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Oxandrolone and High Doses of Metandienone Found in Nutritional Supplements  
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## **Oxandrolone and high doses of metandienone found in nutritional supplements**

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### **Abstract**

Several nutritional supplements were detected, which contain the anabolic androgenic steroids metandienone and oxandrolone. The anabolic androgenic steroids were not declared on the label. The concentrations of metandienone varied from 0,4 to 28,9 mg/g. The oxandrolone concentrations varied from 6,5 to 26,2 µg/capsule.

### **Introduction**

Several recent studies have shown that non-hormonal dietary supplements such as vitamins, amino acids, creatine, etc. may contain anabolic androgenic steroids not declared on the label. The anabolic androgenic steroids mainly found in these studies were so-called prohormones (1-4). Prohormones are legally sold on the US dietary supplement market. The presented cases show that nutritional supplements may also contain anabolic androgenic steroids, which are not admitted as the nutritional supplements and are only available on prescription.

### **Case 1: Nutritional supplements with high doses of the anabolic androgenic steroid metandienone**

#### ***Background***

In a recent follow-up study of a positive doping case a dietary supplement was found to contain the anabolic steroid metandienone, although metandienone was not declared on the label (5). The declared ingredients were “AD-4-Complex Nutrients “, “MetX Synergistic Blend“, “1 – T Matrix“, creatine and ribose. Except for ribose and creatine the names of the other ingredients are not approved. Based on this knowledge an investigation was started to

analyse dietary supplements with similar contents, especially with “1-T matrix“, for anabolic androgenic steroids.

### ***Experimental***

Three nutritional supplements with “1-T matrix “ were purchased by telephone order. The supplier of these supplements is a company from the UK, whereas the manufacturer is a US company.

The sample preparation was performed according to a recently published method (1-3). One gram of each supplement was dissolved in 5 ml methanol. Aliquots of the methanolic layer were evaporated to dryness, and an alkaline extraction with n-pentane was performed. The n-pentane layer was transferred to another test tube and 2 ml of 95 % methanol were added. After mechanical shaking for 5 minutes and centrifugation the n-pentane layer was discarded. The methanolic residue was evaporated to dryness. The dry residue was derivatised with 100 µl N-methyl-N-trimethylsilyltrifluoroacetamide/ $\text{NH}_4\text{I}$ /ethanethiol (1000:2:3 v:w:v) for 15 min at 60°C. Three microlitres of the sample were analysed with gas chromatography/ mass spectrometry (HP6890/HP5973) in the full scan mode. For the quantification of metandienone 10 µl of the methanolic layer were analysed with high pressure liquid chromatography with UV detector (HP 1090) at 245 nm. Metandienone from Sigma Chemical Co St Louis, USA was used as reference compound (one point calibration).

### ***Results***

In all supplements analysed the anabolic-androgenic steroid metandienone was found. Metandienone, only available on prescription, was not declared on the labels. The concentrations of metandienone in the 3 products are presented in table 1.

Tab. 1: Metandienone concentrations in the three dietary supplements

supplement	Concentration of metandienone [mg/g]
1	17,30*
2	0,41
3	0,96

\* concentration of a homogenisate of 10 capsules

In supplement 1 the concentrations of metandienone varied from capsule to capsule. Maximum concentrations of metandienone of 28,9 mg/g were detected (tab. 2).

Tab. 2: Concentrations of metandienone in 5 different capsules of supplement 1

capsule	Concentration of metandienone [mg/g]
1	28,93
2	19,30
3	27,38
4	16,98
5	17,65

### ***Discussion***

The use of metandienone is associated with a large number of adverse effects especially in women, adolescents and children. Due to the fact that metandienone is a 17-methylated compound its use is associated with a high liver toxicity and carcinogenicity. The use of the maximum recommended supplement dosages leads to an administration of 25-43 mg, and 10 and 20 mg metandienone per day, respectively.. These doses are above the recommended therapeutical dose of 5-10 mg per day for this prescription drug (6). In addition to enormous health risks the use of the analysed supplements can lead to positive doping results for metandienone (7).

### **Case 2: Nutritional supplements containing the anabolic steroid oxandrolone**

#### ***Background***

In a follow up study of a positive case with oxandrolone and epioxandrolone several nutritional supplements were analysed on request of the athlete`s federation. All supplement containers contained blue capsules with a white powder and were labelled with adhesive labels with only the two letters “GM”. On the labels were neither lists of ingredients, nor company names, charge numbers or expiry dates. According to the information of the federation, the origin of the supplements was the “Centro Espanol de Medicina y Fisioterapia del Deporte, Madrid, Spain”. In a letter, this medical center gave the following declaration: “We declare that GM (glutamine) is controlled and produced in a chemical and technical high standard, so that no contamination with banned substances occurs.”

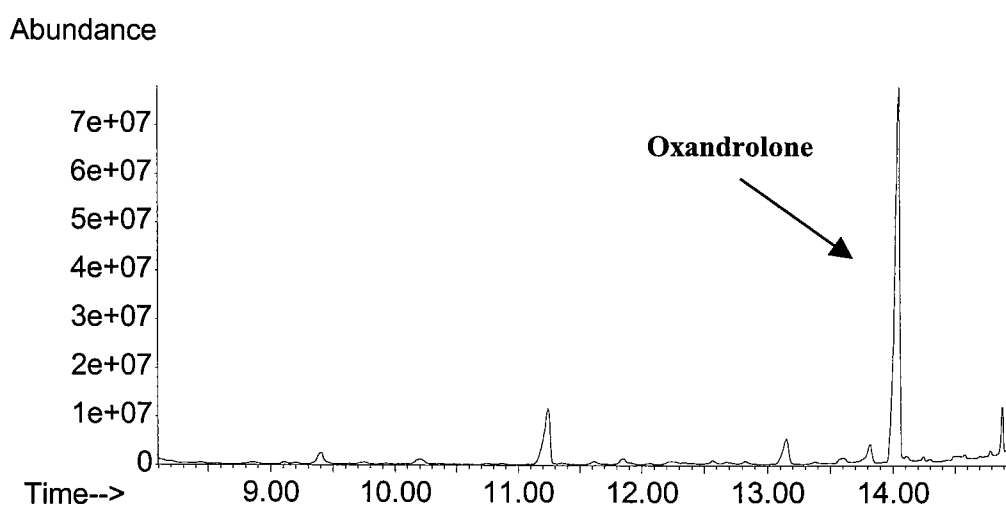
## Experimental

Sample preparation of the nutritional supplements: One gram of the powder was dissolved in 5 ml methanol. The methanolic layer was evaporated to dryness and dissolved in 1 ml phosphate buffer (0,2 M, pH 7). For extraction 5 ml of *tert.*-butylmethyl ether were added. After shaking and centrifugation the ether layer was decanted to another test tube and evaporated to dryness. The dry residue was derivatised with 100  $\mu$ l N-methyl-N-trimethylsilyltrifluoroacetamide/ $\text{NH}_4\text{I}$ /ethanethiol (1000:2:3 v:w:v) for 15 min at 60°C. Three microlitres of the sample were analysed by gas chromatography/ mass spectrometry (HP6890/HP5973) in the full scan mode and SIM mode with the characteristic ions for the TMS derivative of oxandrolone.

Excretion studies with oxandrolone: 2 volunteers (Age 24/45 years; Weight 68/72 kg) administered 20 $\mu$ g of oxandrolone. Before administration and till 17 hours after administration all urine samples were collected and analysed for oxandrolone and epioxandrolone according to the screening procedure for unconjugated anabolic steroids (8).

## Results

In all containers labelled with "GM" and containing blue capsules with white powder oxandrolone was detected (fig. 1). The concentration of oxandrolone varied from about 6,5 to 26,2  $\mu$ g/capsule.



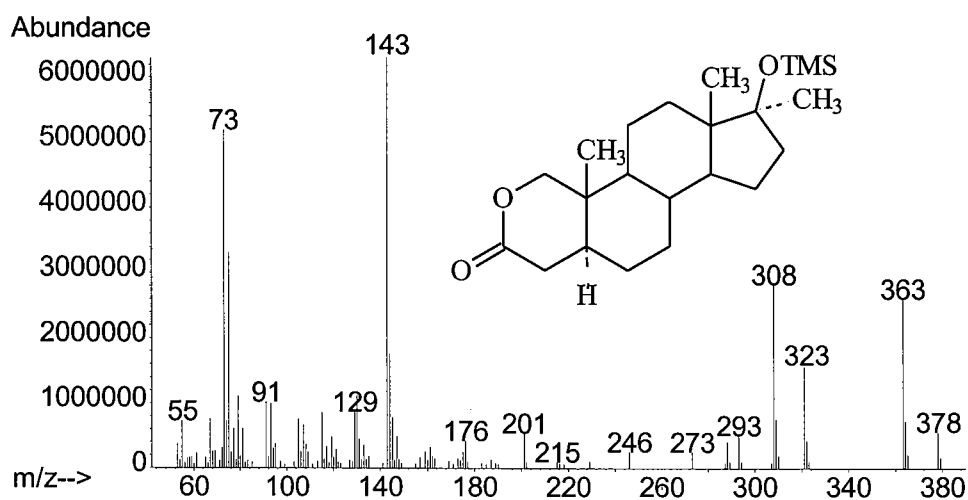


Fig. 1: Detection of oxandrolone in a glutamine supplement. Total ion chromatogram and EI mass-spectrum of oxandrolone-TMS

After administration of 20  $\mu$ g of oxandrolone, oxandrolone and epioxandrolone could be detected in the urines of both volunteers for more than 17 hours (fig. 2 and fig. 3). The excretion rates of oxandrolone and epioxandrolone are shown in figure 4.

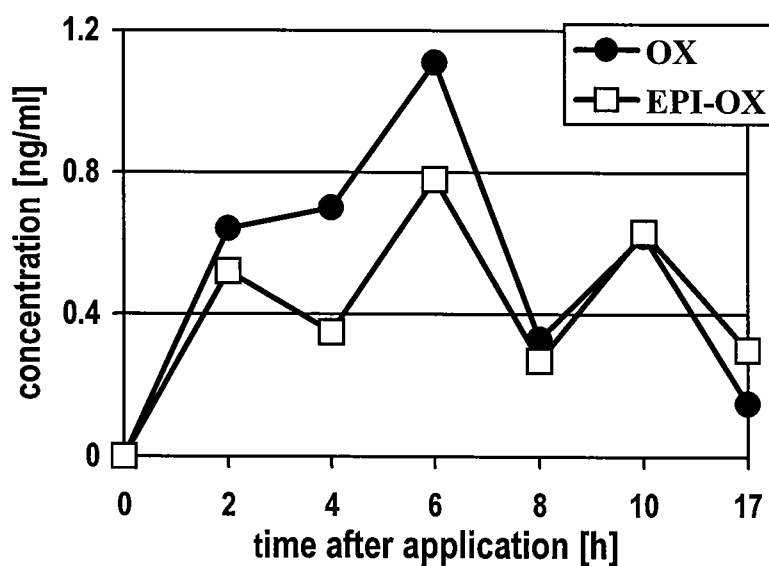


Fig. 2: Urinary oxandrolone and epioxandrolone concentrations in volunteer 1 after application of 20  $\mu\text{g}$  oxandrolone

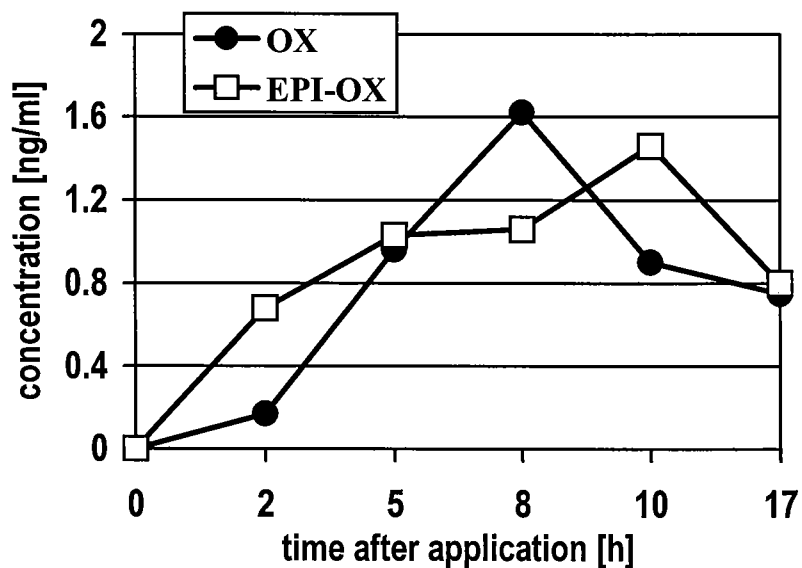


Fig. 3: Urinary oxandrolone and epioxandrolone concentrations in volunteer 2 after application of 20  $\mu\text{g}$  oxandrolone

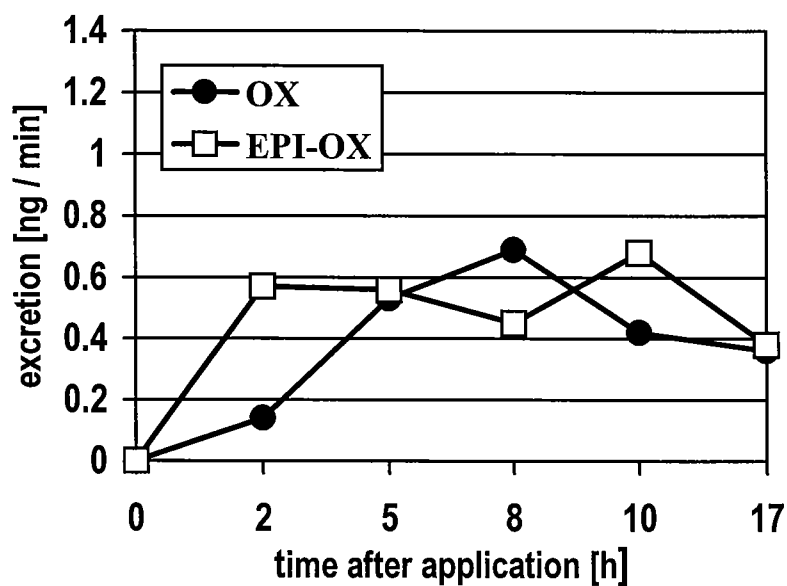


Fig. 4: Oxandrolone and epioxandrolone excretion rates [ng/min] in volunteer 2 after application of 20  $\mu\text{g}$  oxandrolone

## **Discussion**

The anabolic-androgenic steroid oxandrolone is a prescription drug and not admitted as nutritional supplement. The concentrations of oxandrolone in the capsules (about 6,5-26,2 µg per capsule) are about 100 fold lower than the recommended normal therapeutical dose which is 2 mg per day (6). But, as the excretion studies show, such low concentrations (20 µg) can lead to positive doping cases for oxandrolone and epioxandrolone for more than 17 hours (Fig. 2 and 3). As shown in figures 2-4 the unchanged oxandrolone can be detected for at least 17 hours. This observation shows, that oxandrolone is slowly metabolised. In earlier studies, in which high doses of oxandrolone were administered (9), this metabolic pathway was not as obvious as in the present study. Because of this slow metabolism it could be that oxandrolone has also physiological effects in very low concentrations.

The nutritional supplements with oxandrolone were labelled only with 2 letters ("GM"). On the labels were neither lists of ingredients, nor company names, charge numbers or expiry dates. Additionally, the verification of the supplier's address ("Centro Espanol de Medicina y Fisioterapia del Deporte, Madrid, Spain") showed that it was an address of a hotel apartment. The purchase of such non-labelled nutritional supplements from such dubious sources must be declared as grossly negligent behavior, especially for top athletes, who are included in a doping control system.

## **Conclusion**

The results of these study show that it is of major importance to improve the surveillance of the production and trade of dietary supplements. The first step should be a warning to be issued to consumers and the withdrawal of dietary supplements containing prescription drugs.

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