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RESEARCH ARTICLE

Non-allowed Pharmacologically Active Substances in Physical and Sexual Performance Enhancing Products

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Abstract: ***Background*:** Recently, a large amount of physical and sexual performance enhancing products have started to be freely sold mainly on internet web sites as dietary supplements. However, there a high suspicion that pharmacologically active substance, prohibited in these products, can be present to provide the expected effect.

***Methods*:** A simple and rapid systematic toxicological analysis by gas chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry has been applied after a liquid-liquid extraction at acidic, neutral and alkaline pH with chloroform-isopropanol (9:1 v/v). The assays were validated in the range from 10 mg to 250 mg/g products showing a good linearity for the calibration curves ($r^2 \geq 0.99$). Mean extraction recoveries of analytes from different products were always higher than 90% and intra-assay and inter-assay precision and accuracy were always better than 15%.

***Results*:** The developed method was applied to the analysis of products with a high percentage of sales in websites and smart and sexy shops. In twelve of eighty supplements, anabolic steroids, anti-estrogenic drugs, psychoactive substances and sildenafil and analogs were identified and quantified.

***Conclusion*:** Eventual health hazards caused by the hidden presence of pharmacologically active substances in physical and sexual performance enhancing products are reported.

Keywords: Physical performance enhancing products, sexual performance enhancing products, anabolic steroids, sildenafil, psychoactive substances.

1. INTRODUCTION

Public health is witnessing two parallel new drug problems: new psychoactive substances sold as "legal highs", "research chemicals", "plant food" and "bath salts" and the so–called 'enhancement drugs' mainly commercialized as dietary supplements that presume to improve human attributes and abilities [1, 2]. In both cases, much of the drugs or products can be freely sold and mainly by internet websites or by smart and sexy shops.

In the specific case of physical and sexual performance enhancing products, users look for these products with the aim to improve their bodies and minds-to appear younger, well shaped, smarter and sexually powerful [3].

These types of drugs share a few similarities with recreational or addictive drugs but also attract people who do not necessarily perceive themselves as 'drug users' and are vulnerable to cultural pressures to optimize their bodies and sexual performance.

Since many of these substances are drugs (*e.g.* androgenic steroids, hormones, phosphodiesterase type-5 inhibitors) and require medical prescription to be acquired, an illegal market started due to the increasing popularity of reported products [4]. Manufacturers and retailers are able to evade national and international banning laws with convincing and winning marketing strategies *via* the Internet or by using circuits such as "health", "smart" or "sexy" shops, which can freely sell different purposes "dietary supplements". However, according to the United States Food and Drug Administration (FDA), "a dietary supplement is meant to supplement the diet by increasing the total dietary intake of a substance without being or containing any pharmaceutical drug" [5].

Nevertheless, in case of fraudulence, substances contained in the products are not listed and some pharmacologically active substances could be illegally present in order to effectively produce the described effect [6]. In this way,

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customers are unacquainted of the eventual substantial harms associated with consumption of these products, with consequent threat to public health [7, 8].

Recently, to address the concern of many dietary supplements of doubtful origin and composition, the Italian anti adulteration and safety bureau (Carabinieri per la tutela della salute-NAS) seized several products sold *via* Internet web sites or through venues such smart shops, sexy shops and odd stores. Although the ingredients in the products were often not reported, a tough doubt that the pharmacologically active substances could be included illegally in these formulations drove the bureau to request specific analyses of the seized products.

In this concern, a systematic high throughput analysis of non-allowed pharmacologically active substances has been proposed to be generally applied to dietary supplements, sold through the above described channels. Since the compounds surreptitiously added are often unknown, the first analytical step is the identification of compounds of interest. Thus, a procedure should be applied that can simultaneously screen thousands of relevant toxicants using one single procedure. The analytical strategy usually includes a screening test and confirmatory analysis. When unknown compounds of hypothetically low molecular weight (such as the most common non-allowed pharmacologically active substances) have to be screened and consistently identified, particularly in small amounts and/or in complex matrices, gas and liquid chromatography (GC and LC) coupled to mass spectrometry (MS) or with tandem mass spectrometry (MS/MS) detection are the most commonly used techniques [9, 10].

2. MATERIALS AND METHODS

2.1. Chemicals and Materials

All standard solutions (*e.g*. most common drugs of abuse, androgenic steroids, anti-estrogens, sildenafil and analogs, *etc*.) were supplied by LGC Standards (Milano, Italy). The derivatization reagents, a mixture of N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA), ammonium iodide (NH_4I) and dithioerythritol (DTE) (MSTFA/NH_4I/DTE 1000:2:6 v:v:v) and N,O-bis-trimethylsylyl-trifluoroacetamide (BSTFA) + 1% (trimethylsilyl (TMS) were supplied by Sigma-Aldrich (Milano, Italy). All reagents of analytical grade were obtained from Carlo Erba (Milano, Italy).

2.2. Seized Products

Eighty different dietary supplements and herbal preparations (tablets, capsules, powders, herbal powders) seized by the NAS during 2015 were received at the Istituto Superiore di Sanità to be analyzed for the eventual presence of non-allowed pharmacologically active compounds.

2.3. Instrumentation and Conditions

In case of GC-MS, analytes separation was performed on a fused silica capillary column (HP-5MS, 30m×25mm i.d., film thickness 0.25m; Agilent Technologies, PaloAlto, CA, USA). The oven temperature was programmed at 100°C for 2 min and increased to 290°C at 10°C/min. Split injection mode (15:1) and helium (purity 99%)as carrier gas with a flow rate of 1mL/min were used. The injection port, ion source, quadrupole, and interface temperatures were: 260, 230, 150 and 280°C, respectively. The electron-impact (EI) mass spectra were recorded in total ion monitoring mode (scan range 40–550 m/z) to determine retention times and characteristic mass fragments. The full-scan data files acquired by GC–MS system were screened for the presence of peaks and mass spectra of any declared and non declared substance by Data Analysis Agilent Chem. Station software (Agilent Technologies). A first manual screen of the total ion current (TIC) by an experienced toxicologist was followed by identification of unknown or illegal compounds. Several reference standards of Even in the absence of reference substances, identification could be achieved by computer-assisted comparison of the peak underlying mass spectra with those in the mass spectra library.

The LC-MS/MS analyses were performed using an Alliance HPLC system (Waters, Etten-Leur, The Netherlands) interfaced to a Micromass Quattro micro API triple quadrupole mass spectrometer (Waters) equipped with electrospray ionization (ESI) probe. Chromatographic separation was achieved using a Poroshel 120SB-C18 column (100 x 2.1 mm; 2.7 mm) (Agilent Technologies). Chromatography was carried out with mobile phase A (0,1% formic acid) and B (acetonitrile) at a flow rate of 0.2 mL/min. The gradient elution started at 5% phase B, ramped linearly to 20% phase B in 5 min, and maintained at 20% for 3 min, then directly returned to initial percentage and maintained for 4 min. MS/MS characterization of the compounds under investigation was achieved using the triple quadrupole electrospray ionization (ESI) probe. The analytes dissolved in methanol at a concentration of 10 µg/mL, were infused through an integrated syringe pump into the ESI probe at a rate of 10 mL/min for tuning the mass spectrometer and optimizing the acquisition parameters. The following optimized conditions were used: collision energy at 15 V; capillary voltage at 4.5 kV, cone voltage at 30 V, source temperature at 150°C, and desolvation temperature at 450°C. The cone and desolvation gas flows were set at 60 and 800 L/h, respectively. The collision gas was argon at a collision cell pressure of 0.25 Pa (2.5x10-3mbar).

2.4. Sample Preparation and Extraction

Extraction of compounds under investigation was performed by liquid-liquid extraction after suspending 100 mg of each product in 2 mL 0.1 M phosphate buffer at three different pH: acidic (pH = 2.5), alkaline (pH = 10–12) and neutral (pH = 7.0). The samples were placed in an ultrasonic bath for 15 min, and then each of the three solutions was extracted twice with 3 mL chloroform/isopropanol (9:1, v/v).

After centrifugation, the organic layers (three for each product) were divided into two 3 mL aliquots evaporated to dryness at 40°C under a nitrogen stream. The first dry aliquot was derivatized with 100 µl of N,O-bis-trimethylsilyl-trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) at 70°C for 30 min. A second dry aliquot was dissolved in 100 µl ethyl acetate. A 1 µl amount of both underivatized and derivatized acidic, alkaline and neutral extracts were injected into the GC-MS system.

The third dry aliquot was resuspended with 100 µl LC-MS/MS mobile phase and 1 µl amount injected into the LC-MS/MS system.

For investigation of androgenic steroids, a specific method of extraction was applied as the following: 500 µl of methanol-diluted samples in alkaline buffer were extracted with n-pentane with the addition of 200 µl isoamyl alcohol to break the emulsion formed between the two layers, thus facilitating the separation of the organic layer. This last layer was evaporated to dryness at 40°C under a nitrogen stream, derivatized with 100 µl of a mixture of N-methyl- N-trimethylsilyltrifluoroacetamide (MSTFA), ammonium iodide (NH_4I) and dithioerythritol (DTE) (MSTFA/NH_4I/DTE 1000:2:6 v:v:v), and a 1-µL aliquot was injected into the gas chromatographic system.

Similarly, for investigation of phosphodiesterase type-5 inhibitors, a specific liquid-liquid extraction was performed after suspending 25g of each product in 2 mL 0.1 M phosphate buffer, pH 10–12. The aqueous solutions were placed in an ultrasonic bath for 15 min, and then extracted twice with 2 mL chloroform/isopropanol (9:1, v/v). After centrifugation, the organic layer was evaporated to dryness at 40°C under a nitrogen stream and the dry aliquot was re-dissolved in 100 µl LC-MS/MS mobile phase and 1 µl amount injected into the LC-MS/MS system.

2.5. Validation Procedures

Prior to application to real samples, the method was tested in a 5-day validation protocol. Selectivity, linearity, limits of detection (LOD) and quantification (LOQ), recovery, precision, accuracy, and stability were assayed as previously reported using five replicates of three different quality control samples per day for five different days. Both GC-MS and LC-MS/MS assays were validated in the range from 10 mg to 250 mg/g powder preparations [11].

3. RESULTS AND DISCUSSION

3.1. Chromatography and Validation Results

GC run was completed in 34 min, and afterward initial conditions were restored in 2 min; while LC run was completed in 18 min and then initial conditions restored in 2 min. Blank samples injected after the highest point of the calibration curve did not present any traces of carryover. Nonetheless, an injection of methanol was introduced between each injection of the batch.

The validation protocol satisfactorily met the internationally established acceptance criteria [12] with good determination coefficients (r2 $\geq$0.99) for the calibration curves, mean recoveries always higher than 90% and intra-assay and inter-assay precision and accuracy always better than 15%. No relevant degradation was observed after any of the three freeze/thaw cycles, with differences in the initial concentration of less than 10%.

3.2. Non-allowed Pharmacologically Active Substances in Physical and Sexual Performance Enhancing Products and Eventual Health Hazards

Of the eighty analyzed dietary supplements, twelve were found to contain a pharmacologically active substance, which was non-allowed and not declared in product labels (Tables **1** and **2**).

IN DETAILS

Three products (N. 1, 3 and 4) contained non-allowed anabolic steroids (stanozolol, dehydroepiandrosterone and testosterone) which were surreptitiously added to obtain the enhancement of physical (N. 1 and 4) and sexual (N. 3) performance promised from a seemingly natural product. Anabolic steroids are synthetic compounds analogue to the male sex hormones [13]. They show a remarkable anabolic

Table 1. Retention time and qualifying and quantifying (bold) Ions of non-allowed pharmacologically active substances found in twelve out of eighty dietary supplements under investigation.

Compound	Retention Time (Min)	Qualifying and Quantifying (Bold) Ions
Stanozol*	24.9	472,457,342,**143**
Dimethylamilamine (DMAA)*	2.8	**115**,45,28
Dehydroepiandrosterone (DHEA)*	18.7	**432**, 417,327
Testosterone*	17.1	**432**,417,209,73
Clomiphene*	6.1	465,363,**273**,147
Tamoxifen*	16.9	371,252,72,**58**
Yohimbine*	28.2	**404**,99,56
Sildenafil*	30.0	**404**,381,99,56
2a,3a-epithio-17a-methiyl-5a-androstan-17b-ol. (Epistane)*	5.8	**345**,255,143,73
Hydroxythiohomosildenafil**	11.1	**521**,503,461, 327, 299
Thiohomosildenafil**	12.1	**505**,421,327,299

* GC/MS analysis; ** LC/MS/MS analysis.

Table 2. Non-allowed pharmacologically active substances found in twelve out of eighty dietary supplements under investigation.

Item	Product	Formulation Type and Indication	Substances Declared on the Label	Non-allowed Non Declared Pharmacologically Active Substances Found	Mg/g Product (Mg/Tablet or Capsule or Scoops)
1	BCAA-6000®	Tablets for intensive training (1.4 g)	Vitamin B6, vitamin B12, leucine, isoleucine, valine.	**Stanozolol**	5.4 (7.6)
2	BURNERS®	Thermogenic tablets (1 g)	chromium, caffeine, uva ursi, green tea extract, geranium extract, guggul, coleus forkholii, rhodiola rosea, naringin, l-tyrosine, alpha-lipoic acid, ginger	**Dimethylamilamine (DMAA)**	12.6 (12.6)
3	TONGKAT®	Sexual performance enhancing tablets (1 g)	Tribulus terrestris Tongkat Ali	**Dehydroepiandrosterone (DHEA)**	18.2 (18.2)
4	SUPER PUMP MAX®	Pre-workout training powder (1 scoop corresponding to 16 g)	Vitamin C, Vitamin B3, Vitamin B6, Vitamin B12, calcium, phosphorus, magnesium, sodium, potassium, L-taurine, L-citrulline, L-carnitine, L-Leucine, Creatine Monohydrate, L-Tyrosine, Caffeine, Glucuronolactone	**Testosterone**	0.8 (12.8)
5	LEVO PUMP®	Pre-workout training powder (2 scoops corresponding to 12 g)	L-Tyrosine, L-taurine, arginine, caffeine, synephrine, citric acid, capsaicin	**Clomiphene**	41.5 (498.0)
6	X PAND®	Pre-workout training powder (1 scoop corresponding to 20 g)	L-Tyrosine, L-taurine, leucine, iso-small light caffeine, Vitamin B3, Vitamin B6, Vitamin C, folic acid	**Tamoxifen**	1.1 (22.0)
7	LIPO 6 BLACK®	Fat destroyer tablets (0.9 g)	Citrus aurantium, Coleus Forkohlii, Caffeina, Teobromina	**Yohimbine**	52.3 (47.1)
8	SEX INTENSE®	Sexual performance enhancing capsules (1g)	Xanthoparmelia Scabrosa (lichen); Extract Cnidium Monnieri	**Yohimbine**	35.7 (35.7)
9	GOLDEN ROOT®	Sexual performance enhancing capsules (1g)	Herba Epidemi, Cinnamon, TangShen, Angelica, Radix Ginseng, Tuckahoe, Szechuam lovage rhizome, Liquorice	**Sildenafil** **Yohimbine**	98.2 (98.2) 0.1 (0.1)
10	I LOVE YOU®	Sexual performance enhancing powder (1.2g)	Citrullus lanatus, Lepidium meyeni	**Sildenafil**	47.6 (57.1)
11	FUERZA PURA®	Sexual performance enhancing powder (1.2 g)	Not declared	**Hydroxythiohomosildenafil**	55.6 (66.7)
12	HERO®	Sexual performance enhancing powder (1 g)	Not declared	**Thiohomosildenafil**	84.2 (84.2)

effect that promotes muscles growth and their misuse or abuse is often moved by the aspiration to boost muscles, reduce body fat, and improve physical performance. Abuse among elite and recreational bodybuilders and, more in general, among athletes is recognized [14]. Anabolic steroids are consumed orally or by injection of oily preparations or as gels or creams that are rubbed into the skin.

The amount of non allowed steroids found in the products (7.6 mg stanozolol, 18.2 mg dehydroepiandrosterone and 12.8 mg testosterone) was very similar to that contained in pharmaceutical preparations legally sold in pharmacies or illegally (meaning without medical prescriptions) sold on internet.

Indeed, stanozolol tablets usually contain from 5 to 10 mg active principle for a daily use, dehydroepiandrosterone tablets from 30 to 60 mg to be consumed in two separate doses and for testosterone, the daily dose is from 10 to 30 mg [15]. However, abused doses can be up till 100 times greater than medical doses with the purpose to speed anabolic effects. Furthermore, these substances are often taken in combination mixing oral and/or injectable types of different anabolic steroids. Furthermore, abusers often consume different compounds in cycles of 6 to 12 weeks, progressively increasing doses and then slowly decreasing them to zero. Since steroids have to be medically prescribed and mainly to rebuild tissues that have become weak because of serious injury or illness, a black market has been developed to be illegally sold to people interested in enhancing physical aspect and performance [16]. Although steroids smuggling is an internationally pursued crime, it is easy to buy this kind of substances without a prescription in most countries through illegal sources (*e.g.* Asia, Africa and in South America and Europe) or by internet web sites. According to the annual statistic data of the World Anti-Doping Agency (WADA) [17], anabolic androgenic steroids are still the most frequently abused drugs in sports.

The evolution of the analytical technologies to detect these substances for antidoping controls prompted the athletes to use indirect strategies to evade the disclosure of androgen steroids doping, such as the use of oestrogen receptor modulators (SERMs) that bind competitively to oestrogens hypothalamic and pituitary receptors blocking sex steroid negative feedback [18].

Indeed product N. 5, sold as a preworkout training powder contained the non- declared anti-estrogenic clomiphene and product N. 6 the non-authorized selective estrogen receptor modulator tamoxifen.

Anti-estrogen drugs have been found useful to prevent and treat estrogen-dependent breast cancer, post-menopausal osteoporosis and cardiovascular disease. They may cause an increase of the endogenous production of androgens and testosterone by stimulating gonadotrophines release. Generally, clinical daily dosages of these compounds are in the range of 50 to 100 milligrams [15]. In this concern one scoop (minimum dosage suggested) of product n. 6 contained just 22 mg tamoxifen, but the amount of clomiphene present in two scoops of product n. 5 (the dose suggested in the label for daily consumption) was at least five times higher than the maximum clinical dose.

There are no internationally-established clinical indications for anti-estrogens in men [18, 19], but it is known that athletes may use antiestrogenic compounds to compensate a huge abuse of anabolic androgenic steroids trying to re-establish hormonal homeostasis [18]. For these reasons, the use of these substances has been banned in sports disciplines and competitions by the WADA [20].

Three products (N. 7, 8 and 9) were found to contain a non-declared indole alkaloid, yohimbine, derived from the bark of Pausinystalia yohimbe tree and traditionally used as an aphrodisiac. While there are no clinical studies on the use of yohimbe bark extract [21], the pharmacologically active alkaloid has been available for the treatment of male erectile dysfunction with a dose of up to 30 mg per day, but with only modest efficacy [22]. On the other hand, yohimbine is a potent α-2 antagonist and a weaker α-1 antagonist, which presents both peripheral and central nervous system effects [21]. Significant adverse effects including headaches, hypertension, panic attacks and increased frequency of urination have been reported after its use [22-24]. Unexpectedly, yohimbine is an ingredient present in several dietary supplements marketed for sexual and physical enhancement. For this reason, these supplements can pose significant risks to consumers. In the analyzed products, yohimbine amount went from the negligible quantity of 0.1 mg in product N. 10 with no presumable pharmacological effect to the dose of 35.7 mg in product N. 8 comparable to a maximum daily dose, up to 47.1 mg in product N. 7. It is worth of notice that this latter product is not recommended for sexual enhancement, but as slimming product, so that the fraudulence is double: no sexual improvement is required when product is bought and yohimbine is not indicated in the label. Analyzing data from United States Poison Control Centers, it has bee found that in California alone, yohimbine supplements consumption has been associated to more than 130 hospitalizations between 2000 and 2006 [25]. Because of these risks, many American and European countries have banned extracts of yohimbe from supplements and foods [23].

There is growing evidence that the erectogenic effect of yohimbine can be increased by simultaneous administration of phosphodiesterase type-5 inhibitors that augment the release and/or action of nitric oxide in the corpus cavernosum, such as sildenafil and analogues.

Indeed, product N. 9, seized in a sexy shop and sold as a herbal natural "viagra", in reality contained sildenafil and yohimbine, both undeclared in the product label. In addition to that, another sexual performance enhancing product (N. 10), also seized in a sexy shop and sold as herbal dietary supplement contained sildenafil alone, while other two products (N. 11 and 12) from sexy shops sold as dietary supplements without any label contained two sildenafil thio-analogs: hydroxythiohomosildenafil and thiohomosildenafil, respectively. Sildenafil and other inhibitors of phosphodiesterase type 5, are used for the treatment of male erectile dysfunction by enhancing relaxation of the penile corpus cavernosum [26]. These drugs should be used under strict medical control since eventual overdose might cause a series of side effects. For example, rhabdomyolysis and subjective visual perception changes [27] headache, dyspepsia and back pain [28].

Sildenafil is administrated as oral therapy for erectile dysfunction and the usual recommended dose is 50 mg to be taken one hour before sexual activity [29].

Similarly to the above reported surreptitious presence of non allowed active substances in doses compatible with those contained in pharmaceutical preparations, product N.11 contains an hidden concentration of sildenafil matched with the recommended dose, but that in product N. 10 is the maximum dose (*e.g.* 100 mg) for treating medical conditions and in any case since these products are considered "herbal

products" consumers can freely increase the dose as much as they desire.

Differently from sildenafil, which is a registered pharmaceutical product needing a medical prescription to be sold and purchased, the two analogues present in products N.11 and 12 do not result registered as drug substances by the European Medicine Agency and United States Food and Drug Administration (FDA), but were found in pharmaceutical preparations of phosphodiesterase type-5 inhibitors as adulterants [30].

Because these two analogues do not result registered as drug substances, they result as experimental drugs in essence and have no known efficacy or safety profile.

If an equivalent pharmacological action is hypothesized for the two sildenafil analogs, the dosages present in the two sexual enhancers let suppose the possibility of serious adverse effects on cardiovascular function such as arterial systemic blood pressure reduction, headaches, facial flushing, dyspepsia, visual disturbances and back pain [31].

Product N.2, sold as "thermogenic accelerating both metabolism and fat burning" natural dietary supplement actually contained the neurological stimulant methylhexanamine, also known as 1,3-dimethylamylamine (DMAA). Originally developed and sold as a nasal decongestant it was withdrawn from the market in 1983 for its side effects and recently reappeared as a neurological stimulant and party pill. A typical starting dose of 1,3-DMAA to reach stimulant effects is in the 10-20 mg range, like that present in the thermogenic tablets [32]. Since 2006 methylhexanamine has been widely commercialized as a stimulant or energy-boosting dietary supplement, but a number of adverse events have been reported after the use of DMAA-containing supplements including tachycardia, nausea and vomiting, systolic blood pressure increase, especially when co-administered with caffeine and at least 5 deaths have been associated with those dietary supplements [32-35]. It is banned by many sports authorities and governmental agencies and included in WADA prohibited substances and methods list since 2012 [20].

CONCLUSION

The presented results are in agreement with other international studies on black market and internet web sites sales of nutritional supplements faked with doping substances or pharmaceuticals [36-38].

Internet web sites and smart and sexy shops are the principal distribution sources of physical and sexual performance enhancing products. Although there are no official reported statistics, narrative information (often found on websites of bodybuilders, trainers or sex enhancers users) show that:

- physical performance enhancers are commonly consumed by recreational athletes principally from 20 to 45 years of age belonging to sport disciplines (*e.g*. bodybuilding, cycling, swimming) in which it can be useful to increase physical performance with no medical advice or medical prescriptions;

Sexual performance enhancers are used by men in the huge age ranges from 20 to 60 years who look for intense staying power for longer sexual sessions and extreme pleasure.

Both users are likely attracted by the promise of natural herbal products without any pharmacologically active drug. On the contrary, some products are counterfeit with substances different from the ones declared on the labels or they do not contain any of the purported ingredients. The aim is to successfully obtain the promised effect, but using pharmacologically active substances which can cause serious health hazards.

The issue of non-allowed pharmacologically active substances in physical and sexual performance enhancing products is relatively new and the emergency of the problem is comes with the speed of web marketing of non-controlled products. Systematic toxicological analysis by GC-MS and LC-MS/MS, as here presented and in agreement with other studies [36-38], can be a strategic tool in assessing the presence of non-allowed pharmacologically active substances in dietary supplements which can be used by all control laboratories from police forces, customs, and public health laboratories.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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