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Czech Supplements: The Story Continues

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Introduction

Several studies have shown that "non-hormonal" nutritional supplements including vitamins, creatine, minerals, etc. can be contaminated with anabolic androgenic steroids [1-5]. As a consequence of these findings two ISO-validated methods for the analysis of prohormones were developed in our laboratory. Both methods, one for solid and one for aqueous supplements have previously been described [6,7]. These methods allow for the detection of prohormones of testosterone and nandrolone as well as esters of both compounds. Because the latter are only available as injectable formulations, except testosterone undecanoate, their presence was never reported in nutritional supplements. A case of a positive nutritional supplement from Czech origin containing testosterone propionate as well as a supplement containing a never reported anabolic agent is described.

Experimental

Nutritional supplements

Both investigated supplements (A & B) are creatine pyruvate supplements from Czech origin.

Supplement analysis

Sample preparation for both solid and aqueous nutritional supplements is as previously described [6,7]. Briefly, to 1 g of a solid nutritional supplement 5 ml of 1 N NaOH, 50 µl of the internal standard (androsterone, 2µg/ml, MeOH) and 5 ml of a pentane/diethylether mixture (9:1) are added. After rolling for 1h, 1g Na₂SO₄ is added before centrifugation. The organic layer is separated and evaporated under oxygen free nitrogen. The resulting residues are derivatised with 100 µl MSTFA/NH₄I/ethanethiol (320/1/2; v/w/v) for 1 h at 80°C. GC/MS analysis for screening purposes was performed using an Agilent 6890 gas chromatograph, equipped with an HP Ultra 1 column (l=17m, i.d.=0.2 mm, d_f=0.11µm) directly coupled to a Agilent 5973 mass selective detector. The GC oven temperature program was as follows: 120°C (0 min) \rightarrow 70°C/min \rightarrow 181°C (0 min) \rightarrow 4°C/min \rightarrow 234°C (0.1min)

 \rightarrow 30°C/min \rightarrow 300°C (10 min). Detection was performed in the SIM mode. Three diagnostic ions were monitored for each compound [6].

Quantitation of prohormones

The concentration of prohormones in supplement B was determined on the homogenised content of 10 capsules using an equally weighted linear calibration curve constructed in the range 0 to 500 ng/g. Therefore a blank nutritional supplement (Recup Fast lemon, Performance, Nutrico, Belgium) was spiked with 19-nor-4(5)-androstene-3,17-dione and 5 α -androstane-3,17-dione and extracted at 5 different levels in triplicate (0, 50, 100, 250 and 500 ng/g) according to the described method. Quantification was performed using the same GC/MS method, except that the MS was operated in the full scan mode (m/z 40 –780).

Results and Discussion

Calibration curves for 19-nor-4(5)-androstene-3,17-dione and 5 α -androstane-3,17-dione showed a good linearity (R² = 0.988 for 19-nor-4(5)-androstene-3,17-dione and R² = 0.996 for 5 α -androstane-3,17-dione).

Although until now only one ester of testosterone is available for oral administration, the screening method is capable of detecting several other esters of testosterone and nandrolone. Screening of nutritional supplement A revealed a suspicious screening result for DHEA and testosterone, two components frequently found in contaminated nutritional supplements [7]. Both substances could be confirmed using a full scan method. In addition to those two substances, screening results indicated the possible contamination with testosterone propionate, a substance present in injectable preparations such as Sustanon[®]. Full scan results (Figure 1) confirmed the presence of this substance in the creatine pyruvate supplement.

Screening of nutritional supplement B, manufactured by the same company as supplement A, showed the presence of 19-nor-4(5)-androstene-3,17-dione (0.9 μ g/g). Based upon previous findings the presence of such amounts can result in positive doping findings [8].

Besides the nandrolone precursor an unknown peak at a retention time of 12.21 min appeared the total ion chromatogram. Mass spectral information of the underivatised component (Figure 2) and the TMS-derivatised component (Figure 3) were indicative for the presence of 5α -androstane-3,17-dione, and intermediate steroid in the conversion from androstenedione to androsterone. The presence of this substance in nutritional supplements has never been reported. The detected amount of 5α -androstane-3,17-dione was 0.24 µg/ml.



Figure 1: Mass spectrum of TMS-derivatised testosterone propionate in nutritional supplement A.



Figure 2: Structure and mass spectrum of underivatised 5α-androstane-3,17-dione.



Figure 3: Spectrum of TMS-derivatised 5α-androstane-3,17-dione.

Conclusion

Analysis of two nutritional supplements from Czech origin revealed the presence of nonlabelled anabolising agents including the previously never reported testosterone propionate and 5α -androstane-3,17-dione. These results highlight the importance of continuous supplement testing by doping control laboratories.

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