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THE TRACING OUT OF THE CONTAMINATION WITH DOPING AGENTS OF THE NUTRITIONAL SUPPLEMENTS

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Introduction

We used as a starting point the studies of Geyer [2] and others, the first ones who pointed out the contamination of nutritional supplements with doping substances and who established the methodology of extraction and tracing. We have therefore chosen to analyze some of the nutritional supplements distributed on a large scale in Romania in order to identify possible doping agents.

The development of these studies in the Romanian Doping Control Laboratory has also been stimulated by the recent unwanted incidents among the Romanian athletes who were found positive and who blamed the nutritional supplements they used.

In addition, we were stimulated by the studies of nutritional supplements we carried out and which pointed out that sometimes their content is different from what it is written on the label [1,2,3]. A number of four nutritional supplements were analyzed.

Material and methods

The following nutritional supplements, comercialized on the Romanian market, were investigated: PRO ANABOLIC; NATURAL STEROL EXTREME; ANIMAL STAK; TRIBULUS TERRESTRIS.

Two phials of each product were tested, one of them received from the doping charged athlete and the second one, bought from the same company, and belonging to the same series.

Administration

The nutritional supplements were administered to male volunteer subjects, with the age between 20 to 25.

The nutritional supplements were administered in doses of 250 mg, three times per day, during a seven-day period.

The biological samples of urine were prelevated from all the volunteers before the administration and then, after the end of administration, each day, for four days (24h, 48h, 72h, and 96h).

Isolation of steroids

For isolation of anabolic steroids from urine samples, we used the procedure of combined fraction, presented in the previous papers [4,5,6]. The quantity of urine used was 5 ml, hydrolysed with 20 µl β-glucuronidase from Escherichia coli (Roche Molecular Biochemicals, Mannheim) for 12 h at 37 C and there were also used 25 µl d4-norandrosterone (1 µg/ml) and 10µl d4-noretiocholanolone (1 µg/ml) as internal standard obtained from NARL Australia.

For the determination of T/E ratio we realized the same procedure of combined fraction, but we used 20 µl of mixture of deutereted internal standard obtained from the Doping Control Laboratory from Cologne.

Derivatization for GC/MS

The organic phase was evaporated to dryness and then derivatised with 100 µl MSTFA/NH_4/ethanthiol (1000:2:3) v:w:v.

GC/MS Analysis

The biological extracts were analyzed through the computerized system GC/MS Hewlett Packard 6890/5972 in SIM mode.

For each sample we did:

-screening for SAA tracing; T/E ratio; quantification of norandrosterone and noretiocholanolone (in samples in which these metabolites were pointed out).

The working parameters were the following:

-column: CP-SIL 5 CB (methyl silicone) 17 m lengh, I.D. 0.25 mm, film thickness 0.12 µm;

-carrier gas: Helium 0.8 ml/min;

-split injection mode (1:10);

-temperature programe: 160^{0}C, 2 min, 5^{0}C/min, 255^{0}C, 30^{0}C/min, 285^{0}C, 5 min, 60^{0}C/min, 300^{0}C, 2 min;

-injector temperature: 300^{0}C; interface temperature: 300^{0}C.

The quantification of norandrosterone and noretiocholanolone

The quantification of metabolites of nandrolone was performed by GC/MS Hewlett Packard 6890/5972 in SIM mode, using the calibration method with internal standard. We used methanolic solutions for each metabolite standards, in concentration of 1µg/ml, and the concentrations in the samples are shown as in the Table 1.

Table 1 Concentration of working solutions and resulting concentrations per ml of urine

Nr. crt.	norandrosterone (1μg/ml) (μl solution)	sample concentration (ng/ml)	noretiocholanolone (1μg/ml) (μl solution)	sample concentration (ng/ml)
1.	10	2	10	2
2.	20	4	20	4
3.	30	6	30	6
4.	40	8	40	8
5.	50	10	50	10
6.	60	12	60	12
7.	70	14	70	14
8.	80	16	80	16
9.	90	18	90	18
10.	100	20	100	20

Results and discussions

All the urine samples collected from volunteer subjects to whom nutritional supplements commercialized on the Romanian market were administrated, were analysed in SIM mode.

Table 2 Retention times and characteristic ions

Nr. crt.	substance	RT (min)	base peak	other characteristic ions
1.	d4-norandrosterone	12,92	409	424
2.	19-norandrosterone	12,95	405	315;420
3.	d4-etiocholanolone	13,70	409	424
4.	19-noretiocholanolone	13,74	405	315;420
5.	d3-epitestosterone	15,60	435	
6.	epitestosterone	15,62	432	
7.	d3-testosterone	16,21	435	
8.	testosterone	16,24	432	

ANIMAL STAK is a nutritional supplement, commercialized on the Romanian market, labelled with the following compounds: 19-nor-5-androstenedione, 5-androstenediol, DHEA, 4-androstenedione, Tribulus, arginine, ornithine, chrysin and others.

In the biological urine samples analyzed after the administration of **ANIMAL STAK** was detected the presence of nandrolone metabolites, above the admitted limit, and higher T/E ratios. The experimental results are presented in Table 3.

PRO ANABOLIC, the second nutritional supplement tested by us has no hormonal precursors or anabolic steroids inscriptioned on the label.

After administration, it was pointed out that this product induces the growth of endogenous testosterone secretion through cumulative effect, which could lead to a positive result at a doping test. It also contained nandrolone metabolites (Table 4).

Table 3 Urine concentrations of nandrolone metabolites and the T/Epit values after the administration of **ANIMAL STAK**

volunteer	Time (h)	norandrosterone (ng / ml)	noretiocholanolone (ng / ml)	T/E
V_1	0	-	-	0,9
	24	124	63	3,1
	48	96	35	2,5
	72	23	8	2,9
	96	10	-	2,5
V_2	0	-	-	1,2
	24	145	74	3,9
	48	112	42	3,2
	72	54	23	2,5
	96	12	-	2,0

Table 4 Urine concentrations of nandrolone metabolites and the T/E values after the administration of **PRO ANABOLIC**

volunteer	Time (h)	norandrosterone (ng / ml)	noretiocholanolone (ng / ml)	T/E
V_1	0	-	-	1,2
	24	96	31	3,9
	48	82	25	3,5
	72	50	15	2,8
	96	14	-	2,5
V_2	0	-	-	0,8
	24	87	25	3,0
	48	78	12	2,9
	72	43	5	2,5
	96	21	-	2,0

NATURAL STEROL EXTREME

After the application of this product in the mentioned dose, during a period of five days, both subjects showed values for the main nandrolone metabolite, norandrosterone, slightly above the admitted limit: V1-3 ng/ml and V2- 4ng/ml. No modification of T/E ratio was noticed to the both subjects.

TRIBULUS TERESTRIS is the last nutritional supplement tested by us, which has no inscription of hormonal precursors and anabolic steroids.

After the administration and metabolization of this product in the body, it was noticed an increase of norandrosterone above the admitted limit (Table 5). No modifications of the T/E ratio was noticed.

Table 5 Urine concentrations of nandrolone metabolites and the T/E values after the administration of **TRIBULUS TERRESTRIS**

volunteer	Time (h)	norandrosterone (ng / ml)	noretiocholanolone (ng / ml)	T/E
V_1	0	-	-	0,9
	24	10	3.5	1,2
	48	9	-	1,0
	72	5	-	0,9
	96	4	-	0,8
V_2	0	-	-	0,8
	24	9	-	0,9
	48	7	-	1,0
	72	5	-	0,9
	96	3	-	0,9

Conclusions

The studies show that the nutritional supplements do not always contain only what is written on the label and they may be contaminated with hormonal precursors and anabolic androgenous steroids as well.

We consider that there is a need for endorsement of nutritional supplements from the viewpoint of possible content of substances which may generate a positive result at a doping control.

Theoretically, athletes should avoid using nutritional supplements, as among other arguments, there are no scientific proofs of their beneficial effect.

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