Journal of Pharmaceutical and Biomedical Analysis 98 (2014) 327–333

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Qualitative and quantitative analysis of PDE-5 inhibitors in counterfeit medicines and dietary supplements by HPLC–UV using sildenafil as a sole reference

Ida Fejős[a], Gábor Neumajer[b], Szabolcs Béni[a,*], Péter Jankovics[b,**]

[a] Semmelweis University, Department of Pharmaceutical Chemistry, Hőgyes Endre u. 9, H-1092 Budapest, Hungary
[b] National Institute for Quality and Organizational Development in Healthcare and Medicine, Directorate General of National Institute of Pharmacy, Zrínyi u. 3, H-1051 Budapest, Hungary

ARTICLE INFO

Article history:
Received 27 March 2014
Received in revised form 4 June 2014
Accepted 8 June 2014
Available online 14 June 2014

Keywords:
Phosphodieserase-5 inhibitor analogs
Liquid chromatography
Counterfeit
Correction factor
Herbal dietary supplements

ABSTRACT

Due to their popularity, medicinal products containing the phophodiesterase type 5 enzyme (PDE-5) inhibitors sildenafil, vardenafil and tadalafil are often subject to counterfeiting. In addition, illicit herbal dietary supplements adulterated with these substances or their analogs have appeared on the market offering an easy and anonymous sale.

This paper describes an analytical method for qualitative and quantitative screening of sildenafil, vardenafil, tadalafil and 11 of their designer analogs in illegal erectile dysfunction products by high-performance liquid chromatography with UV detection (HPLC–UV). Sildenafil served as a single external standard for both identification and quantification of all analytes. Relative retentions and reference UV spectra were used for qualitative, and correction factors for quantitative analyses, respectively.

The separation was performed on a Kinetex C18 reverse-phased column at 25 °C using gradient elution. Mobile phase A consisted of 200 mM ammonium acetate solution while mobile phase B was a 1:1 (v/v) mixture of methanol and acetonitrile with a flow rate of 0.5 ml/min and injection volume of 5 μl. Detection wavelength was set to 290 nm.

The method was validated in accordance with the appropriate guideline of the International Conference on Harmonization (ICH) in terms of specificity, selectivity, precision, linearity, limit of quantitation, limit of detection, accuracy, robustness and stability, and was successfully applied to the analysis of natural dietary supplements and herbal remedies with an indication for enhanced male sexual potency. The proposed method offers a cheap and simple alternative to LC–MS screening used by control laboratories for routine analysis of suspicious products.

1. Introduction

The phosphodiesterase-5 enzyme (PDE-5) inhibitor sildenafil (SIL) was developed for the treatment of cardiovascular disease (Revatio®, using in therapy of pulmonary arterial hypertension) and was also found to be effective in erectile dysfunction disorders (ED) therapy. SIL (Viagra®) was the only PDE-5 inhibitor approved for the treatment of ED until vardenafil (Levitra®, VAR) and tadalafil (Cialis®, TAD) were also approved in 2003 by the FDA [1].

Erectile dysfunction is a highly prevalent inability to achieve and maintain adequate erection and sexual performance. As the PDE-5 is responsible for the hydrolysis of cGMP in the penis, inhibiting the enzyme results in vasodilatation and penile blood flow maintenance due to the elevated level of cGMP [2]. Owing to the adverse effects such as headache, facial flushing, nasal congestion, back pain and visual disorders [3], and its interactions with nitrates or α-blockers leading to hypotension or syncope [4], PDE-5 inhibitors are under medical doctor's prescription. Since PDE-5 inhibitors are contraindicated in many patients, alternative therapies for ED should be taken into account. Furthermore, the taboo associated with sexual disorders facilitated the increase of cheap, easy-to-access dietary supplement consumption, supported by the internet. There is a widespread belief that dietary supplements and herbal products offer a safe, side effect free and healthy alternative to medicinal products containing synthetic ingredients. However, many of these herbal products were found to contain approved PDE-5 inhibitors, such as SIL, VAR, TAD or unapproved designer analogs with subtle changes in their structures [5–8] (see Fig. 1).

---

[*] Corresponding author. Tel.: +36 1 217 0891; fax: +36 1 217 0891.
[**] Corresponding author.
E-mail addresses: beni.szabolcs@pharma.semmelweis-univ.hu, beniszabi@gmail.com (S. Béni), jankovics.peter@gyemszi.hu (P. Jankovics).

http://dx.doi.org/10.1016/j.jpba.2014.06.010
0731-7085/© 2014 Elsevier B.V. All rights reserved.

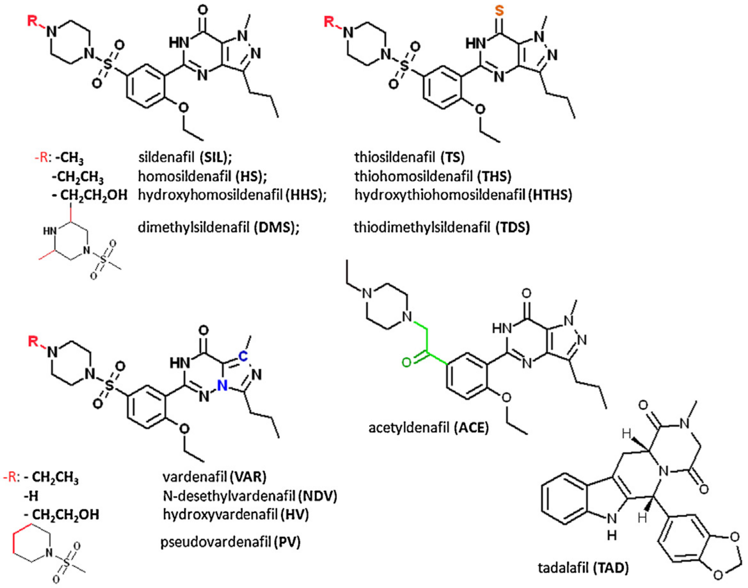

Fig. 1. Chemical structures of the PDE-5 inhibitors examined.

Adulteration with PDE-5 inhibitors and their analogs poses serious health risks in the case of patients who turn to alternative therapies. Further health issues are also evoked by the lack of safety and toxicity data of the numerous unapproved analogs. Due to the popularity of PDE-5 inhibitor drugs, their counterfeit pharmaceutical preparations have appeared in various websites offering an easy and anonymous sale. Therefore, official control laboratories need simple, easy-to-use, inexpensive routine methods to screen and determine the illicit adulterants in herbal dietary supplements and in suspicious pharmaceutical preparations.

Until now, several authors have reported methods for screening and confirmation of synthetic PDE-5 inhibitors in illicit sexual performance enhancer products, including HPLC–MS [1,9,10], HPLC–MS/MS [11–14], UHPLC [15], and UHPLC–MS/MS [2,16]. Even though these methods allowed unambiguous identification of the analytes, quantification required the use of expensive reference standards. In order to overcome this practical difficulty, Poplawska et al. used charged aerosol detection (CAD) [17] to determine the amount of PDE-5 inhibitor analogs in herbal dietary supplements using SIL and TAD as the only reference substances. However, an independent off-line analytical technique (TOF-MS) was necessary to identify the compounds.

Realizing that an easy and cost-effective method capable of both identifying and quantifying PDE-5 inhibitors and their designer analogs in herbal matrices would facilitate the work of control laboratories responsible for screening illicit medicinal products and food supplements, we aimed at designing a simple HPLC–UV method for this purpose. SIL was used as the only external standard to overcome the repeated use of expensive references of the analogs in routine analysis. The method was fully validated based on the guidelines of the International Conference on Harmonization (ICH).

2. Materials and methods

2.1. Standards and reagents

Reference standards of sildenafil citrate, tadalafil and vardenafil dihydrochloride trihydrate were provided by Intas Pharmaceuticals Ltd. (Ahmedabad, India), Eli Lilly and Co. (Indianapolis, IN, USA) and Bayer AG (Leverkusen, Germany), respectively. Certified reference standards of homosildenafil (HS), hydroxyhomosildenafil (HHS), thiosildenafil (TS), thiohomosildenafil (THS), N-desethylvardenafil (NDV), pseudovardenafil (PV) and acetildenafil (ACE)) were purchased from Toronto Research Chemicals (Toronto, ON, Canada) while hydroxythiohomosildenafil (HTHS), dimethylsildenafil (DMS), hydroxyvardenafil (HV) and thiodimethylsildenafil (TDS) were obtained from TLC PharmaChem Inc. (Vaughan, ON, Canada).

Illicit herbal dietary supplement samples used in this work were confiscated and sent for analysis to the laboratory of the National Institute of Pharmacy, Budapest by the National Tax and Customs Office.

Acetonitrile (ACN), methanol (MeOH) (both of gradient purity grade) and ammonium acetate of analytical grade were all purchased from Merck KGaA. (Darmstadt, Germany). Acetic acid (puriss.) was obtained from Sigma–Aldrich GmbH (Seelze, Germany). Water was produced by a Millipore Elix3 (Billerica, MA, USA) water purifying system.

2.2. Instrumentation

HPLC experiments and validation were performed on an Agilent 1200 HPLC system equipped with a diode array detector (Agilent Technologies, Santa Clara, CA, USA). A Kinetex C18 100 Å 2.6 μm 100 mm × 4.6 mm reverse-phased column (Phenomenex, Torrance, CA, USA) was used. Column temperature was maintained at 25 °C.

200 mM ammonium acetate solution was used as mobile phase A. Mobile phase B consisted of a mixture of equal volumes of MeOH and ACN. The gradient program of the optimized method was as follows: 0–9 min 40–50% B; 9–17 min 50–80% B; 17–20 min 80% B; 20–20.5 min 80–40% B; 20.5–25 min 40% B. The flow rate of the mobile phase was 0.5 ml/min. Autosampler temperature was set to 20 °C. The injection volume was 5 μl. The detection of the measurements was performed at 290 nm.

2.3. Standard and sample preparation

2.3.1. Stock and working solutions

Stock solutions were prepared by dissolving precisely weighed amounts of each studied substance in mobile phase B to obtain a concentration of about 1 mg/ml. Working solutions used in the