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# DETECTION AND METABOLISM OF FENCAMFAMINE AND THE INFLUENCE OF ACETAZOLAMIDE ON ITS URINARY EXCRETION

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## ABSTRACT

A gas-chromatographic (g.l.c.) method with electron-capture (e.c.) detection is described for the simultaneous quantitative determination of nanogram concentrations of 2-ethylamino-3-phenyl-norbornane (Fencamfamine, REACTIVAN®) and its metabolite 2-amino-3-phenylnorbornane in urine.

The renal excretion of fencamfamine and its metabolite after oral administration to humans was followed over a period of several days. The excretion of both substances was affected by urinary pH. Excretion peaks were obtained 2–4 h after ingestion and the total amount excreted during 80 h varied from 11·9 to 33·2 per cent. Based on urinary values, the biological half life of fencamfamine was 16 h.

The intake of acetazolamide shortly after fencamfamine resulted in a decrease of the fencamfamine excretion and a suppression of the metabolite output during at least 10 h. Acetazolamide did not influence the percentage of the doses excreted during 80 h.

No changes occurred in urinary fencamfamine or metabolite concentrations during storage of the urine at $-18°$ for 6 weeks.

KEY WORDS Fencamfamine Detection Metabolism Urinary excretion Acetazolamide

## INTRODUCTION

Fencamfamine is a sympathomimetic central stimulant widely abused in sports. In a comparative study of the lipophilic nature and basic strength of amphetamine-like compounds, Vree and Van Rossum[1] found that fencamfamine ($pK_a$: 8·7) is considerably less basic than amphetamine ($pK_a$: 9·97) but at the same time more lipophilic. This means that under alkaline conditions the suppression of its renal excretion might be so great that urinary concentrations might fall below levels detectable with methods currently available such as gas chromatography equipped with flame ionization[1,2] or nitrogen specific detectors.[3]

It is well known that the renal excretion of fencamfamine[1] and other sympathomimetic amines[4–6] can be lowered by the intake of alkalinizing

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substances. Thus, with respect to doping the validity of a test might be negated by the ingestion of such agents. Therefore, it would be advantageous to have a procedure to detect nanogram amounts of fencamfamine in urine. Such a sensitivity can be obtained by derivatization of fencamfamine with fluorine containing compounds and gas chromatography with electron capture (e.c.) detection of the derivatives.

Very little work has been done on the metabolism of fencamfamine in men or animals. Vree and Van Rossum[1] studied the renal excretion of fencamfamine in man and its suppression by the intake of alkalinizing substances. In order to produce an alkaline urine over several hours, however, relative large amounts of sodium bicarbonate are needed.[7] On the contrary, the intake of therapeutic doses of acetazolamide not only results in an alkaline urine for several hours but also produces a diuresis. Both these effects together could lead to a complete suppression of the renal excretion of drugs, such as fencamfamine. Therefore some experiments were undertaken in man to follow the metabolism and excretion of fencamfamine in urine under normal conditions and strong alkaline circumstances obtained after the intake of acetazolamide.

The stability of low amounts of fencamfamine and its eventual metabolites in alkaline urine after a long period of storage at $-18°$ was also studied. An e.c. gas chromatographic method was developed for this purpose.

## METHODS

*Electron capture gas chromatographic determination*

Reagents

Fencamfamine.HCl and 2-amino-3-phenylnorbornane.HCl were gifts of Merck, Darmstadt (GFR). 2-(benzylamino)-norbornane, Aldrich. Cyclohexane (analytical grade) Carlo Erba. Sodium hydroxide (analytical grade) Merck. Pyridine (analytical grade), Merck. Benzene (analytical grade), Merck. Heptafluorobutyric anhydride, Aldrich.

Apparatus and operating conditions

A VARIAN Model 3700 gas chromatograph equipped with a $^{63}Ni$ electron capture detector and a 1·5 m × $\frac{1}{8}$ in silanized glass column packed with 2 per cent OV-17 on 80–100 mesh Chromosorb W was utilized for the g.l.c. analysis.

Column, injector, and detector temperatures were 135, 210, and 350° respectively. Quantitative determinations were done using a VARIAN CDS 101 integrator.

Internal standard

Diethylether saturated with HCl was slowly added to a solution of 2-benzylaminonorbornane in diethylether, resulting in the formation of the insoluble 2-benzylaminonorbornane.HCl. After cooling, the white powder was filtered, washed several times with diethylether and dried in vacuo

at 50° (m.p. 224–226°). A stock solution (0·1 mg ml$^{-1}$) in double distilled water was prepared and stored at 4°. This solution was diluted to 0·01 mg ml$^{-1}$ daily.

Procedure

All glassware was silanized as described previously.[8] To 5 ml alkalinized urine (pH 12) in a silanized glass stoppered 15 ml centrifuge tube, 50 µl of the diluted internal standard solution (0·01 mg ml$^{-1}$) was added.

Extraction was accomplished by adding 7 ml of freshly distilled cyclohexane and shaking the tubes on a rotary shaker for 20 min. After centrifuging for 15 min at 2000 rev min$^{-1}$, the organic layer was transferred into a clean silanized glass-stoppered tube, two drops of diethylether saturated with HCl added, and the contents evaporated to dryness under nitrogen (40°). The residue was dissolved in 250 µl cyclohexane and 20 µl pyridine-benzene (5 : 100), and 20 µl heptafluorobutyric acid anhydride (HFBA) was added. The contents were vortex-mixed and allowed to react for 15 min at room temperature. After vortex-mixing briefly with 3 ml 0·01N NaOH to remove excess anhydride, the phases were separated by centrifugation for 5 min and 1 µl of the cyclohexane layer taken for g.l.c. analysis.

*Human investigations*

In a first series of experiments REACTIVAN® (Merck) was given orally to 4 human volunteers at a dose of 0·4 mg fencamfamine.HCl kg$^{-1}$ bodyweight. Two of them also received the same amount of fencamfamine.HCl in a capsule. Two months later, the same subjects received the same doses of REACTIVAN® followed by 250 mg acetazolamide (DIAMOX®, Lederlé) 2 h later. All the urine was collected at intervals up to 80 or 96 h after the ingestion, and either used immediately or stored at −18° for later analysis. Urinary volume and pH were also measured. Each urine sample was analysed in triplicate. In order to evaluate the extracts correctly (linearity range of the standard graphs) it was necessary to dilute some urine samples. Furthermore, since the peak area ratio in the internal standard method could be influenced by the volume injected,[9] great care was taken to inject the same volume in all experiments.

To study the stability of fencamfamine and its desethylated metabolite during storage at −18°, some urine samples were reanalysed (6 determinations for each sample) 3 and 6 weeks later.

## RESULTS AND DISCUSSION

*Analytical methods*

Several methods for the acylation of amines for g.l.c./e.c. purposes have been published.[10–13] With respect to doping analysis, the quantitative determination of some drugs using HFBA was described by Walle and Ehrsson.[14, 15] A variety

of derivatives of amphetamine and phenmetrazine were prepared for their g.l.c./e.c. analysis by Änggård and Hankey.[16]

Optimal conditions for the derivatization of fencamfamine, the eventual metabolites, and the internal standard were determined experimentally. Pentafluoropropionyl acid anhydride (PFPA) and HFBA were both used for derivatization. By determining the peak height, it was found that the e.c. response of the HFBA derivative was twice the response of the PFPA derivative. Although it has been demonstrated that the use of trichloroacetic anhydride and pentafluorobenzoylchloride (PFBCl) results in derivatives with very good e.c. properties,[12, 13] the e.c. response of the trichloroacetamide and pentafluorobenzamide of fencamfamine is lower than for the analogue heptafluorobutylamide. Moreover, using PFBCl the excess reagent must be removed by evaporation followed by dissolving the residue in cyclohexane and washing with 1N NaOH in order to remove some strongly electron-capturing substances formed by the hydrolysis of PFBCl.

Gas-chromatographic analysis of the residue derivatized with HFBA resulted in two different peaks on the chromatogram. Besides the peak obtained for the derivative of fencamfamine the second one was identified as the derivative of the desethylated metabolite of fencamfamine, 2-amino-3-phenylnorbornane.

The influence of the reaction time for both fencamfamine and its metabolite was also determined. As shown in Figure 1, the peak height ratio did not change much more after 15 min reaction time at room temperature.

Using different concentrations, 0·5, 0·25, 0·10, 0·05, 0·025, and 0·010 μg $ml^{-1}$, of fencamfamine and its desethylated metabolite in pooled urine, a standard graph was obtained by analysing each urine sample in quadruplicate. Data for slopes, $x$-intercept, and correlation coefficient were 0·093, 0·028, 0·994 for fencamfamine and 0·316, 0·040, 0·997 for its desethylated metabolite. For qualitative work, urinary concentrations (5 ml) of 0·025 mg $l^{-1}$ for both substances could easily be detected. However, due to the $x$-intercept values, the quantitative detection limits were 0·04 and 0·05 mg $l^{-1}$ (based on 5 ml urine) for fencamfamine and its metabolite respectively.

A chromatogram representing a derivatized urine extract after the intake of fencamfamine is shown in Figure 2. It is noteworthy that HFBA derivatization of fencamfamine results in two unresolved peaks probably due to isomeric derivatives. The nature of these compounds obtained both with derivatized fencamfamine standard and fencamfamine extracted from urine after intake is currently being investigated.

*Urinary excretion under normal conditions*

An example of urinary production (ml $h^{-1}$), urinary pH, and excretion (μg $h^{-1}$) of both fencamfamine and its desethylated metabolite, 2-amino-3-phenylnorbornane, under normal conditions is given in Figure 3 (Subject 1)

The urinary excretion of weak organic acids or bases depends largely on the lipid solubility of their undissociated form, which is related to the $pK_a$ value and

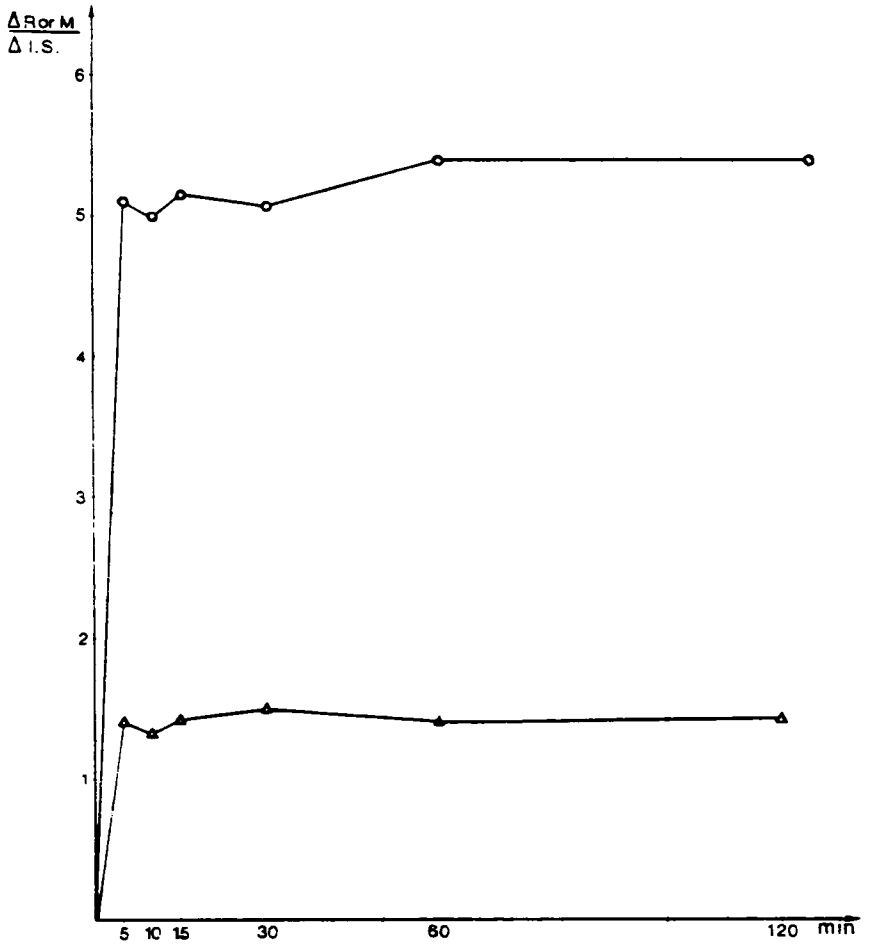


Figure 1. Influence of the reaction time on the HFBA derivatization of fencamfamine (○) and its desethylated metabolite (△)

the pH of the environment. In the study of some physico-chemical properties of amphetamine and related drugs, Vree *et al.*[17] found that 95·24 per cent of fencamfamine was ionized at pH = 7·4 in comparison to more than 99 per cent for amphetamine. Moreover, the non-ionized form of this drug has a high lipid solubility. The partition coefficient between $CHCl_3$ and $H_2O$ was estimated at 4200, against 146 for amphetamine. Therefore, the urinary excretion of fencamfamine and perhaps also its desethylated metabolite should largely depend on the urinary pH value. Indeed, the influence of even minor pH differences could be seen in the excretion diagrams of all subjects. The influence of the urinary pH is clearly demonstrated in the excretion pattern of Subject 1 (Figure 3) for the period 12–80 h during which a relatively constant urine volume was produced.

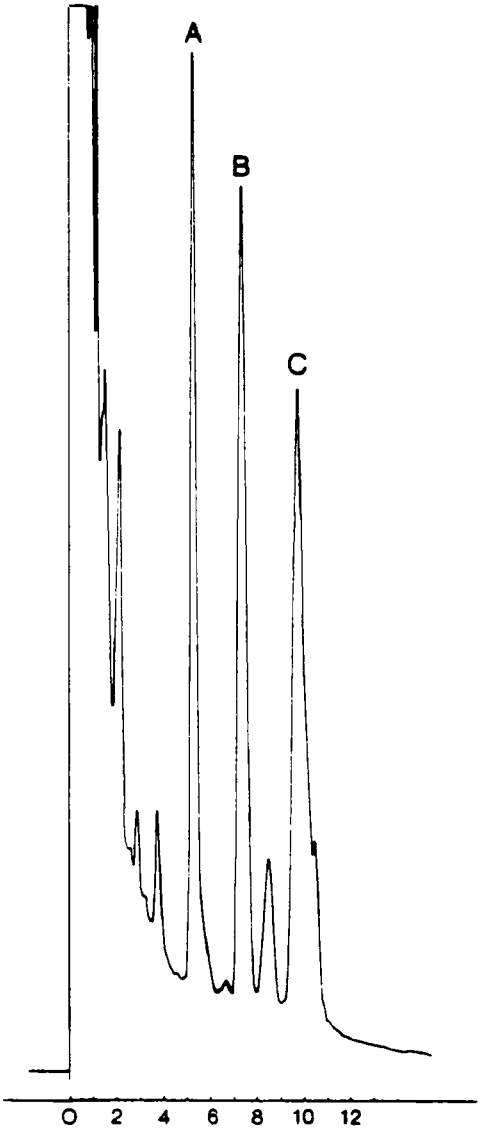


Figure 2. Gas chromatogram of a derivatized urine extract after the intake of fencamfamine. HFBA derivative of (A) 2-amino-3-phenylnorbornane; (B) internal standard; (C) fencamfamine (for g.c. conditions, see text)

The peak time of the fencamfamine excretion and the total excretion of both fencamfamine and its desethylated metabolite during a 80 h period under normal conditions is given in Table 1. The biological half-lives calculated from the excretion values are given in Table 2. As summarized in Table 1, it can be seen that, under normal conditions, the peak time of the fencamfamine excretion

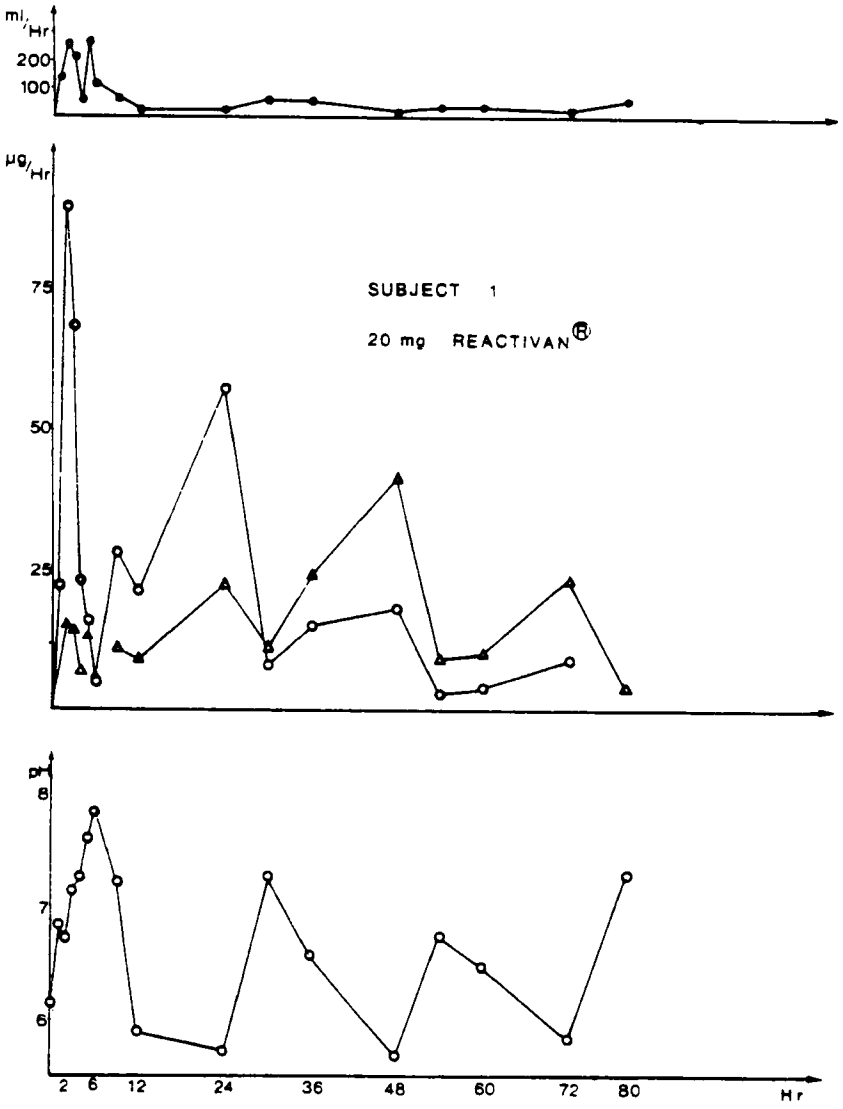


Figure 3. Urinary production (ml $h^{-1}$), urinary pH, and excretion (µg $h^{-1}$) of fencamfamine (○) and its desethylated metabolite (△) under normal conditions (▲: concentrations close to the detection limit)

occurs 2–4 h after the ingestion of the drug. The high renal excretion rate during the first hours proves that fencamfamine is rapidly absorbed from the gastro-intestinal tract. Also, the total excretion seems not to have been influenced by the pharmaceutical formulation in which the active substance was administered, as Table 1 shows no differences between the results for REACTIVAN® and fencamfamine.HCl as such in a capsule.

Table 1. Urinary volume, pH, excretion peak time, and percentage of the dose excreted (80 h) after the intake of fencamfamine under normal conditions

| | Subject | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 1 | 2 | 2 | 3 | 4 |
| Dose form | R | C | R | C | R | R |
| Urinary volume (ml) | 4005 | 5625 | 6006 | 5312 | 4400 | 3756 |
| Mean pH | 6·71 ± 0·69 | 6·47 ± 0·58 | 5·40 ± 0·28 | 5·52 ± 0·57 | 6·22 ± 0·89 | 6·60 ± 0·58 |
| *Excretion* | | | | | | |
| (a) Peak time (h) | 2 | 4 | 4 | 3 | 2 | 3 |
| (b) Fencamfamine (%) | 7·6 | 6·5 | 16·1 | 15·4 | 12·5 | 6·0 |
| (c) Metabolite (%) | 8·3 | 6·4 | 13·7 | 17·8 | 4·3 | 5·8 |
| (d) Total (%) | 15·9 | 12·9 | 29·8 | 33·2 | 16·8 | 11·8 |

R = REACTIVAN®; C = Capsules containing fencamfamine.

Table 2. Biological half-life (h) of fencamfamine under normal and post-acetazolamide conditions

| | Subject | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 1 | 1 | 2 | 2 | 2 | 3 | 3 | 4 | 4 | Mean |
| Dose form | R | C | R | R | C | R | R | R | R | R | |
| Normal conditions | 12·1 | 14·3 | | 15·1 | 21·5 | | 18·3 | | 14·0 | | 15·9 ± 3·4 |
| 7–34 h post-acetazolamide | | | 90·6 | | | 39·3 | | 32·5 | | 23·8 | |
| 34–82 h post-acetazolamide | | | 13·7 | | | 11·8 | | 12·1 | | 10·5 | 12·0 ± 1·3 |

R = REACTIVAN®; C = Capsules containing fencamfamine.

Furthermore, based on the average biological half-life (15·9 ± 3·4 h) one can conclude that fencamfamine is slowly eliminated from the human body. The amount excreted (fencamfamine and metabolite) varies from 11·9 to 33·2 per cent of the ingested dose. Although the exact influence of the total urinary volume or the pH cannot be clearly evaluated, the high recovery from Subject 2 must mainly be attributed to his lower mean urinary pH value.

It is noteworthy that for the 6 volunteers the urinary concentration of the metabolite under normal conditions exceeded the fencamfamine values 32 ± 4 h after the ingestion of the drug.

*Urinary excretion after acetazolamide*

Vree and Van Rossum[1] studied the suppression of the renal excretion of fencamfamine in man after the ingestion of the alkalinizing agents acetazolamide and sodium bicarbonate in one and five volunteers respectively. Using gas chromatography equipped with flame ionization detection (FID) they found that the intake of sodium bicarbonate and/or acetazolamide resulted in a complete suppression of the renal excretion for 8–24 h, depending on the amount of alkali ingested. Furthermore, the effect of sodium bicarbonate was terminated by the ingestion of ammonium chloride. Finally, they concluded that the doping control could lead to false negative results when fencamfamine was used in combination with alkalinizing agents.

Figure 4 (Subject 1) shows that after ingestion of acetazolamide the renal excretion was drastically lowered. Although in some cases the concentration dropped below the sensitivity limit for quantitative purposes, both fencamfamine and its metabolite could still be detected qualitatively, using the more sensitive g.l.c/e.c. method described herein. Generally, the ingestion of acetazolamide resulted in a lowered fencamfamine excretion which is most pronounced during the first 10 h. The period of 2-amino-3-phenylnorbornane suppression, however, could last from 4 to 22 h after the intake of acetazolamide. The influence of acetazolamide is shown in Table 3 where the excreted amount of fencamfamine and its metabolite during the first 12 h are compared with the analogous values under normal conditions.

The biological half-life of fencamfamine under both normal and acetazolamide conditions (Table 2) could be calculated as illustrated in Figure 5 (Subject 2). Indeed, it could be accepted that for the urinary excretion under acetazolamide conditions a distinction should be made between the effect phase and the post-effect phase. As the mechanism for the diuretic action of acetazolamide is based on the inhibition of carbonic anhydrase, resulting in a higher sodium bicarbonate excretion and hence an alkaline urine, it could be accepted that the effect phase coincides with the period of the higher urine pH values and that the post-effect phase starts with the normalization of the pH. With respect to our experiments this appears to be around 36 h. Calculations for the 9–36 h period under acetazolamide suppression gave a biological half-life from 23·8 to 90·6 h. Post-acetazolamide half-life values based on the excretion during the 36–96 h period were similar to the values under normal conditions as shown in Table 2.

Furthermore, with the exception of Subject 1, there were no meaningful differences between normal excretion and that under the influence of acetazolamide up to 78/80 h (Tables 1 and 4).

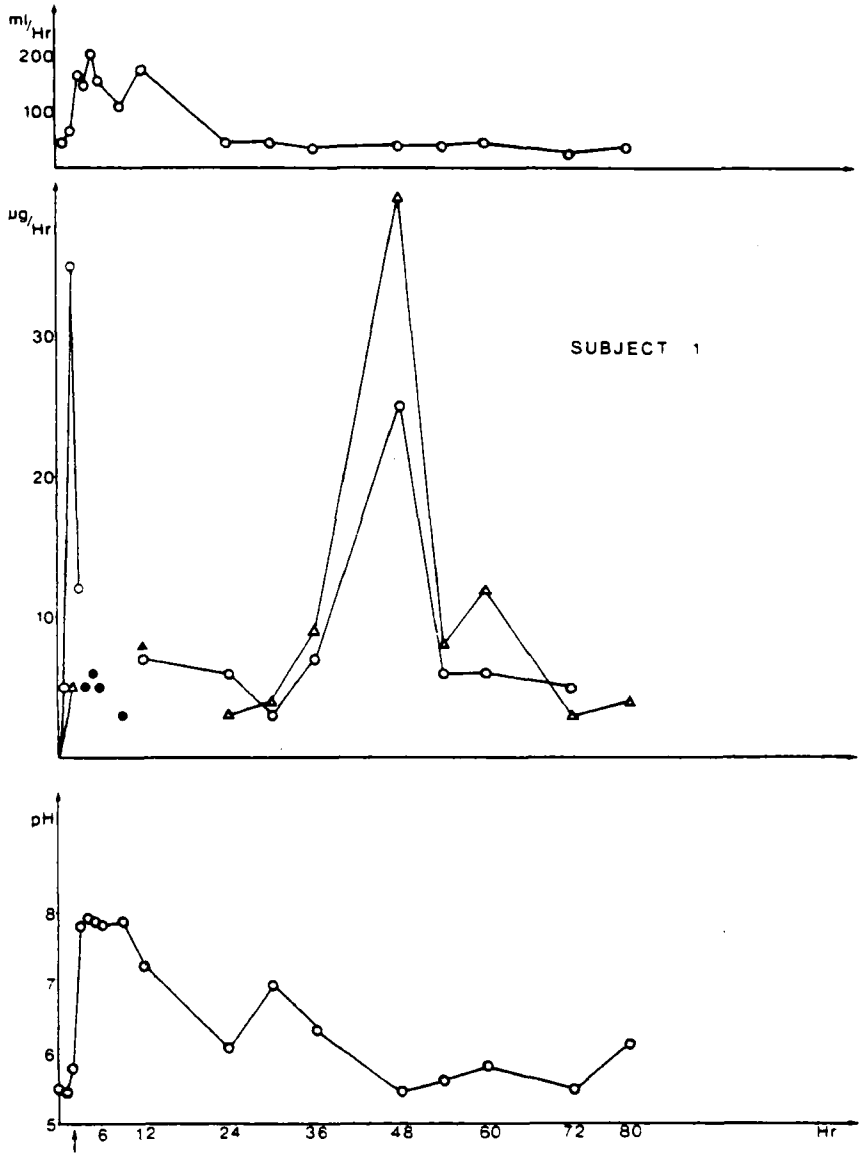


Figure 4. Urinary production (ml $h^{-1}$), urinary pH, and excretion (µg $h^{-1}$) of fencamfamine (○) and its desethylated metabolite (△) after the ingestion of acetazolamide (▲, ●: concentration close to the detection limit)

Finally, for all four subjects, more metabolite than fencamfamine was excreted after the ingestion of acetazolamide whereas this was not always the case under normal conditions. Tentatively, this could be explained by the fact that the higher reabsorption of the non-ionized lipophilic base under strong alkaline conditions

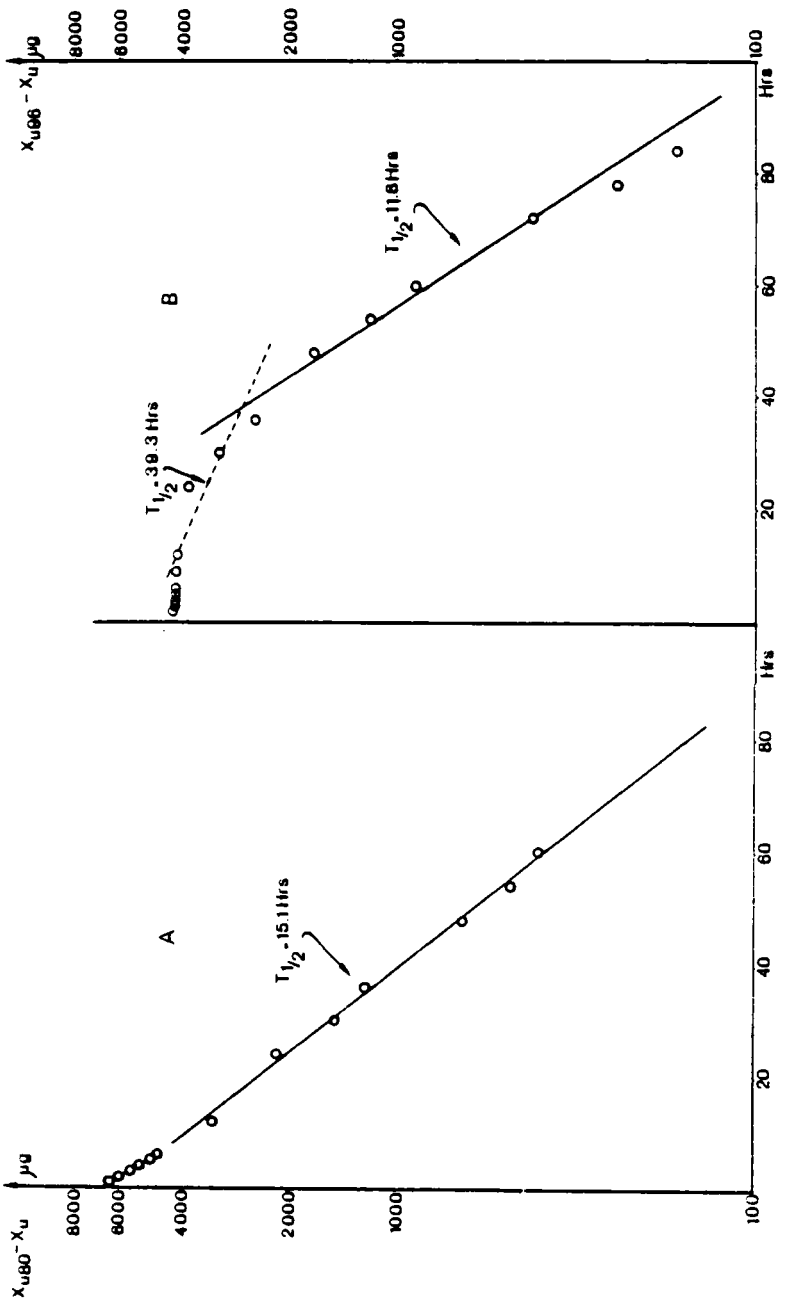


Figure 5. Biological half-life of fencamfamine in Subject 2 under (A) normal and (B) acetazolamide circumstances

Table 3. The excretion of fencamfamine and its desethylated metabolite (μg) during the period 0–12 h, under normal conditions and after the intake of acetazolamide

| | Subject | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 1 | 1 | 2 | 2 | 2 | 3 | 3 | 4 | 4 |
| Dose form | R | C | R | R | C | R | R | R | R | R |
| Fencamfamine | 372 | 313 | 100 | 3131 | 2348 | 100 | 1766 | 118 | 463 | 93 |
| Metabolite | 96 | 82 | 29 | 1155 | 630 | 108 | 174 | 46 | 115 | — |

R = REACTIVAN®; C = Capsules containing fencamfamine.

Table 4. Percentage of the dose excreted as fencamfamine and its metabolite after acetazolamide intake

| | Subject (period) | | | |
|---|---|---|---|---|
| | 1 (80 h) | 2 (78 h) | 3 (80 h) | 4 (78 h) |
| Fencamfamine (%) | 3·3 | 10·0 | 8·3 | 3·9 |
| Metabolite (%) | 4·5 | 18·3 | 8·8 | 6·1 |
| Total (%) | 7·8 | 28·3 | 17·1 | 10·0 |

could lead to a relatively higher metabolism of fencamfamine into 2-amino-3-phenylnorbornane, which at the same time is perhaps less sensitive to pH influences than the parent compound.

## STABILITY OF FENCAMFAMINE AND ITS METABOLITE IN URINE DURING STORAGE AT −18°

It has been claimed by some sportsmen, especially cyclists, that fencamfamine can be degraded in alkaline urine during storage, especially in the presence of acetazolamide. A study was undertaken to evaluate the stability of low amounts of fencamfamine and its metabolite during storage at −18°. Several urine samples were re-analysed after 3 and 6 weeks. Each urine sample was analysed six times and standard deviations calculated. Because of the $x$-intercept values of the regression equations it was not possible to evaluate very low concentrations correctly in some urine samples. In these cases therefore, only the peak ratio is given in Table 5. As can be seen from this table there were no differences in either fencamfamine or metabolite concentrations after storage of the urine for 3 or 6 weeks at −18°. Moreover, these values were not influenced either by the use of acetazolamide or by a slightly alkaline natural pH value (Subject 3).

With respect to doping analysis, some conclusions can be made from these results. Although the experimental conditions in this study were different from real sport circumstances, sports practitioners should be aware of the long biological half-life and thus the prolonged clearance time of fencamfamine.

Table 5. Concentration (μg ml$^{-1}$) of fencamfamine and its metabolite in urine after storage at −18° during 3 and 6 weeks

| Subject | Excretion Time | pH | Fencamfamine | | | Metabolite | | |
|---|---|---|---|---|---|---|---|---|
| | | | 0 | 3 weeks | 6 weeks | 0 | 3 weeks | 6 weeks |
| 1B | 12 | 6·96 | 0·20 ± 0·01 | 0·19 ± 0·02 | 0·20 ± 0·01 | 0·10 ± 0·004 | 0·10 ± 0·01 | 0·08 ± 0·01 |
| 2A | 60 | 6·05 | 0·22 ± 0·01 | 0·24 ± 0·01 | 0·22 ± 0·01 | 0·44 ± 0·06 | 0·47 ± 0·01 | 0·45 ± 0·02 |
| 2B | 60 | 6·92 | 0·12 ± 0·01 | 0·13 ± 0·01 | 0·13 ± 0·01 | 0·23 ± 0·02 | 0·24 ± 0·02 | 0·24 ± 0·02 |
| 3 | 6 | 7·87 | 0·51 ± 0·01 | 0·49 ± 0·02 | 0·49 ± 0·02 | 0·07 ± 0.004 | 0·06 ± 0·004 | 0·07 ± 0·001 |
| 4 | 9 | 6·77 | 0·86 ± 0·04 | 0·84 ± 0·04 | 0·86 ± 0·02 | 0·20 ± 0·01 | 0·18 ± 0·01 | 0·16 ± 0·01 |
| 1* | 36 | 6·32 | 0·21 ± 0·01 | 0·17 ± 0·02 | 0·24 ± 0·01 | 0·24 ± 0·02 | 0·21 ± 0·03 | 0·23 ± 0·01 |
| 2* | 24 | 6·97 | 0·23 ± 0·01 | 0·23 ± 0·02 | 0·24 ± 0·02 | 0·22 ± 0·02 | 0·24 ± 0·05 | 0·19 ± 0·01 |
| 3• | 71 | 5·61 | 0·57 ± 0·03 | 0·61 ± 0·02 | 0·57 ± 0·03 | 1·01 ± 0·05 | 0·92 ± 0·01 | 1·01 ± 0·07 |
| 4* | 5 | 7·74 | 0·13 ± 0·028† | 0·124 ± 0·019† | 0·129 ± 0·020† | — | — | — |

† Peak ratio.

Indeed, when analysed as already described, fencamfamine and/or its desethylated metabolite are detectable in urine for up to 80 h after administration of therapeutic doses. This will be even more true for doping practices where still higher doses are used. Although the total renal excretion for a period as long as 80 h after the administration was not influenced by the simultaneous ingestion of acetazolamide, the excretion during at least the first 10 h after the use of acetazolamide was drastically lowered. As for most kinds of sport urine samples for doping control are collected during this same period, special attention should therefore be paid to alkaline urine samples. In these cases the use of sensitive g.l.c. detection systems other than FID is recommended.

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