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Anabolic Steroids in Plant Medicines for Horses  
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## **Anabolic steroids in plant medicines for horses**

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### **Abstract**

The appearance of steroid contaminations in nutritional supplements for humans has led to the discovery of a plant essence for the treatment of horses containing anabolic steroids. Two samples of an oily plant extract (Mexican Cactus Extract) were prepared according to a sample preparation procedure established for anabolic steroids in nutritional supplements for humans. The sample treatment comprised the extraction and purification of the analytes by means of re-extraction as well as the chemical conversion with N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) to yield the trimethylsilyl-(TMS)-derivatives of the steroids. The analysis by gas chromatography/mass spectrometry (GC/MS) revealed that both samples contained the anabolic androgenic steroids (AAS) stanozolol, 17 $\beta$ -hydroxy-17 $\alpha$ -methyl-5 $\alpha$ -androstane-3 $\beta$ -ol as well as mestanolone without declaration on the label. The concentration of stanozolol, which has been identified as the main component of the detected steroids, was estimated at 10  $\mu$ g per ml of the oily plant extract.

### **Introduction**

During the last few years positive doping tests for steroids have escalated within human sports, and extended research has been conducted to find the reason for this bias. Nutritional supplements have turned out to be one of the sources, and several recent publications [1-3] focus on the study of nutritional supplements and their content of illicit drugs. Since 1996, prohormones have been available on the US sports nutrition market, even though, according to the doping regulations of the International Olympic Committee (IOC), they belong to the

prohibited class of anabolic agents. These recent studies have shown that nonhormonal supplements such as vitamins, amino acids formulations, creatine, etc. can contain prohormones not declared on the label. The administration of such products may lead to positive results in doping tests. In the equestrian world doping regulations are even stricter than in human sports. Besides illegal performance enhancing substances, also compounds that can produce an unnatural effect on horses (non-steroidal anti-inflammatory drugs, sedatives) administered in competition are prohibited.

Equine food contaminated with naturally occurring compounds, e.g. morphine, hordenine, theobromine, salicylic acid derivatives has been reported in various publications since the eighties [4-7].

In contrast to the biosynthesis and incorporation of doping relevant substances in plants, we are now confronted with a new dimension of supplement contamination.

In this study a product called "Energy 5" was investigated regarding forbidden substances. Analyses revealed the content of synthetic androgenic anabolic steroids, which were not declared on the label.

## **Experimental**

Two samples of the product (Energy 5) were delivered by the Federation Equestre National Germany. The samples were prepared according to the sample preparation procedure for nutritional supplements [8]. Briefly: 0.5 ml of the sample were dissolved in methanol and steroids were isolated with n-pentane under basic conditions. After re-extraction by methanol (containing 5 % water), the methanolic layer was dried and the residue derivatised with N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) to yield the trimethylsilyl (TMS) derivatives. For detailed GC-MS conditions refer to Geyer et al. [1,2].

## **Results and discussion**

Figure 1 shows the total ion chromatogram (TIC) obtained after the analysis of Energy 5. The GC-MS data revealed that both samples contained the anabolic androgenic steroids 17 $\beta$ -hydroxy-17 $\alpha$ -methyl-5 $\alpha$ -androstane-3 $\beta$ -ol, mestanolone, and stanozolol. The signal intensity

of stanozolol, which has been identified as the main component of the detected steroids, corresponds to approximately 10 µg per ml (roughly estimated from an one-concentration calibration).

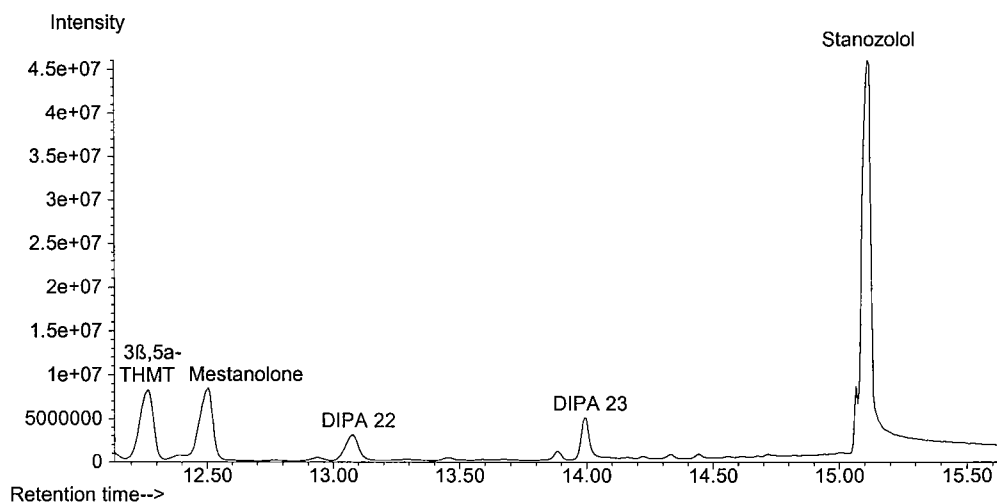


Figure 1: Total Ion Chromatogram (TIC) of the product. The diagram shows stanozolol as the most abundant peak.

The presence of the steroids was confirmed by monitoring their bis-TMS-derivatives in the full scan mode. The electron ionization (EI) mass spectrum of stanozolol-bis-TMS as well as the structures and mass spectra of 17β-hydroxy-17α-methyl-5α-androstane-3β-ol-bis-TMS and mestanolone-bis-TMS are given in figures 2, 3 and 4.

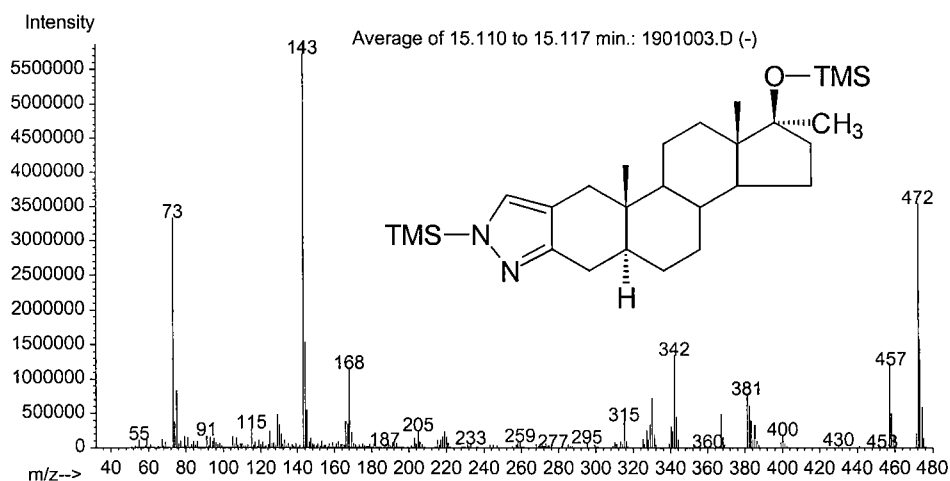


Figure 2: Structure and EI-mass spectrum of stanozolol-bis-TMS (mol wt.: 472).

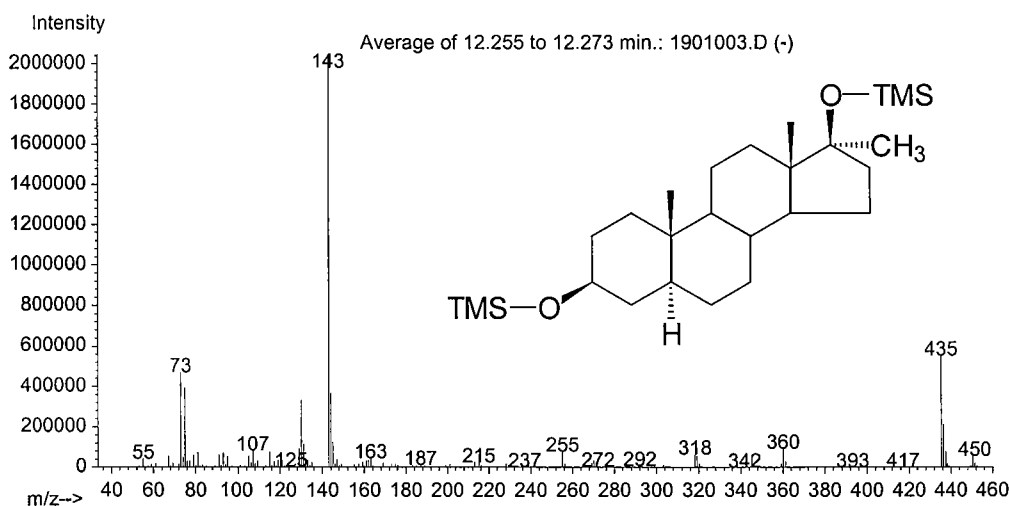


Figure 3: Structure and EI-mass spectrum of 17 $\beta$ -hydroxy-17 $\alpha$ -methyl-5 $\alpha$ -androstane-3 $\beta$ -ol-bis-TMS (mol wt: 450).

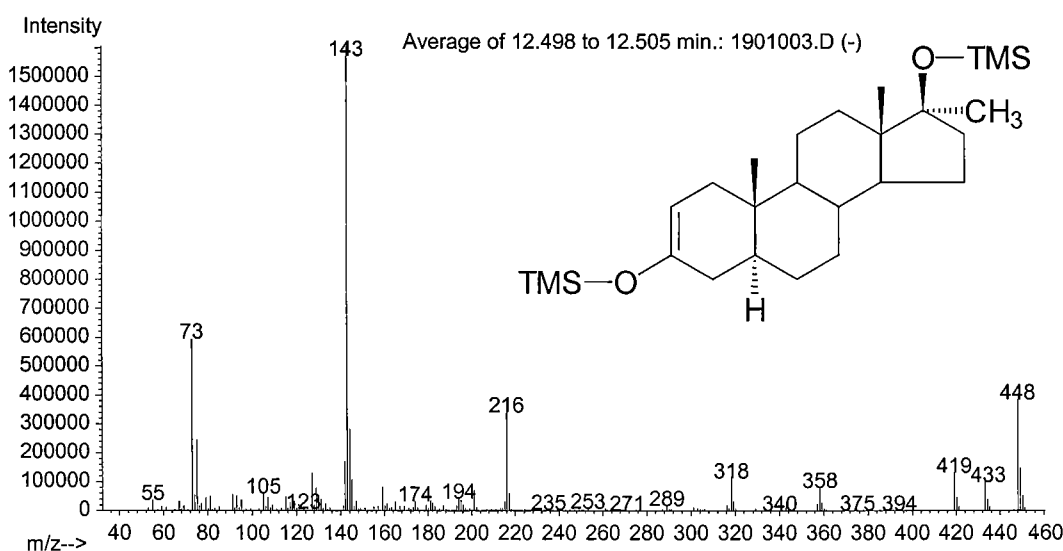


Figure 4: Structure and EI-mass spectrum of mestanolone-bis-TMS (mol wt: 448).

All identified anabolic steroids contain a 17 $\alpha$ -methyl group, which are associated to serious adverse effects, e.g. liver toxicity [9]. The production of 17-alkylated steroids is licensed exclusively by pharmaceutical companies and conducted under good manufacturing practices (GMP conditions). Natural sources for synthetic 17-methylated steroids can be excluded, hence an intentional addition of the steroids or cross contamination may be an explanation for

their presence in the samples. Intentional addition is further confirmed by the fact that both samples contained nearly the same amount of the steroids, which is unusual in a natural preparation. It is known from human studies that low doses of anabolic steroids do not promote muscle growth but fastens the recovery after strong exercises [10]. Horses maintained at a low but permanent level of an Energy 5-steroid cocktail might benefit from this effect.

Our study has demonstrated that the problem of non-hormonal nutritional supplements containing prohibited androgenic anabolic steroids seems to be no longer only a problem in human sports but is also an issue for equestrian sports. Besides health risks (especially in the case of prolonged and repeated use in fillies and mares) pilot excretion studies have shown that the administration of Energy 5 may lead to positive doping cases [11].

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