# Detection of a Tadalafil Analogue as an Adulterant in a Dietary Supplement for Erectile Dysfunction

Jeronimo Ulloa, MSc,* Luis Sambrotta, PhD,† Flavia Redko, PhD,* Osvaldo Nestor Mazza, MD, PhD,‡ Gustavo Garrido, MD, PhD,‡ Edgardo F. Becher, MD, PhD,‡ and Liliana Muschietti, PhD*

*Cátedra de Farmacognosia, Facultad de Farmacia y Bioquímica (UBA), IQUIMEFA (UBA-CONICET), Buenos Aires, Argentina; †LANAIS (UBA-CONICET), Buenos Aires, Argentina; ‡Cátedra de Urología, Hospital de Clínicas "José de San Martin," Buenos Aires, Argentina

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**ABSTRACT**

***Introduction.*** Several cases of adulteration of dietary supplements with tadalafil, sildenafil, and vardenafil, or their unapproved analogues have been reported worldwide. Mainly, the presence of the latter represents a serious health risk to consumers as their efficacy and toxic effects have not been assessed and may result in unpredictable adverse effects.

***Aim.*** To investigate the suspected adulteration with synthetic phosphodiesterase type 5 (PDE-5) inhibitors in a dietary supplement marketed in Argentina for the treatment of erectile dysfunction (ED).

***Methods.*** The content of the capsules of the dietary supplement (sample A) was analyzed by thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC) diode-array detection. From the organic extract of sample A, a major compound was purified by column chromatography (CC). The isolated compound was identified by proton nuclear magnetic resonance (1H NMR) and carbon NMR (13C NMR), heteronuclear single quantum coherence, distortionless enhancement by polarization transfer (DEPT 135), electrospray ionization mass spectrometry, and ultraviolet, and infrared (Fourier transform infrared spectroscopy) spectroscopy.

***Main Outcome Measure.*** Proof of adulteration of herbal products with synthetic PDE-5 inhibitors.

***Results.*** By TLC and HPLC analysis, a major compound was detected in sample A organic extract. The purification of this extract by CC led to the isolation of a pure compound which was identified according to its spectral data as (6R,12aR)-2-amino-6-(1,3-benzodioxol-5-yl)-2,3,6,7,12,12a-hexahydropyrazino [1′,2′:1,6] pyrido [3,4-b] indole-1,4-dione or aminotadalafil.

***Conclusions.*** An unapproved PDE-5 inhibitor analogue, which was identified as aminotadalafil, has been detected in a dietary supplement. This study represents the first report in Latin America and one of the few independent studies of an adulteration with an unapproved PDE-5 inhibitor of an herbal product for ED treatment. **Ulloa J, Sambrotta L, Redko F, Mazza ON, Garrido G, Becher EF, and Muschietti L. Detection of a tadalafil analogue as an adulterant in a dietary supplement for erectile dysfunction. J Sex Med 2015;12:152–157.**

***Key Words.*** Erectile Dysfunction; Dietary Supplements; PDE-5 Inhibitor Analogues; Adulteration; Aminotadalafil

## Introduction

Erectile dysfunction (ED) is a common and widespread health problem. Its prevalence is 1–10% in men younger than 40 years, 2–9% among men between 40 and 49 years, and it increases to 20–40% among men between 60–69 years, reaching the highest rate in men older than 70 years (50–100%) [1]. In addition, it has been estimated that the worldwide prevalence of ED will rise to 322 million cases by the year 2025 [2].

Tadalafil (Cialis™, Eli-Lilly, Indianapolis, IN, USA) is a selective inhibitor of the cGMP-specific phosphodiesterase type 5 (PDE-5) enzyme, which has been approved for the treatment of ED by the European Union in 2002 and by the U.S. Food and Drug Administration in 2003 [3]. It is capable of enhancing the relaxation of the cavernosal smooth muscle, similar to sildenafil citrate (Viagra™, Pfizer, New York, NY, USA) and vardenafil hydrochloride (Levitra™, Bayer, Leverkusen, Germany) and thus has the ability to enhance erection [4]. Compared with sildenafil, tadalafil has an improved PDE-5/PDE-6 selectivity [5].

PDE-5 inhibitors are massively used around the world with a good safety profile. However, these drugs are contraindicated in men taking nitrates and need to be administered carefully in subjects under multiple antihypertensive medications.

In Argentina, dietary supplements (DSs) are incorporated into the Argentina Food Code (CAA) since 1998 and are defined as "products to increase regular dietary intake, supplementing the incorporation of nutrients in the diet of healthy people who, in the absence of pathological conditions, present an unmet dietary need . . ." DSs are sold in pharmacies, natural product stores, health food stores, etc, and do not require a medical prescription. According to their labels, certain DSs are promoted as enhancers of sexual performance and are supposed to contain only natural plant extracts. These kinds of DS are widely consumed around the world, but since they are frequently sold informally and even online, it is difficult to assess the exact market penetration.

In recent years, several cases of adulteration of herbal products with tadalafil, sildenafil, or vardenafil and at least 46 synthetic analogues of these drugs have been reported [6–10]. In particular, new tadalafil analogues such as aminotadalafil, N-octylnortadalafil, 2-hydroxypropylnortadalafil, N-butylnortadalafil, chloropretadalafil, and other analogues are continuously being synthesized [11]. The side effects of these analogues are not fully known, and their safety profiles have not yet been established.

### Aim

The aim of this study was to investigate the suspected adulteration with synthetic PDE-5 inhibitors in a DS marketed in Argentina, which is claimed to be natural and to enhance sexual performance.

### Methods

The DS (sample A) was submitted to our laboratory by professionals from the Urology Department from "Hospital de Clínicas José de San Martín," University of Buenos Aires. Apart from this sample, three different lots of sample A were purchased from the local market. The product is labeled to have the following: *Panax ginseng*, *Astragalus membranaceus*, *Schizandra chinensis*, *Ginkgo biloba* and vitamins. The content (brown powder) of four capsules was ground and extracted with dichloromethane (50 mL/g sample) by ultrasonic shaking at 25°C for 1 hour. The extract was filtered, and the solvent was evaporated under vacuum at 40°C yielding the dried extract from sample A (DESA). Tablets of Cialis™ (tadalafil, 20 mg), Viagra™ (sildenafil, 50 mg), and Levitra™ (vardenafil, 20 mg) were obtained from pharmacies and used as standards and treated in the same way as sample A. Standard solutions were prepared at about 0.1 mg/mL. Thin layer chromatography (TLC) analysis of DESA and standard solutions was performed on silicagel 60 $F_{254}$ using ethylacetate : water : n-buthanol (25:50:100) (upper layer) (system I) and dichloromethane : ammonia : methanol (15:3:2) (lower layer) (system II) as mobile phases. The detection was done with UV light (254 and 366 nm) and by derivatization with anisaldehyde/sulfuric acid reagent. High-performance liquid chromatography-diode-array detection (HPLC-DAD) was performed on a Waters™ 600 liquid chromatographer (Waters, Milford, MA, USA) equipped with a Waters 2996 DAD under the following chromatographic conditions: C18 analytical column (Phenomenex Luna, 250.0 × 4.6 mm, 5 μm), the gradient elution performed with 0.010 M $KH_2PO_4$, pH 3.0 (A)/methanol (B), at a flow rate at 1.0 mL/min, and an injection volume of 20 μL at room temperature. The working solutions were prepared by diluting stock solutions of DESA with MeOH to about 0.4 mg/mL. The elution was initially performed with 43% A and 57% B for 20 minutes. The mobile phase reached 40% A at 24 minutes and 20% at 26 minutes. The initial conditions were restored at 30 minutes and maintained for 5 minutes. Detection was set at 220 nm, and the UV spectrum for each peak was recorded between 190 and 400 nm. The purification of DESA was done by open column chromatography (CC) over silica gel 60 and eluted successively with n-hexane, $CH_2Cl_2$, and MeOH in a gradient of increasing polarity. A total of 70 fractions ($F_1$-$F_{70}$, 20 mL each) were eluted. Accord-