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Incorporation of acidic doping agents to routine screening procedures

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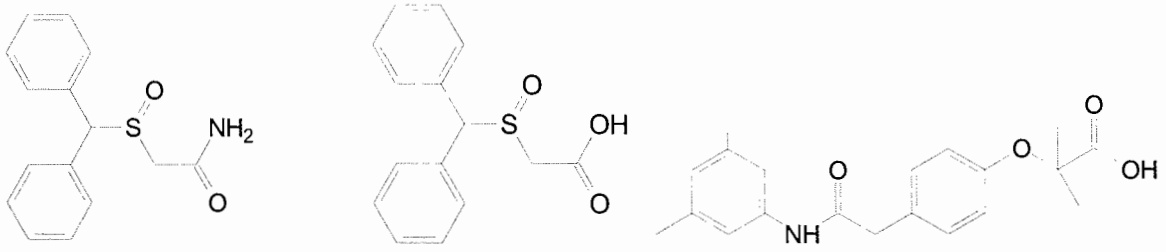
Introduction

Antidoping laboratories are continuously developing new methodologies for the detection of the administration of new substances.

Modafinil (Figure 1), a drug used in the treatment of narcolepsy, has been recently included in the list of prohibited substances as a stimulant. Modafinil is extensively metabolised and its metabolites (modafinilic acid and modafinil sulfone) are predominantly eliminated non-conjugated via urinary excretion [1]. Efaproxiral, also known as RSR13, (Figure 1) is a synthetic allosteric modifier of haemoglobin that might have potential as ergogenic agent due to an increase in muscle oxygen uptake. Its potential use has lead sport authorities to control its abuse and laboratories to include in their analytical procedures [2,3]. In this study, the incorporation of these acidic compounds to routine screening procedures for acidic doping agents such as diuretics is described.

Experimental

Sample preparation: 2.5 μg of the internal standard 7-propyltheophylline was added to 2.5 ml of urine. The urine sample was made alkaline by adding 100 μl of NH_4Cl/NH_3 buffer (pH 9.5) and extracted with 8 ml of ethyl acetate with salting-out effect (1 g of anhydrous NaCl). After mixing and centrifuging, the organic phase was separated and taken to dryness under a stream of nitrogen at 40ºC. Methyl derivatives were formed by redissolving the dry extracts with acetone containing methyl iodide and incubating at 60ºC for 180 min.



Modafinil Modafinilic acid Efaproxiral

Figure 1. Chemical structures of the compounds studied

Instrumental conditions: 2 µl of the extract were injected in a GC/MS system. Separation was performed using a cross-linked 5% phenylmethylsiloxane capillary column (12 m x 0.2 mm i.d., 0.3 µm film thickness, HP Ultra-2). The injection (280°C) was made in split mode (1/10) using He as carrier gas. The temperature column was set at 160°C, increased by 20°C/min to 300°C, and maintained at 300°C for 5 minutes. The interface was set at 280°C, the MS system operated with EI ionisation (70 eV) and acquisition was made in SIM mode.

Qualitative validation of the analytical procedure for modafinil and its acidic metabolite: selectivity and specificity (analysis of 5 blank urine), extraction recoveries (ER, comparison between 4 replicates of a urine spiked with 1 µg/ml of the analytes and 4 replicates of a blank urine extracted and spiked), limits of detection (LOD, lowest amount of the analytes that can be reliably detected) and intra-assay precision (RSD, relative standard deviation obtained for 4 replicates of a spiked urine analysed at the same day) were determined.

Excretion studies: a single oral dose of 100 mg of modafinil was administered. Urine samples were collected prior to the administration and 0-12, 12-24 and 24-32 hours after the administration. The World Association of Anti-doping Scientists (WAADS) provided a urine sample obtained from a clinical study containing 2 µg/ml of efaproxiral; this urine was further diluted with blank urine to a final concentration of 0.4 µg/ml of efaproxiral. All these urine samples were analysed using the method described above.

Results and discussion

Identification of modafinil and its acidic metabolite: the same artefact of modafinil and of its main metabolite modafinilic acid is detected. A chromatogram of a urine obtained after the

administration of a therapeutic dose of modafinil is shown in Figure 2. The characteristic ions of the artefact of modafinil and its metabolite are m/z 167, 152 and 115 (see Figure 3).

Qualitative validation of the analytical procedure for modafinil and its acidic metabolite:

	Selectivity/ specificity	LOD	ER (CV) (%)	Intra-assay precision	
				concentration	RSD (%)
Artefact obtained for modafinil	✓	0.2 µg/ml	86.9 (3.1)	0.2 µg/ml	12.7
				1 µg/ml	3.1
Artefact obtained for the acidic metabolite	✓	0.2 µg/ml	62.6 (5.9)	0.2 µg/ml	21.8
				1 µg/ml	5.9

Identification of efaproxiral: efaproxiral was detected as its methyl ester. Chromatograms of a blank urine and a urine sample obtained after efaproxiral administration are shown in Figure 4. EI mass spectrum of the derivative and the tentative fragmentation pattern is shown in Figure 5.

Conclusions

The results obtained in this work showed that the doping agents modafinil and efaproxiral can be included in a existing routine screening procedure. As one artefact is detected for modafinil and its acidic metabolite, this methodology cannot be used for confirmation purposes but its utility for detecting the drug several hours after the administration has been demonstrated. Efaproxiral was detected as the methyl ester derivative even several days after its administration. For confirmation of samples obtained after recent administration of efaproxiral, previous dilution of the urine may be appropriate to avoid any potential overloading.

References

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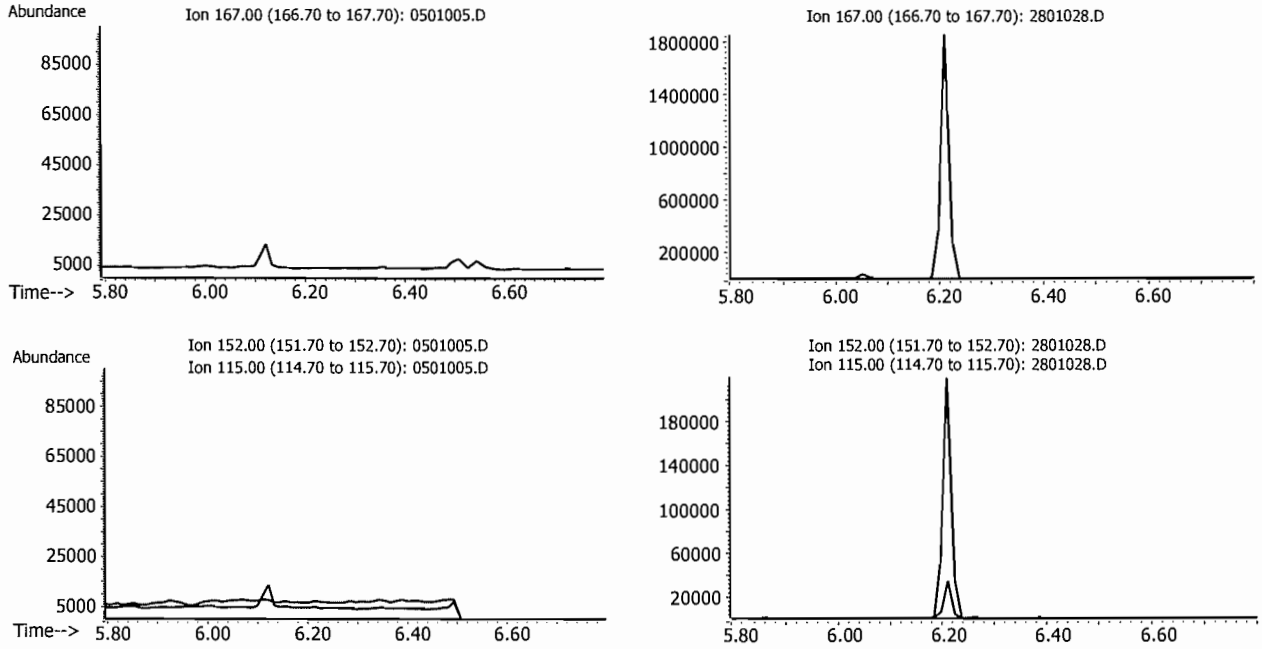

Figure 2. Chromatograms of the characteristic ions of the modafinil and modafinil metabolite artefact. Left: blank urine; right: urine sample collected 12-24 hours after the administration.

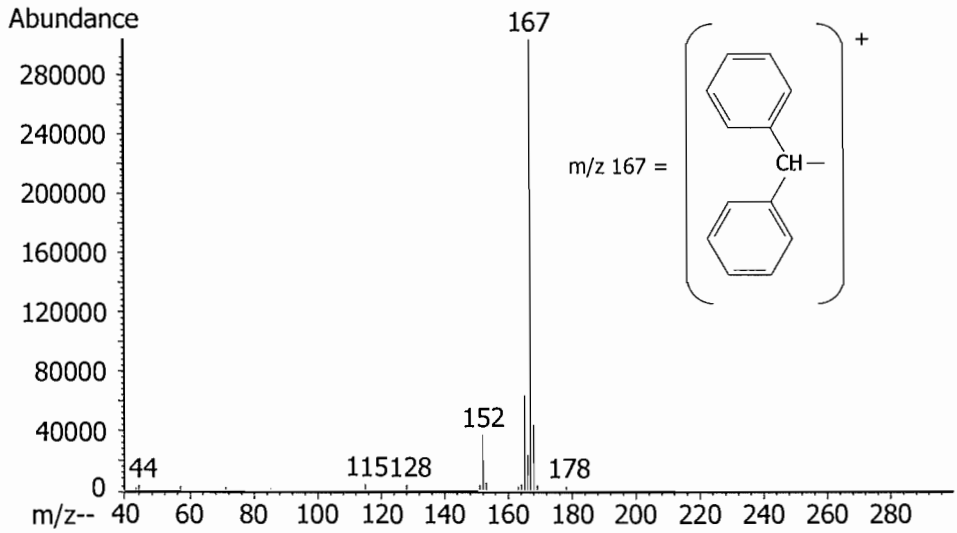

Figure 3. Electron impact mass spectrum of the modafinil and modafinil metabolite artefact.

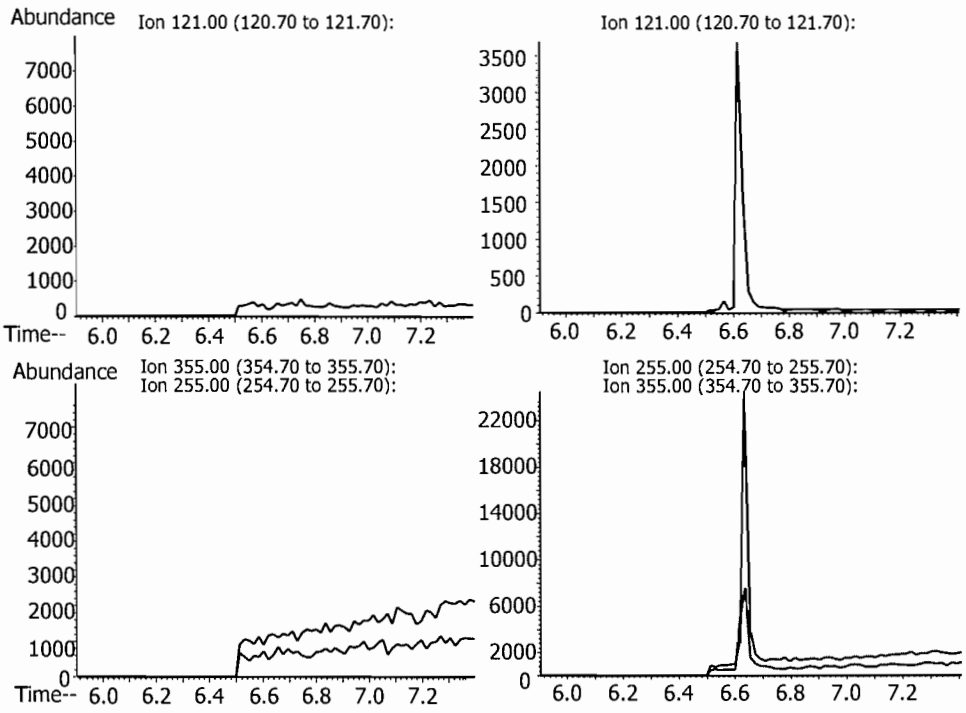

Figure 4. Chromatograms of the characteristic ions of efaproxiral methyl derivative. Left: blank urine; right: urine sample obtained from an excretion study of efaproxiral.

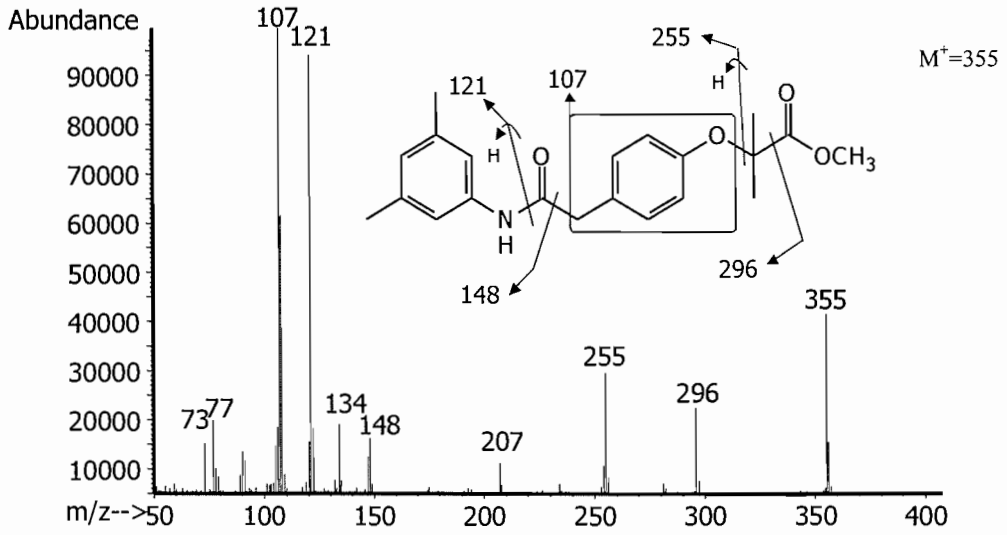

Figure 5. Electron impact mass spectrum and structure of efaproxiral methyl derivative with the proposed fragmentation pattern.

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