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DETERMINATION OF 19-NORANDROSTENEDIOL AND ITS METABOLITES IN HUMAN PLASMA AFTER ORAL ADMINISTRATION OF A NUTRITIONAL SUPPLEMENT

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INTRODUCTION

19-Norandrostenediol is a prohormone of nandrolone. Both substances are included in the WADA´s Prohibited List [1]. Prohormones are provided as nutritional supplements and the plasma metabolites after oral and sublingual administration of two of them have been recently reported [2, 3]. The aim of this study is to determine the plasma levels of 19-norandrostenediol and its metabolites after oral administration of a nutritional supplement containing 19-nor-4-androstenediol. In the present study, concentrations of all the metabolites in free and glucuronide fraction are presented.

EXPERIMENTAL

Clinical study

Two capsules of Norandrodiol Select 300 (Ergopharm, USA) containing 19-nor-4-androstenediol (theoretical content, 150 mg; measured content, 90.7±11.2 mg) were orally administered to 6 healthy male volunteers (protocol AEMPS 04-0012). Plasma samples were collected before administration and 0.5, 1, 2, 4, 8, and 24 h after supplement administration.

Extraction procedure

Samples were extracted at pH 7 with t-butylmethyl ether (TBME) (free fraction). The aqueous phase was applied to Detectabuse cartridges, previously conditioned with methanol and water;

the column was washed with water and analytes were eluted with methanol. After evaporation to dryness and reconstitution with sodium phosphate buffer, enzymatic hydrolysis was performed using β-glucuronidase from *E.coli* (55ºC, 1h). Then, samples were extracted with TBME at pH 9 (glucuronide fraction). Derivatization of both fractions was made with MSTFA: NH_4I: 2-mercaptoethanol (60 ºC, 20 min) and trimethylsilyl derivates were analyzed by GC-MS.

Chromatographic conditions

A dimethylpolysiloxane (17m x 0.2mm, 0.11µm) column was used with helium as carrier gas with a flow rate of 0.8 mL min^{-1} (measured at 180 ºC). The oven was set at a initial temperature of 180 ºC, and then the following rates were programmed: at 3 ºC min^{-1} from 180 ºC to 230 ºC, then at 40 ºC min-1 to 310 ºC and held for 3 min. 2 µl of samples were injected with split mode (10:1). The injector and the detector were set at 280 ºC. Electron ionization (70 eV) and SIM acquisition modes (see table 1) were used.

Compound	Diagnostic ions	RT	RRT
Norandrosterone-d4 (ISTD.)	409, 424, 319	8.9	0.59
Norandrosterone (NA)	405, 420, 315	8.9	0.59
Noretiocholanolone (NE)	405, 420, 315	9.9	0.66
Norandrostenediol (Nol)	420, 405, 330	11.1	0.74
Norandrostenedione (None)	416, 401, 311	11.9	0.79
Nandrolone (N)	418, 403, 194	12.3	0.82
Methyltestosterone (ISTD)	446, 301	14.9	1.00

Table 1**:** Diagnostic ions used for identification and quantification, retention times and relative retention times of the bis-O-TMS derivatives of the compounds in study.

RESULTS AND DISCUSSION

Method validation results

The method was validated to determine linearity, extraction recovery, limit of detection and quantification, intra-day precision and accuracy and inter-day precision and accuracy. Validation parameters obtained are listed in Tables 2 and 3. Results listed for the glucuronide fraction were obtained using the aglycone as reference substance.

	Free fraction					Glucuronide fraction				
	NA	NE	Nol	None	N	NA	NE	Nol	None	N
LOQ (ng/ml)	**0.9**	**1.4**	**0.4**	**0.1**	**0.5**	**1.0**	**0.5**	**0.4**	**0.4**	**0.4**
Mean recovery (SD, n=4)	**100.6 (9.8)**	**94.7 (4.2)**	**95.4 (1.2)**	**92.2 (5.8)**	**84.9 (4.7)**	**52.8 (11.0)**	**51.9 (11.3)**	**78.5 (15.8)**	**40.4 (13.5)**	**46.7 (17.1)**

Table 2: Limits of quantification (LOQ) and extraction recoveries.

			Intra-assay			Inter-assay		
		QC (ng/ml)	n	Precision (RSD, %)	Accuracy (%relative error)	n	Precision (RSD, %)	Accuracy (%relative error)
FREE FRACTION	NA	**6**	**3**	**1.7**	**7.5**	**9**	**5.0**	**7.5**
		30	**3**	**1.4**	**5.1**	**9**	**4.6**	**10.2**
	NE	**6**	**3**	**1.6**	**7.8**	**9**	**4.9**	**9.0**
		30	**3**	**1.1**	**4.3**	**9**	**3.5**	**6.9**
	None	**6**	**3**	**10.6**	**9.5**	**9**	**17.1**	**12.3**
		30	**3**	**6.1**	**4.3**	**9**	**8.7**	**11.7**
	N	**3**	**3**	**10.7**	**16.7**	**9**	**15.7**	**18.2**
		15	**3**	**5.7**	**8.7**	**9**	**8.5**	**6.7**
	Nol	**6**	**3**	**4.2**	**4.8**	**9**	**12.4**	**7.6**
		30	**3**	**1.3**	**12.6**	**9**	**3.1**	**15.3**
GLUCURONIDE FRACTION	NA	**60**	**3**	**0.5**	**2.7**	**9**	**3.3**	**4.2**
		300	**3**	**0.7**	**4.3**	**9**	**5.9**	**5.0**
	NE	**60**	**3**	**3.7**	**5.3**	**9**	**4.8**	**5.7**
		300	**3**	**1.2**	**1.4**	**9**	**5.2**	**5.6**
	N	**6**	**3**	**7.0**	**7.5**	**9**	**6.4**	**8.8**
		30	**3**	**3.0**	**8.4**	**9**	**6.4**	**8.1**
	Nol	**6**	**3**	**6.0**	**6.0**	**9**	**4.6**	**7.1**
		30	**3**	**4.2**	**4.4**	**9**	**9.5**	**8.5**

Table 3: Intra and inter-assay precision and accuracy for free and glucuronide fractions.

Concentrations of plasma metabolites in glucuronide fraction

The main metabolites detected were norandrosterone and noretiocholanolone, mainly in the glucuronide fraction. Nandrolone and norandrostenediol were also detected as conjugates, at lower concentrations (Figure 1).

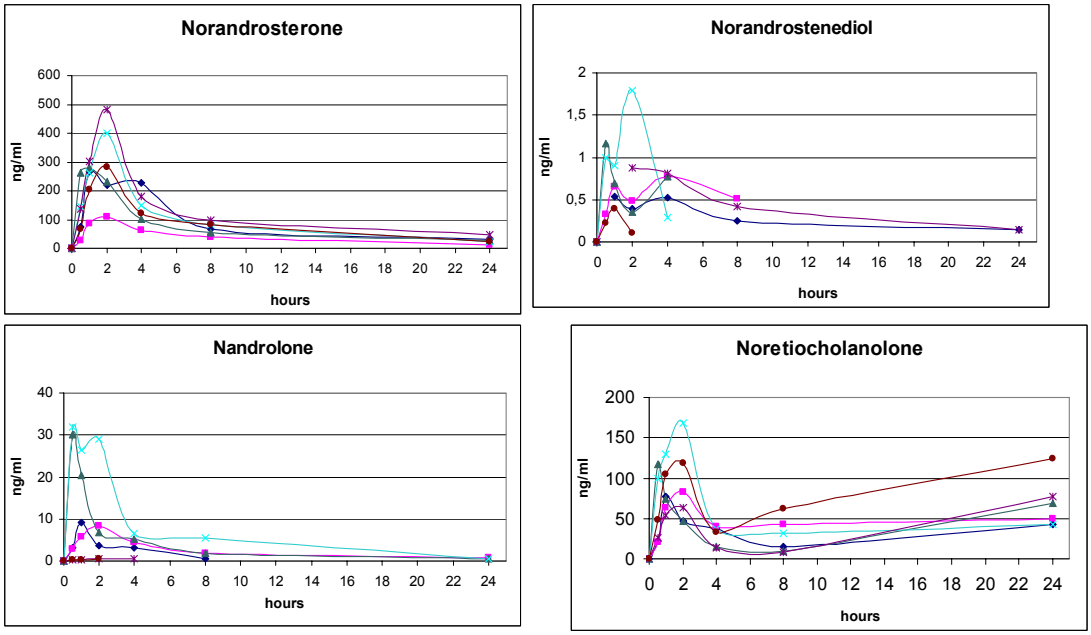

Figure 1: Plasma concentrations of norandrostenediol and metabolites in the glucuronide fraction for each volunteer.

Concentrations of plasma metabolites in the free fraction

Free norandrostenedione was detected in 5 volunteers. The other metabolites were also detected in the free fraction, at low concentrations (Figure 2). Free nandrolone was lower than 0.5 ng/ml, except in 1 volunteer, who had 0.7, 1.1 and 0.6 ng/ml after 1, 2 and 4 h of administration.

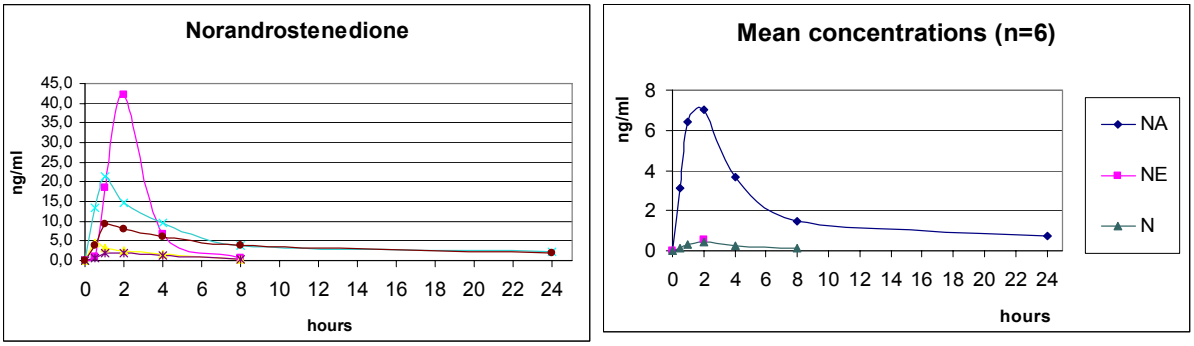

Figure 2: Plasma concentrations in the free fraction: concentrations of norandrostenedione for each volunteer (left) and mean concentrations of the other metabolites (right).

CONCLUSIONS

- A method for quantification of 19-norandrostenediol metabolites in human plasma by GC/MS has been validated.
- After oral administration of the drug, the main metabolites were norandrosterone and noretiocholanolone in the glucuronide fraction.
- Low concentrations of norandrostendione and nandrolone were detected in the free fraction.

REFERENCES

[1] http://www.wada-ama.org/rtecontent/document/2006_LIST.pdf (access date: 29th June 2006)

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[3] Schrader, Y.; Thevis, M.; Schänzer, W. Quantitative determination of metabolic products of 19-norandrostenediol in human plasma using GC/MS. Drug Metab Dispos, 2006 (in press).