

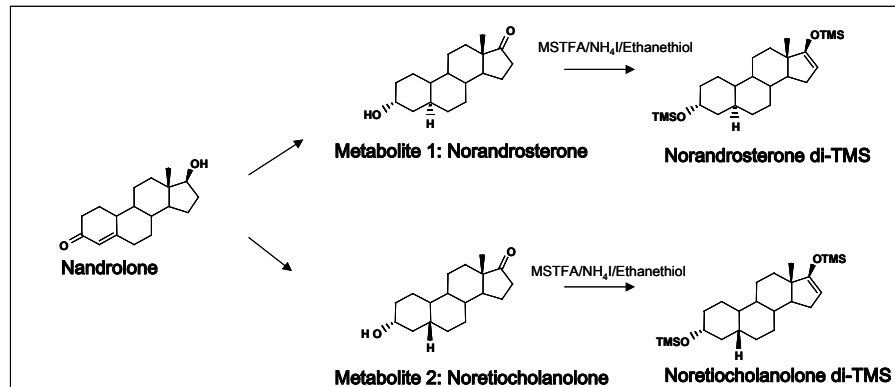
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## Concentrations of Nandrolone metabolites in urine after the therapeutic administration of an ophthalmic solution

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### 1. Introduction

Nandrolone, or 19-Nortestosterone, is an anabolic steroid initially introduced for the treatment of anemia, osteoporosis and breast carcinoma. Nandrolone is available in several pharmaceutical products as 17 $\beta$ -hydroxyester in an oily matrix or as a Nandrolone salt (decanoate or sodium sulfate) in an aqueous solution. The pharmaceutical formulation most widely used is Deca-Durabolin<sup>®</sup>, but other products, as Keratyl<sup>®</sup> eye drops solution, are also currently administered.



*Figure 1: Structure of the investigated compounds*

Nandrolone is one of the most abused anabolic steroid in various sports and the presence of its metabolites in urine at low concentrations is always submitted to discussion, because of possible endogenous production or intake of contaminated nutritional supplements [1-3].

Analyses for Nandrolone according to the World Anti-Doping Agency (WADA) protocol are based on the identification of the Nandrolone two main metabolites, which in humans are glucuronides of 19-Norandrosterone and 19-Noretiocholanolone. For the first and main metabolite, a limit of 2 ng/ml has been set by the anti-doping code.

During 2004, our laboratory has been involved in a Nandrolone positive case which leads to the investigation of the Keratyl® eye drops solution.

## **2. Experimental**

### **2.1. Standards and pharmaceutical**

19-Norandrosterone and 19-Noretiocholanolone (Nandrolone metabolites) were purchased by LGC-Promochem (France). Keratyl® eye drop solution (5 ml, 1%) was obtained from the CHAUVIN SA Laboratory (France) and is used to improve the cornea healing. 100 ml of the solutions contain 1 g of Nandrolone sodium sulphate. The recommended administration is 1 to 2 drops in each eye, 4 to 5 times per day and during 20 days.

### **2.2. Excretion study**

Keratyl® eye drops solution, containing Nandrolone sodium sulphate, was administered at therapeutic levels (2 drops in each eye, 4 times per day ) to a male volunteer (33 years old) during 3 days. All urine samples were collected during 15 days.

### **2.3. Urine extraction**

The extraction is performed with 5 ml of urine. 10 ng/ml of internal standard (Methyl testosterone) is added and the sample is passed through a SPE-C18 column. After enzymatic hydrolysis (E. Coli) and extraction with *n*-pentane, the residue is derivatized with MSTFA/NH<sub>4</sub>I/Ethanol to obtain the TMS-form of Nandrolone metabolites.

### **2.4. Instrumentation and analytical method**

The urinary extracts were both analysed by GC-MS and CG-MS<sup>2</sup> for low concentrations.

#### *GC-MS parameters:*

Analyses were performed by GC-MS with SIM (quantification) and SCAN (identification) modes on a Hewlett-Packard 6890 gas chromatograph (HP Analytical Division, Waldbronn, Germany) and coupled with a HP 5973 mass selective detector (MSD). GC separation was achieved on a ZB-5 (5% phenyl-95% dimethyl-polysiloxane (Phenomenex, St. Torrance, CA, USA) column (15 m x 0.25 mm I.D., 0.25 µm film thickness). Temperature programming: 150°C for 1 min, ramped at 20°C/min to 300°C and held for 2 min. Injections of 1 µl-samples were made at 270°C in the splitless mode.

#### *GC-MS<sup>2</sup> parameters:*

The gas chromatograph was a TRACE GC 2000 series (Thermo Quest, Italy). The GC system was interfaced to a Finnigan GCQ<sup>TM</sup> Polaris ion trap mass spectrometer (USA). Chromatographic separation was performed by using a capillary column (DB-XLB; column length 15 m x 0.25 mm with a 0.25 µm film thickness) from J&W Scientific (Agilent Technologies, USA). The GC temperature program was as follows: the initial temperature was 150 °C for 1 min, then increased with a temperature program of 25 °C/min to a temperature of 300 °C which was held for 4 min. Samples (1 µl) were injected in the splitless mode. The injector temperature was set at 270 °C. The MS instrument was operated in the electron impact ionisation mode at 70 eV and product ion scan was used as detection mode. For collision induced dissociation in MS<sup>2</sup>, helium was used as collision gas. Other MS<sup>2</sup> instrumental conditions for the detection of Nandrolone metabolites: precursor ion m/z 405, q-value = 0.225, 1.40 V excitation voltage, 15 ms excitation time, 8 ms isolation time.

### 3. Results and discussion

#### 3.1. Analytical investigation

Excretion samples were analyzed for quantification purpose together with a calibration curve established with 19-Norandrosterone and 19-Noretiocholanolone spiked urines at concentrations between 2 ng/ml and 500 ng/ml. For Nandrolone metabolites concentrations superior to 10 ng/ml, analyzes were performed by GC-MS and for concentrations inferior to 10 ng/ml, analyzes and identification were performed by GC-MS<sup>2</sup>. Each concentration was corrected with specific gravity.

#### 3.2. Excretion study

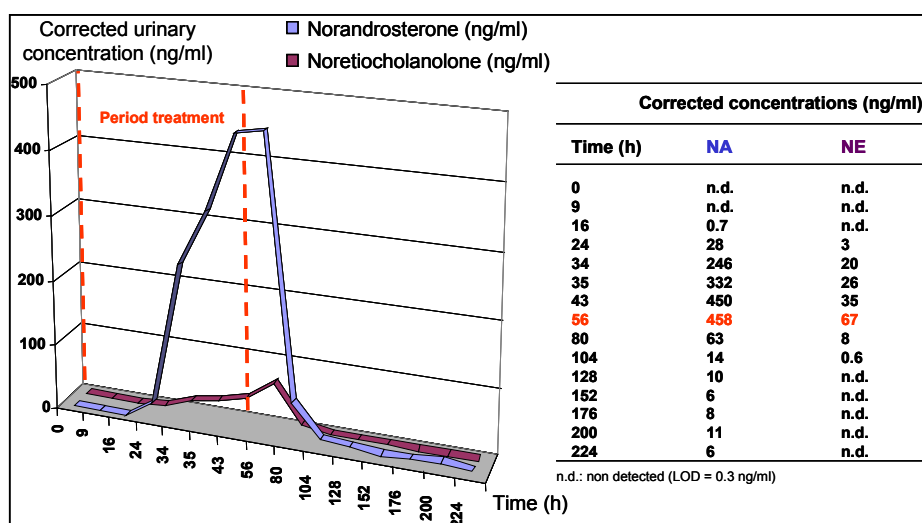


Figure 2: Excretion kinetics for Nandrolone metabolites

After eye drops solution administration, Nandrolone metabolites are excreted since 16 hours and 24 hours for 19-Norandrosterone and 19-Noretiocholanolone, respectively. It can be noticed that, from these preliminary results, the excretion peaks (56h) take place later than after oral or intravenous administrations [4].

In this study, a cumulative phenomenon was observed during the 3 days of treatment, as the excreted amounts increased until the last day of administration. Surprisingly, contrary to all expectations, the concentrations measured in urines reached 450 ng/ml and 70 ng/ml for 19-Norandrosterone and 19-Noretiocholanolone, respectively. Concentrations near 2 ng/ml were found 10 to 15 days after the last administration. In all cases, the excreted amounts of 19-Norandrosterone are clearly superior to that of the second metabolite.

#### **4. Conclusion**

The results of this study, contrary to all expectations, were very instructive as it has been demonstrated that the administration of a Nandrolone containing ophthalmic solution can lead to positive urines. This is a preliminary study and it would be interesting, in the future, to investigate such pharmaceutical with several volunteers.

In the context of this study in relation with a positive Nandrolone case, it appears that these kind of pharmaceuticals is often considered as “harmless medication”. We had the experience that some representatives of medical profession are not aware that such products can lead to a positive urine, even several days after the last administration and do not warn athletes against using this kind of medication.

#### **5. References**

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- [2] N. Baume, L. Avois, PE. Sottas, J. Dvorak, M. Cauderay, P. Mangin, M. Saugy, *Clin. J. Sport Med.*, **15** (2005) 158-166.
- [3] N. Baume, N. Mahler, M. Kamber, P. Mangin, M. Saugy, *Scan. J. Med. Sci. Sport*, (2005) In press.