

Review

Nutritional supplements cross-contaminated and faked with doping substances

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Received 11 April 2008; Accepted 14 May 2008

Since 1999 several groups have analyzed nutritional supplements with mass spectrometric methods (GC/MS, LC/MS/MS) for contaminations and adulterations with doping substances.

These investigations showed that nutritional supplements contained prohibited stimulants as ephedrines, caffeine, methylenedioxymetamphetamine and sibutramine, which were not declared on the labels. An international study performed in 2001 and 2002 on 634 nutritional supplements that were purchased in 13 different countries showed that about 15% of the nonhormonal nutritional supplements were contaminated with anabolic-androgenic steroids (mainly prohormones). Since 2002, also products intentionally faked with high amounts of 'classic' anabolic steroids such as metandienone, stanozolol, boldenone, dehydrochloromethyl-testosterone, oxandrolone etc. have been detected on the nutritional supplement market. These anabolic steroids were not declared on the labels either. The sources of these anabolic steroids are probably Chinese pharmaceutical companies, which sell bulk material of anabolic steroids. In 2005 vitamin C, multivitamin and magnesium tablets were confiscated, which contained cross-contaminations of stanozolol and metandienone. Since 2002 new 'designer' steroids such as prostanazol, methasterone, androstatrienedione etc. have been offered on the nutritional supplement market. In the near future also cross-contaminations with these steroids are expected. Recently a nutritional supplement for weight loss was found to contain the β 2-agonist clenbuterol. The application of such nutritional supplements is connected with a high risk of inadvertent doping cases and a health risk. For the detection of new 'designer' steroids in nutritional supplements, mass spectrometric strategies (GC/MS, LC/MS/MS) are presented. Copyright © 2008 John Wiley & Sons, Ltd.

KEYWORDS: sport; doping; nutritional supplements; GC/MS; LC/MS/MS; designer steroids

INTRODUCTION

The nutritional supplement market has been growing continuously in the last decades. For 2006, the world wide market has been estimated at more than US\$ 60 billion.¹ In athletes, the use of supplements is more widespread than in the general population.^{2,3} For elite athletes, figures of supplement are in the range of 44 and 100%, even though the prevalence greatly depends on type of sport, age, gender, and level of competition.^{3–6}

Some nutritional supplements contain doping substances, which are declared on the labels. Especially products marketed as fat burners or activating agents may contain caffeine (doping substance till 2004) and/or ephedrines. Whereas the use of caffeine is no longer banned since 2004

by the World Anti-Doping Agency (WADA), the use of ephedrines is prohibited during competition.⁷ On the labels of these products are declared caffeine or ephedrines or their natural sources. The natural sources are e.g. Guarana and Ma Huang and ephedra sinica, respectively.

Since 1996 special anabolic androgenic steroids, so-called prohormones, are legally available on the sports nutrition market. These substances are advertised to have enormous properties to increase muscle growth and strength. According to the doping regulations of the WADA, these substances belong to the prohibited class of anabolic agents.⁷ The most common prohormones on the market are the prohormones of testosterone and nandrolone.⁸ The application of a single recommended dose of a nandrolone prohormone (norandrostendione) may lead to positive doping findings for the main metabolite norandrosterone for more than ten days.⁸

Several studies have shown that the labelling of prohormone supplements did not reflect their actual

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content. Many prohormone products contained prohormones as well as concentrations different from those declared on the labels.^{9–13} These mislabelling problems indicated an insufficient surveillance and quality control of dietary supplements and a production, which did not follow Good Manufacturing Practices (GMP) conditions.

On the basis of this knowledge, rumours came up that nutritional supplements such as vitamins, minerals, and amino acids may also contain doping substances not declared on the labels and leading to inadvertent positive doping cases. Therefore several groups started to analyse nutritional supplements with mass spectrometric methods (GC/MS, LC/MS/MS) for nondeclared doping substances.

NUTRITIONAL SUPPLEMENTS CONTAMINATED AND FAKED WITH STIMULANTS

First reports on nutritional supplements containing prohibited stimulants, which were not declared on the product label, were published in 2002. Prior to the Salt Lake City Olympic Games, 14 of 69 products selected by Dutch Olympic athletes contained unlabelled stimulants. In twelve products caffeine was found, three supplements contained ephedrine. Additionally, one supplement was found to contain methylenedioxymetamphetamine (MDMA).^{14,15}

These results were confirmed by a similar study by Parr *et al.* Out of 110 supplements from the international market, 12.7% contained caffeine. Furthermore ephedrine and analogs (pseudoephedrine, methylephedrine) were detected in two products. In all cases, there was no indication of the stimulant content on the product label.¹⁶

The use of caffeine is no longer banned by the World Anti-Doping Agency (WADA) since 2004 but the use of ephedrines and its analogs and MDMA is prohibited during competition.⁷ Besides the apparent doping risk of supplements containing banned stimulants, the potential health risks should not be neglected. Due to structural similarities with amphetamine, ephedrine exhibits similar modes of action and side effects (restlessness, tremor, cardiovascular problems, disturbance of thermoregulation etc.).

Recently several slimming products adulterated with the anorectic drug sibutramine appeared on the market. Only since 2006 sibutramine is on the list of prohibited substances of the World Anti-Doping Agency. In at least three cases, sibutramine was detected in 'pure herbal' Chinese slimming capsules.^{17–19} Additionally, the same compound was also found in Chinese slimming tea, which was labelled to be of solely natural content.¹⁹ Even 50 h following a single administration of the tea, sibutramine metabolites were clearly detectable in a volunteer's urine (Fig. 1) and would have caused a failed doping test.

It should also be considered that severe side effects such as increased blood pressure and heart rate may occur and require regular monitoring of patients using sibutramine.²⁰

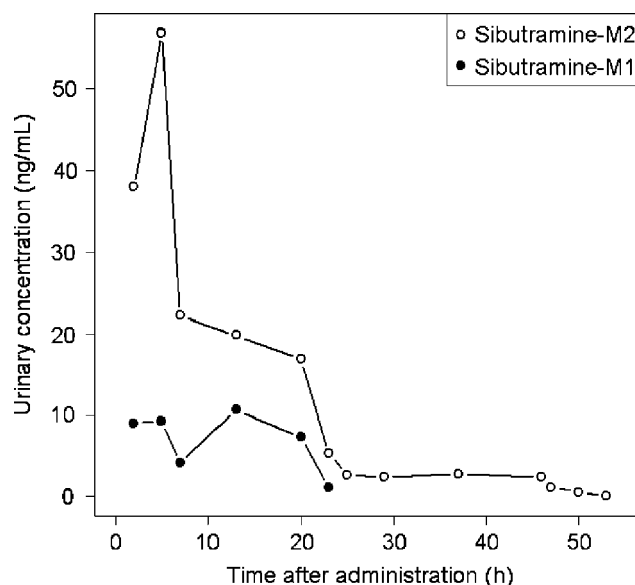


Figure 1. Urinary excretion of sibutramine metabolites following the consumption of faked Chinese slimming tea¹⁹ sibutramine-M1: desmethyl sibutramine, sibutramine-M2: bisdesmethyl sibutramine.

NUTRITIONAL SUPPLEMENTS CONTAMINATED WITH PROHORMONES

In 1999 in connection with follow up studies of positive doping cases with norandrosterone, the first cases of nutritional supplements cross-contaminated with anabolic androgenic steroids (prohormones) were detected.²¹ In the following year further nutritional supplements with non-declared anabolic androgenic steroids were detected in connection with positive doping cases.²² In Fig. 2 are shown results of the GC/MS analysis of a creatine product containing seven different prohibited anabolic androgenic steroids (prohormones) and testosterone not declared on the label. An international study performed in 2001 and 2002 on 634 nutritional supplements purchased in 13 different countries showed that about 15 percent of nonhormonal nutritional supplements, such as minerals, vitamins, proteins, creatine etc. contained anabolic androgenic steroids (mainly prohormones) not declared on the label.^{23,24} In Table 1 the percentages of nutritional supplements cross-contaminated with anabolic androgenic steroids (prohormones) in different countries in 2001/2002 are presented.

On the basis of the very low and varying concentrations of the prohormones their presence was interpreted as cross-contaminations and not as intentional admixtures. The reason for the cross-contaminations was most probably the fact that manufacturers of prohormones (legally marketed as nutritional supplements in the USA till 2004) also manufactured other nutritional supplements such as vitamins on the same production line without a sufficient cleaning of the production line. Another source of cross-contaminations could be unclean transport containers from raw material suppliers of prohormones. The consumption of such contaminated nutritional supplements can lead to inadvertent positive doping cases.²⁴ These results were confirmed by several other investigations.^{25–27}

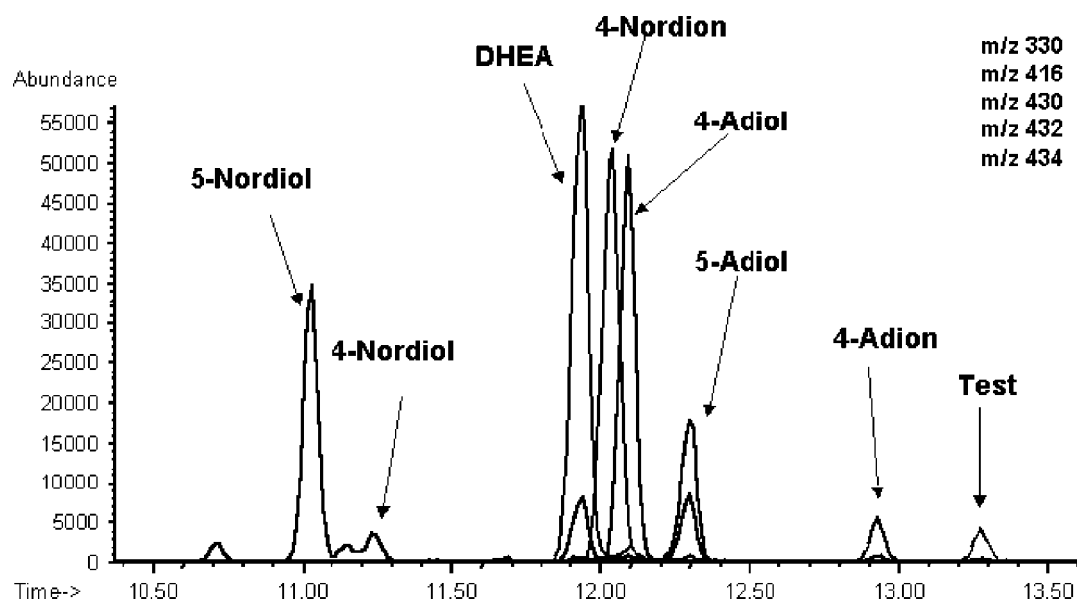


Figure 2. SIM-chromatograms of a creatine product with seven prohormones and testosterone not declared on the label (all steroids are pertrimethylsilylated)²² 5-nordioliol: 19-nor-5-androstene-3 β ,17 β -diol, 4-nordioliol: 19-nor-4-androstene-3 β ,17 β -diol, DHEA: dehydroepiandrosterone, 4-nordion: 19-nor-4-androstene-3,17-dione, 4-adiol: 4-androstene-3 β ,17 β -diol, 5-adiol: 5-androstene-3 β ,17 β -diol, 4-adion: 4-androstene-3,17-dione, test: testosterone.

Table 1. Nutritional supplements cross-contaminated with anabolic-androgenic steroids (prohormones) in different countries in 2001/2002^{23,24}

Country	No. of analyzed products	No. of cross-contaminated products	Percent age of cross-contaminated products
Netherlands	31	8	25.8
Austria	22	5	22.7
UK	37	7	18.9
USA	240	45	18.8
Italy	35	5	14.3
Spain	29	4	13.8
Germany	129	15	11.6
Belgium	30	2	6.7
France	30	2	6.7
Norway	30	1	3.3
Switzerland	13	–	–
Sweden	6	–	–
Hungary	2	–	–

Since that time athletes have been warned by their federations, information systems have been established and legislation towards anabolic androgenic steroids as nutritional supplements has been changed, e.g. by the Anabolic Steroid Act 2004 in the USA.

NUTRITIONAL SUPPLEMENTS FAKED WITH HIGH AMOUNTS OF 'CLASSIC' ANABOLIC ANDROGENIC STEROIDS

Since 2002 nutritional supplements have been detected, which probably were intentionally faked with high amounts

(higher than 1 mg/g) of 'classic' anabolic steroids like metandienone, stanozolol, boldenone, oxandrolone, dehydrochloromethyl-testosterone etc. The anabolic steroids are not declared or declared with nonapproved or fancy names on the labels. In Table 2 some of these faked nutritional supplements are listed.

All anabolic steroids found in these faked supplements are prescription drugs, which are freely available on the nutritional supplement market. The supplements listed in Table 2 can be purchased by telephone order and is delivered by ordinary mail. The consumption of the recommended dose leads to an administration of suprathreshold doses of these steroids. The faked supplements can be purchased without restrictions by everyone, including women, adolescents, and children. Especially for these groups the use of these steroids is associated with a large number of adverse effects. Known symptoms after intake include abnormal liver function, menstrual disorders, virilization, gynecomastia, severe psychological or psychiatric disorders, and an increased risk of cardiovascular disease. Long-term application of large doses has been shown to result in dependence, associated with an acute withdrawal syndrome. The use of these 'classic' anabolic steroids in children can lead to virilization and premature closure of the epiphyses with a derogation of linear growth. The application can also result in irreversible adverse effects. In women these effects are deepening of the voice and growth of the clitoris. During pregnancy the use of such steroids may result in a virilization of the fetus. Due to the fact that most of these steroids are 17-methylated compounds, their use is associated with a high-liver toxicity and carcinogenicity.^{8,34}

With regard to the suprathreshold doses additional health risks can be expected.

In addition to enormous health risks the use of the analyzed supplements can lead to positive doping results.

Table 2. Nutritional supplements faked with 'classic' anabolic androgenic steroids since 2002

Product name (pharmaceutical form)	Company	Declared ingredients	Not declared 'classic' anabolic androgenic steroids and references
Stanozolol II (powder)	Pharmtec	AD-4-Complex nutrients, MetX synergistic blend, 1-T matrix, creatine monohydrate, thermogenic proprietary blend, ribose-4 matrix	Metandienone 4 mg/serving (10 g powder) ²⁸
Parabolon (powder)	Pharmtec	AD-4-Complex nutrients, MetX synergistic blend, 1-T matrix, creatine monohydrate, thermogenic proprietary blend, ribose-4 matrix	Metandienone 10 mg/serving (10 g powder) ²⁹⁻³¹
Met-AD17-diol (capsules)	Pharmtec	Met-AD17-diol/1-T matrix	Metandienone <i>ca.</i> 9 mg/capsule ²⁹⁻³¹
Stanozolol-S (fizzy tablets)	Senesco-Pharma	Several prohormones	Stanozolol <i>ca.</i> 15 mg/tablet, Boldenone, DHT ³²
Parabolon-S (fizzy tablets)	Senesco-Pharma	Several prohormones	Metandienone <i>ca.</i> 17 mg/tablet ³²
OXA 17-DION (capsules)	Muscle Inc. Ltd, Cyprus	5 alpha-androstan-2-oxa-17 alpha-methyl-17, beta-ol-3-one	Oxandrolone <i>ca.</i> 25 mg/capsule ³³
TESTEXX (capsules)	Muscle Inc. Ltd, Cyprus	1,4-Androstadien-4-chloro-17 α -methyl-17 β -ol-3-one	Dehydrochloro-methyltestosterone <i>ca.</i> 25 mg/capsule ³³
Primo 17-acetoxo (capsules)	Muscle Inc. Ltd, Cyprus	17 β -Acetoxo-1-methyl-5-alpha-androst-1-en-3-one	Metenolone acetate <i>ca.</i> 50 mg/capsule ³³
Parabolon-B (blades)	Pharm-Tec	P-Blade 17Tren-1,4dien-3-one Beta-Compound	Metandienone <i>ca.</i> 10 mg/blade ³³
Stanozolol-B (blades)	Pharm-Tec	S-Blade 5-Alpha Pyrazol-Compound	Stanozolol <i>ca.</i> 10 mg/blade ³³

Table 3. Chinese companies which offer anabolic androgenic steroids via internet

Company	Internet address	Online
Tianjin Xinmei Technology Cooperation	http://www.worldpharm.com.cn/xinmei/english/hormones.htm	September 2006
Zyf Pharm Chemical	http://zyongfu.3322.net/	September 2006
Xianju Green Leaf Pharmaceutical Factory	http://www.greenleafpharm.com/	September 2006

The sources of these anabolic steroids are probably Chinese pharmaceutical companies, which offer bulk material of numerous anabolic androgenic steroids via the internet. The knowledge of China as origin of bulk material of anabolic steroids is mainly based on our own experience. After purchase of bulk material, shipment tracking led to Chinese towns as origin of the shipment. In Table 3 some internet addresses of Chinese suppliers of anabolic androgenic steroids are presented. Probably criminal nutritional supplement companies buy anabolic steroids from such sources and admix them to their supplements to make them more effective.

Because the 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 following chapter cases of such cross-contaminations are presented.

VITAMINS AND MINERALS CONTAMINATED WITH 'CLASSIC' ANABOLIC ANDROGENIC STEROIDS

At the end of 2005, a German drug surveillance authority confiscated nutritional supplements faked with high amounts of metandienone and stanozolol from a German manufacturer. The names of the products were Parabolon-S and Stanozolol-S from Pharm-Tec. The analyses of vitamin C, multivitamin, and magnesium tablets, which were produced from the same manufacturer on the same production line within the same time interval showed cross-contaminations of these products with metandienone and stanozolol (Table 4). Metandienone at a concentration of 50 ng/g corresponding to 0.2 µg/tablet

was detected in vitamin C tablets and stanozolol at concentrations of 5–40 ng/g corresponding to 0.06–0.15 µg/tablet was detected in multivitamin and magnesium tablets.³⁵

These vitamin C, multivitamin and magnesium tablets were sold in ordinary German and Spanish groceries and drug stores.³⁵ 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. On the basis of the same considerations health risks for young people and females who consume these vitamins and minerals as nutritional supplements also, cannot be excluded.

NUTRITIONAL SUPPLEMENTS WITH NEW 'DESIGNER' STEROIDS

Since 2002 nutritional supplements with new 'designer' steroids such as prostanazol, methasterone, androstatrienedione etc. have been offered on the nutritional supplement market via the internet. In Table 5 are listed such new findings and the corresponding references.

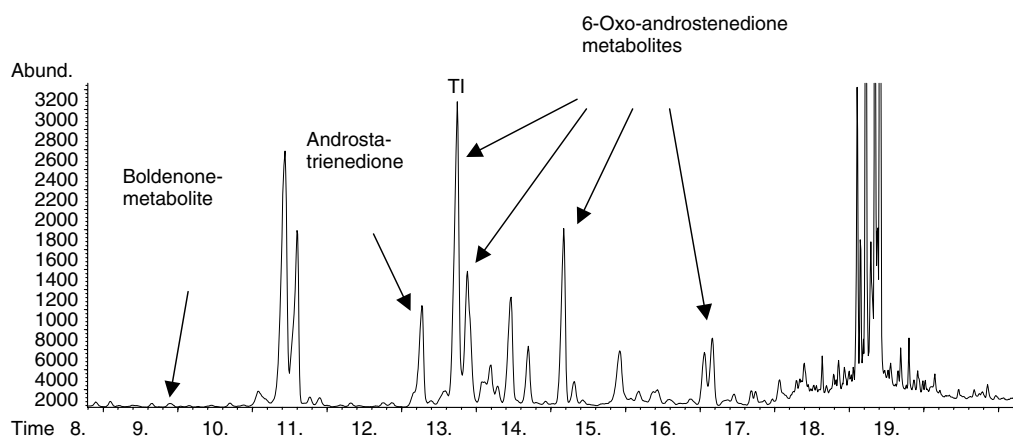
These steroids are neither listed as ingredient in any currently available medicament nor do their names appear in the WADA list of prohibited substances. They are only produced for the nutritional supplement market and are advertised as anabolic steroids or aromatase inhibitors. About effects and side effects of these steroids only little knowledge exists. The legal status of these supplements is not clear. With these 'new' steroids it is possible to circumvent the Anabolic Steroid Control Act 2004.⁵³ According to this act the selling of anabolic steroids as nutritional supplements is prohibited in the USA, however these new steroids are not on the list of banned steroids.⁵³ According to

Table 4. Nutritional supplements from the German and Spanish market cross-contaminated with 'classic' anabolic steroids³⁵

Product	Market	Expiry date	Tube	Cross-contaminations
Vitamin C	Germany	July 2005	Tube 1	Metandienone <i>ca</i> 50 ng/g
			Tube 2	Metandienone <i>ca</i> 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 <i>ca</i> 10 ng/g
			Tube 2	Stanozolol <i>ca</i> 15 ng/g
			Tube 3	Stanozolol <i>ca</i> 10 ng/g
Magnesium	Germany	January 2005	Tube 1	Stanozolol <i>ca</i> 40 ng/g
			Tube 2	Stanozolol <i>ca</i> 40 ng/g
			Tube 3	Stanozolol <i>ca</i> 5 ng/g

Table 5. New steroids detected in nutritional supplements since 2002

Chemical name (IUPAC)	Trivial name	References
17 β -Hydroxy-2 α ,17 α -dimethyl-5 α -androst-3-one	Methasterone	36–40
17 β -Hydroxy-17 α -methyl-5 α -androst-1-en-3-one	Methyl-1-testosterone	36
4-Hydroxyandrost-4-ene-3,17-dione	Formestane	41–43
4,17 β -Dihydroxyandrost-4-ene-3-one	4-Hydroxytestosterone	41–43
5 α -Androstane-3 β ,17 α -diol		44
Androst-4-ene-3 β ,17 α -diol		44
5 β -Androst-1-ene-3 β ,17 β -diol		44
5 β -Androst-1-ene-3 α ,17 β -diol		44
17 β -Hydroxy-5 α -androstano-[3,2-c]-pyrazol	Prostanozol	38, 40
6 α -Methylandrost-4-ene-3,17-dione	6 α -Methylandrostenedione	38, 40, 45
3 β -Hydroxy-5 β -androst-17-one	Epitiocholanolone	40
17 β -Hydroxy-17 α -methyl-5 β -androst-3-one	5 β -Mestanolone	40
17 α -Methyl-5 α -androst-2-en-17 β -ol	Desoxymethyltestosterone	40
4-Chloro-17 α -methylandrost-4-ene-3 ξ ,17 β -diol		39
Androst-4-ene-3,6,17-trione	6-Oxo-androstenedione	46, 47
Androsta-1,4,6-trien-3,17-dione	Androstatrienedione	33, 48, 49
3 β -Hydroxyandrost-4-ene-7,17-dione	7-Keto-dehydroepiandrosterone	50
6 ξ -Bromandrost-4-ene-3,17-dione		51
17 α -Methyl-5 α -androstane-3 α , 17 β -diol		51
17 β -Hydroxy-5 α -androstano-[3,2-c]-isoxazol		52
17 β -Hydroxy-5 α -androstano-[2,3-d]-isoxazol		52
Estra-4,9-diene-3,17-dione		52

**Figure 3.** TIC Chromatogram of a doping control sample containing metabolites of the 'new' steroids androstatrienedione and 6-oxo-androstenedione.³³

European legislation these products have to be classified as nonlicensed pharmaceuticals even if they are marketed as nutritional supplements. In sports these new steroids belong to the prohibited classes of anabolic androgenic steroids or aromatase inhibitors.⁷

In most cases the labeling of these products contains nonapproved or fancy names of the steroids.

The sources of the new anabolic steroids are not known. Most probably Chinese pharmaceutical companies are involved because some of the new steroids detected in nutritional supplements were offered on their product lists on the internet.

The use of such nutritional supplements with new steroids by athletes is shown in the following examples. In September 2006 in several urine samples of athletes,

unknown signals were observed, raising concerns about their relevance for doping control. The mass spectrometric analyses of these signals identified them as metabolites of androstatrienedione and 6-oxo-androstenedione (Fig. 3). The results were attributed to a consumption of the nutritional supplement Novedex Xtreme from Gaspari, which is advertised as testosterone booster and aromatase inhibitor. This product contained androstatrienedione and 6-oxo-androstenedione.³³

Another case is presented in Fig. 4. In a doping control sample was detected hitherto an unknown signal which was identified as metabolite of the steroids 6 α -methyl-androstenedione. Additionally an increased ratio of the endogenous steroids testosterone and epitestosterone was detected. This result was attributed to the administration of

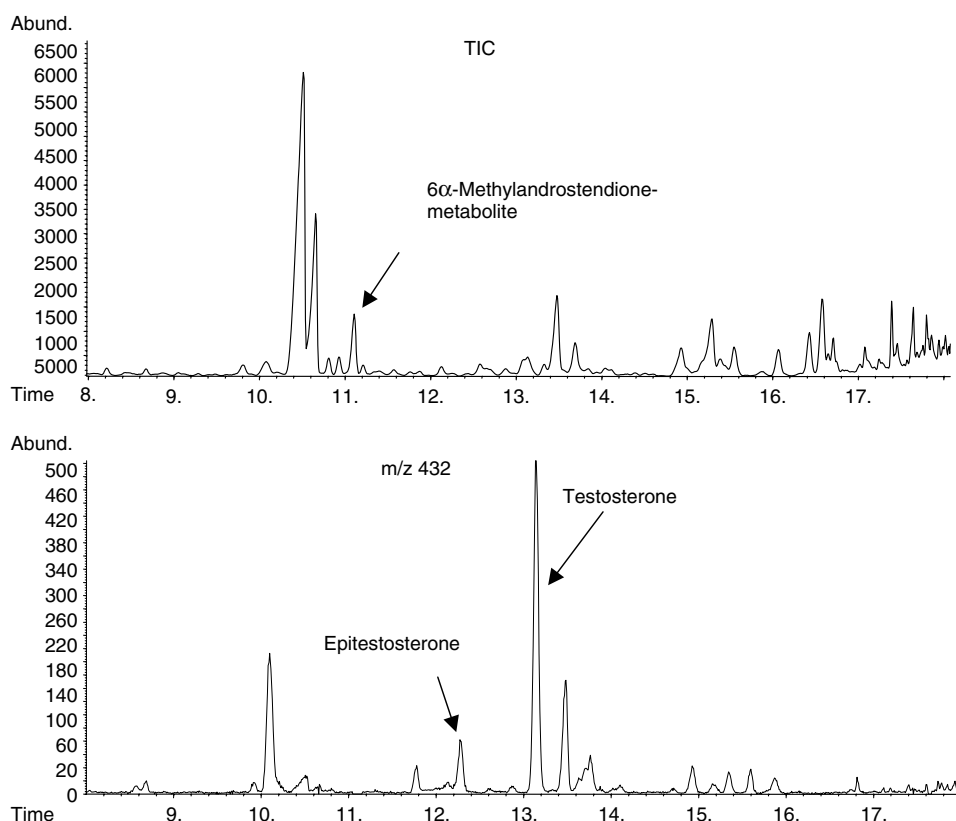


Figure 4. TIC and single-ion chromatograms of a doping control sample containing metabolites of the new steroid 6- α -methyl-androstenedione (upper trace) and showing an increased testosterone/epitestosterone ratio (lower trace).³³

the nutritional supplement methyl-1-pro from Proline, which contains the 'new' steroid 6- α -methyl-androstenedione. Additionally this supplement contains the prohormone androstenedione. As for the steroids in the Novedex product the 6- α -methyl-androstenedione is neither listed on the prohibited list of the WADA nor as ingredient in any currently available medicament and is only produced for the nutritional supplement market.³³

As the manufacturers of these products also manufacture other nutritional supplements on the same production line, cross-contaminations of 'normal' nutritional supplements with 'new' steroids can be expected possibly causing inadvertent doping in the near future.

NUTRITIONAL SUPPLEMENTS WITH THE β 2-AGONIST CLENBUTEROL

Recently nutritional supplements for weight loss, labeled to contain as active ingredient '30 μ g Clenbuterol Enetat' per tablet, appeared on the market. After the GC/MS analysis the tablets were found to contain clenbuterol in an estimated amount of 30 μ g per tablet.⁵⁴ The β 2-agonist clenbuterol is only legally available on prescription and is classified as prohibited doping substance in sports.⁷ After administration of one tablet, clenbuterol was detected in a urine sample, collected 3 h after ingestion, in a concentration of 2 ng/ml.⁵⁴

Table 6. Characteristic fragment ions of selected trimethylsilylated steroids using electron ionization

Steroid nucleus	Representative compound	Common ions (<i>m/z</i>)	
3-Keto	5 α -Dihydrotestosterone-bis-TMS	143	142
3-Keto-4-ene	Testosterone-bis-TMS (androsta-3,5-dien-3,17 β -diol-bis-O-TMS)	208	129
	Testosterone-bis-TMS (androsta-2,4-dien-3,17 β -diol-bis-O-TMS)	194	129
	Nandrolone-bis-TMS (estra-3,5-dien-3,17 β -diol-bis-O-TMS)	194	129
3-Keto-1-ene	1-Testosterone (androsta-1,3-diene-3,17 β -diol-bis-O-TMS)	194	129
3-Keto-1,4-diene	1-Dehydrotestosterone-bis-TMS	206	191
	(androsta-1,3,5-triene-3,17 β -diol-bis-O-TMS)		
3-Hydroxy-1-ene	Androst-1-ene-3 α ,17 β -diol-bis-O-TMS	143	142
17-Methyl	17 α -Methyl-5 α -androstane-3 α ,17 β -diol-bis-O-TMS	143	130
17-Keto	Androsterone-bis-O-TMS	169	105
	Etiocholanolone-bis-O-TMS	169	105

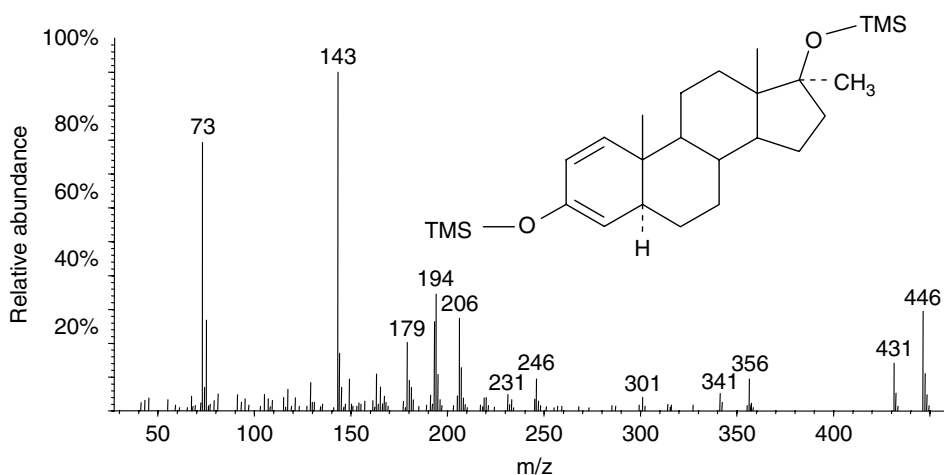


Figure 5. Mass spectrum (EI) of methyl-1-testosterone, bis-TMS ($M^+ = 446$).³⁶

STRATEGIES FOR THE DETECTION OF NEW AND UNKNOWN STEROIDS IN NUTRITIONAL SUPPLEMENTS

The issue of 'designer steroids' has become a serious problem in sports-drug testing, and compounds being available and advertised as nutritional supplements have frequently been found to contain one or more modified steroids with unknown structure, physicochemical and biological properties. Hence comprehensive screening tools to determine the presence of such hormones were required, and mass spectrometry has demonstrated to provide the necessary sensitivity and selectivity to enable the determination of conserved core structures of steroid hormones.^{55,56}

Common screening methods for the detection of steroidal agents are commonly based on either GC/MS or LC/MS/MS methods. GC/MS necessitates the derivatization of most analytes, which is commonly accomplished using trimethylsilylation. Consequently the dissociation behavior of the analytes upon electron ionization (EI) is directed and influenced by the introduced trimethylsilyl (TMS) residues, but characteristic fragment ions or neutral losses are found indicating the principle structure and functional group(s) of steroids. A list of common fragment ions derived from EI-MS analyses of typical steroidal structures is presented in Table 6. Such ions that commonly distinguish a particular steroid structure are for instance m/z 129, 130, 143, 169, 194, and 206 as well as the elimination of 103. The ion at m/z 129 represents a valuable indicator for the presence of a 3- or 17-hydroxyl function^{57,58} while fragments found at m/z 130 and 143 have frequently been observed with 17-methyl-steroids^{59–61} such as methyl-1-testosterone depicted in Fig. 5. A fragment at m/z 169 is a characteristic item of steroids comprising a 17-oxo function, which is converted to its enol-TMS ether,^{62,63} and the ions at m/z 194 and 206 are indicators for α,β -unsaturated 3-keto steroids such as testosterone and metandienone, respectively.^{64–66} A loss of 103 u was observed in cases of hydroxylated methyl residues such as 19-hydroxytestosterone⁶⁷ or 17 β -hydroxymethyl,17 α -methyl-androst-1,4,13-trien-3-one, a long-term metabolite of metandienone.⁶⁸

Table 7. Characteristic product ions of selected steroids using electrospray ionization and CID

Steroid nucleus	Representative compound	Product ions (m/z)	
3-Keto	5 α -Dihydrotestosterone	255	215
	5 β -Dihydrotestosterone	255	215
	17 α -Methyl-5 α -androst-17 β -ol-3-one	269	229
	1 α -Methyl-5 α -androst-17 β -ol-3-one	269	229
3-Keto-4-ene	Testosterone	109	97
	Nandrolone	109	91
3-Keto-1-ene	1-Testosterone	187	145
	Metenolone	187	145
	Androst-1-en-3,17-dione	185	143
3-Keto-1,4-diene	1-Dehydrotestosterone	135	121
3-Keto-4,6-diene	6-Dehydrotestosterone	133	97
3-Keto-4,9-diene	Androsta-4,9(11)-dien-17 β -ol-3-one	147	145
3-Keto-4,9,11-triene	Trenbolone	227	199
	Gestrinone	241	199
	Tetrahydrogestrinone	241	199
	Propyltrenbolone	227	199

The utility of LC/MS/MS in particular for steroids with marginal gas-chromatographic properties was demonstrated several times.^{69–71} Especially employing the precursor ion scanning option of triple-quadrupole mass analyzers provided a useful tool for the detection of unknown steroids when focusing on product ions derived from common steroid structures and nuclei.⁷⁰ For instance, anabolic steroids with 3-keto-4-ene core (e.g. testosterone) commonly yield ions at m/z 97 and 109, 3-keto-1,4-diene nuclei (e.g. metandienone) give rise to abundant ions at m/z 121 and 135, and ions at m/z 227 and 241 are observed with 3-keto-4,9,11-triene structures such as trenbolone and gestrinone, respectively.^{72,73} A summary of characteristic product ions of anabolic steroids is shown in Table 7. The applicability is demonstrated with the product-ion spectrum of androsta-1,4,6-triene-3,17-dione

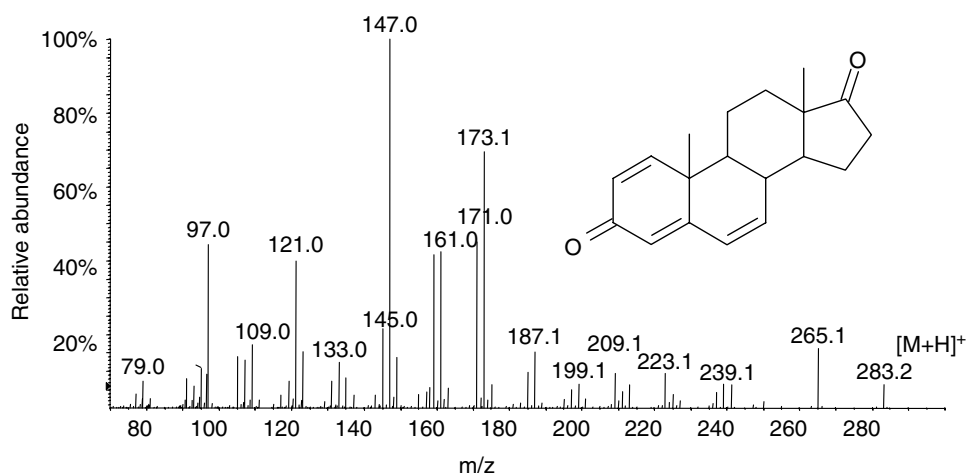


Figure 6. ESI-product-ion spectrum of androsta-1,4,6-triene-3,17-dione, $[M + H]^+ = 283.48$

(Fig. 6). Here the ions at m/z 121 and 133 indicate the combined presence of 1,4- and 4,6-ene structures, which add up to a possible steroid nucleus with three double bonds.

Using the discriminating and identification power of mass spectrometry enables the detection of an enormous variety of unknown steroids based on common structural features. This way more analytes are covered in screening methods than with conventional targeted assays that necessitate the knowledge of the molecular weight.

SOURCES OF 'LOW RISK' SUPPLEMENTS

To prevent inadvertent doping, athletes should take nutritional supplements only from sources of 'low-risk supplements'. Such sources exist, for example, for athletes in the Netherlands and Germany. In these countries athletes have access to databases, which contain nutritional supplements from companies, which perform quality control for anabolic steroids and stimulants and/or guarantee that they have no contact to these substances in the production and transportation processes. In the Netherlands, this data base (NZVT database) was installed by the anti-doping authority of the Netherlands and is available under <http://antidoping.nl/nzvt>. In Germany this database (Kölner Liste = Cologne List) is under the umbrella of the Olympic Center Rhineland (www.koelnerliste.de).

Other sources of 'low-risk supplements' are the lists of therapeutics from pharmaceutical companies. An investigation in Germany has shown that analogs of nutritional supplements listed on the index of therapeutics from the German pharmaceutical industry, the so called 'Rote Liste', did not contain anabolic androgenic steroids.⁷⁴

CONCLUSION

The situation on the nutritional supplement market has got worse because not only cross-contaminations with prohormones and stimulants but also with 'classic' anabolic androgenic steroids and β_2 agonists can be observed. The reason for this situation is the increasing trade and availability of bulk material of anabolic steroids, especially

from Chinese companies. Within the next years cross-contaminations with new 'designer' steroids are expected. With mass spectrometric methods focusing on fragment and product ions from common steroid structures and nuclei it is possible to detect the new 'designer' steroids.

To protect athletes from inadvertent doping, databases of low-risk supplements as in the Netherlands and Germany, should be installed also in other countries.

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