

Reprint from

RECENT ADVANCES  
IN DOPING ANALYSIS  
(10)

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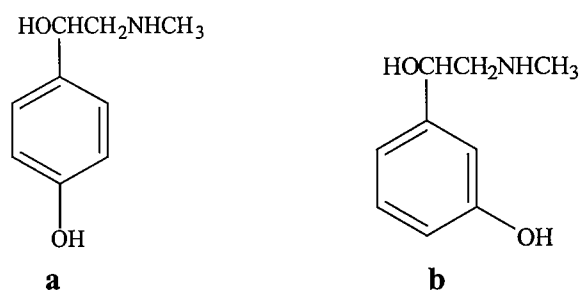
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Synephrine (Oxedrine): Analytical and Pharmacological Issues  
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## Synephrine (oxedrine): analytical and pharmacological issues

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### INTRODUCTION

Synephrine (oxedrine, **a**) is an adrenergic agent with vasopressor activity. Although it is not explicitly considered in the list of prohibited substances and methods, it can be considered a “related substance” to phenylephrine (m-synephrine **b**), that is instead specifically included in the list of examples of the Olympic Movement Antidopin Code [1].



**Figure 1**

Molecular structures of **a**: synephrine (oxedrine) and **b**: phenylephrine (m-synephrine)

Following the recent full reaccreditation of the laboratory of Rome, in the period March 2000 – February 2002 the presence of synephrine was suspected after the screening analysis in a considerable number of samples, but the following confirmation analysis gave negative results. An investigation on the possible causes of this phenomenon was therefore activated, the preliminary results of which are here presented and discussed. The study was structured as follows:

- evaluation of the possible contribution of each step of the pretreatment process of urine samples to the false positive “synephrine-like” signals detected by the screening analysis;

- direct analysis of herbal products, nutraceuticals, galenic preparations containing *Citrus aurantium* and common citrus fruits (in the form of freshly squeezed juices), for the possible presence of synephrine [2];
- preliminary excretion studies of synephrine following ingestion of *Citrus aurantium* extracts and of freshly squeezed orange juice;
- evaluation of the results.

## EXPERIMENTAL SECTION

### a) *Instrumental apparatus and reagents*

All GC-MS assays were performed on a Hewlett Packard 6890-5973 GC-MS-NCI system. All standards and reagents were supplied by Sigma Chemical Co., St Louis (MO, USA). All solvents were analytical grade.

### b) *Urine pretreatment*

enzymatic hydrolysis: 1.0 mL of urine, added with 25  $\mu$ L of a 200  $\mu$ g/ml methanolic solution of diphenylamine as internal standard, hydrolysis by beta-glucuronidase/aryl sulfatase from *H. pomatia* (20  $\mu$ L), liquid/liquid extraction with Et<sub>2</sub>O/tBuOH 9/1, derivatization with 20  $\mu$ L PFPA added with 1 drop of pyridine (incubation at T=70 °C for 40 min);

chemical hydrolysis: 5.0 mL of urine, added with 30  $\mu$ L of a 100  $\mu$ g/ml methanolic solution of bamethane as internal standard, extraction by C18 cartridges, hydrolysis by cysteine/HCl 1N, liquid/liquid extraction and derivatization with PFPA/pyridine as described above.

### c) *Analysis of food supplements, herbal and galenic preparations*

An aqueous solution containing 1  $\mu$ g/mL of the product under investigation was added with 30  $\mu$ L of a 100  $\mu$ g/ml methanolic solution of bamethane as internal standard, and extracted and derivatized according to the procedure described above for chemical hydrolysis;

### d) *Excretion studies*

herbal extract: one capsule of a herbal supplement containing 350 mg of *Citrus aurantium* (labeled to contain synephrine  $\geq$  6%) was administered orally to two female volunteers (age 32 and 31) and the urine was collected for 48 h after administration;

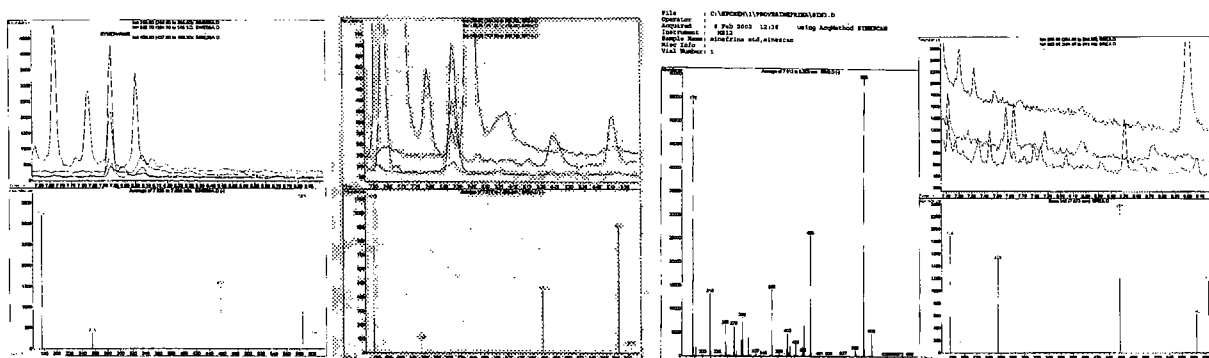
orange juice: four oranges were squeezed, the juice drunk by two female volunteers (age 32 and 30) and the urine was collected for 48 h after administration;

e) *GC-MS conditions*

Carrier gas: He; column: HP1 (cross-linked Methyl Siloxane capillary column, l=17 m; i.d. = 0.20 mm film thickness=0.11  $\mu\text{m}$ ); injector: T=260 °C, constant flow 1.2 mL/min, injection type: split 1:10. Oven temperature program: 4 min at T=100 °C, 27 °C/min to 140 °C, hold 2.5 min, then 35 °C/min to 290 °C, hold 1.5 min. Volume injected: 1  $\mu\text{L}$ . GC-MS-SIM acquisition was carried out on the following ions: m/z 176, 255, 458, 585, 605 for synephrine 3PPF; m/z 315 for the ISTD diphenylamine; and m/z 500 for the ISTD bamethane.

## RESULTS AND DISCUSSION

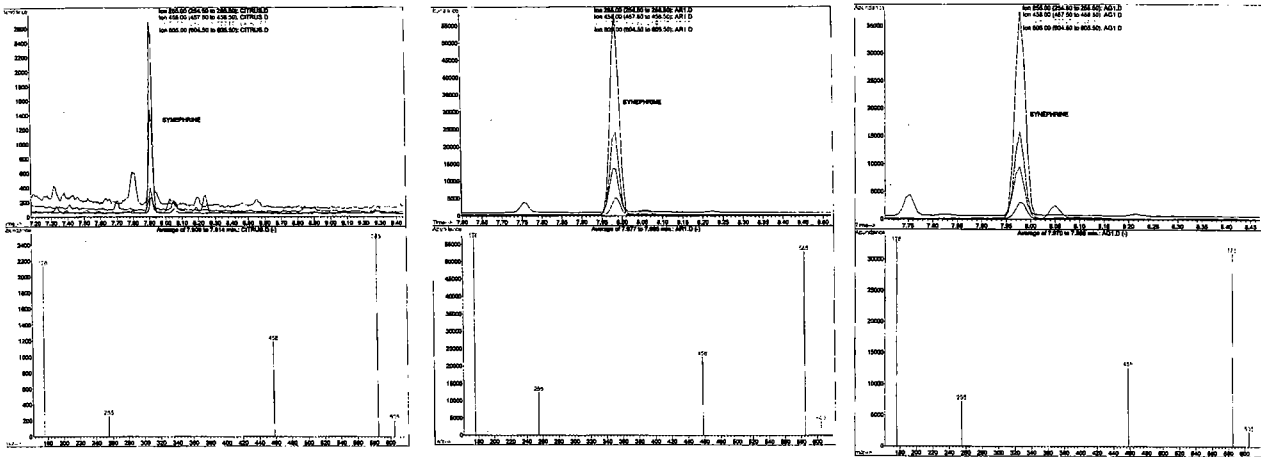
1. The study of the pretreatment procedure of the urine samples showed that some GC-MS signals mimicking the presence of synephrine could be formed following the enzymatic hydrolysis: in many instances, all the diagnostic ions of synephrine-3PPF, including the most abundant ones (m/z 585, 458, and 176), were detected, at the retention time of synephrine-3PPF, in the blank urine and in the reagent blank also; while the same peaks were not present following the chemical hydrolysis (by cysteine/HCl) of the urine extracts (see figure 2).



**Figure 2**

Comparison of the GC-MS chromatograms of the positive reference urine (center-left: SIM; center-right: full scan), with those (SIM) of the reagent blank after enzymatic (far left) or chemical (far right) hydrolysis, at the retention time of synephrine (7.9 min).

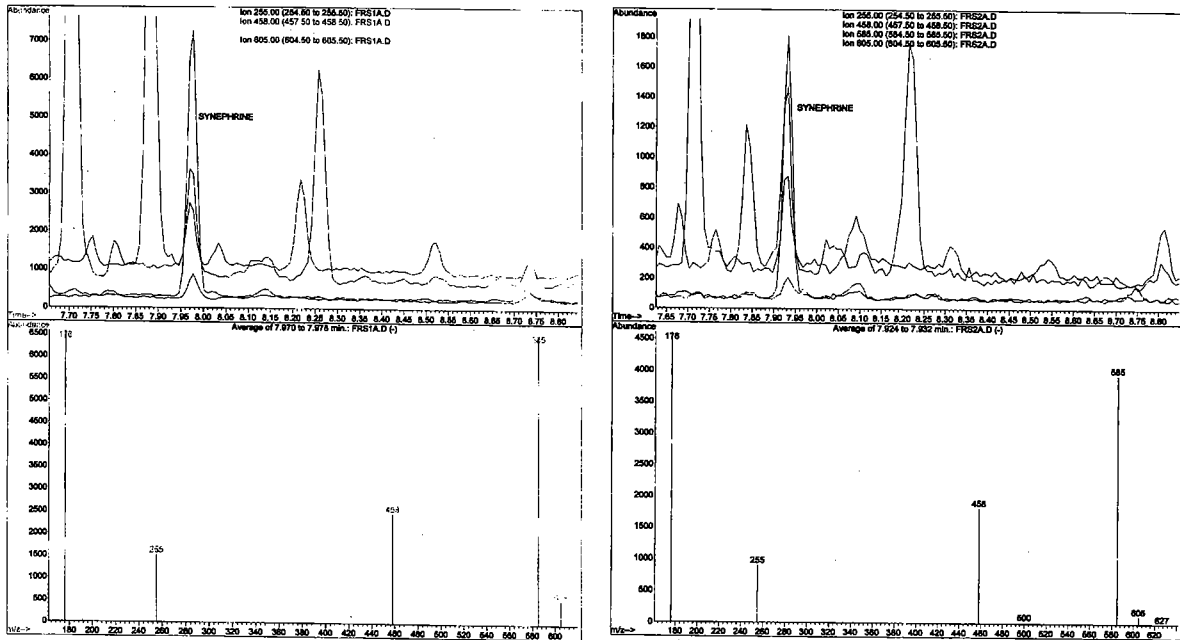
2. Some food supplements, herbal and/or galenic preparations containing extracts of "*Citrus aurantium*" may lead to a positive result, synephrine being present in concentrations  $\geq 6\%$ .
3. Detectable levels of synephrine were also measured in common citrus fruits (figure 3).



**Figure 3**

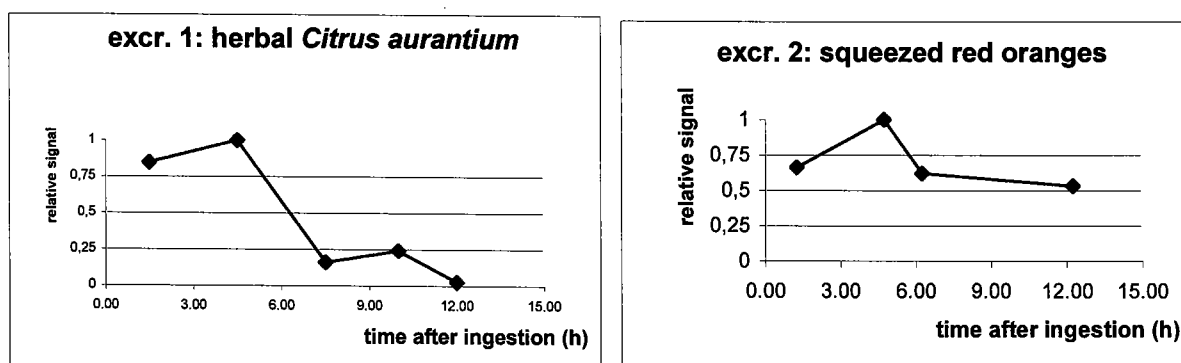
Extracted ion GC-MS chromatograms of an herbal preparation of Citrus Aurantium (left), and of freshly squeezed red oranges (center) and clementines (right).

- Synephrine was detectable in the urine for more than 12 h after a single administration, both of herbal extracts containing *Citrus aurantium* and of orange fruits (figures 4-5).



**Figure 4**

Synephrine signal in urine 5 h after the ingestion of herbal Citrus Aurantium (left) and of freshly squeezed red oranges (right).



**Figure 5**

Trend of the relative concentration of synephrine in urine as a function of time after the administration of herbal Citrus Aurantium (left) and of freshly squeezed red oranges (right).

## CONCLUSIONS

The use of beta-glucuronidase/aryl sulfatase (from *H. pomatia*) for the enzymatic hydrolysis of urine samples is not suitable for the analysis of synephrine: in many instances, the presence of three or more diagnostic ions of synephrine-3PPF was detected, at the same retention time, in the blank urine and in the reagent blank also: the hydrolysis of samples is now being carried out chemically, by cysteine/HCl.

The presence of synephrine in herbal preparations containing *Citrus aurantium* extracts, and, more importantly, in freshly squeezed citrus juices, was confirmed by direct analysis and by excretion studies on volunteers.

Currently in progress is a quantitation study aimed to verify whether it can be possible to fix a concentration threshold in order to preliminarily distinguish the assumption of synephrine with the food from the pharmacological administration of forbidden drugs.

## REFERENCES

1. International Olympic Committee. Olympic Movement Antidoping Code, Lausanne, 2001.
2. Y. Satoh, S. Tashiro, M. Satoh, Y. Fujimoto, J.Y. Xu, T. Ikekawa *Yakugaku Zasshi*. **116**: (1996) 244-50.