Upper limit of the doping risk linked to sports supplements

Study of the presence of prohibited substances
in high-risk sports supplements
available from Dutch web shops

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Summary

Various studies have shown that dietary supplements can contain prohibited substances without this being stated clearly on the label. In response to this risk for elite athletes, the Doping Authority established the Dutch Safeguards System for Dietary Supplements in Elite Sport (NZVT).

It is reasonable to assume that prohibited substances will seldom or never be added *deliberately* to dietary supplements submitted for analysis under the NZVT system. Nevertheless, between 2003 and 2014, eighteen of the product-batch combinations submitted (1.6%) were found to contain prohibited substances. This percentage is considered to be the lower limit for the prevalence of prohibited substances in dietary supplements.

Little or nothing is known about where the upper limit is to be found. International studies have reported maximum upper limits of around 25%. However, the actual upper limit was actually expected to be much higher, especially in a group of high-risk sports supplements specifically targeting hormone regulation, strengthening muscle, weight loss/burning fat, or raising energy levels. This study focused on the question of what percentage of these high-risk sports supplements contain prohibited substances.

After a selection was made from seventeen different web shops, 66 products from 21 different brands were submitted for analysis to the LGC research laboratory (United Kingdom). Twenty-five (38%) of the high-risk sports supplements tested 'positive' and 30 (45%) tested 'negative'. Eight products (12%) were found to contain 'concentrations below the adopted threshold value'. In these cases, the detection of doping substances may be explained by their natural presence in certain ingredients, such as botanical components. However, this cannot be stated with certainty. Specific research in this area is therefore required. Three of the 66 products (5%) were 'not analysable/not fully analysable'.

Three of the 25 'positive' products (5% of the total sample analysed) were found to contain prohibited substances in high concentrations. In addition to the real risk these products pose for athletes in terms of doping violations, there is also a genuine health risk. Given the public health implications, these findings were therefore reported to the Netherlands Food and Consumer Product Safety Authority (NVWA).

This study shows that Dutch elite athletes continue to be exposed to risks if they use product-batch combinations of dietary supplements that have not been tested for the presence of doping substances by the NZVT (or any other recognised system). At the same time, the NZVT is not used by large proportion - or even a majority - of Dutch elite athletes. This is an undesirable situation. It will therefore be important to boost the promotion of the NZVT among these elite athletes.

Glossary

Batch	See *Product-batch combination.*
Cologne List	Dietary supplements control system from Germany.
Concealed declaration	The prohibited substances are listed on the label using a non-standard name or as part of a non-standard name.
Concentrations under the adopted threshold value	Term used in the report. One or more prohibited substances were found, but only in concentrations below the adopted threshold values (reporting level compounds only – see Annex 2). In this case, the detection of prohibited substances may be explained by their natural presence in certain ingredients such as botanical and animal-derived ingredients. The low levels observed do not, in principle, involve a risk of a positive doping test for elite athletes. The supplement would therefore have passed the NZVT screening.
Contamination	The prohibited substance has been included in a product inadvertently.
Cross-contamination	The substances have been used deliberately in certain products but have also ended up inadvertently in other products.
Declared	The prohibited substances are listed on the label using a widespread, often generic, name.
Designer amphetamines	Designer compounds structurally similar to amphetamine.
Designer steroids	Designer compounds structurally similar to anabolic steroids.
Dietary supplements	Food or drink – intended to supplement a normal diet; – that acts as a concentrated source of one or more micronutrients or of other substances with a nutritional or physiological effect; – that are marketed in small unit quantities destined for consumption.
Dietary Supplements (Commodities Act) Decree	Legislation stating requirements applying to dietary supplements. For example, there are requirements for the preparation, composition and labelling of dietary supplements.

Elite athlete	An athlete with a realistic probability of qualifying for a doping control.
HACCP - Plus / NZVT system	The risk assessment system used by participating manufacturers in the NZVT.
High-Risk Dietary Supplement List	Overview released by USADA of dietary supplements that: – list prohibited substances on the label; or – turn out to contain prohibited substances after additional research; or – list ingredients on the label that are often linked to prohibited substances (such as certain herbal ingredients).
Informed-Choice	Dietary supplements control system from the United States.
Informed-Sport	Dietary supplements control system from the United Kingdom.
Negative	Term used in the report. One or more prohibited substances were not found. The supplement would therefore have passed the NZVT screening.
NOC*NSF	Netherlands Olympic Committee*Netherlands Sports Confederation. NOC * NSF is therefore the Dutch Olympic Committee and the Dutch organisation representing the interests of organised sports.
Not analysable/ not fully analysable	Term used in the report. The analysis could (often due to the complex herbal ingredients present) not be completed in full. It was therefore not possible to determine whether one or more prohibited substances were present or absent using the defined method specification. Because of the incomplete test result, the dietary supplement in question would not have passed the NZVT screening.
NZVT	Abbreviation for 'Dutch Safeguards System for Dietary Supplements in Elite Sport'. The NZVT is a system involving the Doping Authority, NPN and NOC*NSF. The system allows manufacturers of dietary supplements to have batches of their supplements checked in exchange for payment.
Phytochemical	Relating to the constituent components of plants.
Prohibited list	The list of prohibited substances and methods.

Positive	Term used in the report. One or more prohibited substances were found. This means that the dietary supplement in question would not have been approved during the NZVT screening process.
Product-batch combination	Dietary supplements are made in 'charges' or 'batches'. Every so often, producers mix the ingredients to form a finished product that is then packaged. The products packaged during a given uninterrupted period are part of the same product-batch combination. Each batch of a product can be identified on the basis of an identical shelf life (often referred to as 'best before'), generally accompanied by an identical batch number or production number.
Proprietary blend	A mixture of ingredients that is not specified on the label except for a statement that it is a *proprietary blend*.
RIVM	Abbreviation for 'National Institute of Public Health and Environmental Protection'. The RIVM's mission is the promotion of public health and a clean and safe environment.
Spiked	The prohibited substances are not listed on the label but they have been deliberately included in the supplement.
Sports supplements	Dietary supplements taken with the aim of improving sports performance
Status athletes	Athletes who have been granted elite status (A, B, or HP status) by NOC*NSF.
Strict Liability Principle	The liability for a positive result of a doping test resides entirely with the athlete.
Testosterone boosters	Supplements that claim to stimulate the body's own testosterone production.
TNO	Research institute.
Trademark substances	Substances or mixtures for which a patent has been obtained (™) or for which a patent is pending (®). These substances or mixtures are often given an invented name, making it unclear which substances are involved.
USADA	Abbreviation for 'United States Anti-Doping Agency'. The USADA is the National Anti-Doping Organisation (NADO) of the United States.

WADA	Abbreviation for World Anti-Doping Agency. WADA leads the global movement for clean sport. In that role, it also draws up doping regulations and the prohibited list for most elite sports.
World Anti-Doping Code	The basic document containing rules for the global fight to keep sport clean published by WADA.

1. Introduction

More than 80% of Dutch elite athletes use dietary supplements (Duiven & de Hon, 2015). Several studies (such as Geyer et al., 2004 and Judkins et al., 2007) have shown that these dietary supplements may contain prohibited substances without clearly saying so on the label. The ingestion of prohibited substances in this way by elite athletes may pose a risk of a positive doping test (Geyer et al., 2004; Baume et al., 2005; WADA[a], undated).

> *The use of dietary supplements by athletes is a concern because in many countries the manufacturing and labeling of supplements may not follow strict rules, which may lead to a supplement containing an undeclared substance that is prohibited under anti-doping regulations. A significant number of positive tests have been attributed to the misuse of supplements and taking a poorly labeled dietary supplement is not an adequate defense in a doping hearing.*
>
> *(World Anti-Doping Agency[a], undated)*

The liability for the result of a doping test resides entirely with the athlete. The World Anti-Doping Code 2015 refers to this as the *Strict Liability Principle*. It leads almost inevitably to an infringement of Article 3 of the World Anti-Doping Code and involves severe penalties for the elite athlete (WADA, 2013).

> *The principle of strict liability is applied in situations where urine/blood samples collected from an athlete have produced adverse analytical results. It means that each athlete is strictly liable for the substances found in his or her bodily specimen, and that an anti-doping rule violation occurs whenever a prohibited substance (or its metabolites or markers) is found in bodily specimen, whether or not the athlete intentionally or unintentionally used a prohibited substance or was negligent or otherwise at fault.*
>
> *(World Anti-Doping Agency[b], undated)*

1.1. NZVT

The doping risk associated with the use of dietary supplements first came to light around the turn of the century. Positive tests by athletes Linford Christie, Merlene Ottey and Troy Douglas (1999) and international football players Edgar Davids, Frank de Boer and Jaap Stam (2001) resulted in widespread commotion. Very low concentrations of the anabolic substance nandrolone were found in all these cases. In response to this discussion, TNO (the Netherlands Organisation for Applied Scientific Research) and RIVM (the Dutch National Institute of Public Health and the Environment) were asked by NOC*NSF (Netherlands Olympic Committee*Netherlands Sports Confederation) to investigate the risk further. The conclusion was that dietary supplements could contain indeed prohibited substances without being listed on the label (Schilt et al., 2002).

This led to the establishment on 21 November 2003 of the *Dutch Safeguards System for Dietary Supplements in Elite Sports (NZVT).* The NZVT is a system involving the Doping Authority, NPN and NOC*NSF. The system allows manufacturers of dietary supplements to have batches of their supplements checked in exchange for payment. A *product-batch combination* which is found to

be 'clean' is then included in the NVZT database. In this way, the NZVT allows Dutch elite athletes to use dietary supplements while virtually eliminating the associated risk of doping.

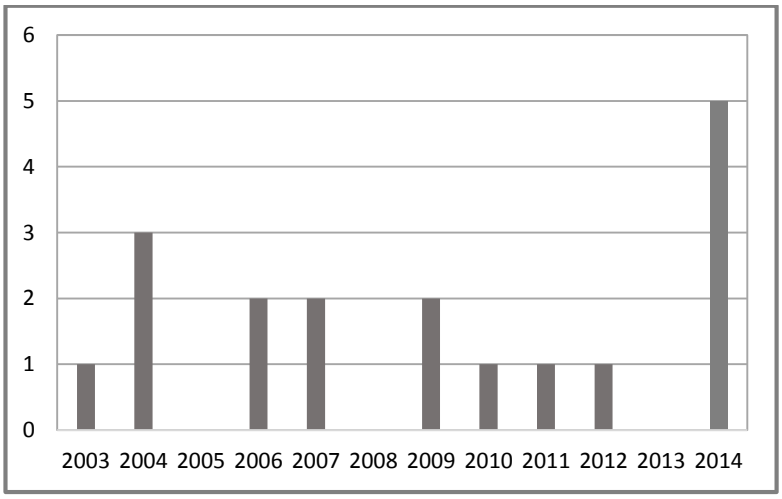
At the time, the NZVT was the first system in the world for checking dietary supplements. In the meantime, a limited number of countries have launched similar alternatives. The principal systems are:

- Informed-Sport (United Kingdom);
- Informed-Choice (United States);
- Cologne List (Germany) (Coumans & de Hon, 2012).

1.2. Lower limit of 1.6%

Prohibited substances will rarely or never be included *deliberately* in dietary supplements submitted to the NZVT for analysis since a producer would then be paying for an analysis without the corresponding benefit (inclusion in the NZVT database). In addition, it is reasonable to assume that the production standards at NZVT manufacturers are higher than average since these companies are required to comply with a dedicated risk assessment system: the *HACCP - Plus/NZVT system* (NZVT, 2015).

Nevertheless, in the period between 2003 and 2014, no fewer than 1.6% of the submitted product-batch combinations (18 samples) were rejected due to the presence of prohibited substances (Doping Authority, 2015). This percentage is considered to be the lower limit for the prevalence of prohibited substances in dietary supplements. It is considered to apply solely to the *inadvertent* addition of prohibited substances in a process referred to as *contamination*.



Graph 1: number of product-batch combinations rejected by NZVT

1.3. Use of unverified dietary supplements

If all Dutch elite athletes were to consider the NZVT as their first port of call, then it is probable that no one would ever test positive as a result of the use of dietary supplements. Unfortunately, however, the NZVT and similar systems are not a comprehensive solution. For example, the NZVT database includes only a relatively limited number of dietary supplements. As a result, athletes in the

Netherlands sometimes use supplements that are not approved by the NZVT. The reasons stated by these athletes are, for example, that another supplement is cheaper, that it tastes better, contains other active substances, or that it is easier to obtain than the best alternative available within the NZVT system (Doping Authority, 2015). Stopping behaviour of this kind is possible to only a limited extent. For example, no fewer than 19% of the NOC*NSF status athletes use unverified supplements. In other groups of elite athletes, this percentage may even rise to 50% (Duiven & de Hon, 2015).

1.4. Deliberate addition of prohibited substances

In the context of doping prevention, it would therefore be useful to determine the risk to which Dutch elite athletes are exposed when they use dietary supplements that have not been checked by the NZVT. This percentage is probably well above the 1.6% reported in the context of the NZVT.

In addition to the contaminated dietary supplements, there are also products to which prohibited substances have been added deliberately. In this respect, the *sports supplements* that place a strong emphasis on the following claims are particularly suspect:

- hormone regulation (*testosterone boosters*, for example (Judkins, 2007));
- strengthening muscle (Judkins, 2007; FDA, 2015);
- weight loss/burning fat (Judkins, 2007; FDA, 2015); and
- boosting energy levels (USADA[a], undated).

In the case of these products, the prohibited substances may be *declared* clearly on the label. This is possible, among other things because not all prohibited substances are subject to a government ban on inclusion in dietary supplements. In addition, the correct listing of the prohibited substances means that there is no infringement of the Dutch Dietary Supplements (Commodities Act) Decree. As a result, and because athletes can at least be expected to check the label for prohibited substances or specific warnings about doping, these products have been excluded from the laboratory analysis in this study. An overview of all declared prohibited substances identified during the selection of the products can be found in Annex 1: overview of declared prohibited substances.

In other cases, the prohibited substances will not be clearly stated on the label. It may be that the product is *spiked*. The prohibited substances have been deliberately added but they are not listed on the label. Another possibility is that the prohibited substances may be included in a *concealed declaration*, for example in a *trademark name* invented by the manufacturer or as a component in a blend that is not further specified. The term often used here is *proprietary blend*. This research focuses in particular on these spiked sports supplements involving a concealed declaration.

1.5. Upper limit unknown

A number of previous studies have also looked at the prevalence of prohibited substances in sports supplements. For example, Judkins et al. (2007) studied a group of sports supplements in the American market. They found a prevalence of

26%, with 9% of the products containing both stimulants and anabolic steroids. A number of years earlier, Geyer et al. (2004) conducted a study with a more international scope. They reported that an average of 15% of the sports supplements (purchased in fifteen different countries) contained traces of anabolic steroids. Products obtained in the Netherlands topped the list with a prevalence of 26%.

Since that time (2004-2007), the prevalence of prohibited substances in sports supplements available in the Netherlands seems to have increased rather than decreased. For example, since the turn of the century, the Netherlands Food and Consumer Product Safety Authority (NVWA) has taken an increasing number of products off the market because they contained substances that are harmful to health. These were often prohibited substances. The first incident was in 2003 when ephedra was banned (Doping Authority, 2003), followed in 2012 by methylhexanamine (DMAA) (Doping Authority, 2012). In 2013, Iomax (Doping Authority[a], 2013) and dexaprine were banned (Doping Authority[b], 2013) and 1.3-dimethylbutylamine (DMBA/nor-DMAA) (NVWA, 2015) followed in 2015. In addition, more and more new amphetamines are being discovered that have been developed specifically for the sports supplement market. An example of a *designer amphetamine* of this kind is N,alpha-diethyl-phenylethylamine, for which four Polish athletes tested positive in 2013 and 2014 (Ergogenics, 2015).

So there is strong evidence that levels of prohibited substances in sports supplements are higher than the percentages found in studies in 2004 and 2007. However, there has been no research since that time to support or invalidate that assumption.

1.6. Research area

What percentage of sports supplements contain prohibited substances when these products:

- are available from Dutch web shops;
- focus on:
    - hormone regulation;
    - strengthening muscle;
    - weight loss/burning fat;
    - raising energy levels;
- no prohibited substances are declared (or carry an explicit doping warning)?

These products will be referred to as *high-risk sports supplements, HR supplements* or *HR products* in the remainder of this study.

1.7 Anonymity

The aim of this study is to determine the upper limit of the doping risk for elite athletes who use dietary supplements. The intention is <u>not</u> to point out their possible shortcomings in public to specific manufacturers of high-risk dietary supplements. The results of this research have therefore been anonymised.

2. Selection process for dietary supplements

This chapter explains the selection process for:

1. the web shops for which the brands were listed;
2. the brands for which products were listed;
3. the products submitted to the laboratory for analysis.

2.1 Selection of web shops

For practical reasons, this study focused specifically on products that were available in the Dutch web shops. A web shop qualified as Dutch if it targeted Dutch consumers and if the language used on the website was also Dutch.

The Dutch web shops were identified in late 2014. The following search terms (in Dutch) were used in Google:

- dietary supplements / supplements;
- dietary supplements online / supplements online;
- buy dietary supplements / buy supplements;
- dietary supplements web shop / supplements web shop.

Only the web shops focusing primarily on sales of dietary supplements were selected. That resulted in 26 unique web shops. Two of them were excluded because they did not provide any overview of the brands they sold.

USADA High Risk Dietary Supplement List

To narrow down the sample, a check was conducted to see whether the web shops sold dietary supplements on the USADA *High Risk Dietary Supplement List* (USADA[b], undated). USADA uses this list as a service for elite athletes. It is the world's most comprehensive, publicly available overview of dietary supplements that:

- list prohibited substances on the label; or
- turn out to contain prohibited substances after additional research; or
- list ingredients on the label that are often linked to prohibited substances (such as certain botanical ingredients).

The web shops that sold these dietary supplements were expected to be most likely to sell large numbers of high-risk sports supplements. After the completion of this second selection procedure, our sample contained seventeen web shops.

2.2 Selection of brands

A list was drawn up of the brands sold by the seventeen suspect web shops. This turned out to contain a total of 469 different brands. It was decided that this was too large a number to allow for full product analysis. We therefore selected only the brands supplied by at least 50% of the web shops (nine or more). This resulted in 34 brands.

Restriction imposed by laboratory

LGC in England was selected to conduct the analysis of the dietary supplements. LGC is a reputable laboratory (with ISO/IEC 17025 accreditation) that also analyses the supplements for the dietary supplement systems in the UK (Informed-Sport) and United States (Informed-Choice).

Of the 34 selected brands, nine were already LGC customers in the context of Informed-Sport or Informed-Choice. These relationships meant it was not desirable to include these brands in our study. In addition, one brand was excluded because the associated web site provided no overview of the products being sold. This reduced the total number of brands to 24.

2.3 Selection of products

A review was conducted of the 24 selected brands looking at all the high-risk sports supplements they sold. It turned out that a number of brands sold so many dietary supplements that it was decided to analyse only the most suspect line of products for the brand concerned. This process resulted in the pre-selection of 190 products from 22 brands. Two brands were found to have no HR supplements in their ranges.

Sixty-six of these 190 products were purchased for laboratory investigation. The criteria for purchase were:

- the selected products preferably had to be on sale in at least four different web shops; and
- no more than five different products were selected per brand.

However, due to practical considerations, these criteria were not always implemented. This led to a relatively skewed distribution of high-risk products among the 22 different brands and it turned out that certain products were not available when orders were placed. In the end, the result was that nine products from one brand were ultimately selected for laboratory analysis, with six products being selected from another brand.

Finally, it emerged that a number of sample packages had also been sent with the products ordered. Three of these samples were considered to be HR supplements. These three were included in the analysis and so the total number of products sent for analysis was, in the end, 69.

3. Laboratory analysis

This chapter will explain:

1. the product assessment;
2. the analysis of high concentrations;
3. the analysis of low concentrations;
4. the overall report for the products;
5. the overall report for the brands;
6. the extra analysis for the three PEA-labelled products.

3.1. Product assessment

Upon arrival in the LGC laboratory, all 69 dietary supplements submitted were assessed prior to the start of the analysis phase. Three products were found to contain components (two, three and four components) with different compositions, all of which had to be analysed separately. The number of laboratory samples was therefore 75.

The following identification codes were assigned to the products and samples comprising several components:

- product H1: samples 57, 58, 58, 60
- product H2: samples 61, 62, 63
- product H4: samples 65, 66

Phenethylamine

In addition, upon closer inspection, three products were found to have declared a prohibited substance (phenethylamine in all cases) on the label after all. Phenethylamine (PEA) was added to the Prohibited List with effect from 1 January 2015 (WADA, 2014). The three dietary supplements in question were therefore officially excluded from the study sample, even though they have been discussed separately in the description of the results.

> *The List now clearly identifies the whole family of phenethylamine derivatives as being prohibited to address the growing number of illegal, designer stimulants derived from phenethylamine.*
>
> (World Anti-Doping Agency, 2014)

The following identification codes were assigned to the products and samples with a PEA declaration:

- product PEA-K4: sample 6,
- product PEA-V1: sample 28
- product PEA-H3: sample 64

-

Only one product with a PEA declaration was submitted for one brand (PEA-V1). The number of brands included in the study was therefore reduced from 22 to 21. The number of products included fell from 69 to 66 (72 samples).

3.2. Analysis of high concentrations

In order to minimise the risk of contamination in the laboratory, it was decided to first analyse all the samples in strongly diluted form. In this way, only the supplements with a high concentration of prohibited substances were identified.

Two different techniques were used for the purposes of this analysis, covering a wide range of prohibited substances:

- *High Resolution Liquid Chromatography Mass Spectrometry (HR-LCMS)* and;
- *Liquid Chromatography Mass Spectrometry – Multiple Reaction Monitoring (LCMS-MRM)*.

Results for high concentrations

Three of the products (5% of all the samples analysed) contained high concentrations of prohibited substances. In two products, these were found to be exactly the same prohibited substances. They belonged to the same brand (brand F), and this may not be entirely coincidental.

Table 1: products with high concentrations of prohibited substances

#	***positive***	***concentration per gram (estimate)***
F1 (18)	β-methylphenethylamine N,β-dimethylphenetylamine Oxilofrine	26 mg 1 mg 11 mg
F3 (20)	β-methylphenethylamine N,β-dimethylphenetylamine Oxilofrine	49 mg 24 mg 40 mg
G3 (44)	Oxilofrine	55 mg

The high concentrations found of the prohibited substances - the results are averages representing the range of results found by the laboratory - mean that it is reasonable to assume that these prohibited substances were deliberately added to the dietary supplement during the production process. Due to these high concentrations, the use of the product may easily result in a positive doping control and, in addition, adverse health effects.

Advanced testing for steroids

In addition to the analysis described above, 23 of 'the products targeting muscle growth most' were also subject to enhanced testing focusing on both anabolic and designer steroids. The Solid Phase Extraction (SPE) technique was used for this test in combination with Gas Chromatography Mass Spectrometry (GCMS) analysis. However, this additional test did not produce any positive results.

3.3. Analysis of low concentrations

After the analysis of the products for the presence of high concentrations of prohibited substances, all 66 products were also checked for the presence of low concentrations of prohibited substances. The presence of these doping substances in low concentrations may present a risk to elite athletes subject to doping controls (Geyer et al., 2004; Baume et al., 2005).

LGC's ISO-accredited supplement screening test (ISO/IEC 17025) was used for the analysis. The prohibited substances covered by this screening test are listed in Annex 2: Prohibited substances analysed by LGC for the purposes of this study.

The screening process consisted of three different tests:

- anabolic steroids: analysis targeting 'anabolic steroids' using Gas Chromatography Mass Spectrometry – Multiple Reaction Monitoring (GCMS-MRM);
- other, alkaline: analysis targeting 'other substances' (alkaline) using LCMS-MRM;
- other, acid/neutral: analysis targeting 'other substances' (acid/neutral) using LCMS-MRM.

It has been decided to split the reporting of the test results into two groups: 'Anabolic Steroids' and 'Other Substances'.

Terms used in description of results
The following terms have been used in the description of the results.

- **'Positive'**
  One or more prohibited substances were found. This means that the dietary supplement in question would not have been approved during the NZVT screening process.

- **'Negative'**
  One or more prohibited substances were not found. The supplement would therefore have passed the NZVT screening.

- ***'Concentrations below the adopted threshold value'***
  One or more prohibited substances were found, but only in concentrations below the adopted threshold values (reporting level compounds only – see Annex 2). In this case, the detection of prohibited substances may be explained by their natural presence in certain ingredients such as botanical and animal-derived ingredients. The low levels observed do not, in principle, involve a risk of a positive doping test for elite athletes. The supplement would therefore have passed the NZVT screening.

- **'Not analysable/not fully analysable'**
  The analysis could (often due to the complex herbal ingredients present) not be completed in full. It was therefore not possible to determine whether one or more prohibited substances were present or absent using the defined method specification. Because of the incomplete test result, the dietary supplement in question would not have passed the NZVT screening.

Report on anabolic steroids
Of the 66 products, fourteen (21%) tested 'positive' and 43 (65%) tested 'negative' for the presence of anabolic steroids and six products (9%) were found

to contain ‘concentrations below the adopted threshold value’. Three products (5%) were ‘not analysable/fully analysable’. The relevant tables can be found in Annex 3: result of analysis for anabolic steroids.

Report on other substances
Of the three products in which a high concentration of prohibited substances had already been found, two (F1-F3 and 18-20) were not included in the analysis looking at the presence of prohibited substances in low concentrations to limit the risk of laboratory contamination. The product that was included (G3-44) was found to be ‘positive’. It contained not only a high concentration of oxilofrine but also four other prohibited substances, namely methyl ephedrine, ephedrine, strychnine and norpseudoephedrine.

The combination of the two tests (looking at high concentrations and low concentrations) then showed that 16 of the 66 products (24%) tested 'positive', with 46 testing (70%) 'negative'. Another four products (6%) contained 'concentrations below the adopted threshold value'. In the case of the 'other substances', all the products were found to be 'fully analysable'. The corresponding table can be found in Annex 4: result of analysis for 'other substances’.

3.4. Overall report on products

Ultimately, 25 (38%) of the total of 66 high-risk products tested 'positive' and 30 products (45%) tested 'negative'. Eight supplements (12%) were found to contain ‘concentrations below the adopted threshold value’ and three products (5%) were ‘not analysable/fully analysable’. Three of the 25 'positive' products (5% of the total sample analysed) were found to contain one or more prohibited substances in high concentrations. The corresponding table can be found in Annex 5: result of analysis by sample / product.

Testing on basis of NZVT admission criteria
The 30 supplements that tested 'negative' and the 8 supplements in which only 'concentrations below the adopted threshold value' were found met the NZVT admission criteria. That is 58% of the total number of tested HR supplements. The 25 supplements that were found to be ‘positive’ and the three supplements that were ‘not analysable/fully analysable’ did not comply with the NZVT criteria. That is 42% of the HR supplements.

3.5. Overall report on brands

The 66 high-risk sports supplements included in this study came from 21 different brands. The highest number of analysed products from one brand was nine. In the case of five brands, just one product was analysed.

This study has shown that at least twelve of these 21 brands (57%) sold HR supplements that tested 'positive' during the analysis for the presence of prohibited substances. None of the brands for which four products or more were analysed (five different brands) got through the test without a 'positive' finding. The highest number of 'positive' findings for one brand was five (out of six

products in total). The corresponding table can be found in Annex 6: result of analysis by brand.

3.6. Additional: analysis of PEA-labelled products

Three of the 69 high-risk sports supplements were found to have listed the prohibited substance phenethylamine (PEA) on the label. Since this meant that the dietary supplements no longer met the inclusion criteria for this study, it has been decided to describe these results from the laboratory analysis separately.

The analysis confirmed that PEA was present in the samples from all three of the PEA-labelled products. No other prohibited substances were found in high concentrations in these samples. However, two of the three samples did contain low concentrations of anabolic steroids.

Table 2: Result *of additional prohibited substances in PEA products*

#	*Positive (low concentrations)*	*concentrations below threshold value*
PEA-K4 (6)	androstenediol	dehydroepiandrosterone (DHEA)
PEA-H3 (64)	1,4-androstadiene-3,17-dione (1,4-ADD)	

4. Analysis of prohibited substances found

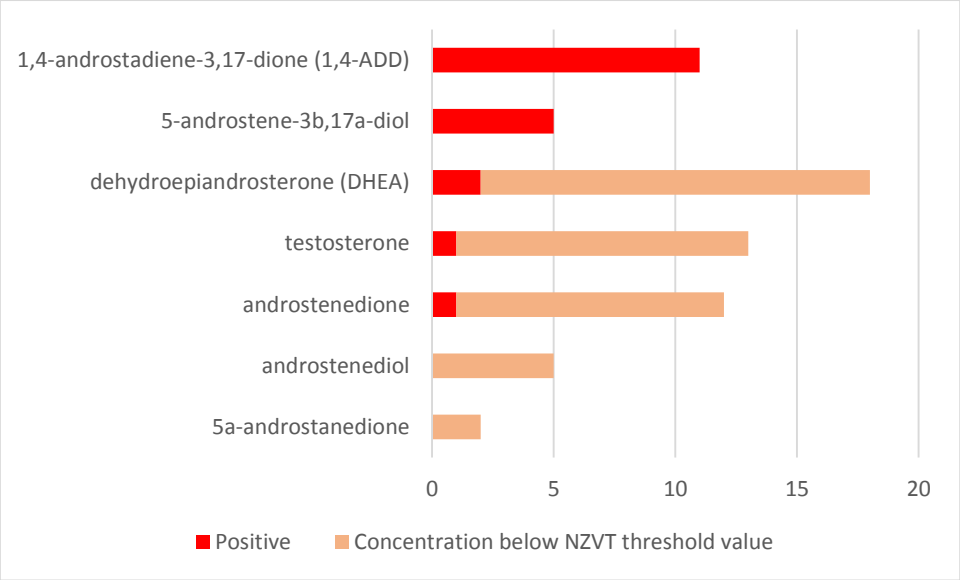
This chapter includes:

1. an overview of the anabolic steroids found
2. a discussion of the anabolic steroids found
3. an overview of the 'other substances' found
4. a discussion of the 'other substances' found

4.1. Anabolic steroids found

Seven different anabolic steroids were found in the 66 analysed products. The substances 1.4-androstadiene-3,17-dione (1.4-ADD) and 5-androstene-3b,17a-diol were found in eleven and five products respectively. Given the fact that no threshold values currently apply to these two substances, all findings in the context of this study were 'positive'.

A threshold value has been adopted for the other five anabolic steroids. Findings below this threshold value may therefore be linked to the possible natural presence of these substances in certain ingredients, such as herbal components. This resulted in 55 findings with 'concentrations below the adopted threshold value'. Dehydroepiandrosterone (DHEA, 2x), testosterone (1x) and androstenedione (1x) were, however, also found in concentrations above the threshold value ('positive'). The 66 findings were related to 21 products (24 samples). The corresponding table can be found in Annex 7: substances found (at product level).



Graph 2: anabolic steroids found (at product level) by type

4.2 Discussion of anabolic steroids

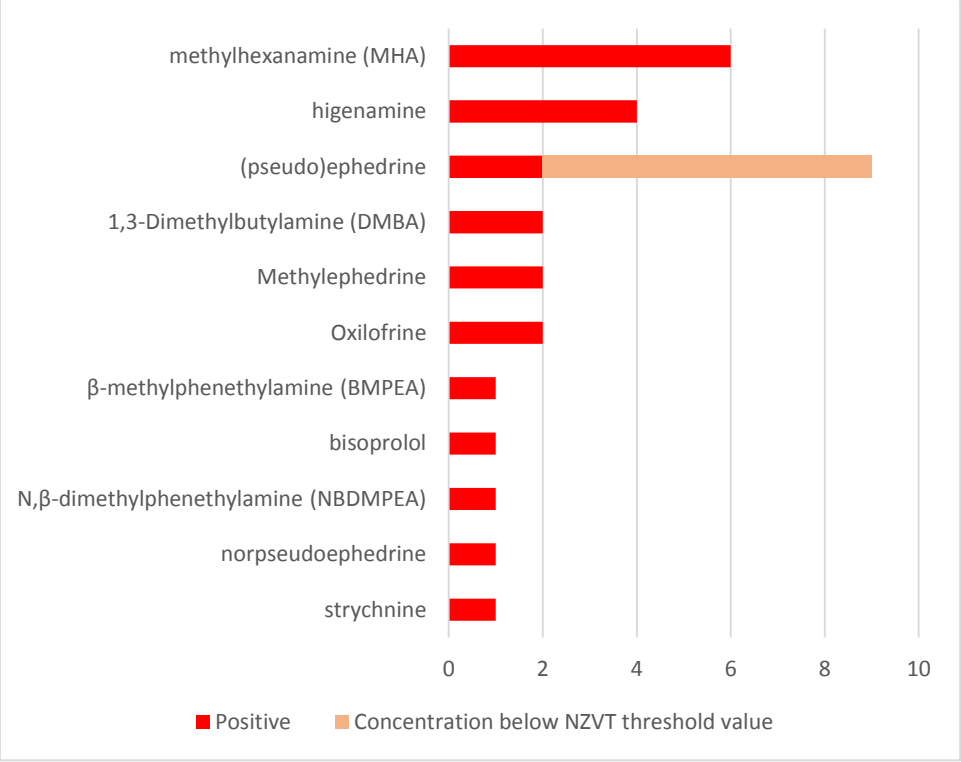
The LGC laboratory notes that dietary supplements have come to contain more and more botanical ingredients in the last six to eight years. Examples of botanical components of this kind are rhodiola rosea and tribulus terrestris. At the same time, the number of findings for a number of specific anabolic steroids such as androstenedione, androstenediol and 1.4-androstadiene-3,17-dione is on the increase. This link between botanical ingredients and anabolic steroids also proved to be a strong feature of this study. Of the 26 products in which anabolic steroids were found (including PEA products), 23 (88%) contained one or more botanical ingredients.

It is still not entirely clear why the addition of some botanical components seems to lead to findings for anabolic steroids. The most logical explanation with the best evidence base is the possible microbial conversion of plant sterols into low concentrations of anabolic steroids. This process may be more pronounced during the production of the supplements because, precisely in the processing of the raw materials, the active botanical components will be present in increased concentrations. More research is needed to confirm or disprove this hypothesis. The deliberate addition of anabolic steroids would not seem plausible given the low concentrations found.

Furthermore, the complex composition of the botanical ingredients may have led to the incomplete / unsuccessful analysis results (not analysable/fully analysable) for samples 14, 22, 53 and 54.

4.3 'Other substances' found

Eleven different 'other substances' were found in the 66 analysed products. Six products contained methylhexanamine and four contained higenamine. (Pseudo)ephedrine was the only one of the substances found for which there was a threshold value. There were seven findings of this substance at ‘concentrations below the adopted threshold value’. In the ‘other substances’ category, there were 30 findings in 20 different products (21 samples). The corresponding table can be found in Annex 7: substances found (at product level).



Graph 3: number of 'other substances' found (at product level), by type

4.4. Discussion of 'other substances'

The analysis of the results for the 'other substances' leads to a number of discussions.

Botanical ingredients and 'other substances'

All dietary supplements with a finding for (pseudo)ephedrine contained one or more botanical ingredients. It is therefore not possible to state whether the findings are a result of the natural presence of these doping substances in the botanical ingredients, whether the raw materials used were contaminated or whether there has been cross-contamination during production. When there is cross-contamination, the prohibited substances are used deliberately as ingredients in certain products inside the production facility. Due to errors in the production process, they then enter other products as well. The same conclusion can be stated for the finding of the prohibited substance higenamine (norcoclaurine). Here again, the four dietary supplements studied were found to contain complex botanical ingredients.

Methylhexanamine (DMAA)

Since methylhexanamine (DMAA) was found in low concentrations only, deliberate inclusion would not seem to be a plausible explanation. The most likely explanation for the six methylhexanamine (DMAA) findings is cross-contamination.

Product F2 (19)

Since products F1, F2 and F3 are marketed under the same brand (brand code F) and since the analysis of products F1 and F3 showed high concentrations of prohibited substances, it is not surprising that four different prohibited substances were found in low concentrations in product F2. Here again, cross-contamination would therefore seem to be the most logical explanation.

5. Conclusion

The main aim of this study was to determine the percentage of high-risk sports supplements containing prohibited substances when these products:

- are available in Dutch web shops;
- focus on:
    - hormone regulation;
    - strengthening muscle;
    - weight loss/burning fat;
    - raising energy levels;
- no prohibited substances are declared (or when there is no explicit doping warning).

Upper limit 38%

This research found a prevalence rate of 38%. This should be seen as the upper limit for the risk for an athlete ingesting prohibited substances with a dietary supplement when there is no clear statement on the label of the product that it may contain prohibited substances. This upper limit of 38% is much higher than reported in previous international studies - targeted at a broader range of sport supplements which found maximum prevalence rates of approximately 25% - such as Geyer et al. (2004) and Judkins et al. (2007). This shows that elite athletes who turn to the internet for HR supplements are actually exposed to a very high risk in the context of doping controls, particularly if it is borne in mind that the majority of the web shops (71%) sell HR products and that it is therefore clear that sports supplements of this kind are widely available.

Of the 66 dietary supplements examined, 25 (38%) tested 'positive' and 30 (45%) tested 'negative'. In eight supplements (12%) 'concentrations below the adopted threshold value' only were found, and three supplements (5%) were 'not analysable/fully analysable'. Three products (5%) were found to contain high concentrations of prohibited substances. These high concentrations would seem to make it very likely that these prohibited substances were deliberately added to the dietary supplement during the production process. Consumption of these products can therefore easily result in a positive doping control and also adverse health effects. In the case of the other 63 products (95%), the deliberate addition of prohibited substances would not seem likely.

Anabolic steroids

In the analysis of anabolic steroids fourteen (21%) of the 66 dietary supplements tested 'positive' and 43 (65%) tested 'negative'. Six (9%) were found to contain 'concentrations below the adopted threshold value' and three (5%) were 'not analysable/fully analysable'. There were 66 findings in total for 21 products. Eleven products (17%) tested positive for 1,4-androstadiene-3,17-dione (17-ADD) and five (8%) for 5-androstene-3b,17a-diol.

Other substances

Analysis of the 'other substances' led to sixteen 'positive' (24%) and 46 (70%) 'negative' results. 'Concentrations below the adopted threshold value' were found

on four occasions (6%). There were 30 findings in total for 20 products. Six products (9%) tested 'positive' for methylhexanamine (DMAA) and four (6%) did so for higenamine. ‘Concentrations below the adopted threshold value’ were reported seven times for (pseudo)ephedrine (11%).

Botanical components

A possible explanation for the numerous findings of prohibited substances in low concentrations ('positive' and 'concentrations below the adopted threshold value') is to be found in the phytochemical processes involving botanical ingredients, which may result in the formation of different anabolic steroids in low concentrations. However, this cannot be stated with certainty. Furthermore, it is known that ‘other prohibited substances’ such as ephedrine and higenamine can be present naturally in botanical ingredients. As a result, it is not possible to state with certainty whether many of these findings are caused by the natural presence of prohibited substances through botanical components or contamination/cross-contamination. Specific research in this area is therefore required as well.

6. Recommendations

NZVT
This study shows that Dutch elite athletes will continue to be exposed to risks if they use product-batch combinations of dietary supplements that have not been tested for the presence of doping substances by the NZVT (or any other recognised system). At the same time, the NZVT is not used by large proportion - or even a majority - of elite Dutch athletes. It is therefore important to give an additional boost to the promotion of the NZVT among elite athletes in the Netherlands. In addition, elite Dutch athletes state that the number of products in the NZVT is limited. Despite the fact that the number of approved product-batch combinations has been growing steadily in recent years, it would therefore also be desirable to encourage the use of the NZVT by manufacturers of dietary supplements available in the Netherlands.

Netherlands Food and Consumer Product Safety Authority (NVWA)
High concentrations of prohibited substances were found in three dietary supplements from two different companies. Using these products could easily result in health problems. Given the public health implications, these findings were therefore reported to the Netherlands Food and Consumer Product Safety Authority (NVWA). This implies a request for the launch of a further investigation into these three dietary supplements.

Botanical components
It would appear to be the case that an increasing number of sports supplements contain botanical ingredients. The addition of these botanical ingredients would also seem to result in findings of low concentrations of steroids. Although phytochemical studies have already demonstrated this principle, the production process used for supplements may lead to an increase in these natural concentrations. This could be a partial explanation for the relatively high number of 'positive' findings for anabolic steroids in this study. Although it is considered to be fairly unlikely that the synthesis of these low concentrations of anabolic steroids could lead to a positive doping test, it would be advisable to investigate this risk further.

HACCP - Plus/NZVT system
A total of 38% of the dietary supplements analysed were 'positive'. These 'positive' products came from 57% of the brands. Although many of the low concentrations found could be explained in part by their natural presence in botanical compounds and/or synthesis in phytochemical processes, it is certainly not inconceivable that contamination or cross-contamination may also have played a role. Manufacturers of dietary supplements are therefore advised to continue the critical monitoring of the entire production process and to make changes where needed in the interest of elite athletes. The use of the risk assessment system HACCP-Plus/NZVT could provide added value in this respect.

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Annex 1: overview of declared prohibited substances

Twenty-six prohibited substances were identified and five specific doping warnings were issued for a total sample of 216 high-risk sports supplements.

- **Androstadienone 1x**
- **Androstenolone 1x**
- **Androsterone 1x**
- **Cannabinomimetics 1x**
- **DHEA 9x**
    - 3.7 keto DHEA 1x
    - 7-keto-DHEA 1x
    - 7-OH-DHEA 2x
    - DHEA 5x

- **Glycerol Monostearate 1x**
- **Higenamine 5x**
    - Higenamine 3x
    - Norcoclaurine HCl 2x

- **Methylhexanamine 1x**
- **Nor-DMAA 2x**
    - 2-amino-4-methyl-pentane citrate
    - 4-amino-2-methyl-pentane citrate

- **Norepinephrine 1x**
- **Octopamine 3x**

- **Specific doping warning (5x)**

Annex 2: prohibited substances analysed by LGC for the purposes of this study

Substances analysed by GCMS	Method Capability*		Reporting Level*	
	Standard Test	Fats/Oils Test[3]	Standard Test	Fats/Oils Test[3]
1,4-androstadiene-3,17-dione	10 ng/g	50 ng/g	-	-
4-androstene-3,17-dione and/or 5(6)-androstene-3,17-dione[1]	-	-	10ng/g (50ng/g)[2]	50 ng/g
4-androstene-3β,17β-diol	-	-	10ng/g	50 ng/g
5α-androstane-3β,17β-diol	-	-	10ng/g	50 ng/g
5(6)-androstene-3β,17β-diol	-	-	10ng/g	50 ng/g
5α-androstane-3,17-dione	-	-	10ng/g	50 ng/g
Dehydroepiandrosterone (DHEA)	-	-	10ng/g	50 ng/g
4-estrene-3,17-dione(19-nor-4-androstene-3,17-dione) and/or 5(10)-estrene-3,17-dione (19-nor-5(10)-androstene-3,17-dione) and/or 5(6)-estrene-3,17-dione (19-nor-5(6)-androstene-3,17-dione) [1]	10 ng/g	50 ng/g	-	-
4-estrene-3β,17β-diol (19-nor-4-androstene-3β,17β-diol) and/or 5(10)-estrene-3β,17β-diol (19-nor-5(10)-androstene-3β,17β-diol) [1]	10 ng/g	50 ng/g	-	-
Nandrolone (19-nor-4-androstene-17β-hydroxy-3-one)	10 ng/g	50 ng/g	-	-
Testosterone	-	-	10ng/g	50 ng/g

* See section titled Reporting Level/Method Capability for full definition of terms.
1 These compounds are isomeric and indistinguishable from each other by this test.
2 Reporting level of 50ng/g applicable to products containing milk or milk derived substances (see additional note relating to "Androstenedione in milk and milk based products").
3 Method capability / reporting levels only applicable to oil based products

Substances analysed by LCMS	Method Capability*	Reporting Level*
	Standard Test	Standard Test
1(3-chlorophenyl)piperazine	100 ng/g	-
Acebutolol	100 ng/g	-
Alfentanil	100 ng/g	-
Alprenolol	100 ng/g	-
Amiphenazole	100 ng/g	-
Amphetamine	100 ng/g	-
Atenolol	100 ng/g	-
Bambuterol	100 ng/g	-
Benzoylecgonine	100 ng/g	-
Benzphetamine	100 ng/g	-
Benzylpiperazine	100 ng/g	-
Bisoprolol	100 ng/g	-
Bumetanide	100 ng/g	-
Bunitrolol	100 ng/g	-
Bupranolol	100 ng/g	-
Buprenorphine	100 ng/g	-
Bupropion	100 ng/g	-
Butofinolol	100 ng/g	-
Canrenone	100 ng/g	-
Carazolol	100 ng/g	-
Carfentanil	100 ng/g	-
Carphedone	100 ng/g	-
Carteolol	100 ng/g	-
Cathine (Norpseudoephedrine)	100 ng/g	-
Celiprolol	100 ng/g	-
Chlorphentermine	100 ng/g	-
Cimaterol	100 ng/g	-
Clenbuterol	10 ng/g	-
Clomifene	100 ng/g	-
Clopamide	100 ng/g	-
Clobenzorex	100 ng/g	-
Clorprenaline	100 ng/g	-
Cocaine	100 ng/g	-
Croethamide	100 ng/g	-

Table continued on next page

Cyclopentamine	100 ng/g	-
Cyproheptadine	100 ng/g	-
Dextromoramide	100 ng/g	-
Diamorphine	100 ng/g	-
Diethylpropion	100 ng/g	-
Dipipanone	100 ng/g	-
Diprenorphine	100 ng/g	-
Doxapram	100 ng/g	
Ephedrine / Pseudoephedrine	-	100 ng/g
Esmolol	100 ng/g	-
Etafedrine	100 ng/g	-
Etamivan	100 ng/g	-
Fenbutrazate	100 ng/g	-
Fencamfamine	100 ng/g	-
Fenfluramine	100 ng/g	-
Fenoterol	100 ng/g	-
Fenozolone	100 ng/g	-
Fentanyl	100 ng/g	-
Fluorophenethylamine	100 ng/g	-
Fluoxetine	100 ng/g	-
Fluvoxamine	100 ng/g	-
Formoterol	100 ng/g	-
Gestrinone	10 ng/g	-
Heptaminol	100 ng/g	-
HMMA	100 ng/g	-
Indapamide	100 ng/g	-
Isometheptene	100 ng/g	-
Labetolol	100 ng/g	-
Levophacetoperane	100 ng/g	-
Mabuterol	100 ng/g	-
MDA	100 ng/g	-
MDMA (ecstasy)	100 ng/g	-
Mefenorex	100 ng/g	-
Mefruside	100 ng/g	-
Mephentermine	100 ng/g	-
Methadone	100 ng/g	-
Methamphetamine	100 ng/g	-
Methoxyphenylpiperazine	100 ng/g	-
Methylephedrine	100 ng/g	-
Methylhexanamine (1,3-dimethylpentylamine)	100 ng/g	-
Methylphenidate	100 ng/g	-
Methyltrienolone	100 ng/g	-
Metoprolol	100 ng/g	-
Modafinil	100 ng/g	-
Moprolol	100 ng/g	-
Nadolol	100 ng/g	-
Nadoxolol	100 ng/g	-
Nalbuphine	100 ng/g	-
Nalorphine	100 ng/g	-
Naloxone	100 ng/g	-
Naltrexone	100 ng/g	-
Nikethamide	100 ng/g	-
Oripavine	100 ng/g	-
Oxprenolol	100 ng/g	-
Oxycodone	100 ng/g	-
Oxymetazoline	100 ng/g	-
Pemoline	100 ng/g	-
Penbutolol	100 ng/g	-
Pentazocine	100 ng/g	-
Pentoxyverine	100 ng/g	-
Pethidine	100 ng/g	-
Phendimetrazine	100 ng/g	-
Phenmetrazine	100 ng/g	-
Phentermine	100 ng/g	-

Table continued on next page

Pindolol	100 ng/g	-
Pirbuterol	100 ng/g	-
Piretanide	100 ng/g	-
Polythiazide	100 ng/g	-
Practolol	100 ng/g	-
Probenecid	100 ng/g	-
Prolintane	100 ng/g	-
Propranolol	100 ng/g	-
Prothipendyl	100 ng/g	-
Quinethazone	100 ng/g	-
Ritodrine	100 ng/g	-
Salbutamol	100 ng/g	-
Salmeterol	100 ng/g	-
Selegiline	100 ng/g	-
Sibutramine	100 ng/g	-
Sildenafil	100 ng/g	-
Sotalol	100 ng/g	-
Spironolactone	100 ng/g	-
Stanozolol	10 ng/g	-
Strychnine	100 ng/g	-
Tamoxifen	100 ng/g	-
Terbutaline	100 ng/g	-
Tetrahydrogestrinone (THG)	10 ng/g	-
Timolol	100 ng/g	-
Torasemide	100 ng/g	-
Toremifene	100 ng/g	-
Trenbolone	100 ng/g	-
Trifluoromethylphenylpiperazine	100 ng/g	-
Tripamide	100 ng/g	-
Tuaminoheptane	100 ng/g	-
Tulobuterol	100 ng/g	-
Xylomatazoline	100 ng/g	-

* See section titled Reporting Level / Method Capability for full definition of terms.

The following tables detail the additional compounds covered within LGC's ISO17025 accredited nutritional supplement screen but which are not themselves covered by the scope of accreditation. The additional compounds include:

†Anabolic and designer steroids
¨Monitoring compounds – Compounds which are routinely screened for but which are not listed on the SLA

Additional Unaccredited LC Compounds	Estimated Method Capability / ngg^{-1}
1,3-dimethylbutylamine¨	100
17-hydroxyprogesterone†	10
1-Androstenedione†	100
1-Testosterone†	10
20-Norstanozolol†	10
4,9-estradiene-17β-ol,3-one (dienolone)†¨	10
4,9-estradiene-3,17-dione (X-tren)†¨	10
7 keto DHEA†	500
Alpha-ethylphenethylamine**	100
Amiloride	100
Andarine	100
Androsta-1,4,6-trien-17b-ol-3-one†	10
Androstatrienedione†	10
Androstenetrione†	500
Beta-methylphenethylamine**	100
Bolasterone	10
Boldenone	10
Clostebol	100
Conivaptan	100
Danazol	100
Dimethamphetamine**	100
Exemestane	100
Fluoxymesterone†	100
Formestane (4-hydroxyandrostenedione)†	100
Higenamine (norcoclaurine)**	100
Lixivaptan	100
MDEA**	100
Methandienone†¨	10
Methyl-1-testosterone†	100
Methylclostebol†	100
Methylnortesoterone†	10
Methylpseudoephedrine**	100
Methyltestosterone†	10
N,alpha-diethylphenethylamine**	100
N,beta-dimethylphenethylamine**	100
Norclostebol†	100
Norephedrine**	100
Norethandrolone†	100
Ostarine	100
Oxabolone†	100
Oxandrolone†	100
Oxilofrine**	100
Oxymesterone†	100
Relcovaptan	100
Stenbolone†	100
Tibolone	100
Tolvaptan	100
Trendione†	100
Triamterene	100
Turinabol†	100

Table 8 Additional non accredited compounds covered by LCMS

Additional Unaccredited GC Compounds	Estimated Method Capability / ngg^{-1}
19-norandrosterone†	10
1-androsten-3β,17β-diol†	10
4-androsten-3β,17α-diol†	50
4-hydroxytestosterone†	200
5α-androstan-3α17α-diol†	10
5α-androstan-3α,17β-diol†	10
5α-androstan-3β,17α-diol†	10
5-androsten-3β,17α-diol†	10
Androsterone†	10
Dihydrotestosterone (5α -DHT)†	10
Dihydrotestosterone (5β -DHT)†	50
Drostanolone†	10
Drostanolone Propionate†	10
Ethylestrenol†	200
Mestanolone†	10
Mesterolone†	10
Methandriol†	10
Methasterone†	10
Methenolone†	10
Methyltestosterone†	50
Mibolerone†	10
Oxymesterone†	200

Table 9 Additional non accredited compounds covered by GCMS

Additional Unaccredited Negative Ion Compounds	Estimated Method Capability / ngg^{-1}
2,4-DNP	100
Acetazolamide	100
Bendroflumethiazide	100
Benzthiazide	100
Chlorthalidone	100
Chlorthiazide	100
Cyclothiazide	100
Ethacrynic Acid	100
Furosemide	100
Hydrochlorthiazide	100
Hydroflumethiazide	100
Methylclothiazide	100
Metolazone	100
Polythiazide	100
Trichlormethiazide	100

Table 10 Additional non accredited negative ion compounds covered by LCMS

Annex 3: result of analysis for anabolic steroids

Product (sample) #	**Anabolic steroids** *Positive*	*Concentration under threshold value*
K1 (3)		dehydroepiandrosterone (DHEA)
A5 (11)		dehydroepiandrosterone (DHEA)
A6 (12)	1,4-androstadiene-3,17-dione (1,4-ADD)	dehydroepiandrosterone (DHEA) testosterone androstenedione
A7 (13)		dehydroepiandrosterone (DHEA)
O1 (16)	1,4-androstadiene-3,17-dione (1,4-ADD) 5-androstene-3b,17a-diol	testosterone dehydroepiandrosterone (DHEA) androstenedione androstenediol
F2 (19)		dehydroepiandrosterone (DHEA)
R1 (25)		testosterone
P1 (26)	5-androstene-3b,17a-diol	testosterone dehydroepiandrosterone (DHEA) androstenedione , 5α-androstanedione
P2 (27)	5-androstene-3b,17a-diol	testosterone dehydroepiandrosterone (DHEA) androstenedione, 5α-androstanedione
E4 (32)	1,4-androstadiene-3,17-dione (1,4-ADD)	dehydroepiandrosterone (DHEA) androstenedione androstenediol
G2 (43)	1,4-androstadiene-3,17-dione (1,4-ADD)	testosterone
G3 (44)	testosterone	
I1 (50)	1,4-androstadiene-3,17-dione (1,4-ADD)	dehydroepiandrosterone (DHEA)
I2 (51)		dehydroepiandrosterone (DHEA) androstenedione
I3 (52)	1,4-androstadiene-3,17-dione (1,4-ADD)	dehydroepiandrosterone (DHEA) androstenedione
H1 (59****)	1,4-androstadiene-3,17-dione (1,4-ADD)	testosterone androstenedione
H1 (60****)	1,4-androstadiene-3,17-dione (1,4-ADD)	testosterone dehydroepiandrosterone (DHEA) androstenedione
H2 (61***)	1,4-androstadiene-3,17-dione (1,4-ADD)	testosterone dehydroepiandrosterone (DHEA) androstenedione androstenediol
H2 (62***)	5-androstene-3b,17a-diol dehydroepiandrosterone (DHEA)	testosterone androstenedione androstenediol
H2 (63***)	1,4-androstadiene-3,17-dione (1,4-ADD)	testosterone dehydroepiandrosterone (DHEA) androstenedione
H4 (65**)	1,4-androstadiene-3,17-dione (1,4-ADD) 5-androstene-3b,17a-diol	testosterone dehydroepiandrosterone (DHEA) androstenedione androstenediol
B1 (67)		testosterone dehydroepiandrosterone (DHEA)
B2 (68)	1,4-androstadiene-3,17-dione (1,4-ADD)	testosterone dehydroepiandrosterone (DHEA) androstenedione
B3 (69)	1,4-androstadiene-3,17-dione (1,4-ADD) 5-androstene-3b,17a-diol androstenedione dehydroepiandrosterone (DHEA)	testosterone androstenediol

//**** product consists of two (H4), three (H2), or four (H1) samples respectively*

Annex 4: result of analysis for 'other substances'

Product (sample) *#*	**Other substances** *Positive*	*Concentrations below threshold value*
Q1 (1)	1,3-dimethylbutylamine (DMBA) oxilofrine	
O2 (17)	higenamine	
F1 (18)	β-methylphenethylamine (BMPEA) *high concentration* N,β-dimethylphenethylamine (NBDMPEA) *high concentration* oxilofrine *high concentration*	
F2 (19)	1,3-dimethylbutylamine (DMBA) β-methylphenethylamine (BMPEA) N,β-dimethylphenethylamine (NBDMPEA) methylhexanamine (DMAA)	
F3 (20)	β-methylphenethylamine (BMPEA) *high concentration* N,β-dimethylphenethylamine (NBDMPEA) *high concentration*) oxilofrine *high concentration*	
E1 (29)		(pseudo)ephedrine
E2 (30)		(pseudo)ephedrine
C1 (36)	bisoprolol	
G2 (43)	higenamine oxilofrine	
G3 (44)	oxilofrine *high concentration* (pseudo)ephedrine methylephedrine strychnine norpseudoephedrine	
D1 (45)	methylhexanamine (DMAA)	
I3 (52)	methylhexanamine (DMAA)	
J2 (55)	methylhexanamine (DMAA)	(pseudo)ephedrine
H1 (59****)		(pseudo)ephedrine
H2 (62***)		(pseudo)ephedrine
H2 (63***)	higenamine	
B1 (67)		(pseudo)ephedrine
B2 (68)	methylephedrine (pseudo)ephedrine	
B4 (70)	methylhexanamine (DMAA)	
B5 (71)	higenamine	
B6 (72)	methylhexanamine (DMAA)	(pseudo)ephedrine

/* *product consists of three (H2) or four (H1) samples respectively*

Annex 5: result of analysis by sample / product

Product (sample)	**Result for sample**				**Result for product**			
#	*Positive*	*<Threshold value*	*Not analysable*	*Negative*	*Positive*	*<Threshold value*	*Not analysable*	*Negative*
Q1 (1)	√				√			
T1 (2)				X				X
K1 (3)		O				O		
K2 (4)				X				X
K3 (5)				X				X
A1 (7)				X				X
A2 (8)				X				X
A3 (9)				X				X
A4 (10)				X				X
A5 (11)		O				O		
A6 (12)	√				√			
A7 (13)		O				O		
A8 (14)			NA				NA	
A9 (15)				X				X
O1 (16)	√				√			
O2 (17)	√				√			
F1 (18)	√				√			
F2 (19)	√				√			
F3 (20)	√				√			
L1 (21)				X				X
L2 (22)				X				X
L3 (23)				X				X
U1 (24)				X				X
R1 (25)		O				O		
P1 (26)	√				√			
P2 (27)	√				√			
E1 (29)		O				O		
E2 (30)		O				O		
E3 (31)				X				X
E4 (32)	√				√			
M1 (33)				X				X
M2 (34)				X				X
M3 (35)				X				X
C1 (36)	√				√			
C2 (37)				X				X
C3 (38)				X				X
C4 (39)				X				X
C5 (40)				X				X
C6 (41)				X				X
G1 (42)				X				X
G2 (43)	√				√			
G3 (44)	√				√			
D1 (45)	√				√			
D2 (46)				X				X
D3 (47)				X				X
D4 (48)				X				X
D5 (49)				X				X
I1 (50)	√				√			
I2 (51)		O				O		
I3 (52)	√				√			
S1 (53)			NA				NA	
J1 (54)			NA				NA	
J2 (55)	√				√			
J3 (56)				X				X

Table continued on next page

Product (sample)	**Result for sample**				**Result for product**			
#	*Positive*	*<Threshold value*	*Not analysable.*	*Negative*	*Positive*	*<Threshold value*	*Not analysable*	*Negative*
H1 (57****)				X				
H1 (58****)				X				
H1 (59****)	√							
H1 (60****)	√				√			
H2 (61***)	√							
H2 (62***)	√							
H2 (63***)	√				√			
H4 (65**)	√							
H4 (66**)				X	√			
B1 (67)		O				O		
B2 (68)	√				√			
B3 (69)	√				√			
B4 (70)	√				√			
B5 (71)	√				√			
B6 (72)	√				√			
N1 (73)				X				X
N2 (74)				X				X
N3 (75)				X				X
Σ	**28**	**8**	**3**	**33**	**25**	**8**	**3**	**30**

- *<Threshold value = 'concentrations below the adopted threshold value'*
- *Not analysable = 'not analysable/fully analysable'*
- **/***/**** = *product consists of two (H4), three (H2), or four (H1) samples respectively*
- *The products PEA-K4 (6), PEA-V1 (28) and PEA-H3 (64) contained phenylethylamine and were therefore excluded from the investigation. They have therefore also been excluded from this table.*

Annex 6: result of analysis by brand

Number of tests	Brand *A-U*	Lab result *result*	 *fraction*	Samples *#*
9	A	**positive**	**1/9 (11%)**	**12**
		<threshold value	2/9 (22%)	11, 13
		not analysable	1/9 (11%)	14
		negative	5/9 (55%)	7-10, 15
6	B	**positive**	**5/6 (83%)**	**68-72**
		<threshold value	1/6 (17%)	67
		not analysable		
		negative		
	C	**positive**	**1/6 (17%)**	**36**
		<threshold value		
		not analysable		
		negative	5/6 (83%)	37-41
5	D	**positive**	**1/5 (20%)**	**45**
		<threshold value		
		not analysable		
		negative	4/5 (80%)	46-49
4	E	**positive**	**1/4 (25%)**	**32**
		<threshold value	2/4 (50%)	29, 30
		not analysable		
		negative	1/4 (25%)	31
3	F	**positive (high)**	**2/3 (67%)**	**18, 20**
		positive	**1/3 (33%)**	**19**
		<threshold value		
		not analysable		
		negative		
	G	**positive (high)**	**1/3 (33%)**	**44**
		positive	**1/3 (33%)**	**43**
		<threshold value		
		not analysable		
		negative	1/3 (33%)	42
	H	**positive**	**3/3 (100%)**	**(57-60), (61-63), 65-66)**
		<threshold value		
		not analysable		
		negative		

Table continues on next page

Number of tests	Brand	Lab result		Samples
	A-U	*result*	*fraction*	*#*
3	I	**positive**	**2/3 (67%)**	**50, 52**
		<threshold value	1/3 (33%)	51
		not analysable		
		negative		
	J	**positive**	**1/3 (33%)**	**55**
		<threshold value		
		not analysable	1/3 (33%)	54
		negative	1/3 (33%)	56
	K	**positive**		
		<threshold value	1/3 (33%)	3
		not analysable		
		negative	2/3 (67%)	4, 5
	L	**positive**		
		<threshold value		
		not analysable		
		negative	3/3 (100%)	21-23
	M	**positive**		
		<threshold value		
		not analysable		
		negative	3/3 (100%)	33-35
	N	**positive**		
		<threshold value		
		not analysable		
		negative	3/3 (100%)	73-75
2	O	**positive**	**2/2 (100%)**	**16, 17**
		<threshold value		
		not analysable		
		negative		
	P	**positive**	**2/2 (100%)**	**26, 27**
		<threshold value		
		not analysable		
		negative		

Table continues on next page

Number of tests	**Brand** *A-U*	**Lab result** *result*	 *fraction*	**Samples** *#*
1	Q	**positive** <threshold value not analysable negative	1/1 (100%)	1
	R	**positive** <threshold value not analysable negative	1/1 (100%)	25
	S	**positive** <threshold value not analysable negative	1/1 (100%)	53
	T	**positive** <threshold value not analysable negative	1/1 (100%)	2
	U	**positive** <threshold value not analysable negative	1/1 (100%)	24

- *<Threshold value = 'concentrations below the adopted threshold value'*
- *Not analysable = 'not analysable/fully analysable'*

Annex 7: prohibited substances found (at product level)

Steroids *Substance*	*Positive*	*Concentrations below threshold value*
5a-androstenedione		2
androstenediol		5
androstenedione	1	11
testosterone	1	12
dehydroepiandrosterone (DHEA)	2	16
5-androstene-3b,17a-diol	5	
1,4-androstadiene-3,17-dione (1,4-ADD)	11	
Total:	**20**	**46**

Other substances *Substance*	*Positive*	*Concentrations below threshold value*
Strychnine	1	
norpseudoephedrine	1	
N,β-dimethylphenethylamine (NBDMPEA)	1	
Bisoprolol	1	
β-methylphenethylamine (BMPEA)	1	
Oxilofrine	2	
Methylephedrine	2	
1,3-Dimethylbutylamine (DMBA)	2	
(pseudo)ephedrine	2	7
Higenamine	4	
methylhexanamine (DMAA)	6	
Total	**23**	**7**