtion maximums, but no change like this appeared in the UV spectrum (Fig.3). Another possibility is that in hydrolyzed urine a decomposed compound was detected. This decomposition is due to the unstability of sydnone imine in so high acidity as nearly 1N HCl. On the other hand the retention time of the eluate of mesocarb tablet is different from the eluates of positive urine, so it is hard to say what

structure change has happened during the metabolism of this drug by the work presented here.

3.2 Could the procedure be used in the doping control ?

Coincidence in the results gotten by HPLC and GC/MSD could give out a report of existing of mesocarb and the results are reproducible in every indivadual analysis.

The sensitivity is high enough for the detection in the urine samples of any time period. On the other hand some uncertainty may come from the fact that the abundance, especially those of higher mass fragments are quite low. Possibly this may cause mistake in detection since many foreign compounds and endogenous material could produce the fragments like m/z 91, m/z 118 and m/z 119. Another problem is that our work is based upon the only one urine sample and no statistical meaning exists in the results.

References

1. The official document of Subcommission Doping and Biochem. of Sport, IOC Dec.19, 1991, Cologne
2. Erdo S. L., Kiss B., Rosdy B.: Identification of dopamine uptake by a new psychostimulant mesocarb, POL J Pharmo 1981, Mar-Apr; 33(2): 141-47
3. Polgar M., Vereczkey L., Szporny L.: Metabolism of mesocarb in the Rat, Xenobiotica (1979) Vol. 9 No. 8 511-20
4. Tamas J., Polgar M., Czira G., Vereczkey L.: Mass spectrometric characterization of sydnocarb and its major metabolites in rat. 1978

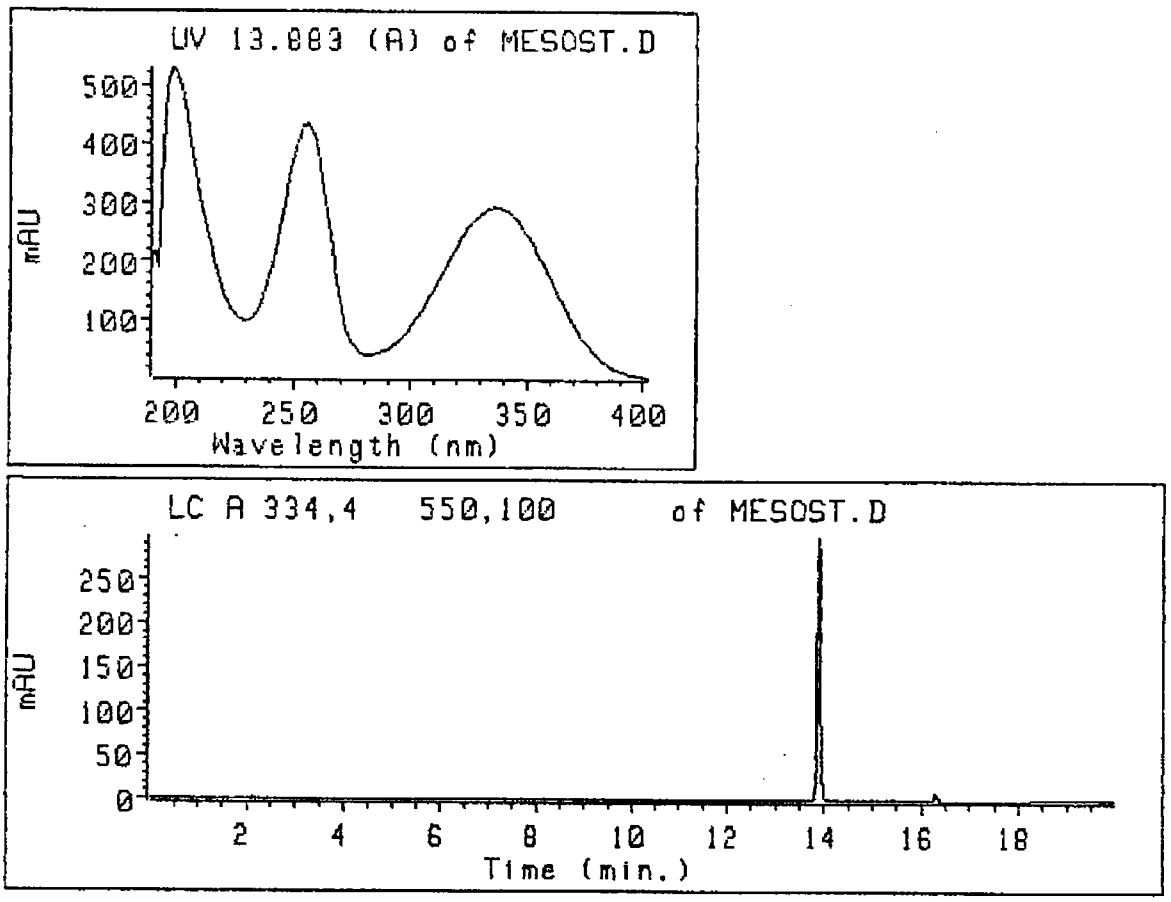

Fig.1 chromatogram and spectrum of the eluate of mesorcarb tablet

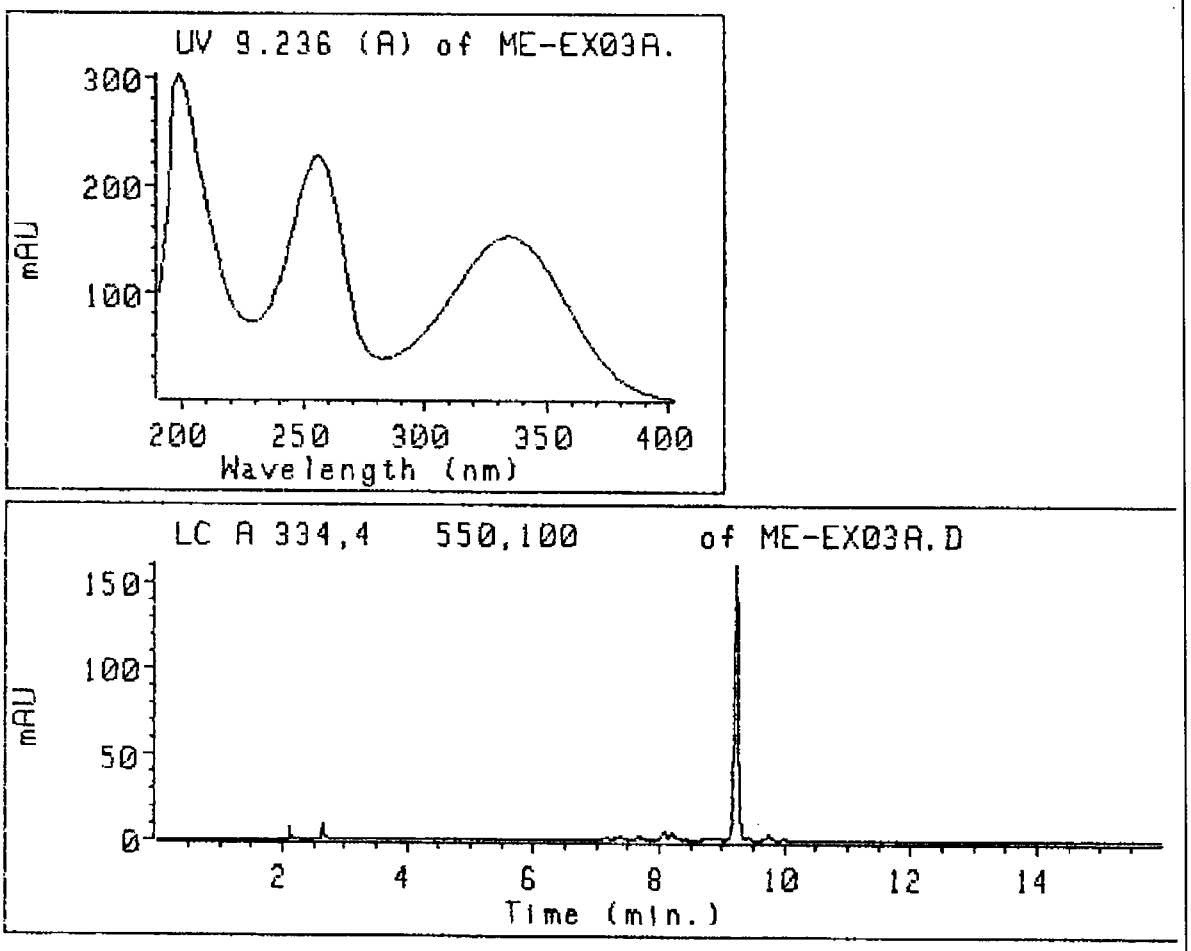

Fig.2 chromatogram and spectrum of the eluate of unhydrolyzed urine

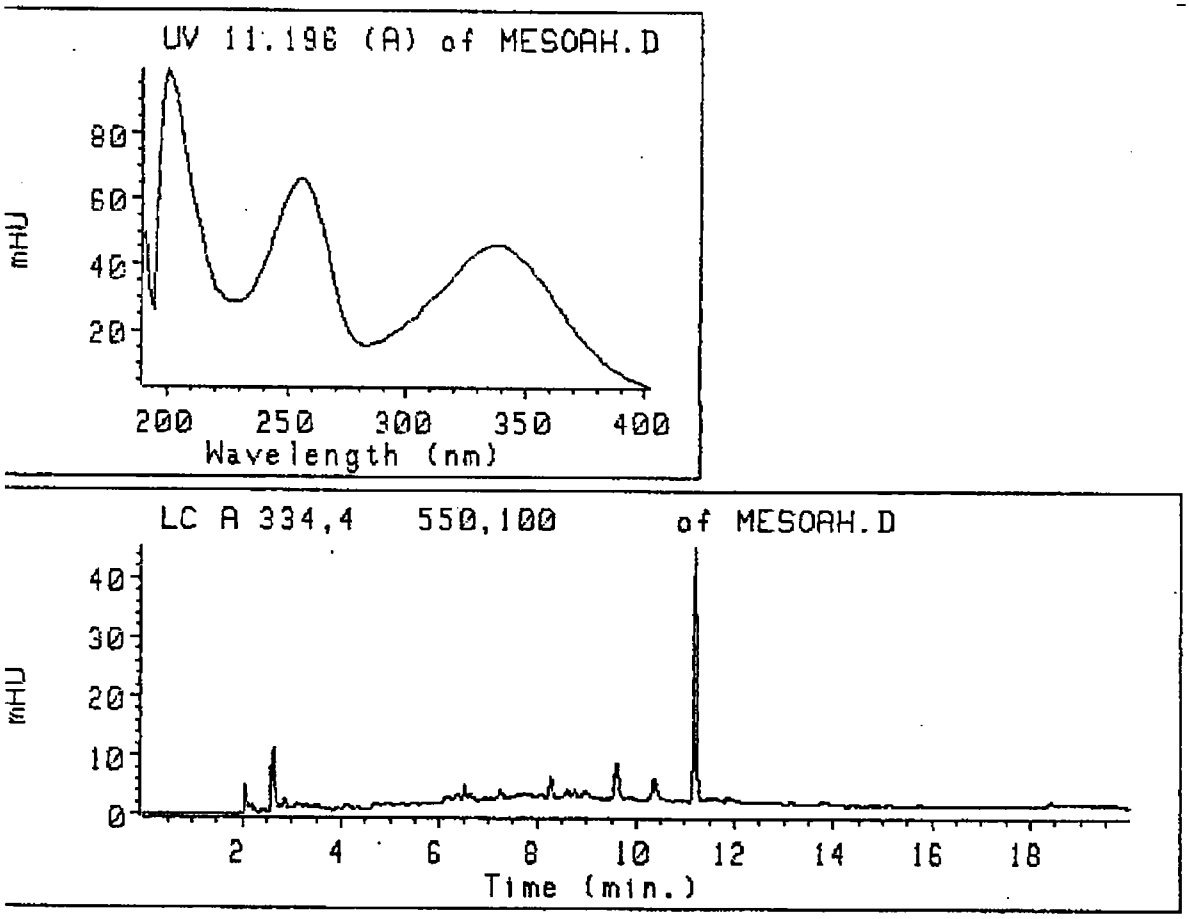

Fig.3 chromatogram and spectrum of the eluate of hydrolyzed urine

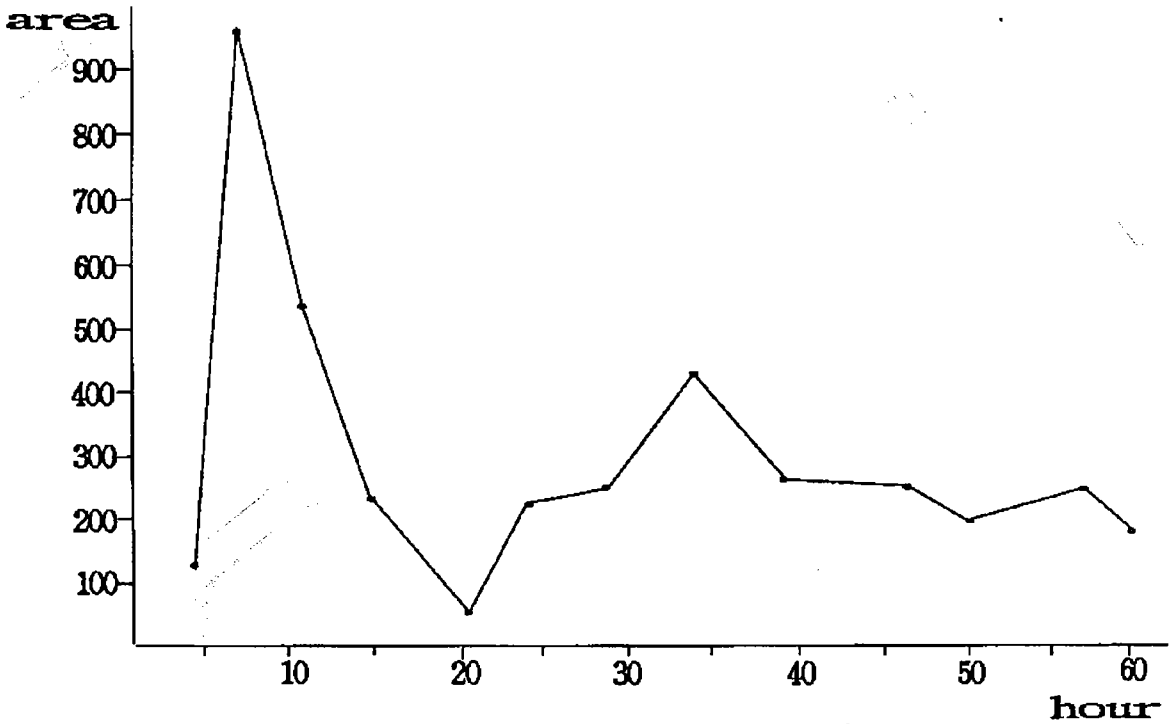

Fig.4 excretion of mesocarb

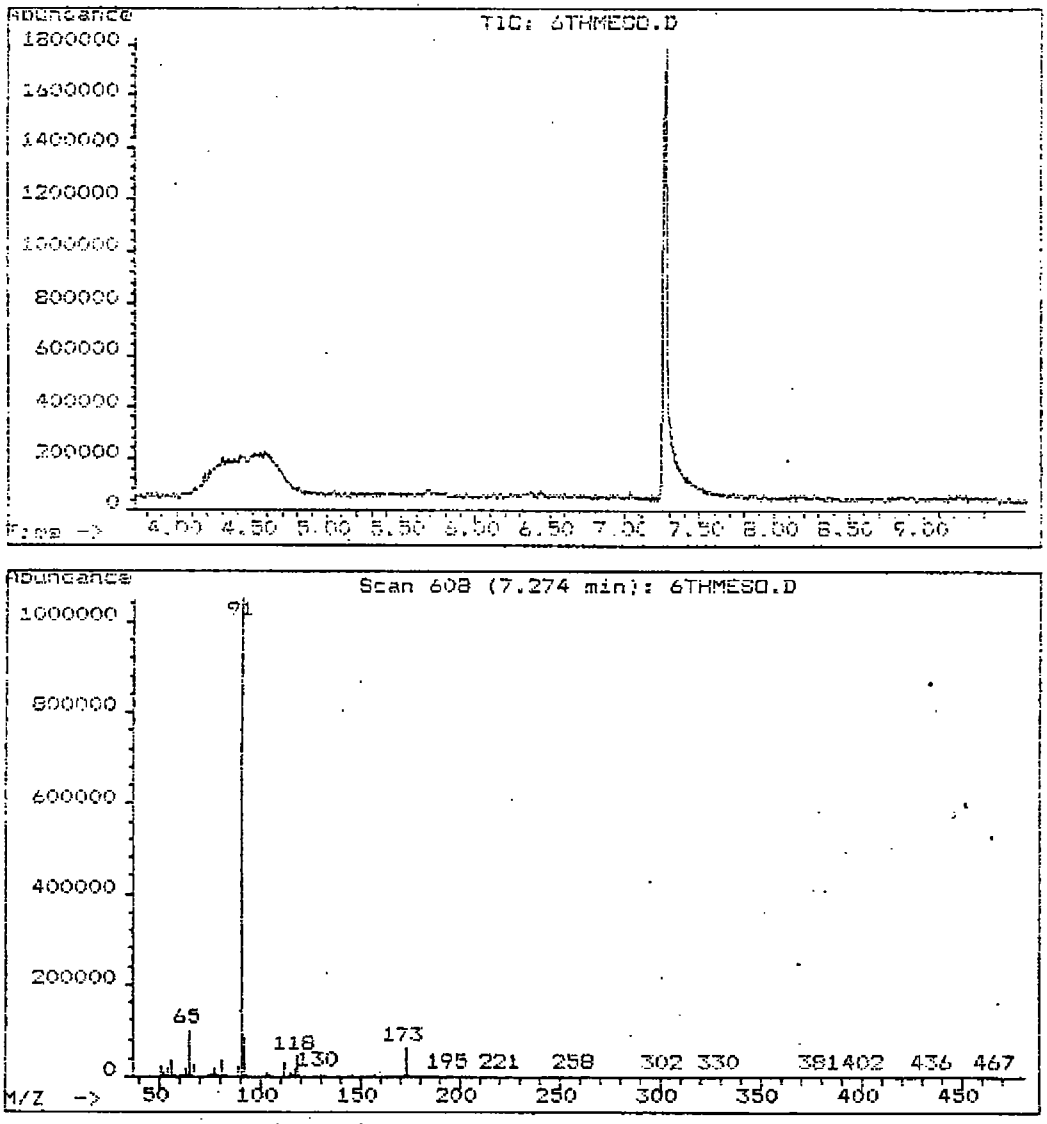

Fig.6 TIC and mass spectrum of pyrolysis product of mesorcarb tablet

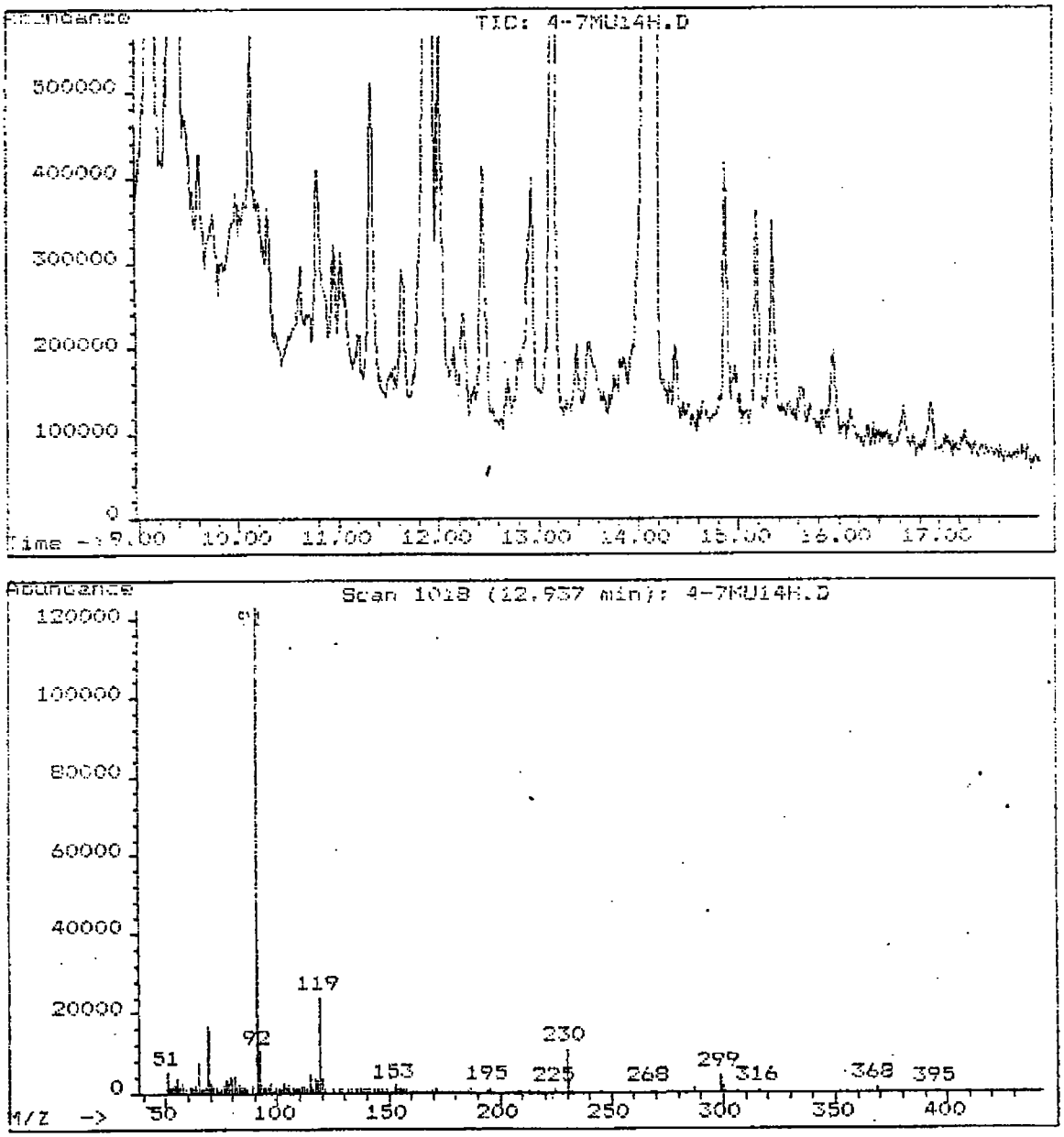

Fig.7 TIC and mass spectrum of pyrolysis product in positive urine treated with TFAA

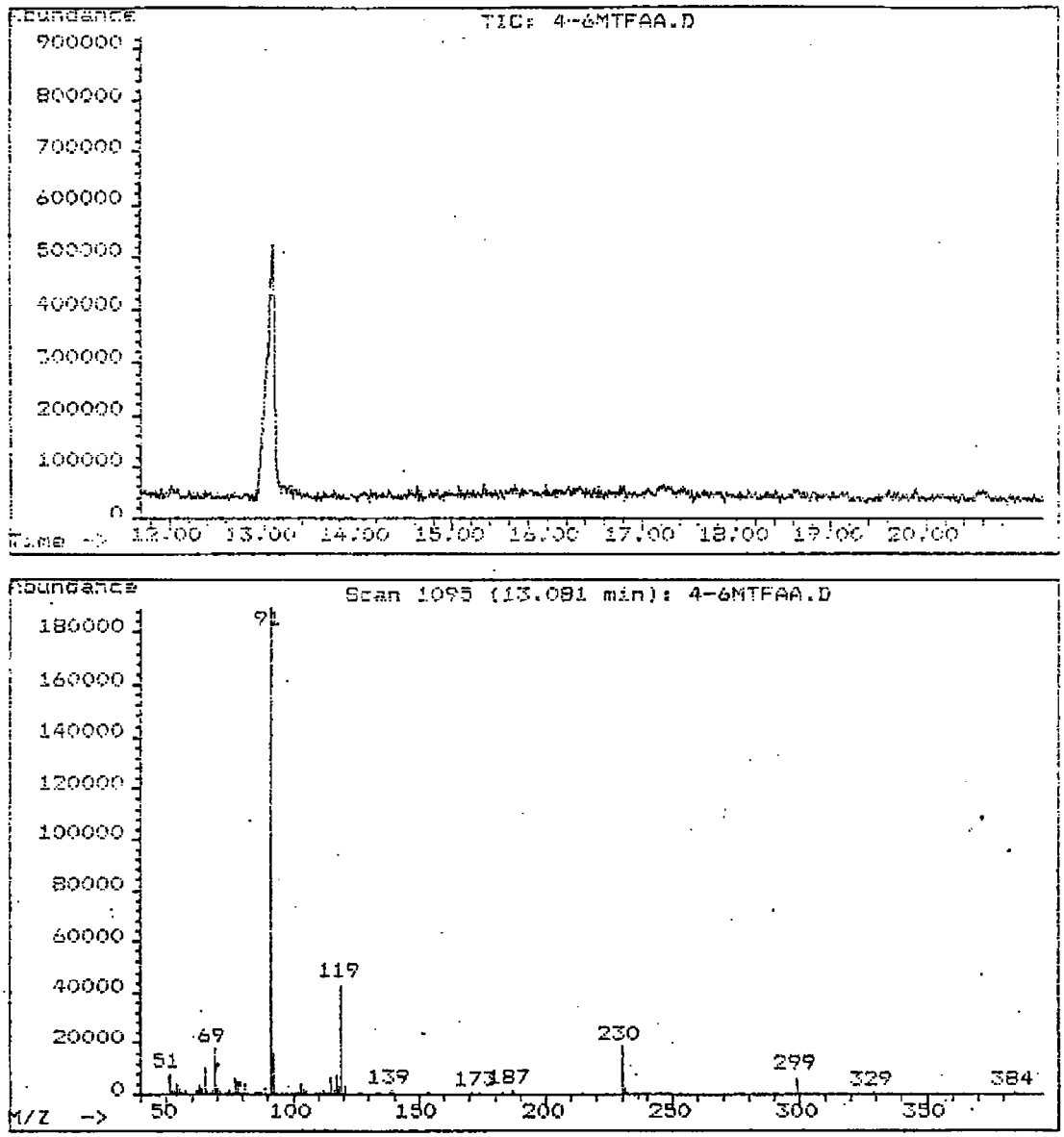

Fig.8 TIC and mass spectrum of pyrolysis product of mesorcarb tablet treated with TFAA

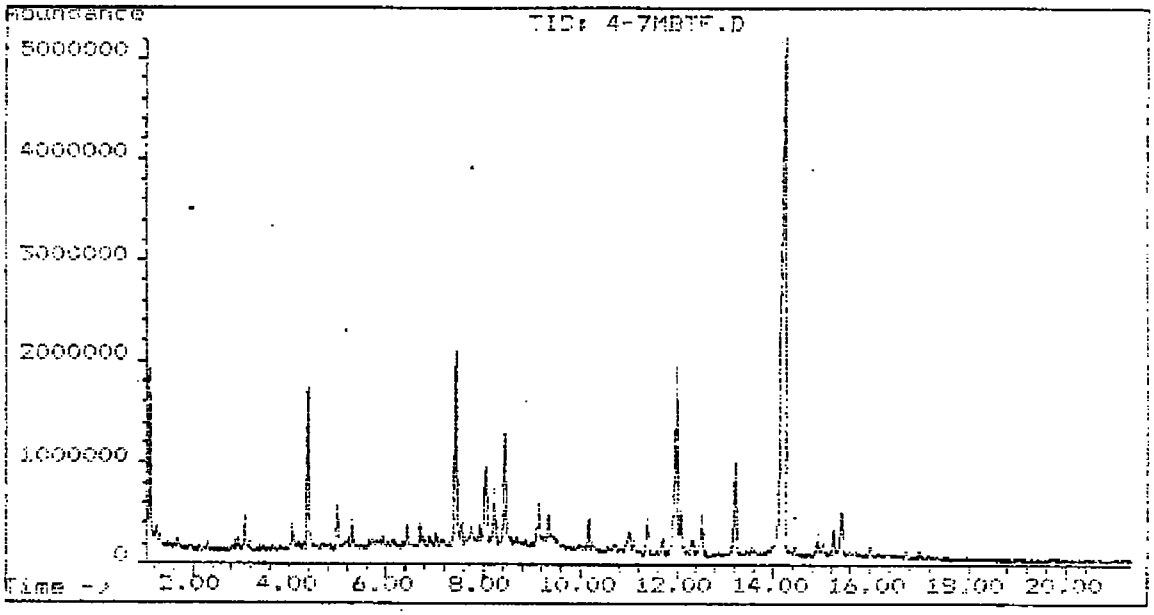

Fig.9 TIC of blank urine treated with TFAA

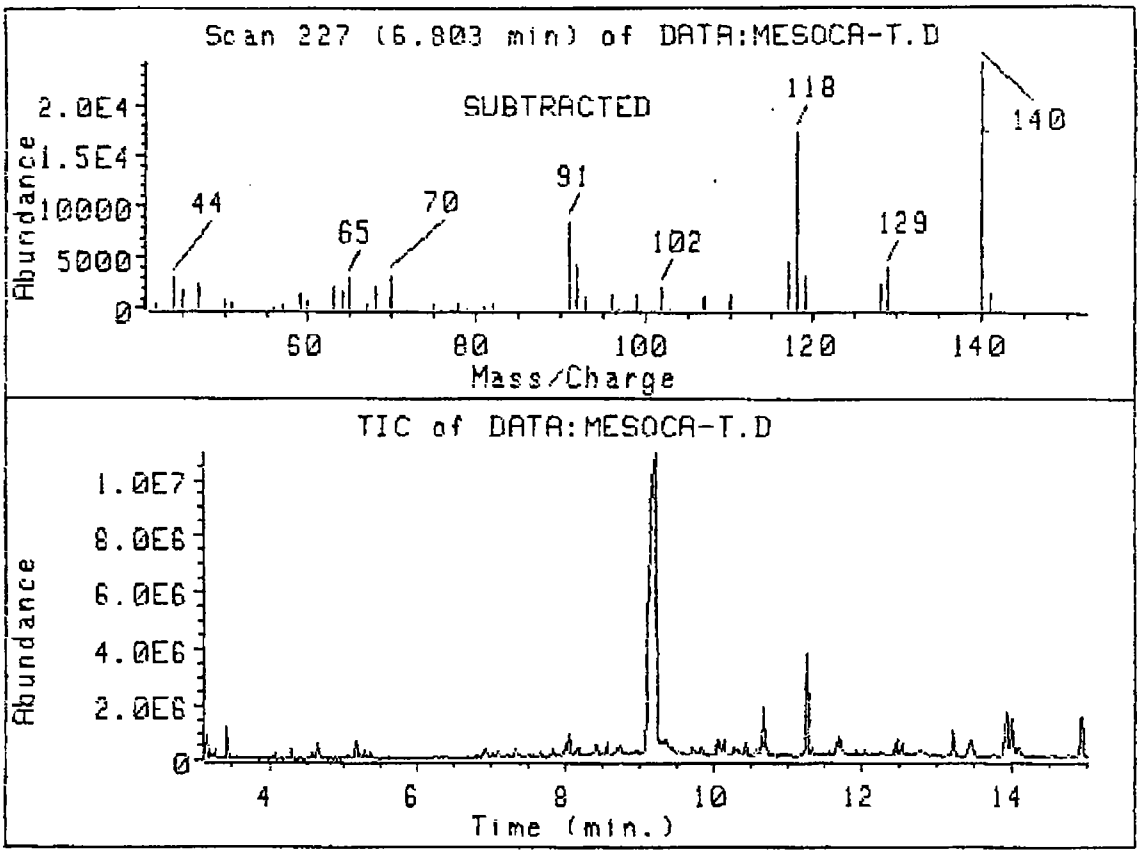

Fig.10 TIC and mass spectrum of amphetamine-TFA