

H. Geyer, K. Köhler, U. Mareck, M.K. Parr, W. Schänzer, M. Thevis

Cross-contaminations of vitamin- and mineral-tablets with metandienone and stanozolol

Institute of Biochemistry, Center for Preventive Doping Research, German Sport University Cologne, Germany

Abstract

At the end of 2005, a German drug surveillance authority confiscated nutritional supplements (effervescent tablets) from a German manufacturer, which contained among other steroids high amounts of metandienone (about 15 mg/tablet) and stanozolol (about 17 mg/tablet). These anabolic steroids were not declared on the labels.

Additionally the drug surveillance authority confiscated other nutritional supplements like vitamin C -, multivitamin -, calcium - and magnesium – tablets, which were produced from the same manufacturer on the same production line within the same time interval. The LC/MS/MS analyses showed cross-contaminations of the vitamin C -, multivitamin - and magnesium-tablets with metandienone and stanozolol. These cross-contaminated products were produced for the sale in ordinary German and Spanish groceries and drug stores.

Introduction

Since 2002 nutritional supplements have been detected, which are intentionally faked with high amounts (higher than 1 mg/g) of “classic” anabolic steroids like metandienone, stanozolol, boldenone, oxandrolone, dihydrotestosterone (1-5) and dehydrochloromethyl-testosterone and metenolone (6). These anabolic androgenic steroids were not declared on the labels. Because manufacturers of these faked products also manufacture other nutritional supplements on the same production line, the risk of cross-contaminations with such “classic” anabolic androgenic steroids is very high. In the present study first cases of such cross-contaminations with “classic” anabolic steroids are described.

Experimental

Nutritional supplements

Two products (Parabolon-S and Stanozolon-S, both effervescent tablets) of the brand name 'pharm-tec' were confiscated by a German drug surveillance authority from a German manufacturer in 2005 and sent to our laboratory for analysis. These products appeared to be prohormone products. The ingredients were labeled as: Parabolon-S: 17 Hydroxy-17-beta-1,4-dien-3-on Matrix, Nor19dion, 4-Adiol; Stanozolon-S: 4-Androstenediol, 1-A-diol, 19-Nor-4-a-dion, 5 α -androsteno-(3,2-c)pyrazol-17-beta Matrix. Except for 4-androstenediol, these names are not approved but indicate steroidal compounds. There was no indication of the quantity of these compounds in the products. According to German and European legislation (7), these products have to be classified as non-licensed pharmaceuticals even if they are marketed as nutritional supplements.

Additionally the food control service confiscated 13 tubes of five different nutritional supplements (all effervescent tablets) which were manufactured at the same time interval and on the same production line as the above mentioned Parbolon-S and Stanozolon-S. According to the label the main ingredients of these five nutritional supplements were: Vitamin C, expiry date: July 2005; Calcium, expiry date: July 2005; Magnesium and Vitamin C, expiry date: January 2006; Multivitamins, expiry date: July 2006; Magnesium, expiry date: January 2005. All products were produced for the for the sale in ordinary German groceries and drug stores except the multivitamins, which were produced for the Spanish market.

Analysis of the nutritional supplements

The sample preparation and the qualitative and quantitative analysis of the Parabolon-S and Stanozolon-S tablets with GC/MS and HPLC-DAD was performed according to a recently described method (8).

The tablets of the five other nutritional supplements were analysed with LC-MS/MS. The samples were prepared according to the screening procedure for anabolic steroids in nutritional supplements used in our laboratory (9) with some modifications.

In brief, one gram of the homogenized nutritional supplement was dissolved in 5 ml methanol and 20 μ l of the internal standard methyltestosterone (25 μ g/ml) were added. After shaking

for 5 min and centrifugation for 5 min at 800g, the methanolic layer was transferred and evaporated to dryness in vacuo. The dried methanolic extract was re-dissolved in KOH (0.1 M) and extracted with *n*-pentane. After re-extraction of the *n*-pentane layer with MeOH–H₂O (95:5, v:v) the methanolic layer was evaporated to dryness and re-dissolved in 100 µl methanol. 10 µl of the methanolic solution were injected in the LC-MS/MS-system.

As quality control samples for the identification and quantification of stanozolol and metandienone 1 g of creatine was spiked with metandienone and stanozolol 10 ng each and 50 ng each respectively. The two quality control samples were prepared according to the above described method.

The LC/MS analysis was performed using a API 2000 LC-MS/MS system (column: Purospher Star 18e, 4.6 mm x 55 mm x 3 mm; mobile phase: A: ammonium acetate (5 mmol/l) 0.1 % acetic acid (pH 3.5), B: acetonitrile; flow: 0 - 7 min: 15 % B to 100 % B, 7 - 8.25 min: 100 % B, 8.25 - 10.5 min: 100 % B to 15 % B; MS parameters: ionization: atmospheric pressure chemical ionization, 475° C, positive measurement mode: multiple-reaction monitoring. For identification and estimation of the quantity of metandienone and stanozolol, the retention time and the most abundant ion transition were compared to the quality control sample. The retention times (RT) and characteristic ion transitions of the monitored steroids were: methyltestosterone (internal standard): 6.92 min, m/z 303.0-109.0; stanozolol: 7.25 min, m/z 329.0-81.0, 329-91, 329-105; metandienone: 7.05 min, m/z 301.0-121.0, 301-173, 301-149.

Results and discussion

In the Parabolon-S and Stanozolon-S high amounts of metandienone and stanozolol respectively and other “classic” steroids were detected (Tab. 1), which were not declared on the labels. Additionally several prohormones were detected. In Parabolone-S: norandrostendione, in Stanozolon-S: norandrostenedione, androst-4-ene-3β,17β -diol, 5β-androst-1-ene-3β,17β-diol, 5β-androst-1-ene-3α,17β-diol, and 1-testosterone. According to German law these steroids are non-licensed pharmaceuticals (7). Therefore the manufacturing of these products in a nutritional supplement company was illegal. According to information of the drug surveillance authority the manufacturer has received pre-mixed bulk powder and labelled tubes from an English client and had the work order to produce effervescent tablets and to send them back to the client.

Tab.1: Effervescent tablets containing high amounts of stanozolol and metandienone

Product name (pharmaceutical form)	Brand name	Company	Not declared “classic” anabolic androgenic steroids
Stanozolon-S (effervescent tablets)	Pharm-Tec	Senesco-Pharma	Stanozolol ca. 15 mg/tablet Boldenone, DHT, Testosterone
Parabolon-S (effervescent tablets)	Pharm-Tec	Senesco-Pharma	Metandienone ca. 17 mg/tablet

The results of the analyses of effervescent tablets, which were manufactured at the same time interval and on the same production line as the above mentioned Parabolon-S and Stanozolon-S are presented in table 2. In the vitamin-C tablets was detected metandienone in a concentration of 50 ng/g corresponding to 0.2 µg/tablet and in the multivitamins- and magnesium-tablets was detected stanozolol in concentrations of 5 – 40 ng/g corresponding to 0,06 – 0,15 µg/tablet.

Based on the very low and varying concentrations of metandienone and stanozolol, their presence was interpreted as cross-contaminations and not as intentional admixtures. The reason for the cross-contaminations was probably the fact that after the production of Stanozolon-S and Parabolon-S the manufacturer did not clean the production line sufficiently. The analyses of urine samples after application of one of these cross-contaminated tablets each with our recently published GC/MS and LC/MS screening procedures for anabolic steroids (10, 11) did not lead to a positive doping results for metandienone- or stanozolol-metabolites. Nevertheless the consumption of such cross-contaminated nutritional supplements could lead to inadvertent positive doping cases, taking into account long term application, individual differences in metabolism and varying concentrations of the contaminants. Based on the same considerations health risks for young people and females, who consume these vitamins and minerals as nutritional supplements, cannot be excluded. These results show, that the appearance of products on the nutritional supplement market with high amounts of “classic” anabolic steroids increase the risk of cross-contaminations with these steroids.

Tab. 2: Nutritional supplements from the German and Spanish market cross-contaminated with "classic" anabolic steroids

Product	Market	Expiry date	Tube	Cross-contaminations
Vitamin C	Germany	July 2005	tube 1	Metandienone ca. 50 ng/g
			tube 2	Metandienone ca. 50 ng/g
Calcium	Germany	July 2005	tube 1	negative
			tube 2	negative
Magnesium + Vitamin C	Germany	January 2006	tube 1	negative
			tube 2	negative
			tube 3	negative
Multivitamins	Spain	July 2006	tube 1	Stanozolol ca. 10 ng/g
			tube 2	Stanozolol ca. 15 ng/g
			tube 3	Stanozolol ca. 10 ng/g
Magnesium	Germany	January 2005	tube 1	Stanozolol ca. 40 ng/g
			tube 2	Stanozolol ca. 40 ng/g
			tube 3	Stanozolol ca. 5 ng/g

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