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

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Abstract

Nandrolone, an anabolic steroid, is used for the treatment of several diseases and is available in various pharmaceutical formulations. The most widely used pharmaceutical formulation is Deca-Durabolin®, but other products, such as Keratyl® eye drops solution, are also currently administered.

Nandrolone is one of the most abused anabolic steroid in sports. Analyses for this anabolic steroid according to the World Anti-Doping Agency (WADA) protocol are based on the identification of the nandrolone two main urinary metabolites which, in humans, are glucuronides of 19-norandrosterone and 19-noretiocholanolone. A positive cut off limit of 2 ng/mL has been set by the anti-doping code for the first metabolite, 19-norandrosterone.

In this preliminary study, an eye drops solution (Keratyl®) containing a therapeutic dose of a nandrolone sodium sulphate was administered to several male volunteers during 3 days and urines were collected during 3 weeks. Surprisingly, contrary to all expectations, the urinary concentrations measured in urines reached 450 ng/mL and 70 ng/mL for norandrosterone and noretiocholanolone, respectively. Moreover, concentration levels near to 2 ng/mL were found, more than 2 weeks after the last administration, depending on individual metabolism. Inter-variability as well as intra-variability of nandrolone excretion kinetic, regarding this particular administration mode, were also evaluated.

Quantification of nandrolone metabolites was performed by GC–MS. The method was previously validated in terms of specificity, precision, linearity, LOD, LOQ, robustness, accuracy and the expanded uncertainty was also evaluated.

Keywords: Nandrolone; Anabolic steroids; Urine; Doping; Ophthalmic solution; GC–MS

1. Introduction

Nandrolone, or 19-nortestosterone, is an anabolic steroid initially introduced for the treatment of anemia, osteoporosis and breast carcinoma [1]. Nandrolone is available in several pharmaceutical products as 17β-hydroxyester in an oily matrix or as a nandrolone salt (decanoate or sodium sulfate) in an aqueous solution. The most widely used pharmaceutical formulation is Deca-Durabolin®, but other products, such as Keratyl® eye drops solution, are also currently administered.

Nandrolone is one of the most abused anabolic steroid in sports and the presence of its metabolites in urine at low concentrations is always subject to discussion, because of a possible endogenous production or intake of contaminated nutritional supplements [2–7].

Analyses for nandrolone according to the World Anti-Doping Agency (WADA) [8] protocol are based on the identification of nandrolone two main metabolites, which in humans are glucuronides of 19-norandrosterone (NA) and 19-noretiocholanolone (NE) (Fig. 1). For the first and main metabolite, a cut off limit of 2 ng/mL has been set by the anti-doping code.

Recently, the laboratory was involved in a Nandrolone positive case which led to preliminary investigations on metabolism and excretion kinetic of the Keratyl® eye drops. The pharmaceutical solution was administered to several volunteers and the amounts excreted were evaluated in order to assess the inter-individual variability in excretion kinetic and pattern. The intra-individual variability was also evaluated with one volunteer which repeated the Keratyl® administration at 6-months interval. The dosage of nandrolone metabolites was performed

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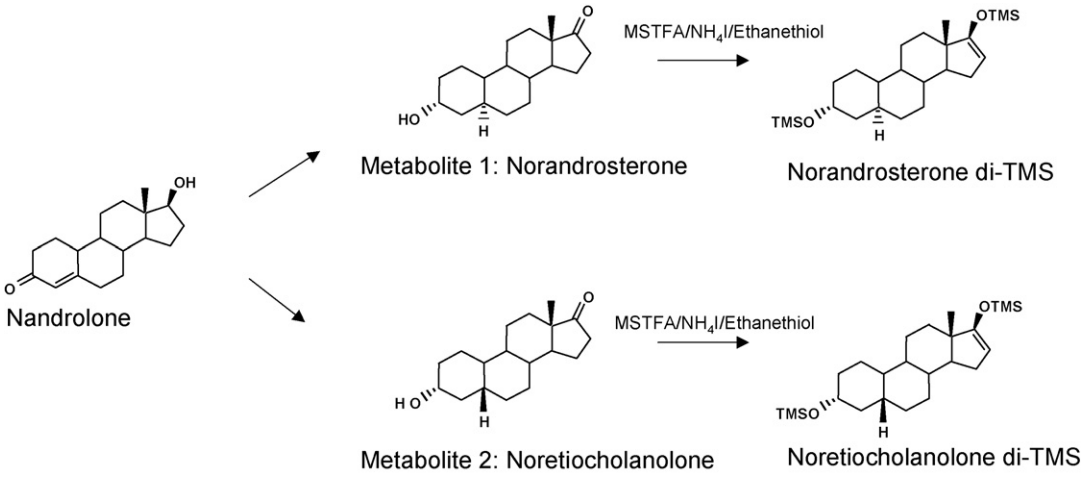

Fig. 1. Structure of investigated steroids.

by GC–MS with a method which has been previously validated in terms of specificity, precision, linearity, limits of detection and quantification (LOD and LOQ, respectively), robustness and accuracy. In this context, the expanded uncertainty of the overall method was also determined.

2. Experimental

2.1. Equipment

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 J & W Scientific HP-1 capillary column (100% dimethylpolysiloxane, 25 m × 0.2 mm I.D., 0.11 μm film thickness) from Agilent Technologies (Basel, Switzerland). Temperature programming: 180 °C initial temperature, ramped at 3 °C/min to 230 °C, ramped at 40 °C/min to 310 °C and held for 2 min. At these conditions, the retention time for norandrosterone, noretiocholanolone and metyltestosterone (IS) was 13.8 min, 14.4 min and 18.4 min, respectively.

Samples (1 μL) were injected in the splitless mode. The injector temperature was set at 270 °C. Helium was used as carrier gas at a flow rate of 1 mL/min. The transfer line, quadrupole and ion source temperatures were set at 180 °C, 150 °C and 230 °C, respectively. The MS instrument was operated in the electron impact ionization mode at 70 eV. In SCAN mode, the scan range was *m/z* 50–650 with a scan rate set at 2.48 scan/s. In SIM mode (dwell times at 30), the diagnostic ions were *m/z* 225, 315, 420 and 405 for nandrolone metabolites and *m/z* 301 and 446 for methyltestosterone (IS). Ions *m/z* 405 and *m/z* 301 were selected for quantification purpose, respectively. In all cases, the MS acquisition was started at 10 min.

2.2. Materials and reagents

All chemicals were of analytical grade: *n*-pentane was purchased by Fluka Chemie (Buchs, Switzerland). Sodium carbonate (Na_2CO_3) was obtained from Merck (Darmstadt, Germany) and sodium hydrogen carbonate ($NaHCO_3$) form Acros (Geel, Belgium). *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide (MSTFA) was provided by Macherey-Nagel (Düren, Germany), ammonium iodide (NH_4I) from Sigma (St. Louis, MO, USA) and Ethanethiol from Fluka Chemie (Buchs, Switzerland). *E. coli* β-glucuronidase was purchased by Roche Diagnostics (Mannheim, Germany). 19-norandrosterone (NA) and 19-noretiocholanolone (NE) (nandrolone metabolites) were purchased by LGC-Promochem (France), whereas methyltestosterone was provided by Sigma (St. Louis, MO, USA). Bakerbond SPE-C18 cartridge (500 mg/6 mL) were obtained from J.T. Baker (Deventer, Holland).

Keratyl® eye drops solution (5 mL, 1%) was purchaised by CHAUVIN SA Laboratory (France) and is used to improve the cornea healing. 100 mL of the solution contain 1 g of Nandrolone sodium sulphate. The recommended administration is 1–2 drops in each eye, 3–5 times per day and during 20 days.

2.3. Excretion study

Keratyl® eye drops solution, containing Nandrolone sodium sulphate, was administered at therapeutic levels (2 drops in each eye, 3 times per day during only 3 days) to four male volunteers aged between 24 and 50, during 3 days. The administration was regularly done by each volunteer, between 6 and 8 a.m., between 1 and 3 p.m. and between 7 and 9 p.m. All urine samples were collected during 2 weeks after the first administration, whereas only the first urine in the morning was collected during the third week. One of the volunteers performed twice the same protocol, at 6 months interval in order to have an idea of the intra-variability of the excretion kinetic and urinary concentrations of nandrolone metabolites.

2.4. Nandrolone metabolites isolation from urine

The extraction, was performed with 5 ml of urine. 10 ng/mL of internal standard (methyl testosterone) was added and the sample was passed through a SPE-C18 column. The residue

was then added with 1 mL phosphate buffer (0.2 M, pH 7.0) and 70 μL β-glucuronidase from *E. coli* (200 U/mL specific activity) for the enzymatic hydrolysis during 1 h at 50 °C.

After addition of approximately 200 mg of solid carbonate buffer (Na_2CO_3/$NaHCO_3$, 1/10), the sample was extracted with 5 mL *n*-pentane by shaking during 10 min. After centrifugation (2500 × *g* for 5 min), the organic phase was collected, dried with Na_2SO_4 and the residue was derivatized with 50 μL MSTFA/NH_4I/ethanethiol (10:0.010:0.015, v/w/v) during 30 min at 60 °C. Similar methods are currently used by the whole anti-doping laboratories for the extraction of anabolic steroids from urine [9–14].

2.5. Analytical method performance

The validation was carried out in a similar way to what is generally adopted in analytical chemistry fields: procedures generally require the assessment of migration time and peak area precision, detector response linearity with sample concentration, specificity, sensitivity and accuracy [15–21]. In this study, the analytical method for qualitative and quantitative analysis of NA and NE by gas chromatography–mass spectrometry (GC–MS) was validated in terms of specificity, sensitivity, precision, linearity and accuracy. The robustness of the extraction procedure and the potential sources of variability on the analytical method were also evaluated and expressed as expanded uncertainty of the method [16,17,22].

3. Results and discussion

3.1. Validation study

3.1.1. Specificity

Samples containing single analytes in methanol were derivatized and analysed by GC–MS in full scan mode for spectral characterization. Ions chosen for the identification of nandrolone metabolites were *m/z* 225, 315, 420 and 405. For each urine specimen containing nandrolone metabolites, those ions and their relative ratios were systematically established and compared with methanolic standards. Elsewhere, several negative urines from the volunteers were also investigated and in all cases, no interference from the urinary matrix was observed. The ion chosen for quantification was *m/z* 405 and was determined in selected ion monitoring mode.

3.1.2. Precision

Instrument precision was determined by measuring repeatability (intra-day precision) and intermediate precision (between-day precision) of relative migration times and normalized peak areas for each compound. In order to evaluate the instrumental performance regarding the injection, replicate injections ($n = 6$), of an urinary sample spiked with nandrolone metabolites at the concentration set by WADA (2 ng/mL) and methyltestosterone used as internal standard (IS) at 10 ng/mL, were carried out. In Table 1, relative standard deviations (R.S.D.) are given for migration time and peak area ratio. In all cases, repeatability was better than 1% for the migration time and 2% for the peak area ratio. The intermediate precision was evaluated over 3 days by performing six successive injections daily. Results (Table 1) show that R.S.D. values are in this case slightly superior than those obtained for repeatability.

Table 1
Precision of GC–MS analysis expressed as R.S.D. values for relative migration time and peak area ratio

	Norandrosterone (%)	Noretiocholanolone (%)
Repeatability		
Relative migration time[a]	0.04	0.05
Peak area ratio[b]	0.99	1.88
Intermediate precision		
Relative migration time[a]	0.04	0.06
Peak area ratio[b]	4.90	5.30

[a] The relative migration time is defined as the analyte migration time divided by the internal standard (methyltestosterone) migration time.
[b] The peak area ratio is defined as the analyte peak area divided by the internal standard (methyltestosterone) peak area.

3.1.3. Linearity

Detector response linearity was evaluated during one year by preparing calibration curves ($n = 10$) with five NA and NE spiked urines covering the concentration range 1–50 ng/mL. Each sample was injected in duplicate. Regression curves were obtained by plotting peak area ratios (analyte peak area divided by internal standard area) versus concentration, using the least square method. In all cases, the correlation coefficient R^2 (Table 2) was superior to 0.99 and was improved by using peak area ratios. Each time, quality controls (NA spiked urines at 2 ng/mL and 5 ng/mL) were analysed and the concentrations obtained from each dosage are listed in Table 2. The relative standard deviation calculated for each quality control was equal to 5.1% and 2.7%, respectively. It has to be noted that, in this study, regression curves were used for the quantification of relatively low concentrations of labelled nandrolone metabolites (between

Table 2
Regression data obtained during one year for norandrosterone

Run	Regression parameters		Dosage	
	Equation	R^2	QC (2 ng/mL)	QC (5 ng/mL)
1	$y = 0.040x + 0.006$	0.9976	2.06	4.71
2	$y = 0.043x + 0.008$	0.9986	1.98	4.90
3	$y = 0.036x + 0.036$	0.9990	1.83	5.17
4	$y = 0.040x + 0.001$	0.9993	2.00	5.01
5	$y = 0.043x + 0.014$	0.9972	1.81	4.98
6	$y = 0.078x + 0.010$	0.9985	2.06	4.96
7	$y = 0.046x + 0.025$	0.9898	1.90	4.84
8	$y = 0.043x + 0.004$	0.9949	2.06	4.93
9	$y = 0.037x + 0.001$	0.9994	2.07	4.78
10	$y = 0.034x + 0.028$	0.9997	2.07	5.04
Mean ($n = 10$)		0.9974	1.99	4.93
S.D.[a] (ng/mL)		0.0030	0.10	0.13
R.S.D.[b] (%)		0.3	5.08	2.68

[a] S.D. is the standard deviation.
[b] R.S.D. is the relative standard deviation.

1 and 50 ng/mL), whereas, higher concentrations (superior to 50 ng/mL) were calculated by mean of three calibration points at 100, 300 and 500 ng/mL introduced in every extracted batch (one point calibration).

3.1.4. Detection limit and quantification limit

The limit of detection (LOD), defined as the lowest concentration of analyte that can be detected above the baseline signal, is estimated as three times the signal to noise ratio. The LOD for NA and NE was determined by analysing spiked urines with decreasing concentrations of nandrolone metabolites. The estimated limit of detection was determined as equal to 0.1 ng/mL, giving a limit of quantification equal to 0.3 ng/mL for both compounds. Between 1 and 50 ng/mL, urinary concentrations were calculated with a calibration curve, whereas, for lower concentrations, an estimation was performed with the calibration point at 1 ng/mL.

3.1.5. Accuracy (recovery method)

In order to evaluate the accuracy of the method for the dosage of nandrolone metabolites, inter-laboratory tests are regularly performed and the obtained results were evaluated (results for norandrosterone shown in Table 3). Indeed, no certified urines, containing known amounts of nandrolone metabolites, are available. Consequently, since several years, the World Association of Anti-Doping Scientists (WAADS) and more recently the World Anti-Doping Agency (WADA) frequently organize proficiency tests to allow anti-doping laboratories to evaluate the accuracy of their quantification method. Each laboratory is provided with the same urine sample for the dosage. Regarding nandrolone, proficiency tests are applied to norandrosterone which is the main nandrolone metabolite and for which a cut off limit of 2 ng/mL has been fixed by the antidoping code for male and female athletes. For each proficiency test, statistical evaluations are performed and each laboratory is informed of its accuracy performance. For all tests performed by our laboratory since 1999, the obtained results were similar to the scope values, which demonstrates the good accuracy of the used method and are in accordance with the required performances for dosage in biological matrices (Table 3).

3.1.6. Robustness

Since few years and in various domains, the robustness of a method (extraction, analysis) has become an important parameter to consider for method validation. According the International Conference on Harmonization (ICH) guidelines [16,17], robustness is defined as the capability of a procedure to remain unaffected by small but deliberate variations in the method parameters (nominal value ± 10%). Consequently, for nandrolone extraction, the most relevant parameters, which could affect analytical performances, were individually examined: volume of β-glucuronidase, temperature and length of enzymatic hydrolysis, *n*-pentane liquid–liquid extraction length, volume of MSTFA/TMSI/DTE, temperature and length of derivatization. Each experiment test was performed in triplicate. Consequently, small changes in the main extraction parameters did not drastically affect the analytical result, as the most important relative standard deviation (R.S.D.) calculated was equal to 8.5% for peak area ratios (obtained during the variation of the derivatization reactive volume). Moreover, 20 aliquots of the same urine spiked with NA and NE at 2 ng/mL were simultaneously extracted by several technicians at the stated conditions. For all these extracts, repeatability of the overall extractions was better than 10% for the peak area ratio. Consequently, all these results show that the extraction method used for nandrolone metabolites dosage is robust.

3.1.7. Expanded uncertainty

More recently, anti-doping laboratory have been asked by WADA and ISO guidelines to evaluate the expanded uncertainty of their quantification methods. Then, the overall uncertainty of the method for the dosage of Nandrolone metabolites has been calculated, based on the evaluation of the variability of the urine sample preparation, the standards preparation, the reproducibility of the extraction and the precision of the analytical procedure. For nandrolone metabolites, the total uncertainty is expressed, such as given below:

$$U_{\text{total}} = \sqrt{(U_{\text{sample}}^2 + U_{\text{std}}^2 + U_{\text{extraction}}^2 + U_{\text{precision}}^2)} = 21\%$$

were U_{sample} is the uncertainty related to the preparation of the urinary sample, U_{std} uncertainty related to the preparation of the

Table 3
Accuracy data for norandrosterone dosage (inter-laboratory tests)

Inter-laboratory test	Involved laboratories		Norandrosterone dosage		
	n	Scope (ng/mL)[a]	Amount found (ng/mL)[b]	S.D. (ng/mL)[c]	Recovery (%)[d]
Test 1 (1999)	21	9.7	10.2	0.40	103
Test 2 (2001)	24	3.4	3.2	0.03	94
Test 3 (2002)	25	4.2	4.4	0.05	105
Test 4 (2003)	29	4.3	4.6	0.10	107
Test 5 (2004)	31	6.1	6.7	0.10	110
Test 6 (2005)	33	8.4	8.6	0.20	102

[a] The scope is defined as the mean value calculated on the mean values obtained from all the laboratories involved in the study.
[b] Mean of three extracted aliquots, injected twice.
[c] S.D. is the standard deviation (three extracted aliquots, injected twice).
[d] Accuracy is determined as the recovery, calculated in %, between the obtained value (amount found) and claimed value (scope).

standard solutions, $U_{extraction}$ uncertainty related to the robustness of the extraction and $U_{precision}$ uncertainty related to the analytical precision.

For the sake of simplicity, detailed calculation for uncertainty of sample and standards preparation, as well as for robustness and analytical precision are not presented.

3.2. Analytical investigations

3.2.1. Nandrolone dosage in the ophthalmic solutions

Nandrolone, as the free base, was first quantified in all ophthalmic solutions in order to verify that the same pharmaceutical preparation, in terms of qualitative and quantitative aspects, was administered to each volunteer. Nandrolone *bis*-TMS derivative was analysed by GC–MS (*m/z* 194, 403 and *m/z* 418, the last ion being used for quantification purpose), after chemical hydrolysis (methanolysis) (see references [23] and [24]). All ophthalmic solutions contained the labelled 1% nandrolone.

3.2.2. Quantification of nandrolone metabolites in excretion studies

Excretion samples were analyzed for quantification purpose together with a calibration curve established with 19-norandrosterone and 19-noretiocholanolone spiked urines at various concentrations between 1 ng/mL and 50 ng/mL. For nandrolone metabolites concentrations inferior to 1 ng/mL or superior to 50 ng/mL, the estimated concentration was performed with a one point calibration at 1 ng/mL and by means of three calibration points at 100, 300 and 500 ng/mL introduced in every extracted batch, respectively. Urinary concentrations were corrected with specific gravity, in the case of specific gravity values greater than 1.020.

3.2.3. Inter-variability in excretion kinetic

Surprisingly, after the therapeutic eye drops solution administration, no metabolites were found in the urines of two volunteers (the Keratyl® administration was repeated twice), whereas great amounts (superior to 400 ng/mL, see Table 4) were measured in the urines of other volunteers. Consequently, nandrolone sulphate was checked in the urines of the two volunteers for which no nandrolone metabolites were found, in case of metabolism deficiency. No nandrolone free base was detected, as well.

Drug delivery in ocular therapeutics is a challenging problem [25]. First of all, the major problem is the attainment of an optimal drug concentration at the site of action. Poor bioavailbility of drugs from ocular dosage form is mainly due

Table 4
Excreted amounts of nandrolone metabolites (concentrations are corrected with specific gravity)

Volunteer 1			Volunteer 2 (test 1)			Volunteer 2 (test 2)		
Time (h)	NA (ng/mL)	NE (ng/mL)	Time (h)	NA (ng/mL)	NE (ng/mL)	Time (h)	NA (ng/mL)	NE (ng/mL)
0	n.d.	n.d.	0	n.d.	n.d.	0	n.d.	n.d.
8	4.2	n.d.	8	4.3	n.d.	9	n.d.	n.d.
13	16.7	0.6[a]	14	40.9	2.8	16	0.7[a]	n.d.
20	120.3	3.6	24	167.1	3.6	24	28.3	3.4
25	326.2	9.8	29	213.3	4.8	34	246.1	19.7
27	415.3	11.0	37	154.7	3.5	35	331.7	25.5
30	320.3	11.3	46	177.3	4.5	43	449.7	34.8
32	7.8	0.2[a]	54	221.6	3.5	56	458.1	66.9
35	95.6	2.2	56	94.0	1.8	59	349.5	57.2
43	192.8	4.8	61	66.7	1.3	65	267.8	34.9
47	293.8	7.6	63	252.1	4.3	69	181.7	26.6
53	69.1	1.5	70	242.0	3.7	73	93.5	13.7
56	202.0	3.7	78	148.9	2.8	80	62.9	7.4
67	132.2	2.6	89	103.5	2.3	104	13.8	0.6[a]
70	97.4	2.0	110	18.8	1.0	128	10.3	n.d.
95	75.8	2.3	143	14.6	n.d.	146	8.7	n.d.
116	17.5	0.5[a]	158	16.9	n.d.	152	6.2	n.d.
138	7.6	n.d.	184	9.4	n.d.	176	7.6	n.d.
166	8.7	n.d.	209	14.8	n.d.	200	10.7	n.d.
190	2.5	n.d.	232	7.6	n.d.	224	6.2	n.d.
218	14.6	n.d.	258	6.2	n.d.	250	3.8	n.d.
237	5.5	n.d.	283	3.5	n.d.	276	1.9	n.d.
263	4.4	n.d.	307	2.0	n.d.	289	2.1	n.d.
290	4.5	n.d.	331	2.3	n.d.	323	2.2	n.d.
317	1.3	n.d.	355	2.6	n.d.	347	1.8	n.d.
332	5.8	n.d.	379	1.0	n.d.	371	0.9[a]	n.d.
356	1.2	n.d.	403	0.1[a]	n.d.	396	0.3[a]	n.d.
380	5.1	n.d.	427	n.d.	n.d.	421	0.1[a]	n.d.
404	2.8	n.d.	451	2.3	n.d.	443	n.d.	n.d.
428	0.4[a]	n.d.	475	n.d.	n.d.	468	n.d.	n.d.

n.d.: non detected (LOD = 0.1 ng/mL). Time 0: urine collected just before the first administration of Keratyl®.

[a] Estimated concentration with the calibration point at 1 ng/mL.

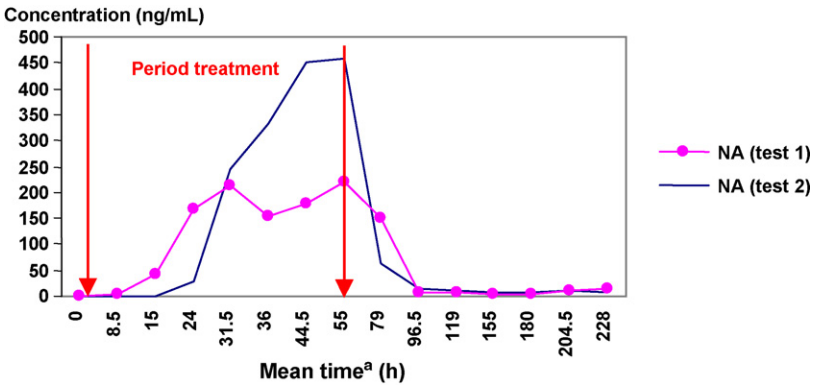

Fig. 2. Excretion kinetics for norandrosterone (Keratyl® administration to the same volunteer at 6 months interval, selected values). [a]Mean time (between both excretions in the same time period).

to the precorneal loss factors which include tears dynamics, non-productive absorption, transient residence time in the cul-de-sac, and the relative impermeability of the corneal epithelial membrane. Due to these physiological and anatomical constraints, only a small fraction of the administered drug, 1% or even less of the instilled dose, is ocularly absorbed. Secondly, the observed results can also be explained by the way the eye drop solutions are assimilated, mainly at the nasal wall, in relation with the ability to tolerate the solution in situ (eye dryness, inability to tolerate excipient or preservative, which induce ocular reaction, production of tears and then, bad absorption of the drug). Moreover, the ocular space do not accept more than 30 μL of solution and only a small part of the administered volume is assimilated, the volume in excess being wasted. It is noteworthy that those two volunteers were the most affected by the pharmaceutical drug (ocular reaction), which can explain that no nandrolone metabolites were found in their urines.

For the other two volunteers, nandrolone metabolites appeared rapidly in the urines. Indeed, 19-norandrosterone and 19-noretiocholanolone were detected from 8 h and 13 h, respectively. It can be noticed that, the excretion peak (near 30 h) took place later than after oral or intravenous administrations, as already published [26].

Contrary to all expectations, the concentrations measured in urines reached 415 ng/mL and 11 ng/mL for 19-norandrosterone and 19-noretiocholanolone, respectively (Table 4). Indeed, regarding the poor bioavailability of this kind of medication, it was not expected to measure so high urinary concentrations of nandrolone metabolites. Moreover, for the first metabolite, concentrations near 2 ng/mL were found beyond 2 weeks after the last administration. In all cases, the excreted amounts of 19-norandrosterone were clearly superior to that of the second metabolite and are widely above the WADA positive limit. Finally, even if the excretion kinetics were similar for the volunteers, the excreted amounts varied significantly.

3.2.4. *Intra-variability in excretion kinetic*

For one volunteer, the Keratyl® administration was repeated twice, within 6 months interval in order to evaluate the intra-individual excretion variability. Also in this case, even if the excretion kinetics were similar (excretion profile, excretion peak time) the excreted amounts varied significantly between both administrations (see Fig. 2 for norandrosterone and Table 4), as well as the ratio between both metabolites, as already described [26]. Indeed, for this second test, the excreted amounts reached 450 ng/mL and 70 ng/mL for norandrosterone and noretiocholanolone, respectively. In this case, as well, the discrepancy in the urinary concentrations regarding both tests was also attributed to the hazardous bioavailability of ophthalmic solutions, due to several biological factors which consequently limit the entry of ocular drugs and lead to uncertain measures.

From these results, it can be said that it is very difficult to estimate the bioavailability of the eye drop solutions in general and overall the concentrations that will be found in urines, even in the same individual.

4. Conclusion

The results of this very preliminary study, contrary to all expectations, were very instructive as it was demonstrated that the administration of an ophthalmic solution containing nandrolone can lead to positive urines. Indeed, due to poor bioavailability of ophthalmic solutions, it was not expected to obtain such high urinary concentrations and such discrepancies between individuals. It has to be noticed that the poor bioavailability of ocular drugs has already been documented in the literature [25]. The results of quantification indicated a great variability in terms of inter- and intra-individual excretion of nandrolone metabolites, particularly regarding this kind of medication.

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, as physicians, 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. Consequently, it can be useful in the future to extend this study to

more volunteers and to investigate more deeply the individual metabolism. Additional and comprehensive investigations are being processed in our laboratory.

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