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RECENT ADVANCES IN DOPING ANALYSIS (9)

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Detection of bupropion and metabolites

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Introduction

Bupropion ((+-)-1-(3-chlorophenyl)-2-[(1,1-dimethylethyl)amino]-1-propanone hydrochloride) or amfebutamon is a relatively new antidepressant of the aminoketone class which is used in withdrawal therapy of smokers. An additional stimulating effect on the central nervous system has also been observed. Its structure is related to the phenylethylamines and is very simular to that of diethylpropion. It is an inhibitor of neuronal serotonine and norepinephrine uptake. The mechanism of its antidepressant effect is not yet known. In january 2001 it has been added to the IOC list of prohibited substances. This poster presents a method to detect bupropion and its metabolites in human urine.

Experimental

Excretion study

The excretion study was performed by one male volunteer (49 years, 68 kg) who took a single oral dose of 150 mg of bupropion hydrochloride (1 tablet Zyban®). Urine samples were collected for 400 hours after administration.

Sample preparation

The urine samples were prepared according to the screening 1 procedure:

5 ml urine
add
25µl of internal standard solution (1 mg of diisopropylaminododecan/ml of methanol) 0.5 ml 5N KOH (pH 14)
vortex mix thoroughly
add
2 ml of tert.-butyl methyl ether 3 g of anhydrous sodium sulphate
mix thoroughly shake mechanically 20 min. and centrifuge 5 min.
organic layer is injected to the GC/MS and GC/NPD

GC/MS and GC/NPD parameters

A combined GC/MSD and GC/NPD system was used.

GC/MSD, GC/NPD system: Hewlett Packard 5890/5973

Carrier gas: Helium (1ml/min. flow), split ratio: 1:10

Analytical column: HP-5 MS f.s. cap.column, 0.25 mm i.D.,0.25 µm film thickness

length: MSD: 24 m, NPD:19 m

Temperature program: 100°C, 22°C/min. till 320°C, 1 min. const.

Results

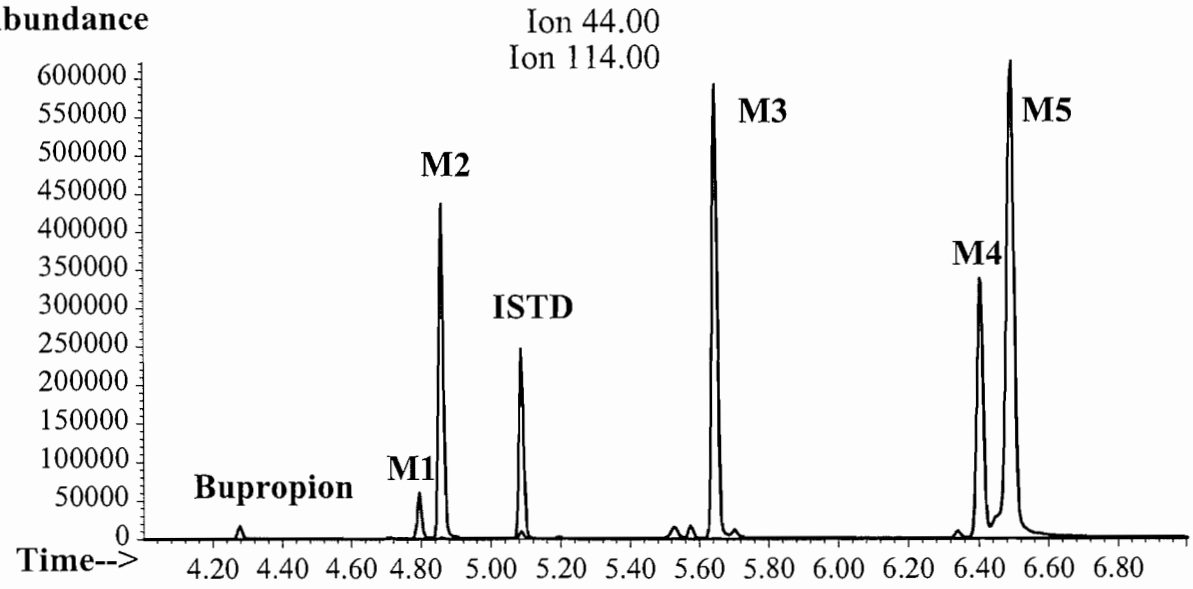

Fig.1: EIP m/z 44 and 114 (ISTD) of a urine sample 18 hours after oral application of 150 mg of bupropion hydrochloride

	RT [min.]	*MW*	*Compound*
Bupropion	4.28	239	parent compound
M 1	4.80	241	erythro- and threo-dihydrobupropion
M 2	4.86	241	
M 3	5.65	257	erythro- and threo-hydroxy-dihydrobupropion metabolites
M 4	6.41	257	
M 5	6.50	275	

The parent compound can only be detected in a very small amount. The metabolites 1 and 2 are physiologically active metabolites which are formed by reduction of the carbonyl group. They are isomers with the same mass spectrum and can only be distinguished by the retention time.

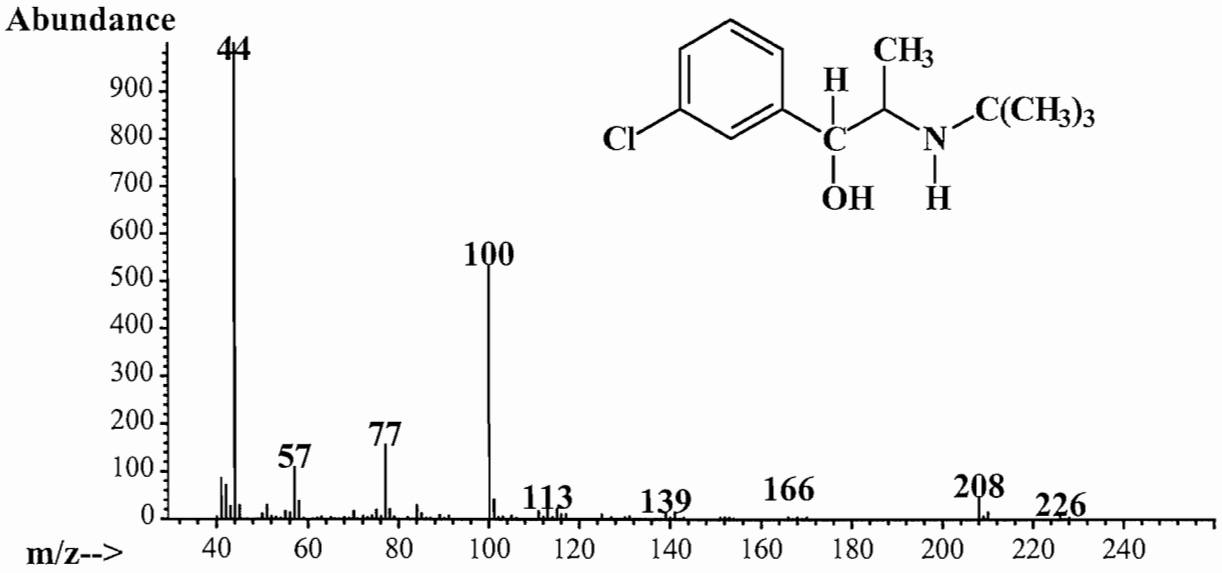

Fig. 2: EI-mass spectrum of M1and M2, erythro- and threo-dihydrobupropion (mol wt = 241)

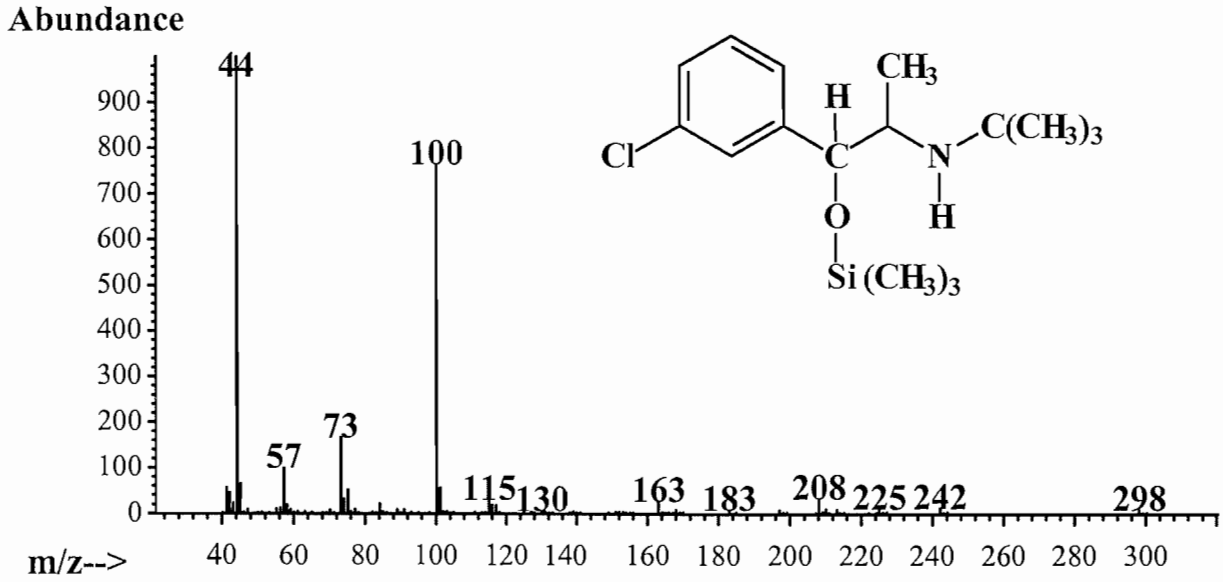

Fig. 3: EI-mass spectrum of silylated M1 and M2, erythro-and threo-dihydrobupropion mono-TMS (mol wt = 313)

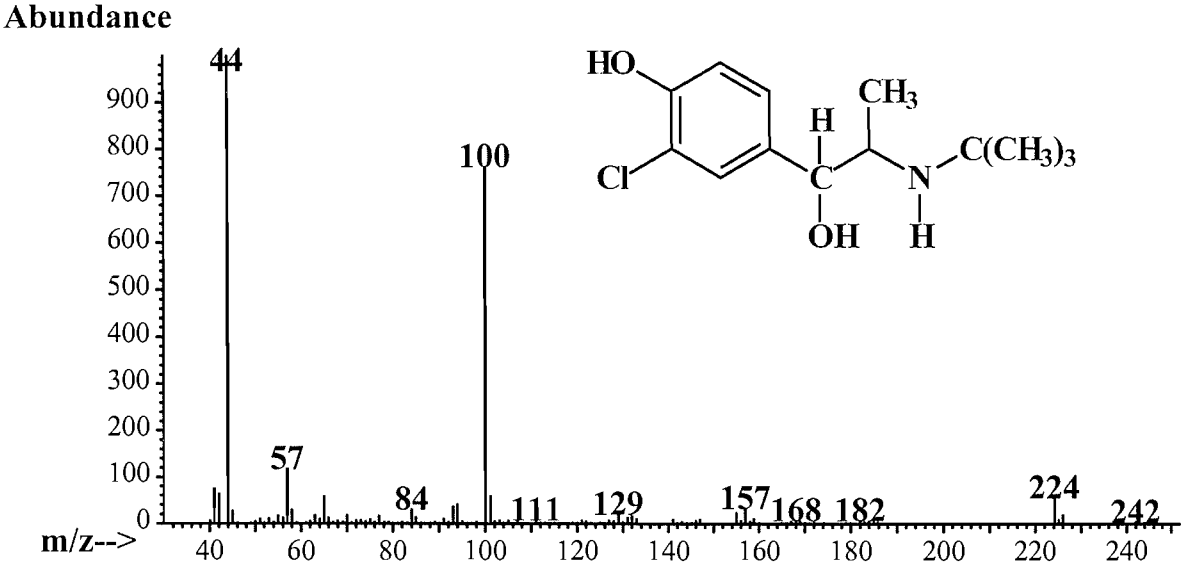

Fig. 4: EI-mass spectrum of M3, erythro-or threo-hydroxy-dihydrobupropion (mol wt 257)

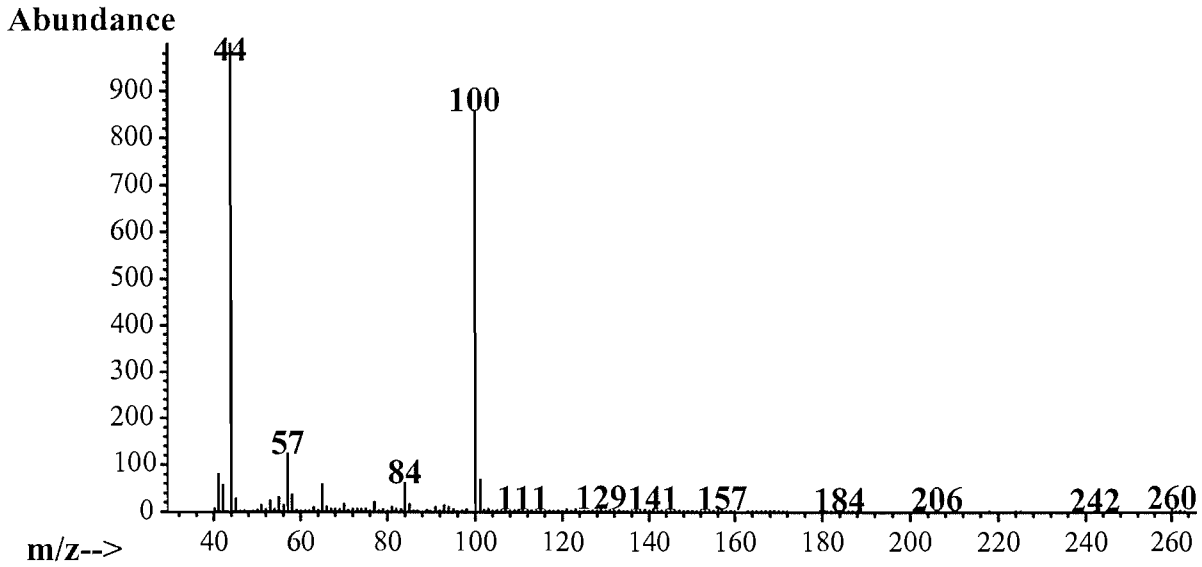

Fig. 5: EI-mass spectrum of M5 (mol wt 275)

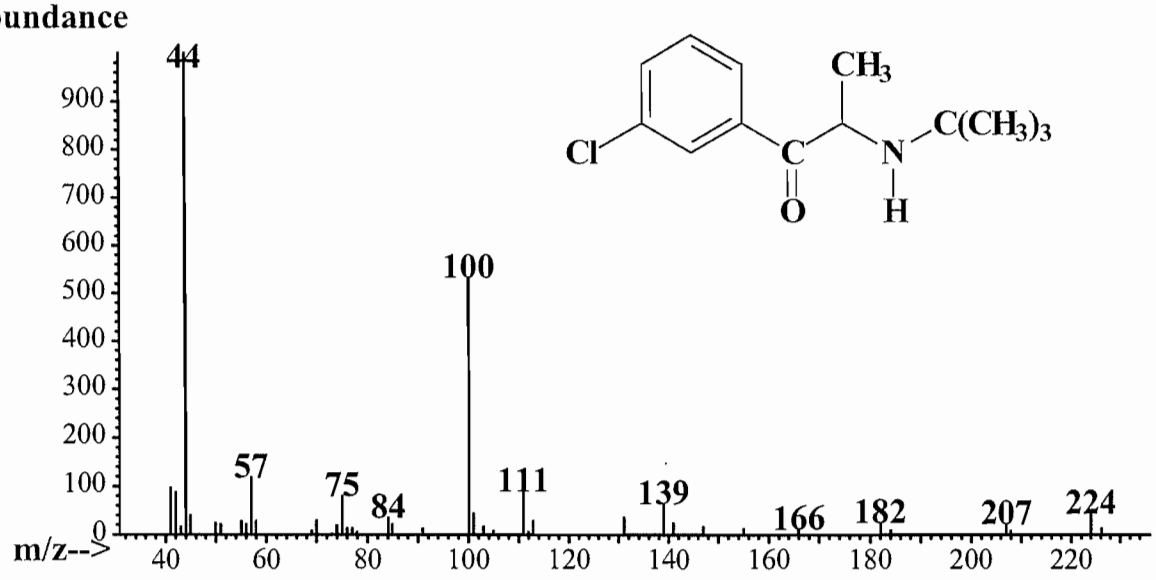

Fig. 6: EI-mass spectrum of bupropion (mol wt = 239)

Results and Conclusion

Bupropion is extensively metabolized and only a small amount is excreted unchanged in urine. The main metabolites are the stereoisomeres erythro- and threo-dihydrobupropion and erythro- and threo-hydroxy-dihydrobupropion. The fragmentation shows only small differences between the metabolites. The maximal concentration (> 10µg/ml) of dihydrobupropion in the excretion study is obtained at about 18 hours after administration. Till about 300 hours ion traces of the main fragments m/z 44 and 100 are detectable in screening 1 (full SCAN mode).

References

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