I - 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 formulations as 17β-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®. But other products, as Keratyl® eye drops solution, are also currently administered.

II - EXPERIMENTAL CONDITIONS
II.1 - Keratyl® product
Keratyl® eye drops solution (5 ml, 1%) was obtained from the CHAUVIN SA Laboratory (France). This ophthalmic solution is used to improve the cornea healing and 100 ml of the solution contains 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.

II.2 - Urine extraction
The extraction is performed with 5 ml of urine. 10 µg/ml of internal standard (methyltestosterone) 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/NH4/Ethanol to obtain the TMS-form of nandrolone metabolites.

II.3 - Analytical equipments and conditions
The urinary extracts were both analyzed by GC-MS and GC-MS² for low urinary concentrations.

GC-MS parameters:
Column: ZB-5 capillary column, film thickness 0.25 µm, ID 0.25 mm, length: 15 m
Carrier gaz: Helium, pressure 8.6 Psi, splitless
Injector temperature: 270°C
Emission: 230°C, MS source: 150°C
Oven: 1 min 150°C, 20°C/min up to 300°C, 2 min.

GC-MS² parameters:
Ion trap (GCO Plus-Finnigan)
Electron impact ionisation: 70 eV.
Column: DB-5 capillary column, film thickness 0.25 µm, ID 0.25 mm, length: 15 m
Carrier gaz: Helium, pressure 8 Psi, splitless.
Injector temperature: 270°C
Emission: 230°C, MS source: 150°C
Oven: 1 min 150°C, 25°C/min up to 300°C, 1 min.
Precursor ion: 405, q value: 0.225, excitation voltage: 1.4 eV, scan time: 0.48 sec.

III - ANALYTICAL INVESTIGATION
Excretion samples were analyzed for quantification purpose together with a calibration curve established with 19-nortestosterone and 19-noretiocholanolone spiked urines at concentrations between 2 ng/ml and 500 ng/ml. For nandrolone metabolites concentrations superior to 10 ng/ml, analyses were performed by GC-MS² and for concentrations inferior to 10 ng/ml, analyses and identification were performed by GC-MS. Each concentration was corrected with specific gravity.

IV - 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.

V - 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.

Figure 1: Structure of the investigated compounds

Figure 2: Photo of Keratyl® pharmaceutical

Figure 3: Mass spectra of 19-Norandrostenedione obtained by A) GC-MS and B) GC-MS²

Figure 4: Excretion kinetics for nandrolone metabolites