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Short communication

# Identification of a novel vardenafil analogue in herbal product

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**Abstract**

A new herbal health product marketed for enhancing erectile function, namely Power58 Platinum, was purchased over-the-counter in Hong Kong. The product was tested for adulteration with sildenafil, tadalafil, and vardenafil as well as their structurally modified analogues. A new analogue of vardenafil, in which the *N*-ethylpiperazine ring and the sulphonyl group were removed from the vardenafil structure, was identified in the product.


*Keywords:* Vardenafil analogue; Erectile dysfunction; Herbal product; PDE-5 inhibitor; Liquid chromatography–mass spectrometry (LC–MS)

## 1. Introduction

Sildenafil (Viagra; Pfizer, NY, USA), tadalafil (Cialis; Elli Lilly, IN, USA), and vardenafil (Levitra; Bayer Pharmaceuticals Co., Wuppertal, Germany) are the only three phosphodiesterase-5 (PDE-5) inhibitors licensed for the treatment of erectile dysfunction [1,2]. These drugs have documented side effects and must be used under medical supervision [3].

The introduction of PDE-5 inhibitors was associated with a proliferation of herbal products purporting to enhance male sexual function. However, some of these 'natural' products contain concealed substances, which are structurally modified analogues of the PDE-5 inhibitors [4–6]. The adverse effects of these chemicals remain largely unknown and unpredictable. In a previous study, the authors surveyed the over-the-counter male erectile dysfunction health products available in convenience stores and pharmacies in Hong Kong [7]. Of 26 products studied, one (4%) was found to contain undeclared sildenafil while 14 (54%) contained drug analogues of different kinds. The latter included acetildenafil, hydroxyacetildenafil, hydroxyhomosildenafil, and piperidenafil. The first three were analogues of sildenafil and the last was an analogue of vardenafil. One young patient presented with ataxia after taking an acetildenafil-containing product.

In the current study, a herbal product for male erectile dysfunction, known as Power58 Platinum, was bought over-the-counter in Hong Kong. The product was tested for adulteration with sildenafil, tadalafil, and vardenafil as well as their structurally modified analogues, making use of previously published methods [6,8]. A novel analogue of vardenafil was identified. The structure was elucidated as 2-(2-ethoxy-phenyl)-5-methyl-7-propyl-3H-imidazo(5,1-f)-(1,2,4)triazin-4-one which is a hydrolysis product of vardenafil.

## 2. Experimental

### 2.1. Chemicals and reagents

Vardenafil hydrochloride trihydrate was purchased from International Laboratory (CA, USA). Toxi-Tube A was purchased from Toxi-Lab Inc. (Irvine, CA, USA), potassium dihydrogen phosphate from Merck (Damstadt, Germany), phosphoric acid (analytical reagent) from Mallinckrodt (St. Louis, MO, USA), formic acid (reagent grade, min. 98%) from BDH (Poole, England), acetonitrile (HPLC-grade) from LabScan (Bangkok, Thailand). 18 MΩ purity grade water was prepared from Milli-Q Water System (Millipore, Bedford, MA, USA). All other reagents were purchased from Sigma (St. Louis, MO, USA).

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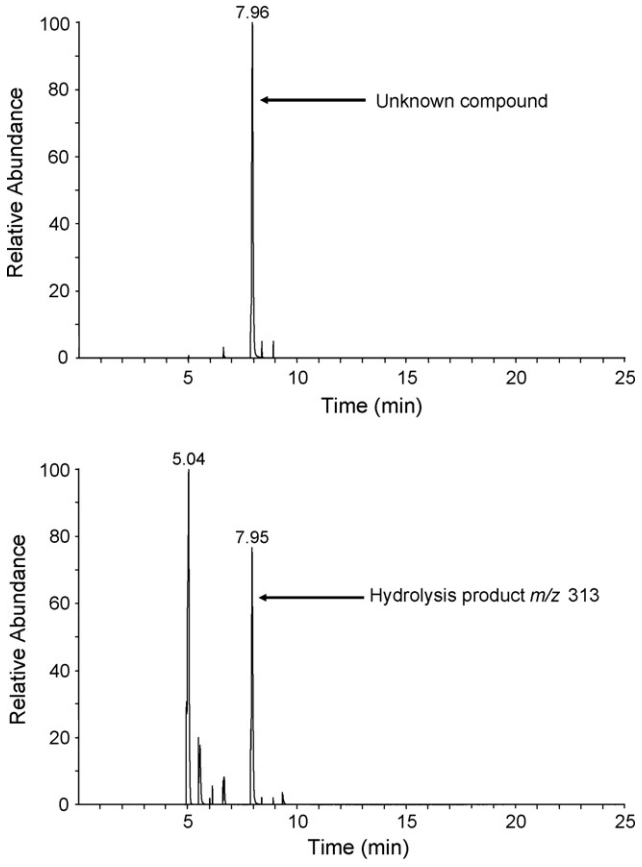


Fig. 1. Total ion chromatograms of the unknown compound in the herbal product, and the hydrolysis products *m/z* 313 of vardenafil.

### 2.2. Standard and sample preparation

Stock solution of vardenafil hydrochloride was prepared in 70% acetonitrile at concentration 1 mg/mL and was stored at 4 °C. The content of the herbal product was extracted by sonication in 70% acetonitrile at concentration 0.1 g/mL for 30 min. 0.5 mL supernatant was then extracted by Toxi-Tube A liquid/liquid extraction for 30 min with shaking. Toxi-Tube A contains sodium carbonate and bicarbonate to give a pH of 9.0 in a mixture of dichloromethane and dichloroethane. The hydrolysis products of vardenafil was also extracted by Toxi-Tube A liquid/liquid extraction to serve as the compensation of the extraction efficiency. The organic phase was dried under compressed air and dissolved in 0.8 mL of 30% acetonitrile. Ten microliters of the sample was injected into high-performance liquid chromatography–diode array detection (HPLC–DAD) system.

### 2.3. HPLC–DAD

An Agilent 1100 HPLC system was used and was controlled by ChemStation software, version Rev. A.10.02 [1757]. The stationary phase consisted of Zorbax XDB-C8, 5 μm-particle size (150 mm × 4.6 mm) with guard column Eclipse XDB C8 (12.5 mm × 4.6 mm) (Agilent Technologies, Wilmington, DE, USA) and was operated at 25 °C. The system was pumped at a flow rate of 1.0 mL/min and full UV spectra were recorded on-line during the 20 min chromatographic run. Two mobile solvents consisted of acetonitrile:50 mM potassium dihydrogen phosphate (A) (adjust to pH 2.5 with phosphoric acid) (10:90, v/v) and acetonitrile:50 mM potassium dihydrogen phosphate (B) (adjust to pH 2.5 with phosphoric acid) (70:30, v/v). The mobile phase consisted of 100% A, changed linearly to 100% B over 0–20 min, maintained at 100% B for 5 min, returned to 100% A in 5 min and maintained at 100% A for 7 min.

### 2.4. LC–MS–MS

An Agilent 1100 HPLC system linked with an API 4000 Q TRAP mass spectrometer (Applied Biosystem, Foster City, CA, USA) was used with electrospray ionization in the positive ion mode. The column used was a Zorbax XDB-C8, 5 μm-particle size (150 mm × 4.6 mm) (Agilent Technologies, Wilmington, DE, USA) and was operated at 25 °C. Two mobile solvents consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The mobile phase consisted of 10% A, changed linearly to 80% B over 0–15 min, maintained at 80% B for 5 min and returned to 10% A in 5 min. The system was pumped at a flow rate of 1.0 mL/min.

### 2.5. Acid hydrolysis of the vardenafil standard

The vardenafil standard was hydrolyzed according to the previously published methods [2]. After hydrolysis, 1 mL portion was neutralized with concentrated sodium hydroxide. The neutralized reaction solutions was subjected to liquid/liquid extraction by Toxi-Tube A as mentioned above in the sample preparation, and analyzed by HPLC–DAD and LC–MS–MS.

### 2.6. NMR

The target fractions of the unknown compound *m/z* 313 in the herbal product and the hydrolysis product *m/z* 313 of vardenafil were collected in HPLC–DAD and dried to yield the purified compounds. Both of these purified compounds were analyzed with $^{1}$H NMR.

## 3. Results and discussion

For the herbal product, a single large unknown peak was present at 14.7 min in the HPLC chromatogram. The protonated molecular mass of this unknown compound was found to be *m/z* 313 by direct flow injection electrospray ionization tandem mass spectrometry. In a previous study performed by Reepmeyer and Woodruff [6], a product of protonated molecular mass of *m/z* 313 was produced by acid hydrolysis of vardenafil. In view of the findings, the unknown compound was suspected to be a hydrolysis product of vardenafil.

After acid hydrolysis of vardenafil reference standard according to the published method, chromatographic separation was performed. A hydrolysis product with retention time (14.7 min) and UV spectrum identical to that of the unknown com-

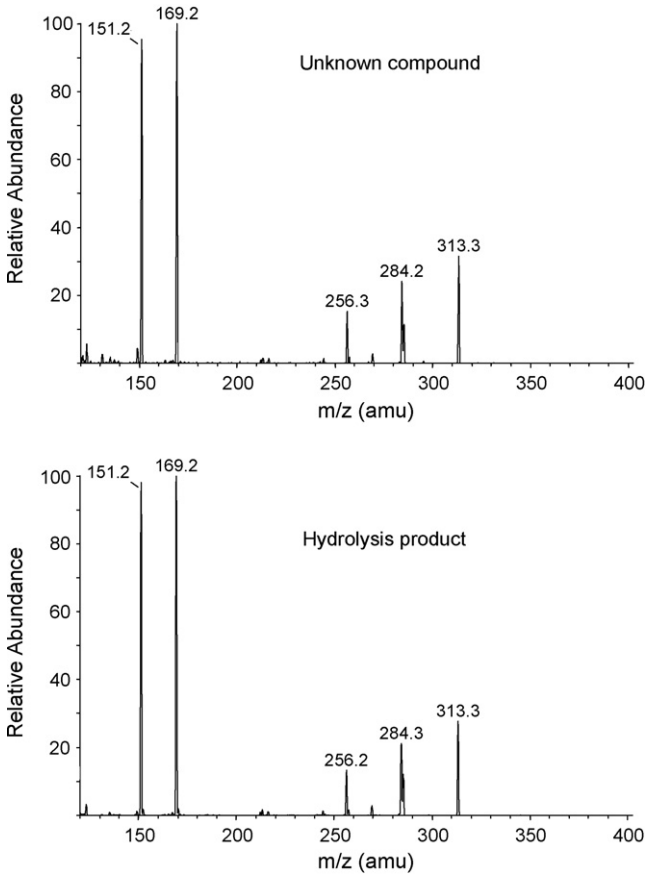


Fig. 2. Tandem mass spectra of the unknown compound in the herbal product, and the hydrolysis product *m/z* 313 of vardenafil.

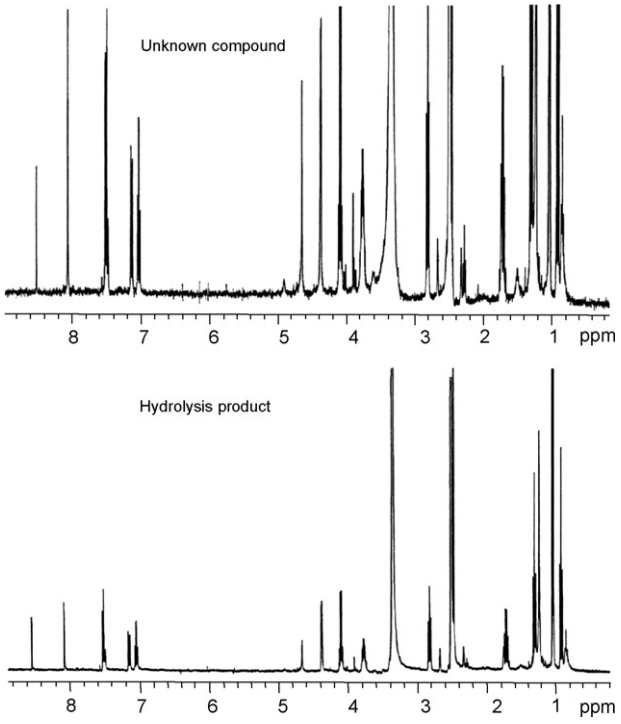


Fig. 3. $^{1}$H NMR spectra of the unknown compound in the herbal product, and the hydrolysis product *m/z* 313 of vardenafil.

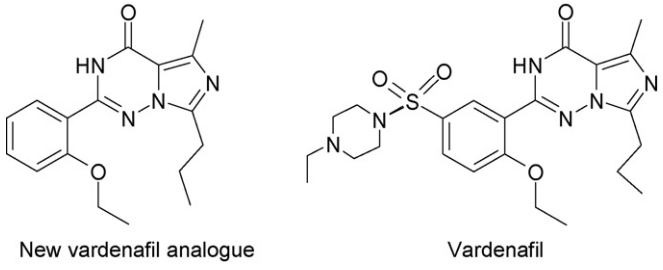


Fig. 4. Structures of the new vardenafil analogue and vardenafil.

pound was observed. This suggests that chromophores of the two compounds are identical and arranged in the same position.

The total ion chromatogram showed that hydrolysis of vardenafil generated a compound with signal at *m/z* 313 and retention time of 7.9 min which was identical to that of the unknown compound in the herbal product (Fig. 1). It has been proposed by Reepmeyer and Woodruff [6] that the hydrolysis product *m/z* 313 is produced from the cleavage of vardenafil at the nitrogen–sulphur bond, followed by the subsequent loss of $SO_3$ molecule from the sulphonic acid compound. The mass spectral fragmentation pattern of this hydrolysis product *m/z* 313 and the unknown compound in the herbal product both generated prominent product ions of *m/z* 284, 256, 169, and 151 (Fig. 2). The results are consistent with the published data [6,9]. The $^{1}$H NMR spectra of the unknown compound *m/z* 313 in the herbal product and the hydrolysis product *m/z* 313 of vardenafil were found to be identical (Fig. 3).

Based on the same UV spectra, the mass spectral fragmentation patterns and the $^{1}$H NMR spectra of the unknown compound in the herbal product and the hydrolysis product *m/z* 313 of vardenafil, we concluded that the structure of the two molecules is the same. The unknown compound in the herbal product is an analogue of vardenafil with systematic name 2-(2-ethoxy-phenyl)-5-methyl-7-propyl-3H-imidazo(5,1-f)-(1,2,4)triazin-4-one and protonated molecular mass of *m/z* 313. The structures of vardenafil and the new analogue are shown in Fig. 4.

## 4. Conclusions

A new herbal product for male erectile dysfunction was found to contain a synthetic analogue of vardenafil, in which the *N*-ethylpiperazine ring and the sulphonyl group had been removed. The structure of this compound was confirmed to be a hydrolysis product of vardenafil by HPLC–DAD, LC–MS–MS and $^{1}$H NMR.

Such analogues are difficult to identify by ordinary laboratory methods and might be used as an attempt to evade regulatory inspection. Our findings showed that surveillance of the over-the-counter health products is necessary and should be extended to cover the registered pharmaceuticals as well as their illicit analogues.

## References

[1] N.K. Terrett, A.K. Bell, D. Brown, P. Ellis, Bioorg. Med. Chem. Lett. 6 (1996) 1819–1824.
[2] S.R. Gratz, C.L. Flurer, K.A. Wolnik, J. Pharm. Biomed. Anal. 36 (2004) 525–533.
[3] E. Wespes, E. Amar, D. Hatzichristou, F. Montorsi, J. Pryor, Y. Vardi, Eur. Urol. 41 (2002) 1– 5.
[4] M.H. Shin, M.-K. Hong, W.S. Kim, Y.J. Lee, Y.C. Jeoung, Food Addit. Contam. 20 (2003) 793–796.
[5] L. Blok-Tip, B. Zomer, F. Bakker, K.D. Hartog, M. Hamzink, J.T. Hove, M. Vredenbregt, D. de Kaste, Food Addit. Contam. 21 (2004) 737–748.
[6] J.C. Reepmeyer, J.T. Woodruff, J. Chromatogr. A 1125 (2006) 67–75.
[7] W.T. Poon, Y.H. Lam, C.K. Lai, A.Y.W. Chan, T.W.L. Mak, Hong Kong Med. J. 13 (2007) 359–363.
[8] J.C. Reepmeyer, J.T. Woodruff, D.A. Avignon, J. Pharmaceut. Biomed. Anal. 43 (2007) 1615–1621.
[9] P. Zou, S.S.-Y. Oh, P. Hou, M.-Y. Low, H.-L. Koh, J. Chromatogr. A 1104 (2006) 113–122.