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Anabolic steroids detected in bodybuilding dietary supplements – a significant risk to public health

V. Abbate,[a] A. T. Kicman,[b] M. Evans-Brown,[c] J. McVeigh,[d] D. A. Cowan,[b] C. Wilson,[e] S. J. Coles[e] and C. J. Walker[b]*

Twenty-four products suspected of containing anabolic steroids and sold in fitness equipment shops in the United Kingdom (UK) were analyzed for their qualitative and semi-quantitative content using full scan gas chromatography-mass spectrometry (GC-MS), accurate mass liquid chromatography-mass spectrometry (LC-MS), high pressure liquid chromatography with diode array detection (HPLC-DAD), UV-Vis, and nuclear magnetic resonance (NMR) spectroscopy. In addition, X-ray crystallography enabled the identification of one of the compounds, where reference standard was not available. Of the 24 products tested, 23 contained steroids including known anabolic agents; 16 of these contained steroids that were different to those indicated on the packaging and one product contained no steroid at all. Overall, 13 different steroids were identified; 12 of these are controlled in the UK under the Misuse of Drugs Act 1971. Several of the products contained steroids that may be considered to have considerable pharmacological activity, based on their chemical structures and the amounts present. This could unwittingly expose users to a significant risk to their health, which is of particular concern for naïve users. Copyright © 2014 John Wiley & Sons, Ltd.

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Introduction

In recent years, a large global uncontrolled market in human enhancement drugs has developed.[1] This market is a result of entrepreneurs exploiting demand from consumers, and is tightly coupled to increased manufacturing capacity of pharmacologically active substances in countries with emerging economies, globalized free trade, and modern communication and transport networks. Consumers looking to get a better body, empower themselves and increase their well-being can now choose from a broad range of drugs that claim to allow them to lose weight, brighten their mood, modify their social and sexual behaviour, lighten or tan their skin, enhance their cognitive function, and build muscle.[1] In order to boost sales of these drugs, entrepreneurs have used a range of creative and apparently persuasive marketing strategies,[1] including the sale of these drugs as off-the-shelf 'dietary', 'food', or 'herbal' 'supplements'.[1–4] One example of this practice in the United Kingdom is the sale of such drugs as bodybuilding 'dietary supplements' which are often marketed as 'safer' and 'legal' replacements to established anabolic steroids and other performance-enhancing drugs.[1] Similar to the 'legal highs' phenomenon,[5,6] and to some degree overlapping,[7–12] this market has been driven by opportunists[13] who have searched the scientific and patent literature for active substances, which were often originally synthesized as part of academic and pharmaceutical research programmes but were not commercialized as medicines.[14–20] Importantly, when such products containing anabolic steroids first emerged in the mid to late 1990s in the United States, the substances were also selected on the basis that they were not subject to drug control laws at that time.[21,22]

Most of the anabolic steroids used in these products are produced in bulk and obtained from wherever they can be cheaply and reliably sourced, imported into countries such as the United States and the United Kingdom where they are processed and packaged into finished products and sold in bricks-and-mortar and online fitness equipment shops; their sale as 'dietary supplements' appears to be a ploy to circumvent regulatory systems such as those governing medicines. Consumers may find such products attractive given the marketing practices used by manufacturers and distributors, as well as their ease of availability. Of particular concern here is that potentially vulnerable groups such as teenage boys as well as young men who face growing pressures to conform to a more stylized muscular body image,[23] may also use such products; such a scenario has been seen before in the United States

* *Correspondence to: Chris Walker, Drug Control Centre, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NH, UK. E-mail: chris.walker@kcl.ac.uk*

a *Institute of Pharmaceutical Science, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NH, UK*

b *Drug Control Centre, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NH, UK*

c *European Monitoring Centre for Drugs and Drug Addiction, Lisbon, Portugal*

d *Centre for Public Health, Liverpool John Moores University, Liverpool, L3 2ET, UK*

e *EPSRC UK National Crystallography Service, School of Chemistry, University of Southampton, Southampton, SO17 1BJ, UK*

when supplements containing androstenedione were available from high street and online shops during the late 1990s and early 2000s.[21,24] The primary aim of the current investigation was to identify the specific active substance(s) and estimate the dose present in a series of bodybuilding 'dietary supplements' that were suspected to contain anabolic steroids. Often publications have predominantly focused on the accuracy of the container label with regard to whether that steroid is present, without verification of amount. Little, if anything, is known about the toxicity or efficacy of many of these anabolic steroids but, with the presumption that they are biologically active on the basis of their structures, knowing the dose can give an insight into whether these products are likely to be pharmacologically significant. Using spectroscopic and spectrometric approaches any steroid present in the supplements was characterized and, when estimated to be present in more than 1 mg, the amount also determined.

Materials and methods

A total of 24 products were purchased from two fitness equipment shops one in Merseyside (n=4 products) and one in Cheshire (n=3) and three online shops (n=5; n=6; n=6) (Table 1). The products were selected after a review of information collected from Internet monitoring of online shops, bodybuilding websites discussing these products, as well as from members of the gym community familiar with the use of these products. In order to examine any potential variation in composition between different samples of the same product, four products bearing the same name as those purchased from the bricks-and-mortar retailers were also purchased via the Internet, making a total of 20 different products that were analyzed.

Potassium hydroxide (KOH), anhydrous sodium sulfate, sodium chloride, ethyl acetate, chloroform and hexane, all analytical reagent grade, and high-performance liquid chromatography (HPLC) grade methanol, dichloromethane, acetonitrile, and trifluoroacetic acid were purchased from Fisher Scientific (Loughborough, UK). Dodecane, deuterochloroform, N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA), ethanethiol, androst-4-ene-3,17-dione, adrenosterone (androst-4-ene-3,11,17-trione), dehydroepiandrosterone (DHEA) and ammonium iodide (NH_4I) were purchased from Sigma-Aldrich (Poole, UK). Formic acid Analar grade was purchased from VWR (Lutterworth, UK). Furazabol, desoxymethyltestosterone (DMT, madol) and methasterone were obtained through the World Association of Anti-Doping Scientists (WAADS) as part of its educational programme. Purified water was obtained from ELGA Purelab Maxima (Vivendi Water Systems, High Wycombe, UK). Methyl-1-testosterone and [19,19,19-$^2H_3$]-testosterone (d_3T) was purchased from LGC Promochem (Teddington, UK).

Table 1. List of the products analyzed with the name of the active ingredient, dose and recommended serving as listed on the packaging. (* spelling errors in the nomenclature in addition to 'a' and 'b' used for α and β)

#	Product Name	Manufacturer/Supplier	Labelled Contents	Labelled Dose per tablet or capsule (mg)	Recommended serving of tablet or capsule
1	Super Tren-MG	Black China Labs	13-ethyl-3mthoxy-gona-2,5(10) diene-17-one*	25	1/day
2	19-Nor Tren	Tri-City Chemicals	19-Norandrosta-4,9-diene-3,17-dione	35	2-3/day
3	Tren Bomb	Pharma Labs	17b-hydoxy-androstan-4-one*	10	2/day
4	Super Halo	Black China Labs	polydehydrogenated, polyhydroxylated halomethetioallocholane	25	1/day
5	Straight Epi	Black China Labs	2,3a-Epithio-17a-methyletioallo cholane-17b-ol	10	1/day
6	Straight Drol	Black China Labs	17a-dimethyl-5a-androst-3-one-17b-ol	10	1/day
7	Straight Phlexed	Black China Labs	17a-methyletioallocholan-2-ene-17b-ol	10	1/day
8	Protodrol	I Force Nutrition	Protodrol	25	2/day
9	Cynostane	AI	2-cyano-17a-methyl-17b-hydroxy-androst-3-one	10	1/day
10	Furazadrol	Axis Labs	5a-etioallocholan[2,3-c]furazan-17b-tetrahydropyranol ether	50	2-3/day
11	Trenavol-V	Chaparral Labs.	Estra-4,9-diene-3,17-dione	30	1-3/day
12	Ultra Mass	APS	19-Norandrosta-4,9-diene-3,17-dione 17a-methyletioallocholan-2-ene-17b-ol	40 10	2-3/day
13	11-Sterone	Competitive Edge Labs.	Androst-4-ene-3,11,17-trione	75	3-6/day
14	P-MAG	Competitive Edge Labs.	4-Chloro-17a-methyl-andro-4-ene-3,17b-diol	25	2-3/day
15	Furuza-A	Competitive Edge Labs.	5a-etioallocholan[2,3-c]furazan-17b-tetrahydropyranol ether	50	2-3/day
16	Epivol	Chaparral Labs.	Estra-4,9-diene-3,17-dione 2a,3a-Epithio-17a-methyletioallocholanol	30 10	1-2/day
17	M-LMG	Competitive Edge Labs.	13-ethyl-3methoxy-gona-2,5(10) diene-17-one	25	2-3/day
18	X-Tren	Competitive Edge Labs.	19-Norandrosta-4,9-diene-3,17-dione	30	3-6/day
19	Epivol Black	Black China Labs	2,3a-Epithio-17a-methyl-5a-androstan-17b-ol	10	1-3/day
20	S-Drol-17-Black	Black China Labs	2a,17a-dimethyl-17b-hydroxy-etiocholan-3-one	10	1-3/day
21	Straight Phlexed	Black China Labs	17a-methyletioallocholan-2-ene-17b-ol	10	1/day
22	Super Halo	Black China Labs	polydehydrogenated, polyhydroxylated halomethetioallocholane	25	1/day
23	Super Tren-MG	Black China Labs	13-ethyl-3mthoxy-gona-2,5(10) diene-17-one*	25	1/day
24	Tren Bomb	Pharma Labs	17b-hydoxy-androstan-4-one*	10	2/day

Gas chromatography-mass spectrometry (GC-MS) conditions

GC-MS analysis was carried out on an Agilent 5973 mass selective detector coupled to an Agilent 6890 GC system with an Agilent 7683 autosampler. The GC was fitted with a cross-linked polymethylsiloxane capillary column (HP-1; length 25 m; internal diameter 0.2 mm; film thickness 0.11 μm). The initial column temperature was 180 °C for 1 min, ramped to 220 °C at 8 °C /min, then from 220 °C to 250 °C at 3 °C /min and from 250 °C to 320 °C at 14 °C /min. The final temperature, 320 °C, was held for 5 min. Injections (1 μL) were made in the splitless mode; the injection port and transfer line temperatures were 250 and 280 °C, respectively. Helium was used as the carrier gas at a flow rate of 0.7 mL/min. The mass spectrometer when operated in the full scan mode used the mass range *m/z* 80–650. When operating in the selected ion monitoring (SIM) mode, the instrument condition were the same, but specific ions were selected for the compounds of interest. These ions were *m/z* 432 for DHEA (bis-TMS derivative), *m/z* 435 for d_3T (bis-TMS derivative), *m/z* 446 for methyl-1-testosterone (bis-TMS derivative), *m/z* 430 for androstenedione (bis-TMS derivative), *m/z* 501 for androst-4-ene-3,11,17-trione (tris-TMS derivative), and *m/z* 143 for furazabol (TMS derivative).

Accurate mass liquid chromatography-mass spectrometry (LC-MS)

This analysis was performed on a high resolution mass spectrometer (Exactive, Thermo Scientific, Hemel Hempstead, UK) that was coupled to an ultra-performance liquid chromatograph (Acquity UPLC® with 2777C Sample Manager, Waters, Manchester, UK). The source parameters were sheath gas flow 50; aux gas flow 10; spray voltage 4.5 kV; capillary temperature 250 °C and heater temperature 300 °C. The mobile phase was acetonitrile:water (50:50) containing 0.1 % formic acid run isocratically at 0.2 mL/min. An aliquot (~2 μL) of the methanolic extract was transferred to an autosampler vial containing 1 mL of acetonitrile:water containing 0.1 % formic acid. Flow injection (10 μL) was performed with positive ESI and alternating full scan and HCD fragmentation (30 V) both from *m/z* 100-1000, using the $[M+H]^+$ ion of caffeine at *m/z* 195.08765 as the lock mass ensuring a mass accuracy within 1 ppm.

Analytical HPLC

The HPLC analysis was conducted on a Hewlett-Packard 1050 system equipped with an autosampler, a reversed-phase HPLC column (Agilent Zorbax 300Å, C18, 5 μM, 150 x 2 mm) and a diode array detector (DAD) set to monitor 210-280 nm. Linear gradients (20 % B to 90 % B in 25 min) were run using solvents A (0.1 % (v/v) trifluoroacetic acid (TFA) aqueous solution and B (methanol) at a column temperature of 35 °C and flow rate of 0.2 mL/min. Sample injections of 5 μL and 25 μL of the analytes (~0.7 mg/mL in methanol) were performed in triplicate. Average retention times and standard deviations were determined (see supplemental data Table S1). The chromatograms can also be found in the supplemental data.

Nuclear magnetic resonance (NMR)

The ^{1}H and ^{13}C NMR measurements were obtained with an Avance 400 MHz NMR spectrometer (Bruker, Coventry, UK). The compounds were dissolved in deuterated chloroform containing tetramethylsilane as the internal reference and spectra recorded at room temperature (20 °C). ^{13}C Distortionless Enhancement by Polarization Transfer (^{13}C DEPT-135) was used, if necessary, to help assign spectra. Depending on the sample concentration, 16-128 scans and 512-4096 scans were integrated for ^{1}H and ^{13}C spectra, respectively.

Analysis of products

The products were opened and the contents (tablets or capsules) were counted, photographed, and weighed, as well as five randomly selected tablets or capsules from each product which were weighed individually. From this, the total weight of the contents and a mean tablet/capsule weight were calculated. The mean tablet/capsule weight was multiplied by the number of tablets/capsules present to give an extrapolated total weight of the contents of each product. This was compared with the total weight of the contents as a measure of uniformity. These data are summarized in Table 2.

Analysis of tablets and capsules

Five tablets or capsules from each product were placed into a tube containing methanol (20 mL) and mixed thoroughly by sonication then centrifuged at 1320 g for 5 min. A portion (2 mL) of the methanolic layer was transferred to a clean tube and the solvent evaporated to dryness under a steady stream of nitrogen at 60 °C. To the residue, potassium hydroxide (5 mL, 0.1 M), sodium chloride (~1g) and hexane (10 mL) were added. The contents were mixed thoroughly, centrifuged at 1320 g for 5 min and the hexane layer transferred to a clean tube. Hexane (10 mL) was added to the aqueous fraction and the extraction was repeated. The hexane fractions were combined and a 10 μL aliquot transferred to a glass tube and the solvent evaporated to dryness under a steady stream of nitrogen at 60 °C. The residues were derivatized with enol-reagent (0.06 mL, MSTFA:NH_4I:ethanethiol solution, 1000:3:9, *v/w/v*) and heated at 60 °C for 15 min to form ether-TMS and/or enol-TMS derivatives. After cooling, dodecane (0.04 mL) was added, transferred to a vial and analyzed using full scan GC-MS.

The remaining hexane extract was evaporated to dryness under a steady stream of nitrogen at 60 °C. The residues were dissolved in chloroform, transferred to glass vials and the solvent evaporated to dryness under a steady stream of nitrogen. These extracts were submitted for gravimetric estimation of steroid amount, HPLC-DAD, UV-Vis spectroscopy and NMR analysis.

Acid hydrolysis

Preliminary analysis suggested that products 15 and 17 contained esterified or ethereal compounds; as a result hydrolysis was performed using the methanolic supernatant from the extract of one capsule of these products. The solvent evaporated to dryness under a steady stream of nitrogen at 60 °C, the residue was dissolved in hydrochloric acid solution (2 mL, 1 M) and heated at 90 °C for 1 h. The solution was allowed to cool, then neutralized with KOH (5 M) and extracted with ethyl acetate (5 mL) three times. The ethyl acetate fractions were combined and evaporated to dryness under a steady stream of nitrogen at 60 °C.

Gravimetric estimation of steroid amount

Gravimetric estimation was performed when the authentic standard was not available. The extracted material was dissolved in dichloromethane and transferred to a pre-weighed glass vial. Following

Table 2. Data on the appearance and uniformity of the products, total weight of the contents, mean weight of a tablet/capsule (n=5), the extrapolated weight and the percentage difference between the total weight, the extrapolated total weight and number of tablets/capsules versus that claimed on the packaging. (Products were capsules unless otherwise stated)

#	Appearance	Total weight of contents (g)	Mean weight (n=5) (g)	Estimated Total weight (g)	Estimated weight/ total weight (%)	Number claimed	Number Counted
1	white tablet, unmarked	54.25	0.608	54.75	100.9	60	**90**
2	white, unmarked	59.38	0.648	58.30	98.2	90	90
3	off-white, unmarked	23.40	0.390	23.42	100.1	60	60
4	white, unmarked	18.49	0.283	18.08	97.8	60	**64**
5	white, unmarked	26.86	0.364	26.93	100.2	75	**74**
6	cream tablet, unmarked	30.20	0.492	29.51	97.7	60	60
7	white tablet, unmarked	32.69	0.546	32.74	100.2	60	60
8	orange, unmarked	39.75	0.701	42.09	105.9	60	60
9	red, unmarked	43.36	0.480	43.67	100.7	90	**91**
10	blue/white, unmarked	53.51	0.887	53.20	99.4	60	60
11	white, unmarked	38.56	0.431	38.79	100.6	90	90
12	blue, unmarked	43.99	0.490	44.10	100.3	90	90
13	white, unmarked	18.16	0.233	17.96	98.9	75	**77**
14	white, unmarked	13.86	0.232	13.90	100.3	60	60
15	white, unmarked	13.77	0.228	13.67	99.3	60	60
16	white, unmarked	59.55	0.648	59.00	99.1	90	**91**
17	white, unmarked	37.49	0.408	37.10	99.0	90	**91**
18	white, unmarked	39.69	0.423	40.59	102.3	90	**96**
19	orange, unmarked	47.53	0.636	47.70	100.4	75	75
20	orange, unmarked	36.27	0.641	38.44	106.0	60	60
21	cream tablet, unmarked	31.41	0.497	31.28	99.6	60	**63**
22	white, unmarked	39.95	0.650	39.02	97.7	60	60
23	cream tablet, unmarked	54.89	0.611	54.98	100.2	60	**90**
24	off-white, unmarked	24.02	0.402	24.10	100.3	60	60

evaporation of the solvent, the container was weighed again and the amount of extract was calculated by difference in weight.

Estimation of the amount of steroid using GC-MS

For those steroids where authentic standards were available, the steroid amount was calculated by comparison of the peak height ratio of the compound to that of the internal standard (d_3T) between the products being analyzed and a known amount of steroid standard. Five tablets or the contents of 5 capsules from products containing DHEA (1, 2, 4, and 23), methyl-1-testosterone (3 and 24), androstenedione (6, 7, 19, and 21), androst-4-ene-3,11,17-trione (13) and the residue from 15 (furazabol) following acid hydrolysis were diluted with methanol, sonicated, allowed to cool and the solution made up to an accurate volume with methanol. Portions of these solutions were taken and analyzed against known amounts of their respective standards using d_3T as the internal standard by full scan GC-MS as their ether-TMS and/or enol-TMS derivatives.

Results

Fourteen of the products contained the number of tablets or capsules claimed on the packaging, of the remaining only two products (1 and 23, these are the same brand) differed from the amount claimed by greater than ±10 % (Table 2). Visibly the products appeared uniform and this was supported by comparison of their total and estimated weights where the largest difference found was +6 % (range 97.7 to 106 %), (Table 2). The variation, as measured by the relative standard deviation, for the weighed tablets/capsules ranged from 0.8 to 8.4 %.

The results from the repeated full scan GC-MS analysis were consistent with the preliminary findings and these were supported by HPLC-DAD, and NMR analysis with reference to authentic standards (Table 3). Accurate mass LC-MS confirmed that steroids consistent with androstenedione, methyl-1-testosterone and adrenosterone were detected in the respective products. In the absence of reference standard, the putative identifications were made based on the full scan GC-MS spectra being consistent with the known structure of the named compound and were supported by the additional analyses. No steroid was detected in product 10.

Product 8

The compound detected in product 8 had a mass spectrum similar to that of desoxymethyltestosterone (DMT, Figure 1(2)), but many of the ions were +2 amu; for example the major ions of DMT were at *m/z* 360, 345, 270, 255 and 143 whereas those of the compound were at *m/z* 362, 347, 272, 257and 143. The *m/z* 143 is characteristic of the TMS derivative of 17α-methyl substituted steroids and the increase in mass is probably due to reduction of the double bond between C2 and C3 of DMT. This compound did not absorb UV-Visible light, even at increased sample concentration. The ^{1}H NMR had a similar profile to that of DMT, the major differences being the disappearance of the characteristic multiplet at 5.59 ppm, probably due to the 2-H and 3-H of DMT as well as of the olefinic peaks at around 125 ppm in the ^{13}C spectrum (which is in line with the loss of UV absorbance). Furthermore, the methyl signals appear shifted downfield with a proposed inversion of chemical shift for

Table 3. The steroid(s) identified in the products against reference standard and the estimated amount (* gravimetric estimation, ♦combined total = gravimetric estimation where more than one steroid detected)

#	Product Name	Steroid Identified	Estimated amount per tablet/capsule
1	Super Tren-MG	DHEA	28 mg
2	19-Nor Tren	DHEA	16 mg
3	Tren Bomb	Methyl-1-Testosterone	7 mg
4	Super Halo	DHEA	20 mg
5	Straight Epi	DMT	12 mg*
6	Straight Drol	Androstenedione	9 mg
7	Straight Phlexed	DMT	7 mg♦
		Androstenedione	4 mg
12	Ultra Mass	DMT	17 mg♦
		Methasterone	
13	11-Sterone	Androst-4-ene-3,11,17-trione	61 mg
15	Furuza-A	Furazabol	31 mg
16	Epivol	DMT	36 mg♦
19	Epivol Black	Androstenedione	~1 mg
		Methasterone	2.4 mg♦
20	S-Drol-17-Black	Methasterone	8 mg*
21	Straight Phlexed	Androstenedione	9 mg
22	Super Halo	Methasterone	5 mg*
23	Super Tren-MG	DHEA	21 mg
24	Tren Bomb	Methyl-1-Testosterone	7 mg

C18 and C19. In DMT the protons of the C19 methyl are affected by the electrophilic nature of the double bond between C2 and C3 and, as a result, they are more downfield when compared to the protons in the C18 methyl. However, in the compound present in product 8, the absence of the double bond removes this influence leading to the protons on the C18 methyl being more deshielded, due to the hydroxyl group on C17, than those on the C19 methyl. These data leads us to conclude the identity of the steroid component of supplement 8 as dihydro DMT (Figure 1(**5**)).

NMR Product 8

^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.79 (3H, s, 19-H), 0.84 (3H, s, 18-H), 1.21 (3H, s, 17α-H) 0.61-1.82 (residual CH and CH_2) ppm.

^{13}C NMR ($CDCl_3$, 100.62 MHz): δ 12.27, 14.0, 20.44, 22.2, 23.25, 25.8, 26.83, 29.0, 29.06, 31.73, 31.91, 36.37, 36.43, 38.76, 39.0, 45.55, 47.17, 50.81, 54.82 and 81.79 ppm. The NMR data were consistent with literature values.[25] (See supplementary data for the NMR spectra, Figures S61–S65)

Product 9

The mass spectrum of the compound, as the bis TMS, derivative detected in product 9 is shown in Figure 2; it has *m/z* 143 as the most intense ion that is characteristic of the TMS derivative of 17α-methyl substituted steroids. The apparent molecular ion is an odd mass, *m/z* 473, strongly suggesting that the compound contains an odd number of nitrogen atoms. The study by Parr *et al.*[26] on steroid isoxazoles, structurally similar to the proposed compound but without the 17α-methyl, showed that the configuration of the isoxazole ring affects the properties of the steroid. Steroids with the [3,2-c] configuration e.g. 17β-hydroxyandrostano [3,2-c] isoxazole and androisoxazol form only mono-TMS derivative by reacting with the 17β hydroxyl group, whereas those with [3,2-d] arrangement; for example, 17β-hydroxyandrostano [3,2-d] isoxazole and danazol form a bis-TMS derivative. The compound detected in product 9 using GC-MS is probably as the bis-TMS derivative and therefore likely to have the configuration shown in Figure 3 (structure 2). The use of accurate mass LC-MS confirmed that this product contain a compound whose protonated molecule differs by ≤1 part per million from that calculated (330.2428 versus 330.2425) for the putative chemical formula $C_{21}H_{31}NO_2$ proposed following GC-MS analysis. Of course, these data cannot give information on the exact conformation of the steroid detected and NMR was not successful for this compound (see supplementary data for the NMR spectra, Figures S53).

Products 11, 16, 18, and 23.

Products 16 and 23 have already been shown to contain DMT and DHEA respectively, but also a second compound was detected in these products that was consistent with that detected in products 11 and 18. The mass spectrum of the compound, as the bis-TMS derivative, detected in these products has *m/z* 414 as the highest mass ion. Comparison of this spectrum with those of closely associated steroids, 19-nortestosterone and 19-norandrostenedione support the notion that the compound detected is a 19-norsteroid with further conjugation and the likelihood that *m/z* 414 is the molecular ion. The relative abundance of this ion is consistent with that of a steroid with a '4-ene, 3-one' structure, following enolization as the TMS derivative. The bis-TMS derivative would be a highly conjugated molecule with four carbon – carbon double bonds in the structure that resists extensive fragmentation forming a stable molecular ion. The ^{1}H NMR for extracts from all these products had a methyl signal observed at 1.03 ppm, this is likely to be due to the C-18 group. The singlet detected at 5.71 ppm strongly suggests the presence of a 4-ene unsaturated system. Moreover, the ^{13}C NMR is consistent with the presence of 2 carbonyl peaks, these probably belong to the 3-oxo and 17-oxo system and the four peaks in the alkene region support the presence of two carbon –

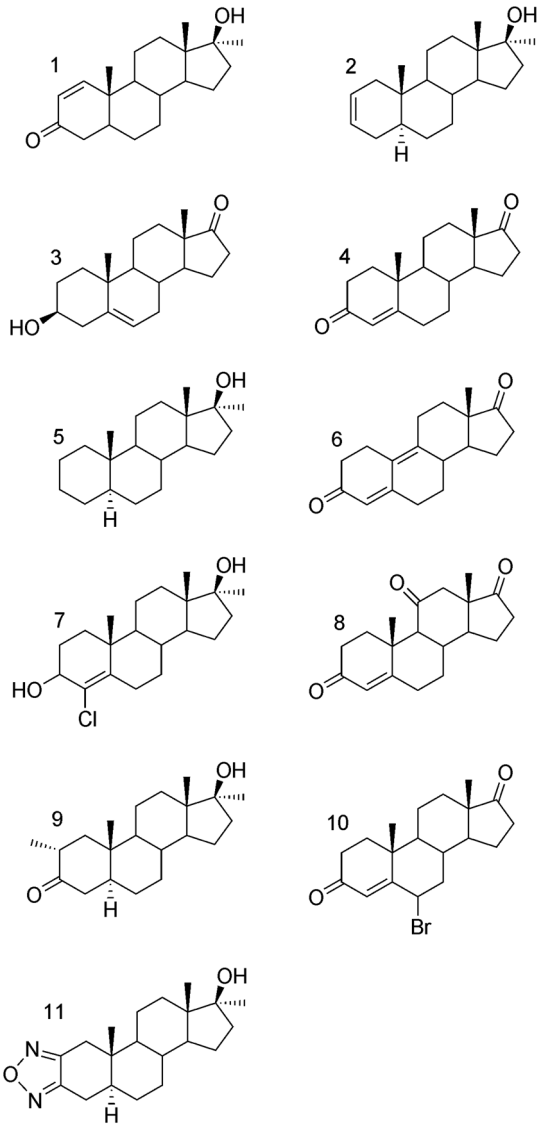

Figure 1. Structures of: methyl-1-testosterone (1); DMT (2); DHEA (3); androstenedione (4); reduced DMT (5); 19-norandrosta-4,9-dien-3,17-dione (6); 4-chloro-17α-methyl-androst-4-ene-3,17β-diol (7); adrenosterone (8); methasterone (9); 6-bromo-androstenedione (10); furazabol (11).

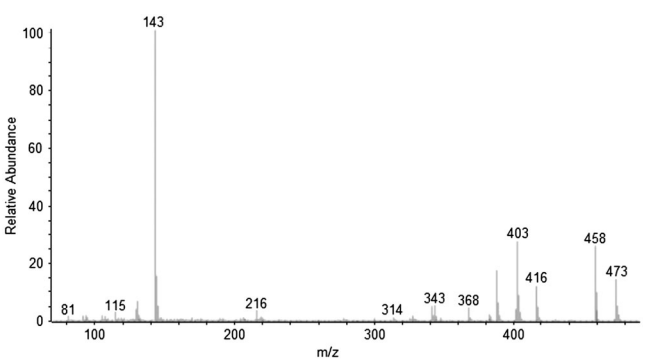

Figure 2. Full Scan GC-MS mass spectrum of the compound in product 9 as the trimethylsilyl derivative.

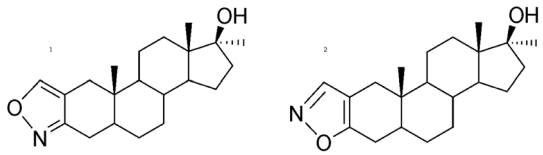

Figure 3. Androisoxazol ([3,2-c]isoxazole) (1) and the ([3,2-d]isoxazole isomer (2) believed to be in product 9.

carbon double bonds in the underivatized steroid structure. These data leads us to conclude that this steroidal component was estra-4, 9-dien-3,17-dione (or 19-norandrosta-4,9-diene-3,17-dione, $C_{18}H_{22}O_2$) (Figure 1(**6**)). Accurate mass LC-MS confirmed that these products contain a compound whose protonated molecule differs by ≤1 part per million from that calculated for estra-4,9-dien-3,17-dione. Although these data cannot confirm the exact conformation of the steroid detected, this same compound was detected in all these products according to the LC-MS data.

NMR Product 11 and 18

^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.03 (3H, s, 18-H), 1.33-2.92 (residual CH and CH_2) and 5.71 (1H, s, 4-H) ppm.

^{13}C NMR ($CDCl_3$, 100.62 MHz): δ 13.18, 21.9, 25.14, 25.9, 26.53, 30.67, 31.44, 35.86, 37.04, 38.74, 47.51, 51.19, 122.62, 126.3, 144.63, 156.51, 199.61, and 219.7 ppm. The NMR data were consistent with literature values.[27–29] (See supplementary data for the NMR spectra, Figures S31-S34 and S37-S39.)

Product 14

Full scan GC-MS analysis of this product showed the presence of 2 isomers, with *m/z* 482 presumed to be the molecular ion of the bis-TMS derivative. The other major ions were *m/z* 467 [M-15], 447 [M-35 i.e., Cl], 357 [M-35-90], and 143, the latter characteristic of the TMS derivative of 17α-methyl substituted steroids. The use of accurate mass LC-MS confirmed that this product contains a compound that gave a low response for the protonated molecule ($C_{20}H_{31}O_2Cl$, 339.2085) but it fragmented in the source to give protonated ions that differs by ≤1 part per million from that calculated for the loss of one and two molecules of water chemical formulae $C_{20}H_{29}OCl$ and $C_{20}H_{27}Cl$, respectively. These data supported by ^{1}H and ^{13}C NMR indicates that product 14 contains probably 2 isomers (1 major and 1 minor) of 4-chloro-17α-methyl-andro-4-ene-3,17β-diol.

NMR Product 14

^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.88 (3H, s, 18-H), 1.16 (3H, s, 19-H), 1.19 (3H, s, 17α-H), 0.78-2.12 (residual CH and CH_2), 2.92 (1H, ddd, *J* 14.4 Hz, 4.2 Hz, 2.8 Hz, 3α-H) and 4.15 (1H, ddd, *J* 8.9 Hz, 6.2 Hz, 1.8 Hz, 3β-H) ppm.

^{13}C NMR ($CDCl_3$, 100.62 MHz): δ 13.91, 19.28, 20.91, 23.18, 25.78, 27.1, 27.99, 31.31, 31.53, 33.72, 36.32, 38.95, 40.52, 45.39, 50.28, 54.13, 69.61, 81.58, 128.27 and 142.65 ppm (see supplementary data for the NMR spectra, Figures S41 S43).

Product 15

Full scan GC-MS analysis of this product showed five peaks (Figure 4), the first was identified as furazabol. Furazabol has a molecular mass of 330 amu, the spectra of the two pairs of isomeric peaks all contain *m/z* 313 probably as the result of cleavage of the bond between the steroid and ether group.

The product claimed to contain a steroid as an ether derivative so as a result of these findings a portion of the extracted material was submitted to acid hydrolysis. Following acid hydrolysis a single peak was detected (Figure 5) with a retention time and mass spectrum (Figure 6) consistent with furazabol TMS derivative (Figure 7). (See supplementary data for the NMR spectra, Figures S45-S46 and GC-MS data Figures S47-S52.)

Product 17

Full scan GC-MS analysis of this product showed two peaks, the first, and larger, had the highest mass ion at *m/z* 372 with *m/z* 155, 251, 253 and 343 as abundant ions (Figure 8). The second had the highest mass ion at *m/z* 370 with *m/z* 155, 173, 251 and 341 as abundant ions. The following assumes that *m/z* 372 and *m/z* 370

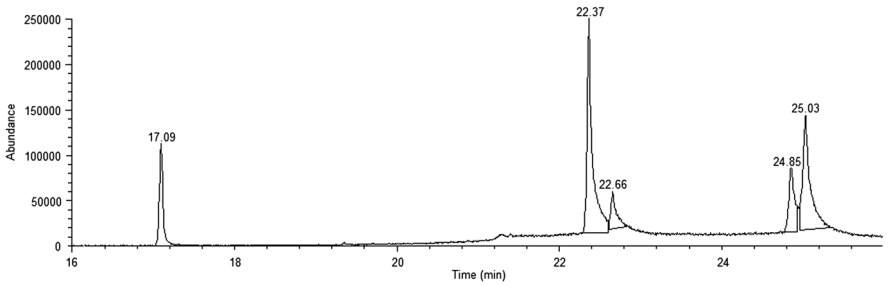

Figure 4. Total ion chromatogram from full scan GC-MS analysis of a derivatised extract of Product 15.

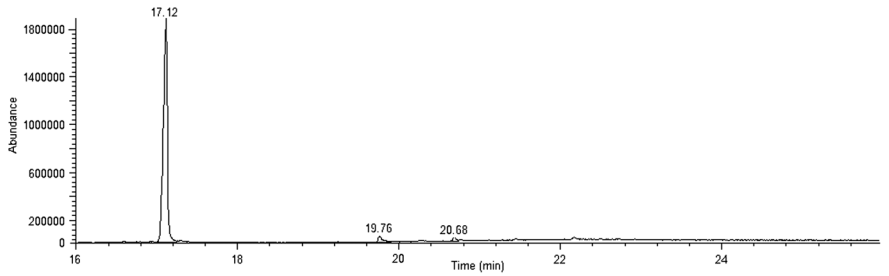

Figure 5. Total ion chromatogram from full scan GC-MS analysis of a derivatised extract of Product 15 following acid hydrolysis.

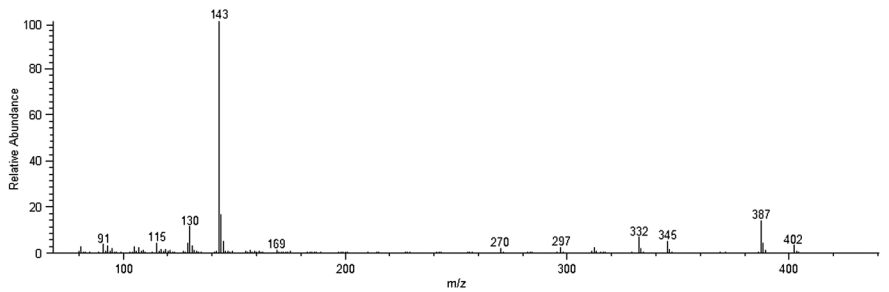

Figure 6. Full scan spectrum at retention time of 17.12 minutes in Figure 5.

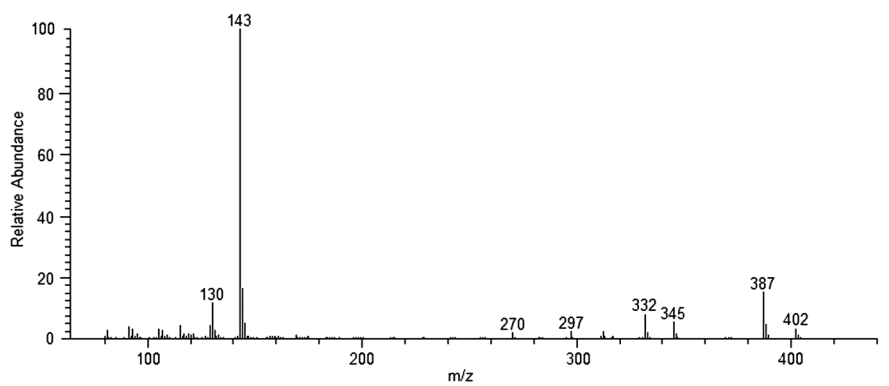

Figure 7. Full scan spectrum of furazabol TMS derivative.

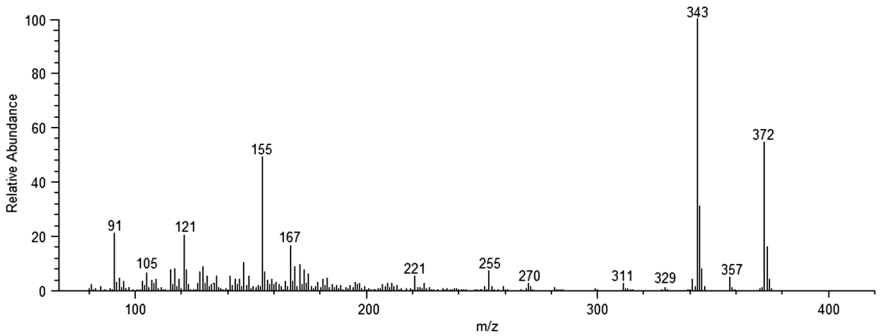

Figure 8. Full scan spectrum from the principal peak in the derivatised extract of Product 17.

are the molecular ions, respectively. These data suggest that both of these products contain ethyl groups, based on the loss of 29 amu, *m/z* 372 to 343 and *m/z* 370 to 341. The [M-29] ions were the most abundant in their respective spectra and both products showed loss of 90 amu from this; i.e., *m/z* 253 and 251 consistent with the loss of trimethylsilanol [$C_3H_{10}OSi$]. Following extraction with hexane under basic conditions, additional peaks were detected two with the highest mass ion at *m/z* 430 and one at *m/z* 428. More thorough hydrolysis, using acid, produced a major product with the highest mass ion at *m/z* 430 and *m/z* 415, 401, 194, 328, and 311 (Figure 9), and a minor product ion at *m/z* 428. These data suggest that a methyl group that was bound to an oxygen atom was removed by hydrolysis, which enabled the resulting hydroxyl to be derivatized with TMS, producing the bis-TMS for both compounds. The mass spectral data also suggests that both of these products contain ethyl groups, based on the loss of 29 amu, *m/z* 430 to 401 and *m/z* 428 to 399. Although in these compounds the highest mass ions *m/z* 430 and *m/z* 428 were the most abundant in their respective spectra. Both products showed losses of 90 amu consistent with the loss of trimethylsilanol [$C_3H_{10}OSi$]. The presence of *m/z* 194 is a characteristic fragment formed by the cleavage of the A and B ring containing two carbon-carbon double bonds. The use of accurate mass LC-MS confirmed that this product before hydrolysis contains a compound with a protonated molecule that differs by $\leq$1 part per million from that calculated for $C_{20}H_{28}O_2$ and after hydrolysis contains a compound with a protonated molecule that differs by $\leq$1 part per million from that calculated for $C_{19}H_{26}O_2$ supporting the idea that the hydrolysis involved conversion of a methoxy group to a hydroxyl.

The product of hydrolysis was crystallized from methanol/cold water and single X-ray crystallography[30] revealed the crystal structure to be 13-ethyl-gona-4-ene-3, 17-dione (Figure 10), which could be readily formed from the hydrolysis of 13-ethyl-3-methoxy-gona-2,5-(10)-diene-17-one (also known as methoxydienone, CAS No. 2322-77-2) that this product claimed to contain.

Product 19

GC-MS analysis of extracts from supplement 19 was found to contain small amounts of androstenedione (Figure 1(**4**)), methasterone (Figure 1(**9**)), plus a compound for which we had no reference standard that gave *m/z* 508 and 510 of similar intensity as the most intense signals. This isotopic pattern is consistent with the possible presence of a bromine atom in the molecule and the intense molecular ion is consistent with a steroid with a '4-ene, 3-one' following enolization as the TMS derivative. The putative identification given to this compound was 6-bromo-androstenedione (Figure 1(**10**)). HPLC-DAD detected 2 peaks that eluted with a distinctive bathochromic effect in the UV-Visible profile, suggesting a conjugated halogenated system, and other distinctive features from NMR, such as the 2 multiplets at 4.9 and 5.02 ppm due to 6-Hα and 6-Hβ geminal protons of a 6-halogenated system and the 2 singlets at 5.02 and 5.71 ppm indicative of conjugated alkene protons led us to identify the major components of this product to be 2 isomeric forms of 6 bromo-androstendione (Figure 1 (**10**)).

NMR Product 19

^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.92 (s, 18-H of 6α), 0.98 (s, 18-H of 6β), 1.25 (s, 19-H of 6β), 1.56 (s, 19-H of 6α), 0.67-2.62 (residual CH and CH_2), 4.9 (m, J 13.1 Hz, 6-H of 6β), 5.02 (m, J 4.0 Hz, 6-H of 6α), 5.93 (s, 4-H of 6β), and 6.45 (d, J 1.8 Hz, 4-H of 6α) ppm.

^{13}C NMR ($CDCl_3$, 100.62 MHz): δ 13.71, 17.38, 20.31, 21.75, 30.76, 31.27, 32.56, 33.91, 35.15, 35.70, 35.75, 38.64, 47.50, 50.84, 53.81, 124.15, 170.3, 199.3, and 220.4 ppm. NMR data for the putative 6α/β-bromo-androstenedione were consistent with literature values.[31] (See supplementary data for the NMR spectra, Figure S57.)

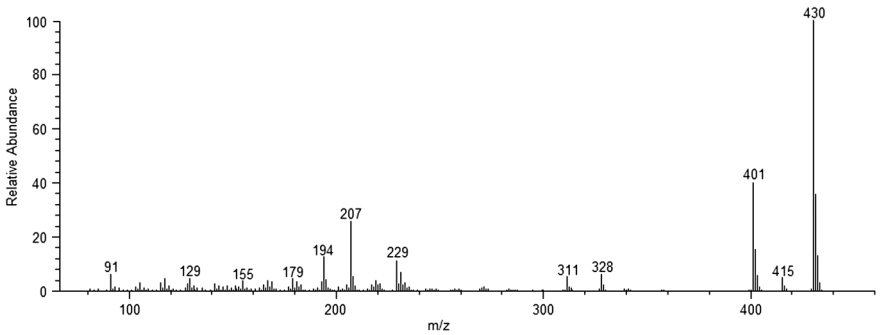

Figure 9. Full scan spectrum from the principal peak in the derivatised extract of Product 17 following acid hydrolysis.

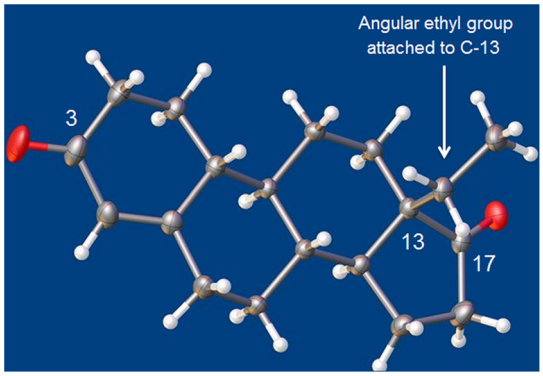

Figure 10. Crystal structure of the hydrolyzed product 17 from X-ray crystallography; numbers of particular carbon atoms are displayed.

Discussion

Examination of the appearance and weights of the tablets and capsules from the products tested showed reasonable uniformity, suggesting a level of competency in production, though the number of tablets and capsules supplied often differed from that claimed on the packaging. Of the 24 products tested, 23 of them contained steroids 16 of these contained steroids that were different to those indicated and one product contained no steroid at all. This is not unusual, often only an obscure reference is made to the substance(s); this includes using a chemical name(s), which may be misspelled and likely to have the effect of hiding the true ingredients from both consumers and regulators.[1,3,4,32] There are clear examples of this in this study for example 'mthoxy' instead of methoxy for products 1 and 23; 'hydoxy' instead of hydroxy for products 3 and 24 and replacing androstane with etioallocholane as in products 4, 5, 7, 10, 12, 15, 16, 21, and 22.

Many of the steroids detected have been previously described in the patent and scientific literature and originally synthesized as part of academic and pharmaceutical research programmes but for unknown reasons were never commercialized as medicines. For example, methasterone was first synthesized in the 1950s[15] and shown to be a potent anabolic agent in animal models[14,17], DMT was first synthesized in the 1960s[18] and shown in animal models to be a potent anabolic agent[33,34], while methyl-1-testosterone was first synthesized in the 1960s.[18] Despite the fact that many of these 'supplements' have been advertised as legal replacements for anabolic steroids, all of the substances identified in Table 3 are controlled in the United Kingdom under the Misuse of Drugs Act 1971[35–37], while 6 substances putatively identified in Table 4 would also be controlled.

Given the limited data on the pharmacology and toxicology of the substances identified in this study, it is difficult currently to estimate the potential for acute and chronic harm posed by their use. That said, some of the products contained steroids that may be considered to have considerable pharmacological activity when administered chronically, based on their chemical structures and the doses present. Products 12, 19, 20, and 22 marketed as 'Ultra Mass', 'Epivol Black', 'S-Drol-17-Black', and 'Super Halo', respectively, all contained methasterone which is a 17α-alkylated compound. This substance emerged as a dietary supplement in the United States around 2005, marketed as 'Superdrol'; subsequently clinical case reports of serious hepatotoxicity associated with its use have been reported in the United States and the United Kingdom.[38–44] Although causal assessment of these cases is, in part, impeded by a lack of analytical identification of the actual substance used (either from toxicological screening of a biological sample from the patient or analysis of the product used), in our opinion it is likely that methasterone is capable of causing hepatotoxicity given the clear temporal relationship between exposure to the product and the onset of symptoms as well as pharmacological plausibility based on the accepted causal link between this structural feature and hepatotoxicity.[45,46] This could also be true of the other 17-alkylated steroids detected: DMT, methyl-1-testosterone, [2,3 d]-androisoxazol, 4-chloro-17α-methyl-andro-4-ene-3,17-diol and furazabol. The doses estimated for these 17-alkylated anabolic steroids (Table 3) ranged from 3 mg-31 mg (the 36 mg of steroid calculated for product 16 is the combined amount of DMT and 19-norandrosta-4,9-diene-3,17-dione), these equal or exceed the typical therapeutic doses of 2.5–20 mg daily recommended for established 17-alkylated anabolic steroids that are, or were, licensed as medicines. Not only does this raise a major concern that naïve users, notably teenage boys, would be exposed to doses of these steroids more associated with 'hardcore' bodybuilding, whilst being unaware that such doses are likely to have adverse effects when administered chronically, but more broadly there are major concerns about the use of such substances on which there is little or no data on their pharmacology and toxicology outside of limited studies that were conducted during the 1950s and 1960s.

In recent years a global uncontrolled market in enhancement drugs has flourished.[1] Consumers are now able to choose from a broad range of substances and products that claim to allow them to enhance their bodies.[1] In some cases the active substances that are used are the same as those in authorized medicines, in others the substances have been withdrawn from use in medicines due to safety concerns, while a growing number of substances available on the market – such as many of those detected in this study – have never been tested in humans.[1,2,4]

The analytical methods described here can play an essential role in the public health response to these drugs by providing

Table 4. Putatively identified steroids from GC-MS. († spelling errors in the nomenclature apart from 'a' and 'b' used for α and β)

#	Product Name	Labelled Contents	Putative identification of steroid detected
8	Protodrol	Protodrol	Reduced DMT
9	Cynostane	2-cyano-17a-methyl-17b-hydroxy-androst-3-one	[2,3 d]-androisoxazol
11	Trenavol-V	Estra-4,9-diene-3,17-dione	19-norandrosta-4,9-diene-3,17-dione
14	P-MAG	4-Chloro-17a-methyl-andro-4-ene-3,17b-diol	4-chloro-17α-methyl-andro-4-ene-3,17-diol
15	Furuza-A	5a-etioallocholan[2,3-c]furazan-17b-tetrahydropyranol ether	Ethers/Esters of furazabol
16	Epivol	Estra-4,9-diene-3,17-dione	19-norandrosta-4,9-diene-3,17-dione
17	M-LMG	13-ethyl-3methoxy-gona-2,5(10) diene-17-one	Unknown
18	X-Tren	19-Norandrosta-4,9-diene-3,17-dione	19-norandrosta-4,9-diene-3,17-dione
19	Epivol Black	2,3a-Epithio-17a-methyl-5a-androstan-17b-ol	bromo-androstenedione
23	Super Tren-MG	13-ethyl-3mthoxy-gona-2,5(10) diene-17-one†	19-norandrosta-4,9-diene-3,17-dione

methodologies to identify and quantify the active substance(s) present. This helps develop our understanding of this market, as well as allowing us to monitor the composition of 'supplements' sold and the hazards that they may pose. When considered with other data, such as prevalence of use, these types of study play a central role in assessing and quantifying the risks to individual and public health.

References

[1] M. Evans-Brown, J. McVeigh, C. Perkins, M.A. Bellis. *Human Enhancement Drugs - The Emerging Challenges to Public Health*. North West Public Health Observatory, Liverpool, **2012**.
[2] U.S. Food and Drug Administration. Tainted_Supplements_CDER. Available at: http://www.accessdata.fda.gov/scripts/sda/sdNavigation.cfm?sd=tainted_supplements_cder [6 June 2014].
[3] R. Kazlauskas. Designer steroids, in *Doping in Sports: Biochemical Principles, Effects and Analysis. Handbook of Experimental Pharmacology, Vol. 195*. (Eds: D. Thieme, P. Hemmersbach), Springer, Berlin, Heidelberg, **2010,** pp. 155–185.
[4] M. Evans-Brown, A. Kimergård, J. McVeigh, M. Chandler, S.D. Brandt. Is the breast cancer drug tamoxifen being sold as a bodybuilding dietary supplement? doi: 10.1136/bmj.g1476.
[5] L.A. King, A.T. Kicman. A brief history of 'new psychoactive substances. *Drug Test. Anal.* **2011**, *3*, 401.
[6] P. Griffiths, M. Evans-Brown, R. Sedefov. Getting up to speed with the public health and regulatory challenges posed by new psychoactive substances in the information age. *Addiction* **2013**, *108*, 1700.
[7] European Monitoring Centre for Drugs and Drug Addiction. *EMCDDA–Europol 2010 Annual Report on the Implementation of Council Decision 2005/387/JHA*. European Monitoring Centre for Drugs and Drug Addiction, Lisbon, **2011.**
[8] Twitter. *@toxicovigilance.* Available at: https://twitter.com/toxicovigilance/status/442231055719084032 [14 May 2014].
[9] B.J. Venhuis, D. de Kaste. Scientific opinion on the regulatory status of 1,3-dimethylamylamine (DMAA). *Eur. J. Food Res. Rev.* **2012**, *2*, 93.
[10] Medicines and Healthcare products Regulatory Agency. MHRA determines that a product containing 1,3-Dimethylamylamine (DMAA) is a medicinal product. Available at: http://www.mhra.gov.uk/Howweregulate/Medicines/Medicinesregulatorynews/CON180713 [16 June 2014].
[11] P.A. Cohen, J.C. Travis, B.J. Venhuis. A methamphetamine analog (N,α-diethyl-phenylethylamine) identified in a mainstream dietary supplement. *Drug Test. Anal.* **2013**, 10.1002/dta.1578
[12] J. Lee, B. Venhuis, S. Heo, H. Choi, I. Seol, E. Kim. Identification and quantitation of N,α-diethylphenethylamine in preworkout supplements sold via the Internet. *Forensic Toxicol.* **2014**, *32*, 148.
[13] S. Assael. *Steroid Nation: Juiced Home Run Totals, Anti-Aging Miracles, and a Hercules in Every High School*. ESPN, New York, **2007**.
[14] J.A. Vida. *Androgens and Anabolic Agents. Chemistry and Pharmacology*. Academic Press, London, **1969**.
[15] H.J. Ringold, G. Rosenkranz. Steroids. LXXXIII. Synthesis of 2-methyl and 2,2-dimethyl hormone analogs. *J. Org. Chem.* **1956**, *21*, 1333.
[16] H.J. Ringold, E. Batres, O. Halpern, E. Necoechea. Steroids. CV.1 2-methyl and 2-hydroxymethylene-androstane derivatives. *J. Am. Chem. Soc.* **1959**, *8*, 427.
[17] F.A. Kincl, R.I. Dorfman. Anabolic-androgenic potency of various steroids in a castrated rat assay. *Steroids* **1964**, *3*, 109.
[18] R.E. Counsell, P.D. Klimstra, F.B. Colton. Anabolic agents. Derivatives of 5α-androst-1-ene. *J. Org. Chem.* **1962**, *27*, 248.
[19] E. Rohrmann, H.A. Shonle. Aminoalkanes as pressor substances. *J. Am. Chem. Soc.* **1944**, *66*, 1516.
[20] J. Knoll. New psychostimulant agent. Patent WO 88/02254. World Intellectual Property Organization, **1988**. Available at: http://worldwide.espacenet.com/publicationDetails/biblio?CC=EP&NR=0284621B1&KC=B1&FT=D [9 June 2014].
[21] Subcommittee on Crime and Drugs of the Committee on the Judiciary United States Senate. *Body building products and hidden steroids: Enforcement barriers: Hearing before the Subcommittee on Crime and Drugs of the Committee on the Judiciary United States Senate*. One Hundred Eleventh Congress, US Government Printing Office: Washington D.C., **2009**.
[22] Drug Enforcement Administration. Classification of two steroids, prostanozol and methasterone, as Schedule III anabolic steroids under the Controlled Substance Act. *Fed. Reg.* **2012**, *77*, 44456.
[23] T. Cash, L. Smolak. *Body Image: A Handbook of Science, Practice, and Prevention*. Guilford Press, New York, **2011**.
[24] L.D. Johnston, P.M. O'Malley, J.G. Bachman. *Monitoring the Future National Survey Results on Drug Use, 1975-2001. Volume I: Secondary School Students*. National Institute on Drug Abuse, Bethesda, **2002**.
[25] J.B. Jones, J.D. Leman. Steroids and steroidases. XI. synthetic approaches to C-17 Bis(2-hydroxyethyl)-amino compounds as potential precursors of 17-Hydroxyandrostane nitrogen mustards. *Can. J. Chem.* **1971**, *49*, 2420.
[26] M.K. Parr, M. Guetschow, J. Daniels, G. Opfermann, M. Thevis, W. Schänzer. Identification of steroid isoxazole isomers marketed as designer supplement. *Steroids* **2009**, *74*, 322.
[27] H.L. Holland, E. Riemland. Microbial hydroxylation of steroids. 10. Rearrangement during epoxidation and hydroxylation, and the stepwise nature of these enzymic reactions. *Can. J. Chem.* **1985**, *63*, 1121.
[28] K. Krohn, I. Vinke, H. Adam. Transition-metal catalyzed oxidations .7. Zirconium-catalyzed oxidation of primary and secondary alcohols with hydroperoxides. *J. Org. Chem.* **1996**, *61*, 1467.
[29] M. Rosenberger, R. Borer, G. Saucy. Steroid total synthesis. 11. (+)-Estr-4-ene-3,17-dione from a chiral lactone. *J. Org. Chem.* **1978**, *43*, 1550.
[30] S.J. Coles, P.A. Gale. Changing and challenging times for service crystallography. *Chem. Sci.* **2012**, *3*, 683.
[31] N.V. Lukashev, G.V. Latyshev, P.A. Donez, G.A. Skryabin, I.P. Beletskaya. 6-Chloro- and 6-bromo-substituted steroids in the Suzuki-Miyaura cross-coupling reaction. A convenient route to potential aromatase inhibitors. *Synthesis*, **2006**, *3*, 533.
[32] H. Geyer, M.K. Parr, K. Koehler, U. Mareck, W. Schänzer, M. Thevis. Nutritional supplements cross-contaminated and faked with doping substances. *J. Mass Spectrom.* **2008**, *43*, 892.
[33] M.H. Sekera, B.D. Ahrens, Y.C. Chang, B. Starcevic, C. Georgakopoulos, D. H. Catlin. Another designer steroid: Discovery, synthesis, and detection of 'madol' in urine. *Rapid Commun. Mass Spectrom.* **2005**, *19*, 781.
[34] P. Diel, A. Friedel, H. Geyer, M. Kamber, U. Laudenbach-Leschowsky, W. Schänzer, M. Thevis, G. Vollmer, O. Zierau. Characterisation of the pharmacological profile of desoxymethyltestosterone (Madol), a steroid misused for doping. *Toxicol. Lett.* **2007**, *169*, 64.
[35] Misuse of Drugs Act 1971. Her Majesty's Stationery Office (HMSO), London, **1971**. Available at: http://www.legislation.gov.uk/ukpga/1971/38/contents [9 June 2014].
[36] The Misuse of Drugs Act 1971 (Modification) Order 1996. Her Majesty's Stationery Office (HMSO), London, **1996**. Available at: http://www.legislation.gov.uk/uksi/1996/1300/article/2/made [9 June 2014].
[37] The Misuse of Drugs Act 1971 (Amendment) Order 2009. Her Majesty's Stationery Office (HMSO), London, **2009**. Available at: http://www.legislation.gov.uk/ukdsi/2009/9780111486610/article/2 [9 June 2014].
[38] B. Jasiurkowski, J. Raj, D. Wisinger, R. Carlson, L. Zou, A. Nadir. Cholestatic jaundice and IgA nephropathy induced by OTC muscle building agent superdrol. *Am. J. Gastroenterol.* **2006**, *101*, 2659.
[39] M.I. Kafrouni, R.A. Anders, S. Verma. Hepatotoxicity associated with dietary supplements containing anabolic steroids. *Clin. Gastroenterol. Hepatol.* **2007**, *5*, 809.
[40] N.L. Shah, I. Zacharias, U. Khettry, N. Afdhal, F.D. Gordon. Methasteron-associated cholestatic liver injury: Clinicopathologic findings in 5 cases. *Clin. Gastroenterol. Hepatol.* **2008**, *6*, 255.
[41] V. Singh, M. Rudraraju, E.J. Carey, T.J. Byrne, H.E. Vargas, J.E. Williams, V. Balan, D.D. Douglas, J. Rakela. Severe hepatotoxicity caused by a methasteron-containing performance-enhancing supplement. *J. Clin. Gastroenterol.* **2009**, *43*, 287.
[42] J. Nasr, J. Ahmad. Severe cholestasis and renal failure associated with the use of thedesigner steroid Superdrol™ (Methasteron™): A case report and literature review. *Dig. Dis. Sci.* **2009**, *54*, 1144.
[43] P.V. Krishnan, Z.-Z. Feng, S.C. Gordon. Prolonged intrahepatic cholestasis and renal failure secondary to anabolic androgenic steroid-enriched dietary supplements. *J. Clin. Gastroenterol.* **2009**, *43*, 672.
[44] Y. El Sherrif, J.R. Potts, M.R. Howard, A. Barnardo, S. Cairns, A.S. Knisely, S. Verma. Hepatotoxicity from anabolic androgenic steroids marketed as dietary supplements: Contribution from ATP8B1/ABCB11 mutations? *Liver Int.* **2013**, *33*, 1266.
[45] H.L. Krüskemper. *Anabolic Steroids*. Academic Press: San Diego, **1968**.
[46] H.M. Behre, E. Nieschlag.Testosterone preparations for clinical use in males, in *Testosterone: Action, Deficiency, Substitution,* (Eds: E. Nieschlag, H.M. Behre, S. Nieschlag), Springer: Berlin Heidelberg, **1998** pp. 309–335.

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