

Pharmaceutical doses of the banned stimulant oxilofrine found in dietary supplements sold in the USA

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Oxilofrine (4-[1-hydroxy-2-(methylamino)propyl]phenol) is a pharmaceutical stimulant prescribed in dosages of 16 to 40 mg to stimulate the heart and increase blood pressure. It has never been approved for use in the USA as a prescription drug or as a dietary supplement. Several athletes, however, have been banned from sport for testing positive for oxilofrine and have claimed that they inadvertently consumed oxilofrine in sports supplements. Consumption of supplements containing oxilofrine may also pose serious health risks. For example, one brand of supplements containing oxilofrine has been linked to serious adverse events including vomiting, agitation, and cardiac arrest. We designed our study to determine the presence and quantity of oxilofrine in dietary supplements sold in the USA. A validated ultra-high performance liquid chromatography-quadrupole time of flight-mass spectrometry method was developed for the identification and quantification of oxilofrine. The separation was achieved using a reversed phase column, mass spectrometry detection, and a water/acetonitrile gradient as the mobile phase. The presence of oxilofrine was confirmed using a reference standard. We analyzed 27 brands of supplements labelled as containing a synonym of oxilofrine ('methysynephine') and found that oxilofrine was present in 14 different brands (52%) at dosages ranging from 0.0003 to 75 mg per individual serving. Of the supplements containing oxilofrine, 43% (6/14) contained pharmaceutical or greater dosages of oxilofrine. Following instructions on the label, consumers could ingest as much as 250 mg of oxilofrine per day. The drug oxilofrine was found in pharmacological and greater dosages in supplements labelled as containing methysynephine.

Additional supporting information may be found in the online version of this article at the publisher's web site.

Keywords: oxilofrine; methysynephine; hydroxyephedrine; dietary supplements; UHPLC-QToF-MS

Introduction

Oxilofrine, 4-[1-hydroxy-2-(methylamino)propyl]phenol (also known as methysynephine, *p*-hydroxyephedrine, oxyephedrine, 4-HMP and suprifin), is a pharmaceutical drug developed in Europe in the 1930s to stimulate the heart, increase blood pressure and improve oxygen exchange.^[1,2] In countries where oxilofrine has been used to treat medical conditions, it has been formulated in 16 mg, 20 mg, 32 mg, and 40 mg dosages.^[3,4] Oxilofrine has cardiac stimulatory effects similar to ephedrine, one of the most active alkaloids in *Ephedra* species (Figure 1).^[1,2]

In the USA, oxilofrine has never been approved for use as a medication or as a dietary supplement. Nevertheless, since 2009, several professional athletes have been banned from sports for testing positive for oxilofrine on doping tests and claimed that they were exposed to oxilofrine when consuming sports supplements sold in the USA.^[5–8] Oxilofrine is prohibited from use in sport by the World Anti-Doping Agency (WADA),^[9] and recent research has described several analytical methods for detecting oxilofrine in biological matrices.^[10–13] Görgens *et al.* demonstrated that after a volunteer consumed one capsule of a dietary supplement (Blackbombs, Dorian Yates Nutrition Inc.), both oxilofrine and its metabolite were detectable in urine.^[10]

In addition to the risks posed to athletes' careers, oxilofrine might pose health risks to consumers. Recently, one of us, as part of an investigation of 26 adverse events in the Netherlands, found

oxilofrine in supplements (Dexaprine, iForce Nutrition) linked to serious adverse events including nausea, vomiting, agitation, tachycardia, chest pain, and cardiac arrest.^[14]

The US law regulating supplements does not permit dietary supplements to contain unapproved pharmaceutical drugs such as oxilofrine.^[15] Dietary supplements may contain certain extracts of botanical ingredients, but, to our knowledge, oxilofrine has never been identified in, or extracted from, any plant. However, a synonym for oxilofrine, 'methysynephine', may mislead consumers and regulators. Synephine, a constituent of bitter orange and other citrus species, is a legal supplement ingredient. When reading a label, consumers may confuse the term 'methysynephine' with 'synephine'.

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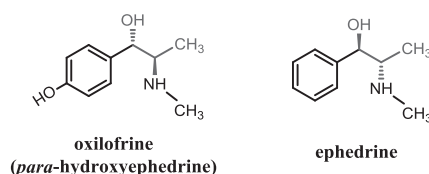


Figure 1. Chemical structures of oxilofrine and ephedrine. The compounds depicted represent the chirality of the registered drug oxilofrine and ephedrine from *Ephedra sinica*.

It is not currently known if consumers are exposed to pharmacological doses of oxilofrine from dietary supplements purchased in the USA. Therefore, we designed our study to determine the presence and quantity of oxilofrine in dietary supplements labelled as containing methylsynephrine sold in the USA.

Experimental

Materials

Supplements were identified by (1) searching the National Institute of Health's Dietary Supplement Label Database^[16] for supplements listing methylsynephrine or oxilofrine on their labels, and (2) querying Google search engine for 'diet supplement containing methylsynephrine' or 'fat burning containing methylsynephrine' and filtering the results with the key words 'methylsynephrine' or 'methylsynephrine HCl'. (The search was also repeated with methylsynephrine written as two separate words, i.e., methyl synephrine, as well as by replacing 'methylsynephrine' with 'oxilofrine'.) Supplements found in this initial screen were then individually reviewed to determine if methylsynephrine, methyl synephrine, or oxilofrine were listed on the supplement label posted on the distributor's website. In total, 57 brands of supplements were identified listing methylsynephrine or methyl synephrine on the label (none listed oxilofrine on the label). We attempted to purchase these 57 supplements from online retailers in August 2015. Twenty-four brands were unavailable (23 brands were listed as unavailable at the time of purchase; 1 brand had been discontinued). Thirty-three brands of supplements were purchased. Labels of the 33 brands were inspected for the presence of methylsynephrine or methyl synephrine as a listed ingredient. None of the synonyms (methylsynephrine, methyl synephrine, or oxilofrine) were listed on 6 brands. These 6 brands were excluded from the analysis. We analyzed the 27 brands of supplements that listed methylsynephrine or methyl synephrine on the label. (The 27 brands of supplements included two brands that listed 'methyl synaphrine' as an ingredient which we interpreted as an inadvertent misspelling of 'methylsynephrine'.)

The actual amount of oxilofrine a consumer would ingest in a single day following the labelled directions was calculated by multiplying the quantity of oxilofrine in the recommended serving size by the maximum number of recommended servings per day.

The standard compound, oxilofrine (Figure 1), was purchased from Chromadex (>98% purity) (Santa Ana, CA, USA).

Instrumentation

An Agilent Series 1290 ultra-high performance liquid chromatographic system (UHPLC) coupled to a quadrupole time of flight-mass spectrometry (QToF-MS) (Model #G6530A, Agilent Technologies, Santa Clara, CA, USA) was used for this analysis. Separation was achieved on a Zorbax SB-C8 (2.0x100 mm, 1.8 μ m) column. The flow rate was 0.20 mL/min and the column

temperature was set at 35 °C. Two microliters of sample were injected. The mobile phase consisted of water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B), with the following gradient elution: 0 min, 2% B to 25% B over 5 min, and in next 2 min to 100% B. Each run was followed by a 3.5 min wash with 100% B and an equilibration period of 5 min with 98% A/2% B.

The QToF-MS was equipped with an electrospray ionization (ESI) source with Jet Stream technology with the following parameters: drying gas (N_2) flow rate, 9.0 L/min; drying gas temperature, 250 °C; nebulizer, 35 psig; sheath gas temperature, 325 °C; sheath gas flow, 11 L/min; capillary, 3500 V; skimmer, 65 V; Oct RF V, 750 V; fragmenter voltage, 100 V. All operations, acquisition and analysis of data were controlled by Agilent MassHunter Acquisition Software Ver. A.05.00 and processed with MassHunter Qualitative Analysis software Ver. B.05.00. Each sample was analyzed in positive full scan mode in the range of m/z 100–1000. Accurate mass measurements were obtained by internal standard mass calibration using reference masses at m/z 121.0509 (protonated purine) and 922.0098 [protonated hexakis (1H, 1H, 3H-tetrafluoropropoxy) phosphazine or HP-921], introduced into the ESI source via a T-junction using an Agilent Series 1200 isocratic pump (Agilent Technologies, Santa Clara, CA, USA) and a 100:1 splitter set at a flow rate of 20 μ L/min. Ion chromatograms were extracted from the full scan data at 182.115 with a ± 5 millimass unit window for quantitation of oxilofrine. The peak of oxilofrine in all samples was identified by comparing the MS spectrum obtained with the standard, which showed the protonated molecule $[M+H]^+$ and a characteristic fragment ion $[M+H-H_2O]^+$.

Preparation of standard solutions

A stock solution of the standard compound oxilofrine was prepared at a concentration of 1.0 mg/mL in methanol. The calibration curve was prepared at seven different concentration levels. The range of the calibration curve was 5.0–10 000 ng/mL. Quantitation of oxilofrine in the present study was performed using the external standard method.

Sample preparation

For capsules, five samples were weighed, opened, and the contents mixed and triturated with a mortar and pestle. For tablets, five tablets were weighed and then pulverized with a mortar and pestle. One hundred milligrams were then weighed and sonicated in 2.0 mL of methanol:water (80:20) for 30 min followed by centrifugation for 30 min at 959 \times g. The supernatant was transferred to a 10 mL volumetric flask. The procedure was repeated four more times with 2.0 mL methanol:water (80:20) combining the respective supernatants. The final volume was adjusted to 10 mL with methanol:water (80:20) and mixed thoroughly. An adequate volume (ca. 2 mL) was passed through a 0.45 μ m polytetrafluoroethylene membrane filter prior to injection, discarding the first 1.0 mL and collecting the remaining volume in a sample vial. Dilutions were performed as required using methanol:water (80:20). If oxilofrine was not detected in the 100 mg sample mass, the extraction was repeated using the average capsule/tablet mass for the analysis.

Method validation

Linearity and Range, LOD and LOQ

The seven-point calibration curve for oxilofrine showed a linear correlation between concentration and peak area. Calibration data indicated linearity ($r^2 > 0.998$) of the detector response for

Table 1. Information provided on the label and actual oxilofrine content in 27 brands of dietary supplements

| Code # | Product name (manufacturer) | Label description of methylsynephrine [labelled quantity of methylsynephrine/serving] | Claim provided on label | Serving size listed on label | Maximum recommended daily dose listed on label | Number of other ingredients listed on the label* | Average weight per tablet or capsule (mg) | Measured amount (mg/serving size) | Measured amount (mg/maximum recommended daily dose)** |
|---------|---|---|--|------------------------------|--|--|---|-----------------------------------|---|
| DS17364 | HyperDrive® 3.0 (ALR industries) | methylsynephrine | hardcore diet and energy aid | 1 capsule | 4 servings | 7 | 751.8 | 61 | 250 |
| DS17381 | Shredder (TBN Total Body Nutrition) | methyl synephrine | a killer ABS formula | 1 capsule | 3 servings | 11 | 735.1 | 75 | 220 |
| DS17360 | Fastin® (Hi-Tech Pharmaceuticals) | methylsynephrine HCl | pharmaceutical-grade weight loss aid for optimal diet and energy fat burning | 1 tablet | 4 servings | 7 | 830.4 | 48 | 190 |
| DS17367 | Lean Pills™ (Line One Nutrition) | methyl synephrine | | 1 capsule | 4 servings | 6 | 501.9 | 25 | 98 |
| DS17383 | Tummy Tuck (TBN Total Body Nutrition) | methyl synephrine | a killer ABS formula | 1 capsule | 3 servings | 11 | 702.6 | 15 | 44 |
| DS17355 | Methyl Drive™ 2.0 (ANS Advanced Nutrition Systems) | methylsynephrine | powerful thermogenic rush | 1 capsule | 1 serving | 6 | 712.7 | 35 | 35 |
| DS17357 | Drop Factor™ (MTS Nutrition) | methylsynephrine HCl | thermogenic powerhouse | 2 capsules | 2 servings | 14 | 671.7 | 16 | 33 |
| DS17359 | Exile (AmericanMuscle Sports Nutrition Company) | methyl synephrine | euphoric weight loss | 1 capsule | 3 servings | 20 | 750.4 | 8.4 | 25 |
| DS17356 | China White 25 Ephedra (Cloma Pharma Laboratories) | methylsynephrine | energy, thermogenesis, alertness, fat burning | 1 tablet | 2 servings | 12 | 1124.5 | 11 | 23 |
| DS17376 | Phenadrine™ (APS) | <i>Acacia rigidula</i> extract leaves (methylsynephrine) | world strongest diet and energy aid | 1 capsule | 3 servings | 5 | 671.6 | 3.8 | 11 |
| DS17363 | Hypercor™ (Kat-a-lyst Nutraceuticals) | methyl synephrine HCl | metabolic formula | 3 capsules | 2 servings | 30 | 772.5 | 0.60 | 1.2 |
| DS17372 | MethylDrene™ 25 Ephedra Elite stack (Cloma Pharma Laboratories) | methylsynephrine [20 mcg] | super intense-hardcore version | 1 capsule | 2 servings | 15 | 735.8 | 0.025 | 0.050 |
| DS17373 | Miami Lean (Skyline Nutrition) | methylsynephrine HCl [10 mg] | fat burner | 1 capsule | 2 servings | 29 | 754.1 | 0.010 | 0.020 |
| DS17379 | Eliminator X (Rok Hard Body Sports Nutrition) | methyl synephrine | maximum strength formula | 2 capsules | 1 serving | 9 | 521.2 | 0.0003 | 0.0003 |
| DS17385 | Ultimate Burn (Schwartz Laboratories) | methylsynephrine HCl [25 mg] | the ultimate thermogenic fat burner | 2 capsules | 3 servings | 19 | 670.7 | ND | ND |
| DS17369 | Matrix-Xtreme (One Body Nutrition) | methyl-synephrine HCl [8 mg] | fat loss formula | 3 capsules | 1 serving | 10 | 708.6 | ND | ND |
| DS17358 | Ephedra X (Xcel sports nutrition) | methyl synephrine | advanced weight loss formula | 1 capsule | 2 servings | 6 | 781.9 | ND | ND |
| DS17362 | FireStorm™ (GE Pharma) | methyl synephrine | hardcore thermogenic | 1 capsule | 2 servings | 11 | 485.2 | ND | ND |
| DS17365 | | methyl synephrine | thermogenic fat burner | 1 capsule | 2 servings | 9 | 575.5 | ND | ND |

Table 1. (Continued)

| Code # | Product name (manufacturer) | Label description of methylsynephrine [labelled quantity of methylsynephrine/ serving] | Claim provided on label | Serving size listed on label | Maximum recommended daily dose listed on label | Number of other ingredients listed on the label* | Average weight per tablet or capsule (mg) | Measured amount (mg/serving size) | Measured amount (mg/maximum recommended daily dose)** |
|---------|---|--|--|---------------------------------|---|--|--|---|--|
| DS17366 | Incineration (EN Enraged Nutrition) Burn ² (M4 Nutrition Companies) | methyl synephrine HCl | fat loss and detox complex | 2 capsules | 1 serving | 14 | 651.5 | ND | ND |
| DS17371 | MethylDrene [™] 25 Ephedra ECA stack with cayenne and ginger (Cloma Pharma Laboratories) | methylsynephrine | extra-potent fat burner | 1 capsule | 2 servings | 16 | 700.9 | ND | ND |
| DS17378 | Burn [™] (Pinnacle Sports Nutrition) | methyl synephrine | fat burning formula | 2 capsules | 2 servings | 25 | 658.4 | ND | ND |
| DS17380 | RX 90 (MuscleSport Nutrition) | methyl synephrine | rapid fat loss accelerator | 2 capsules | 1 serving | 9 | 700.9 | ND | ND |
| DS17382 | ThermoBombs (NutraClipse Premier Sports Supplements) | methylsynephrine | accelerates metabolism (body recomposition agent) | 1 capsule | 2 servings | 11 | 695.9 | ND | ND |
| DS17384 | Nciner 8 (Twisted Genetix) | methyl synephrine | advanced fat burner | 1 capsule | 2 servings | 9 | 600.3 | ND | ND |
| DS17386 | Ultra Fire (Live 4 Life Health) | methyl synephrine | thermogenic metabolic booster | 1 capsule | 2 servings | 8 | 538.1 | ND | ND |
| DS17387 | Burn-FX (Tokkyo Nutrition) | methylsynephrine | weight loss | 2 capsules | 2 servings | 8 | 771.2 | ND | ND |

ND, not detected.

* Figure S2 has photographs of labelled ingredients.

** The calculated amount was rounded to two significant figures.

oxilofrine from 500 to 10 000 ng/mL and 5 to 500 ng/mL. The limit of detection (LOD) and limit of quantification (LOQ) were determined by injecting a series of dilute solutions with known concentrations for each standard. The LOD and LOQ were defined as the signal-to-noise ratio equal to 3 and 10, respectively. The LOD and LOQ in solution were found to be 0.3 ng/mL and 1 ng/mL, respectively.

Solution stability

Standard and sample solutions were prepared, stored at room temperature and analyzed over a 24 hour period. No significant changes were observed in the concentration of oxilofrine during this time frame.

System suitability

System suitability tests are used to ensure reproducibility of the instrument. The test was carried out by injecting 2 μ L of an oxilofrine standard (500 ng/mL) at least seven times. The relative standard deviation (RSD) was found to be 0.3%.

Precision

Intra- and inter-day variation of the analysis was performed on three products. Analysis was executed in duplicate at three concentrations (500, 2500, and 5000 ng/mL) on three different days. The intra-day RSD for the replicates were between 0.02 and 0.1% and inter-day RSD for the replicates were between 0.01 and 0.05%.

Accuracy

The accuracy of the method was determined by fortifying three products with a known amount of oxilofrine. These samples were extracted as discussed under the section on Sample preparation, dried and then fortified with known amounts of the standard compound at three different concentrations, extracted again, and analyzed. The accuracy of the assay method was evaluated in duplicate at three concentration levels 500, 2500, and 5000 ng/mL. The percentage recovery of these samples ranged from 97 to 103%.

Results and discussion

Of the 27 supplement brands tested, 14 (52%) contained oxilofrine (Table 1). Figure 2 shows an example of the extracted ion chromatograms (EIC) obtained for dietary supplements as compared to that of an oxilofrine standard at 0.5 μ g/mL. Some of the EIC exhibited peaks with a different retention time compared to oxilofrine (Figure S1 in the Supporting Information). These were determined not to be diastereomers or positional isomers of oxilofrine due to differing accurate mass (Figure S2). For those supplements containing oxilofrine, the amount that would be consumed in a single serving of the supplement ranged from 0.0003 to 75 mg, and the amount of oxilofrine consumed in a day following the labelled directions ranged from 0.0003 to 250 mg (Table 1). In Germany, where oxilofrine was developed as a pharmaceutical drug used to treat low blood pressure, the manufacturer produced 16 mg to 40 mg dosages of oxilofrine.^[3,4,17] The usual adult dose is 32 mg, and the recommended pediatric dosage ranges from 8 mg to 24 mg.^[17] Of the supplements we tested that contained oxilofrine, 43% (6/14) contained adult pharmaceutical or greater dosages of oxilofrine. One supplement contained more than twice the usual adult dose and three times the usual adolescent dose.

One supplement brand (Phenadrine, APS) listed methylsynephrine as an extract of a shrub, *Acacia rigidula*. *Acacia rigidula* has been previously listed on supplement labels as the source of another synthetic stimulant, β -methylphenylethylamine (BMPEA); however, we are not aware of a single study in which oxilofrine or BMPEA has been detected in the *Acacia rigidula* plant.^[18,19]

In countries in which oxilofrine is sold as a pharmaceutical drug, the label provides a specific quantity of the drug. In the USA, dietary supplements are not required to provide specific quantities of individual ingredients. In the current study, only 15% of the supplements (4/27) listed a specific quantity of methylsynephrine on the label, and 75% (3/4) of these brands were inaccurately labelled (Table 1).

Since it was first synthesized in the early 1930s, oxilofrine has been studied in animals and humans. Dozens of studies in a variety of animal models including dogs,^[20–24] cats,^[22,25,26] rabbits,^[23,26] rats^[26,27] and guinea pigs^[28] have been consistent in demonstrating sympathomimetic properties of oxilofrine similar to ephedrine. The US Food and Drug Administration (FDA) banned ephedrine from supplements in 2004 due to serious side effects.^[29,30] Since then, many synthetic drugs, such as oxilofrine, have been introduced into supplements in an effort to replace the stimulant effects of ephedra.^[14,31–35]

Beyond animal studies, oxilofrine has been developed as a drug for human use. While there have been no large, blinded randomized controlled trials of oxilofrine that would provide sufficient data of efficacy and safety for the drug to be considered for approval by the FDA, there have been smaller studies of oxilofrine in humans^[1,2,36–57] (a summary of key human studies is provided in Table 2). Although the human studies varied widely in the quality of their design, the route and dose of oxilofrine, and the health of the subjects, the clinical studies published since the 1930s point to several key characteristics of oxilofrine. Oxilofrine has been found (1) to act predominantly as a β_1 agonist increasing the speed and force of heart muscle contraction (inotropic effects), specifically, increasing left ventricular ejection fraction and stroke volume^[2,37,39,40]; (2) to increase blood pressure^[1,38–41]; (3) to have variable effects on heart rate^[1,2,38,41]; and (4) to potentially increase oxygen uptake by the lungs.^[1,2,41] Given these pharmacological properties in humans, oxilofrine has been used in a variety of medications in countries outside the USA to treat low blood pressure,^[38] to manage asthma exacerbations,^[58] to suppress coughing,^[59] and to enhance cardiac function during anesthesia.^[40]

The potential adverse health effects of inadvertently consuming up to 250 mg of oxilofrine per day, as would be possible with supplements in our study, are entirely unknown. Human studies using lower dosages suggest that the increased demand on the heart muscles might lead to palpitations, arrhythmias, increased blood pressure, or other adverse effects.^[50,54] In the investigation into adverse events from supplements in the Netherlands, oxilofrine was found at doses of 1 to 5 mg in combination with several other stimulants.^[14] It is not known which stimulant, or combination of stimulants, might have been responsible for the reported adverse events including tachycardia, chest pain, and cardiac arrest. In the USA, concern has been raised about the safety of weight loss and sports supplements.^[60,61] Of particular concern with respect to the safety of oxilofrine in sports supplements is that more than one million youth athletes in the USA use sports supplements.^[62,63] Their relatively small developing bodies might be particularly vulnerable to adult dosages of oxilofrine. Usual oxilofrine doses for children and adolescents range from 8 to 24 mg;^[17] therefore, adolescents using supplements analyzed in our study might

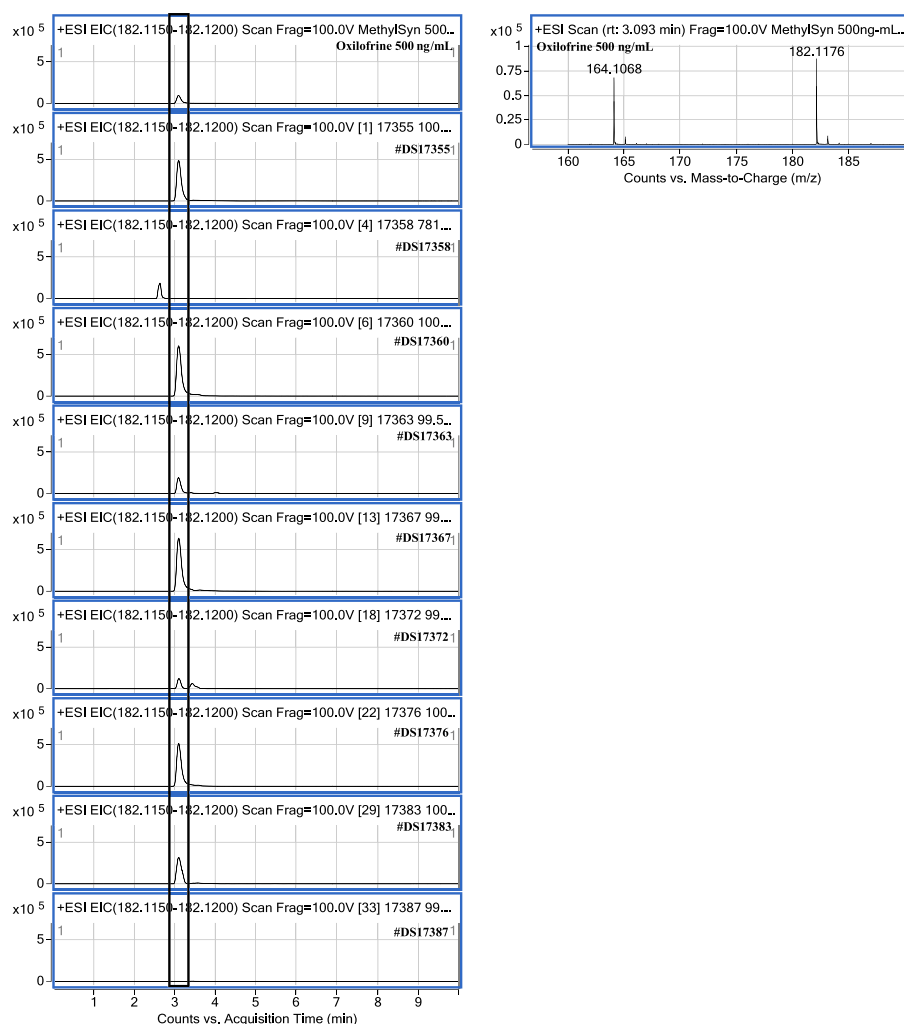


Figure 2. Typical extracted ion chromatograms and mass spectrum of oxilofrine from dietary supplements. (Figure S1 gives extracted ion chromatograms of all 27 samples).

Table 2. Key clinical studies of oxilofrine. Human studies of oxilofrine were not included if no specific dosage of oxilofrine was provided^[42–46,56]; if hemodynamic measurements were not reported^[36,47]; or if oxilofrine was only given in combination with another drug such as adenosine.^[48–57]

| Source | Country (Language) | No. of subjects | Subjects: volunteers or patients | Study design | Intervention | BP | HR | Cardiac output | Pulmonary function |
|--|--------------------|-----------------|----------------------------------|-----------------------------|---|----|----|----------------|--------------------|
| Liljestrand et al, ^[1] 1933 | Sweden (German) | 1 | Unknown | Tolerability study | oxilofrine 7 mg, 10 mg & 14 mg SC | ↑ | ↓ | n/a | ↑ |
| Oremus, ^[2] 1938 | France (French) | 4 | Volunteers | Tolerability study | oxilofrine 5 mg SC x 1 | — | — | ↑ | ↑ |
| Dominiak et al, ^[37] 1992 | Germany (English) | 15 | Volunteers | Tolerability study | oxilofrine 20 mg of IV x 1 | — | — | ↑ | n/a |
| Pohl et al, ^[38] 1991 | Germany (German) | 60 | Patients with hypotension | Randomized controlled trial | oxilofrine 32 mg orally three times a day x 3 weeks | ↑ | — | n/a | n/a |
| Kauert et al, ^[39] 1988 | Germany (English) | 8 | Volunteers | Pharmacokinetic study | oxilofrine 120 mg PO x 1 | ↑ | — | ↑ | n/a |
| Kemmotsu et al, ^[40] 1979 | Japan (Japanese) | 20 | Anesthetized patients | Randomized controlled trial | oxilofrine 10 mg IV | ↑ | — | ↑ | — |
| Matthes et al, ^[41] 1944 | Germany (German) | 6 | Volunteers | Tolerability study | oxilofrine 20 mg IV x 1 | ↑ | ↑ | n/a | ↑ |

↑ increase, ↓ decrease, — no change, n/a, not assessed, SQ subcutaneous, IV intravenous, BP blood pressure, HR heart rate.

* (Studies of Carnigen® should be interpreted with care as this proprietary brand by Albert-Roussel Pharma was a combination product of oxilofrine and adenosine from at least the 1950s until 1983.^[54,55,57,66] In 1984, Carnigen® was reformulated as only oxilofrine,^[4,38] Some studies do not describe the precise formulation of Carnigen® used^[48–53]).

consume more than 3 times the highest pediatric dose of oxilofrine. The health effects of sports supplements containing oxilofrine have never been studied in children or adolescents, so the health risks when consumed alone, or when combined with exercise, are entirely unknown.

Our study has several limitations. We analyzed only one sample of each supplement, and the presence and quantity of oxilofrine might vary in different samples of the same brand.^[64]

Secondly, we determined only the identity and quantity of oxilofrine. It is likely that a wide range of other active ingredients are also present in these supplements, potentially increasing the risks these supplements pose to consumers.^[65] In fact, all supplements we tested listed many additional ingredients on the label (Figure S2 for ingredients listed on the labels). Based on preliminary analyses, there were peaks in the total ion chromatograms that might suggest the presence of yohimbine, 1,3-dimethylamylamine, caffeine, synephrine, phenethylamines, isopropylorsynephrine, and other pharmacologically active ingredients listed on the labels. Further information about the safety of these brands of supplements would be gained by identifying all major ingredients as well as substances present but not included on the label. Such an analysis was beyond the scope of the current study in which we focused our attention on oxilofrine as an individual ingredient rather than focusing on the safety of any specific brand of supplement.

Lastly, our study only tested supplements labelled as containing 'methylsynephrine' or 'methyl synephrine'; therefore, it is possible that other brands of supplements on sale in the USA may also contain oxilofrine without listing methylsynephrine on the label.

Despite these limitations, our study demonstrates that oxilofrine is currently present in pharmaceutical and greater-than-pharmaceutical dosages in dietary supplements sold in the USA.

Conclusions

To our knowledge, this is the first study to systematically test for oxilofrine in dietary supplements sold in the USA. We analyzed 27 brands of supplements labelled as containing methylsynephrine and found that 14 of these brands (52%) contained oxilofrine in dosages from 0.0003 to 75 mg per serving. Of the supplements containing oxilofrine, 43% (6/14) contained pharmaceutical or greater dosages of oxilofrine. Following instructions on the label, consumers could ingest up to 250 mg of oxilofrine per day.

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Conflict of interests

At the time of submission, Dr. Cohen was a defendant in a civil suit brought by one of the product manufacturers alleging defamation and trade practices claims based on a publication discussing BMPEA in that manufacturer's products. Dr. Cohen denies the claims. The claims have since been dismissed by the court for failure of jurisdiction. Ikhlal Khan reported receiving grants from the US

Food and Drug Administration, the National Institute of Health and the US Department of Agriculture. Mr. Khan is also the coordinator of the International Conference on the Science of Botanicals which receives support for conference-related expenses from multiple supplement-related companies. John Travis is an employee of NSF International. Some of NSF International's clients are dietary supplement manufacturers. Bastiaan Venhuis, Bharathi Avula and Yan-Hong Wang do not report any conflicts of interest.

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Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web site.

Figure S1. Extracted ion chromatograms and total ion chromatograms of oxilofrine and 27 supplements.

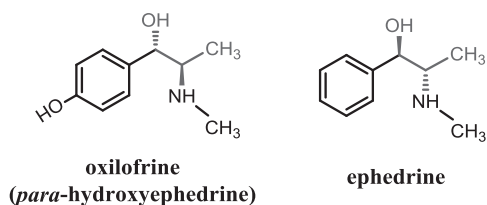
Figure S2. Mass spectra of oxilofrine.

Figure S3. Label information.

Short communication

Pharmaceutical doses of the banned stimulant oxilofrine found in dietary supplements sold in the USA

Pieter A. Cohen, Bharathi Avula, Bastiaan Venhuis, John C. Travis, Yan-Hong Wang and Ikhlas A. Khan



Oxilofrine is a drug with cardiac effects similar to ephedrine. We analyzed 27 brands of supplements labelled as containing a synonym of oxilofrine ('methysynephrine') and found that 14 of 27 brands (52%) contained oxilofrine in dosages from 0.0003 to 75 mg per serving. Of the supplements containing oxilofrine, 43% (6/14) contained pharmaceutical or greater dosages of oxilofrine. Following instructions on the label, consumers could ingest up to 250 mg of oxilofrine per day.