
**INVESTIGATION INTO SUPPLEMENT CONTAMINATION LEVELS IN THE
US MARKET**

HFL 2007



1 PROJECT DETAILS

Testing Facility: HFL Ltd
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Study Objectives: To investigate levels of steroid/ stimulant contamination in popular supplements available on the US market

Dates of Study: July 2006 to January 2007

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2 SUMMARY

58 supplement samples were purchased from popular retail outlets and internet sites in the US and sent to HFL for analysis using HFL's ISO 17025 accredited banned substance screen for supplements. The banned substance screen is used to detect the presence of low levels of steroid and stimulant contaminants that are considered prohibited by the World Anti-Doping Agency (WADA) in a range of supplement formulations.

Samples were extracted using a combination of liquid and solid phase extraction techniques and analysed by GCMS and LCMS using an Agilent 5973 GCMS instrument and a LCQ XP LCMS instrument.

Approximately one quarter of the samples analysed showed the presence of low levels of steroid and/or stimulant contamination that were not declared on the label.

3 INTRODUCTION

Between 2000 and 2002 a study was undertaken by the International Olympic Committee (IOC) accredited lab in Germany to investigate claims that many supplement products available on the market could contain steroids and prohormones that were not declared on the label (Geyer et al). Such steroid contaminants are considered prohibited by the sports regulatory authorities and professional sports people are banned from using these substances. Geyer's research involved the purchase and analysis of 634 supplement products, bought from 13 different countries. These samples were tested for a range of steroids and prohormones - almost 15% of the products tested contained steroids and prohormones that were not declared on the label. If consumed by professional sports people, such contaminants could result in them failing a drug test.

Geyer suggested in his report that poor quality control procedures were the likely cause of the contamination and cited cross contamination of shared production lines as the main likely cause. Unless supplement products are manufactured in line with strict regulations and good quality control processes, there is a risk that the final products may be contaminated.

The aim of this present survey was to re-evaluate these findings in today's market in the US by testing a range of popular supplement products available to athletes.

4 EXPERIMENTAL

4.1 Sample Purchasing

A range of supplement products was randomly selected and purchased from internet health food outlets, as well as general stores. Product categories (e.g. recovery products, weight loss products, etc.) were selected based on HFL's knowledge of products that are commonly used by male and female athletes. Samples from the "best-selling" brands of these product types were then purchased. Products were selected from manufacturers who were not believed to regularly carry out banned substance screening as part of part of their routine quality control processes.

4.2 Analytical Methods

All samples were analysed qualitatively for the presence of a range of steroids and stimulants that appear on the WADA prohibited list (see Appendix 1 for the list of substances screened for and methods used to screen for these substances). All samples were analysed by both GCMS and LCMS.

- Samples were analysed using a validated and ISO 17025 accredited method developed for the qualitative analysis of steroids and stimulants in supplements
- The limits of detection (LOD) for the tests are 10 ng/g for steroids and 100 ng/g for stimulants (see Appendix 1 for detection levels of each substance)
- A representative portion of each sample was weighed and spiked with internal markers (used to monitor the extraction process)
- Internal markers used were: deuterated testosterone, deuterated androstenediol, deuterated cocaine, deuterated ephedrine and deuterated stanozolol
- Alongside each batch of samples analysed, two positive control samples and one negative control sample were analysed
- Positive control samples were prepared by spiking a blank matrix with known levels of all the substances screened for (levels equal to the LOD for each substance)
- Negative control samples comprised of blank matrix that did not contain any of the substances screened for
- All test samples and control samples were extracted into methanol
- Further “clean-up” of the methanolic extract was performed using both liquid and solid phase extraction techniques in accordance with HFL’s standard operation procedures
- Prior to analysis by GCMS the eluate was treated to form the enol-tertiarybutyldimethylsilyl (TBDMS) derivative
- No derivatisation was carried out on samples analysed by LCMS

GCMS analysis was carried out on an Agilent 5973 instrument in SIM mode. Data was analysed using ChemStation software. GCMS/MS analysis was carried out as needed on a Varian 1200 instrument.

LCMS analysis was carried out on a LCQ XP instrument. Data was analysed using Xcalibur software.

5 RESULTS AND DISCUSSION

5.1 Sample Analysis

Each of the 58 samples analysed underwent analysis by both LCMS and GCMS. Of the 58 samples analysed by LCMS conclusive data could not be

obtained for 4 of the samples due to matrix effects. Of the 58 samples analysed by GCMS, conclusive data could not be obtained for 6 of the samples analysed due to matrix effects.

The results for these tests will not be considered further.

Of the 54 samples that were successfully analysed by LCMS, 6 showed the presence of stimulant contamination. This corresponds to 11.1%. Of the 52 samples that were successfully analysed by GCMS, 13 showed the presence of steroid contamination. This corresponds to 25.0%.

Appendix 2 summarises the analysis results for all product types. The highest incidence of contamination appeared to be in the “testosterone booster” product category type – 6 of the 9 products in this category (including product “stacks” which contained a testosterone booster component) contained steroid and/or stimulants as contaminants. This is equivalent to 67%.

The second highest predominance of contamination was found in the “weight loss” products, with 29% of products tested (including “stacks”) containing steroid and/or stimulant contamination.

The third highest incidence of contamination was found in “muscle building” products. In this category (including “stacks” that contained muscle building components), 24% contained steroid and/or stimulants contaminants.

Table 1 shows the frequency of the substances found across all tests. Androstenedione was the most common finding across all product types tested, with 27% of the GCMS tests showing the presence of this substance. DHEA and 1,4 androstadiene-dione were also common findings, with 23% and 21% of products containing these substances, respectively.

Ephedrine remained the most common stimulant contaminant, with 15% of products showing the presence of ephedrine contamination.

Steroid	Occurrence	Stimulant	Occurrence
1,4 androstadiene-dione	11	ephedrine	8
androstenediol	8	methylephedrine	5
androstenedione	14		
DHEA	12		
estrenediol	1		
estrenedione	1		

Table 1 Frequency of substances found

6 CONCLUSIONS

This study shows that 4 years on from the initial research that highlighted the issue of supplements and contamination, the presence of steroids and stimulants in supplement products is still very much an issue. It is clear that not all supplement manufacturers follow good manufacturing practices (though GMP regulations do not specifically address banned substance screening - so many manufacturers who do follow GMP are still prone to contamination issues) and that the necessary controls are not always implemented to ensure the safety of athletes and the general public who use supplement products. Banned substance screening of products is an essential part of the quality control process and athletes need to be aware of the ongoing risks associated with using products made by manufacturers who do not regularly screen products for these contaminants.

7 MAINTENANCE OF RECORDS

All data generated during the course of this study is archived at HFL, Newmarket Road, Fordham, Cambridgeshire, CB7 5WW, England.

8 REFERENCES

Geyer H, Parr MK, Mareck U, Reinhart U, Schrader Y, Schanzer W. Analysis of non-hormonal nutritional supplements for anabolic-androgenic steroids – results of an international study. *Int J Sports Med.* 2004; 25, 124-129

Appendix 1 – Substances screened for (including analytical method used and LOD)

Substance	Method	Reporting Level
Amphetamine	LCMS	100 ng/g
1,4-androstadiene-3,17-dione	GCMS	10 ng/g
4-androstene-3,17-dione and/or 5(6)-androstene-3,17-dione ¹	GCMS	10 ng/g
4-androstene-3 β ,17 β -diol	GCMS	10 ng/g
5 α -androstane-3 β ,17 β -diol	GCMS	10 ng/g
5(6)-androstene-3 β ,17 β -diol	GCMS	10 ng/g
5 α -androstane-3,17-dione	GCMS	10 ng/g
Cathine (norpseudoephedrine)	LCMS	100 ng/g
Clenbuterol	LCMS	100 ng/g
Cocaine	LCMS	100 ng/g
Dehydroepiandrosterone (DHEA)	GCMS	10 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	GCMS	10 ng/g
4-estrene-3 β ,17 β -diol (19-nor-4-androstene-3 β ,17 β -diol)	GCMS	10 ng/g
5(10)-estrene-3 β ,17 β -diol (19-nor-5(10)-androstene-3 β ,17 β -diol)	GCMS	10 ng/g
Ephedrine	LCMS	100 ng/g
Gestrinone	LCMS	10 ng/g
Methylephedrine	LCMS	100 ng/g
MDMA (ecstasy)	LCMS	100 ng/g
Nandrolone (19-nor-4-androstene-17 β -hydroxy-3-one)	GCMS	10 ng/g
Salbutamol	LCMS	100 ng/g
Stanozolol	LCMS	10 ng/g
Strychnine	LCMS	100 ng/g
Terbutaline	LCMS	100 ng/g
Testosterone	GCMS	10 ng/g
Tetrahydrogestrinone (THG)	LCMS	10 ng/g

¹ These compounds are isomeric and indistinguishable from each other by this test.

Appendix 2 – Table of Results by Product Type

Product Type	Product ID	GCMS analysis result	LCMS analysis result
energy drink	43	Negative	Negative
energy drink	58	Negative	Negative
hormone regulator	29	<i>Inconclusive</i>	Negative
hormone regulator	40	Negative	Negative
hormone regulator	41	Positive - androstenedione, DHEA	Negative
hormone regulator	46	Negative	Negative
muscle builder	1	Negative	Negative
muscle builder	2	Negative	Negative
muscle builder	4	<i>Inconclusive</i>	Negative
muscle builder	14	Negative	Negative
muscle builder	15	Negative	Negative
muscle builder	18	Negative	Negative
muscle builder	26	Negative	Negative
muscle builder	33	Negative	Negative
muscle builder	34	<i>Inconclusive</i>	Positive - ephedrine
muscle builder	35	Negative	Negative
muscle builder	50	Negative	Negative
muscle builder	51	Positive - androstenedione, DHEA, 1,4 androstadiene-dione	Negative
muscle builder	53	Negative	Negative
muscle builder	54	Negative	Negative
post work out/recovery	36	Negative	Negative
post work out/recovery	39	Negative	Negative
post work out/recovery	44	Negative	Negative
pre workout	3	Negative	Negative
pre workout	7	Negative	Negative
pre workout	12	Negative	Negative
pre workout	37	Negative	Negative
pre workout	49	Negative	Negative
protein	42	Negative	Negative
protein	47	Negative	Negative
protein	55	Negative	Negative
protein	56	Negative	Negative
protein	57	Negative	Negative
rehydration/protein	38	Negative	Negative
testosterone booster	21	Positive - DHEA, androstenedione, 1,4 androstadiene-dione, androstenediol (trace)	<i>Inconclusive</i>
testosterone booster	22	Positive - androstenedione, 1,4 androstenedione, androstenediol, DHEA	Positive - ephedrine
testosterone booster	23	Positive - DHEA, androstenedione, 1,4 androstadiene-dione, androstenediol (trace)	Negative
testosterone booster	24	Positive - androstenedione, 1,4 androstadienedione, androstenediol, DHEA	Negative

testosterone booster	25	Negative	Negative
testosterone booster	27	<i>Inconclusive</i>	Negative
testosterone booster	28	Positive - androstenediol 1,4 androstadiene-dione, some DHEA (low)	Negative
testosterone booster	45	Positive - androstenedione, 1,4 androstadiene-dione, DHEA, androstenediol (low)	Positive - ephedrine
weight loss	5	Negative	Negative
weight loss	8	Negative	<i>Inconclusive</i>
weight loss	9	Negative	Negative
weight loss	10	Positive - estrenedione, DHEA, possible estrene-diol and androstenedione	Negative
weight loss	16	Negative	Negative
weight loss	17	Negative	Negative
weight loss	19	Positive - DHEA, possible androstenedione, 1,4 androstadiene-dione	Positive - ephedrine and methylephedrine
weight loss	20	<i>Inconclusive</i>	<i>Inconclusive</i>
weight loss	30	Negative	Negative
weight loss	31	Positive - androstenedione, 1,4 androstadiene-dione	Positive - ephedrine and methylephedrine
weight loss	32	Negative	<i>Inconclusive</i>
weight loss	48	Negative	Negative
weight loss	52	<i>Inconclusive</i>	Negative
Stack (2 products): a) post work out/recovery b) muscle builder	6	Negative Negative Negative	Negative
Stack (3 products): a) weight loss b) muscle builder c) diuretic	11	Positive - androstenedione, trace DHEA in overall product	Positive - ephedrine and methylephedrine, in overall product
Stack (3 products): a) testosterone booster b) muscle builder c) muscle builder	13	Positive - DHEA, androstenedione, 1,4 androstadiene-dione, androstenediol (trace) in overall product	Negative