Sildenafil Determination by Using UPLC in Different Italian Viagra Tablets

Nicola Giarretta[1], Maria C. Mezzacapo[1], Antonella M. A. Di Giuseppe[1], Nicola Landi[1], Martina Lippert[1] and Antimo Di Maro[1*]

[1]*Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, Second University of Naples, Via Vivaldi 43, I-81100 Caserta, Italy.*

Authors' contributions

This work was carried out in collaboration between all authors. Author NG designed the study, performed the statistical analysis and wrote the protocol. Authors MCM, AMADG and NL managed the analyses of the study. Author ML managed the literature searches. Author ADM designed the study, wrote the protocol, and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Aims: The introduction of generics drugs has brought the need for more control of their quality and purity. In Italy from June 23, 2013 the Pfizer® has no longer the patent for the Viagra's production and other industries produce equivalent products containing Sildenafil citrate.
Study design: Thus, in this work, the chemical profiles of both Viagra Italian Pfizer® and 3 Italian commercial Sildenafil citrate tables (generic pharmaceutical manufacturers) for male erectile dysfunction were obtained by using UltraPerformanceLiquid Chromatography (UPLC).
Methodology: UPLC methodology was successfully used for the assay of Sildenafil citrate in different products in Italy which are under the cover of alternative systems of medicine.
Results: The results show that: i) the chromatographic profiles obtained from Italian Sildenafil citrate tablets are identical and not present other active pharmaceutical ingredients; ii) the commercial samples have a quantity of Sildenafil citrate comparable with the corresponding labelled amounts.

*Corresponding author: Email: antimo.dimaro@unina2.it;

> **Conclusion:** The UPLC method can be used for determination of Sildenafil citrate tables marketed by generic pharmaceutical manufacturers in Italy.

1. INTRODUCTION

Male erectile dysfunction (ED), known as impotence, is a type of male sexual dysfunction [1]. It is characterized by the inability to develop and maintain the erected penis during sexual performance [1,2]. It is generally accepted to adversely affect the quality of life and some indication it is frequently associated with some disorders such as depression, anxiety which can have several psychological consequences tied to relationship difficulties and masculine self-image generally [3]. The causes may be different. In general it is divided two types: generally psychological, physical and organic, related to the drugs itself or to the lifestyle and diseases [4].

Medical therapy for this condition was unsatisfactory for invasiveness or inefficacy before the introduction of Sildenafil citrate knows as Viagra [5]. Sildenafil citrate was patented in 1996 and launched in May 1998 as first oral medical drug approved by Food and Drug Administration (FDA) to treat this type of male dysfunction. It's a white to off-white crystalline powder with a molecular weight of 666.7 Dalton. Molecular formula is $C_{22}H_3N_6O_4S$, chemically, designed as 1-[(3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H pyrazolo[4,3-d]pyrimidin-5yl)-4-ethoxyphenyl]sulfonil]-4-methylpiperazine citrate. The parasympathetic nerves are stimulated when man arouses sexually, leading to penile erection as result of release of nitric oxide (NO) which active enzyme guanylateciclase responsible for converting guanosine triphosphate (GTP) to 3',5' cyclic guanosine monophosphate (cGMP). This last is a potent vasodilatator vital erection of penis. Sildenafil citrate inhibits the enzyme PDE-5A (phosphodiesterase-5A) that idrolize cGMP. Thus, it increases level of cGMP by preventing it from breaking down. Consequently smooth muscle relaxation leads to vasodilation and increased inflow of blood into the spongy tissue of the penis causing an erection by facilitating the signaling actions of nitric oxide (NO) in penile smooth muscle [6].

For many years after being originally developed by British scientists, Sildenafil citrate was a substance marketed exclusively by Pfizer® as a pharmaceutical company Pfizer® [7]. Pfizer®, until 23 June 2013 had the patent of substance Sildenafil citrate to sell medicines containing the active substance. After the patent was expired and other companies have had the opportunity to sell products containing Sildenafil citrate with the same results on patients. Thus, there are few information on the quality and purity (chemical profile) of these products.

In this context, we develop our research that for the first time compares the Sildenafil citrate marketed by generic pharmaceutical manufacturers in Italy trough using the reproducible and sensitive method of UPLC system.

2. MATERIALS AND METHODS

2.1 Chemicals, Drugs and Buffers

The standard of Sildenafil citrate is purchased from Sigma-Aldrich (Milan, Italy). The UPLC grade methanol, triethanolamine, phosphoric acid were provided by Carlo Erba (Milan, Italy).

the 50 mg tablets Viagra produced by Pfizer® (leader, Pfizer® Italia Srl, Latina, Italy) was used as reference. Three Italian Sildenafil citrate generic drugs [Sildenafil DOC generics (50 mg; DOC generic Srl, Milan, Italy), Silderafil EG (50 mg; EG SpA, Milan, Italy) and Sildenafil Ranbaxy (50 mg; Ranbaxy Italia SpA, Milan, Italy)] which are sold under the cover of alternate system of medicines in Italy were purchased from local pharmacy of Caserta. The purified water prepared by Milli-Q system was used for the preparation of buffer and other aqueous solutions.

The following eluents were used: eluent A, 70 mL triethanolamine in a purified water sufficient to make 1000 mL.The pH 4.0 was adjusted by use of 85% phosphoric acid; eluent B, methanol. Eluents was filtered through 0.22 µm filter.

2.2 Instrumentation

The LC system used for the analyses consisted of an Acquity UPLC with PDA detector (Waters, Vimodrone, Milan, Italy), and a separated module with an BEH C-18 column (50 x 2.1 mm, 1.7 µm particle size; Waters), set at 45ºC operating temperature with a 0.22 µm pre-column filters. Empower software (Waters) was used for data treatment. The RP-HPLC method of Mahmood et al., 2010 [8] was been adapted for UPLC by using Waters software tool. The analysis was carried out using an isocratic elution as previously mentioned containing 92% eluent A and 8% eluent B at flow rate of 0.8 mL/min. Eluted Sildenafil citrate was monitored at 290 nm. The injection volume was 3.0 µL. In the case of necessity, buffer A was used for sample dilution. Separation of all drugs was achieved with a total run time of 5 minutes. The retention time of Sildenafil citrate is normally 1.78 min. Solutions of standard: The solution was prepared with external standard Sildenafil citrate by diluting with mobile phase. The samples at concentration of 4.6, 4.1, 3.6, 3.1 and 2.6 µg/mL were also prepared by diluting stock solution of external standard sildenafil citrate in mobile phase for constructing standard curve, in quintuplicate [9]. We also mixed these samples and ultrasonicated them for 30 minutes. At the end, we filtered the solution through 0.22µm.

2.3 Degradation of Sildenafil Citrate Drugs

Degradation of Sildenafil citrate samples (leader and the generic drugs) with chemical reagent was carried out as already described [10]. Typically, 30 mg of Sildenafil citrate were treated with chemical treatment in 2 mL and then the mixture neutralized (final volume 50 mL). The following chemical treatments were employed: (i) 30% H_2O_2; (ii) 37% HCl; (iii) 10 M NaOH. After the chemical treatments all samples were stored for 15 minutes at the room temperature and analysed by UPLC. Temperature stability of medicines dissolved in water was also tested storing Sildenafil citrate samples for 12 hour at 80°C in dark. Then we analyses the samples using UPLC analyses. As reference (standard preparation), 30 mg of Sildenafil citrate (leader and the generic drug samples) were dissolved in neutralized without treatment (final volume 50 mL).

3. RESULTS AND DISCUSSION

3.1 Linearity of UPLC Method Achieved

The retention time of Sildenafil citrate was 1.78 min in the new adapted method respected to the elution time of 7.8 min as it is previously reported by Mahmood using RP-HPLC [8].

Moreover, there was no peak when placebo mobile phase was run. The calibration curve for Sildenafil citrate was found to be linear over the range of 2.6- 4.6 µg/mL (Fig. 1A).
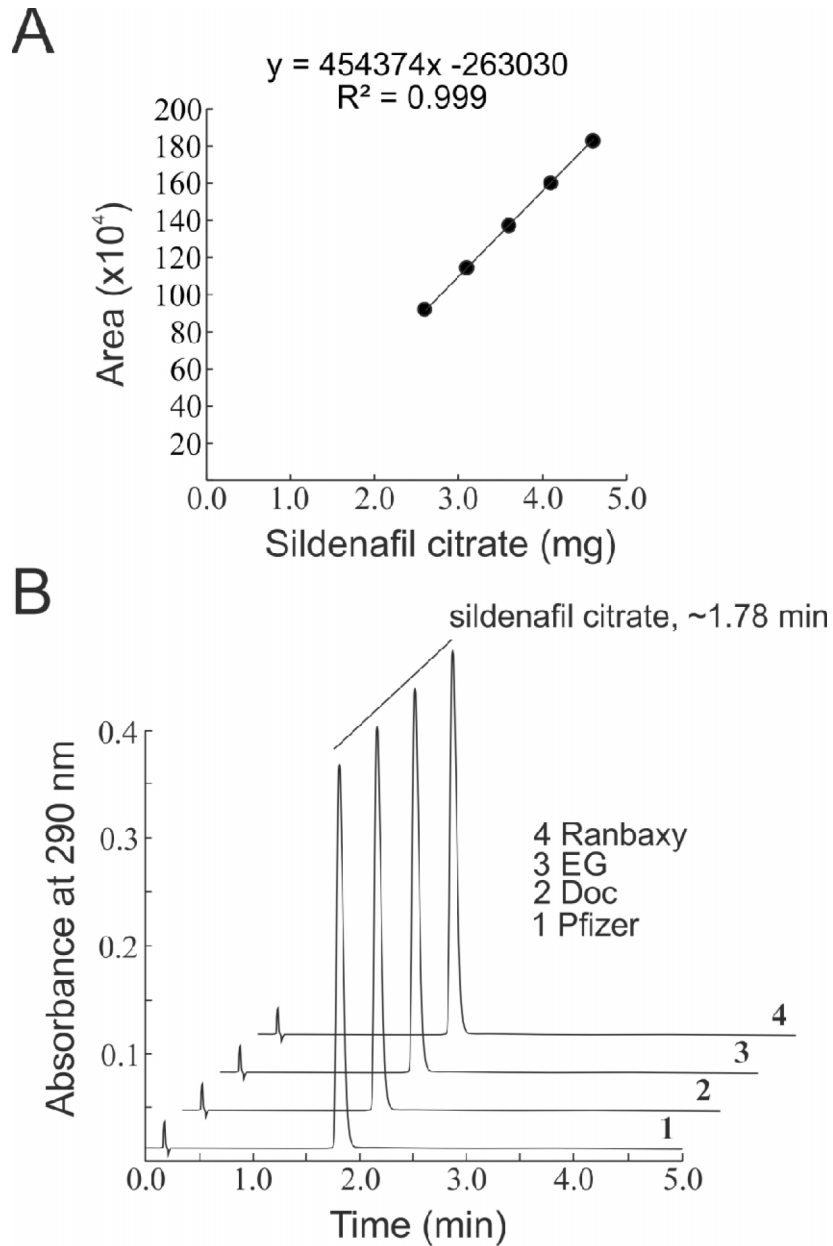
A

$$y = 454374x - 263030$$
$$R^2 = 0.999$$

B

sildenafil citrate, ~1.78 min

4 Ranbaxy
3 EG
2 Doc
1 Pfizer

Fig. 1. A, standard calibration curve and linearity plots for sildenafil citrate (Sigma-Aldrich) quantitation. In the inset, the equation and R^2 values of calibration. B, UPLC elution profiles of Italian Viagra tables (50 mg tablets from Pfizer®, DOC generic, EG, Ranbaxy Italia) on an acquity UPLC with PDA detector from reverse-phase chromatography using a C-18 column. Experimental conditions are described in the text

3.2 Analyses of Sildenafil Citrate Samples in Pure form Using UPLC

A set of 4 Sildenafil citrate samples (Viagra 50 mg tablets from Pfizer®, DOC generic, EG SpA, Ranbaxy Italia SpA) were analysed by UPLC. Fig. 2B showed the typical chromatogram profiles of Sildenafil citrate samples (leader and the generic drugs). In all samples, Sildenafil was eluted with the same elution time at the presence of the high purity content, without any interference peak. In all tables the recovered percentage of Sildenafil citrate was about 99.80% with Relative Standard Deviation (R.D.S.) of 0.5%. The obtained results for the four samples were comparable with to the corresponding labelled amounts of the five replicate injections of standard preparation (data not shown).

3.3 Degradation of Sildenafil Citrate Samples to Verify Specificity

The specificity was demonstrated by the induced degradation of Sildenafil citrate samples we treated them with either 37% HCl and storing them at 25ºC for 15 min, or 10 M NaOH, and storing at 25ºC for 15 min, 30% H_2O_2 and storing the sample at 25ºC for 15 min or heating dissolved medicines in water and testing the samples stored at 80º for 12 hour. In the case of 37% M HCl (Fig. 2A) and 10 M NaOH (Fig. 2B), respectively, the recovery of Sildenafil in all samples was 101.7 and 99.3 % with a total degradation of 0.2 and 0.4 %.
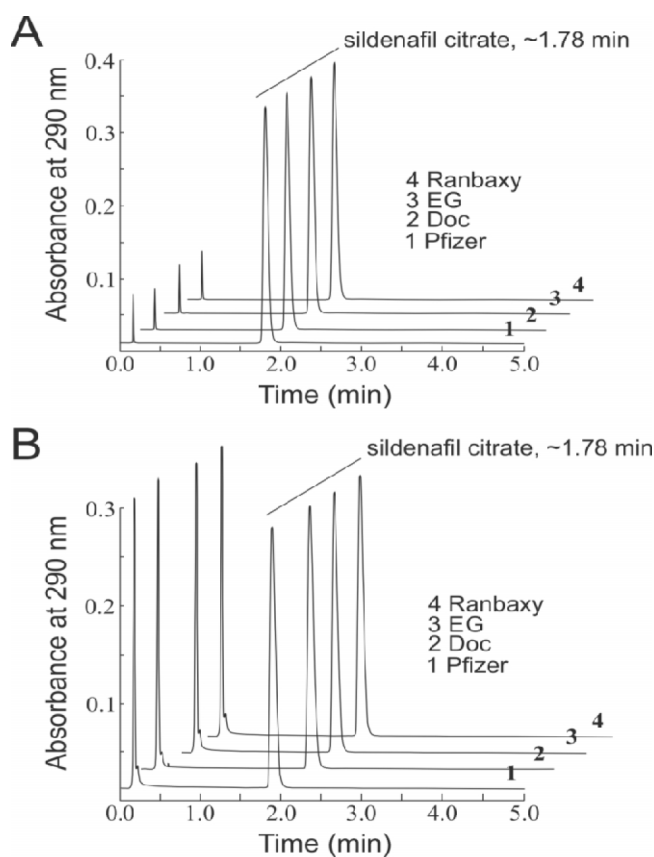

Fig. 2. Elution profiles of Italian market formulation of sildenafil citrate samples (50 mg tablets from Pfizer®, DOC generic, EG SpA, Ranbaxy Italia SpA) after chemical treatment under acid (A) or basic (B) conditions

Vice versa Sildenafil citrate samples were found to be susceptible to H_2O_2 (Fig. 3A). We detected the impurity B (5-[2-ethoxy-5-[(4-methyl-4-oxidopiperazin-1-yl)sulfonyl]phenyl]-1-methyl-3-propyl-1,6-dihydro-7Hpyrazolo[4,3-d]pyrimidin-7-one) in all Sildenafil citrate samples as previously reported [11]. However, the decomposition showed a Sildenafil recovery of 65.0 % and also the presence of impurity B of about 35%. Considering the heating treatment the chromatographic profiles show the presence of unknown peak which represents degradation of 0.2% (Fig. 3B). Therefore the characterization of this degradation was not carried out for the minimal amount.
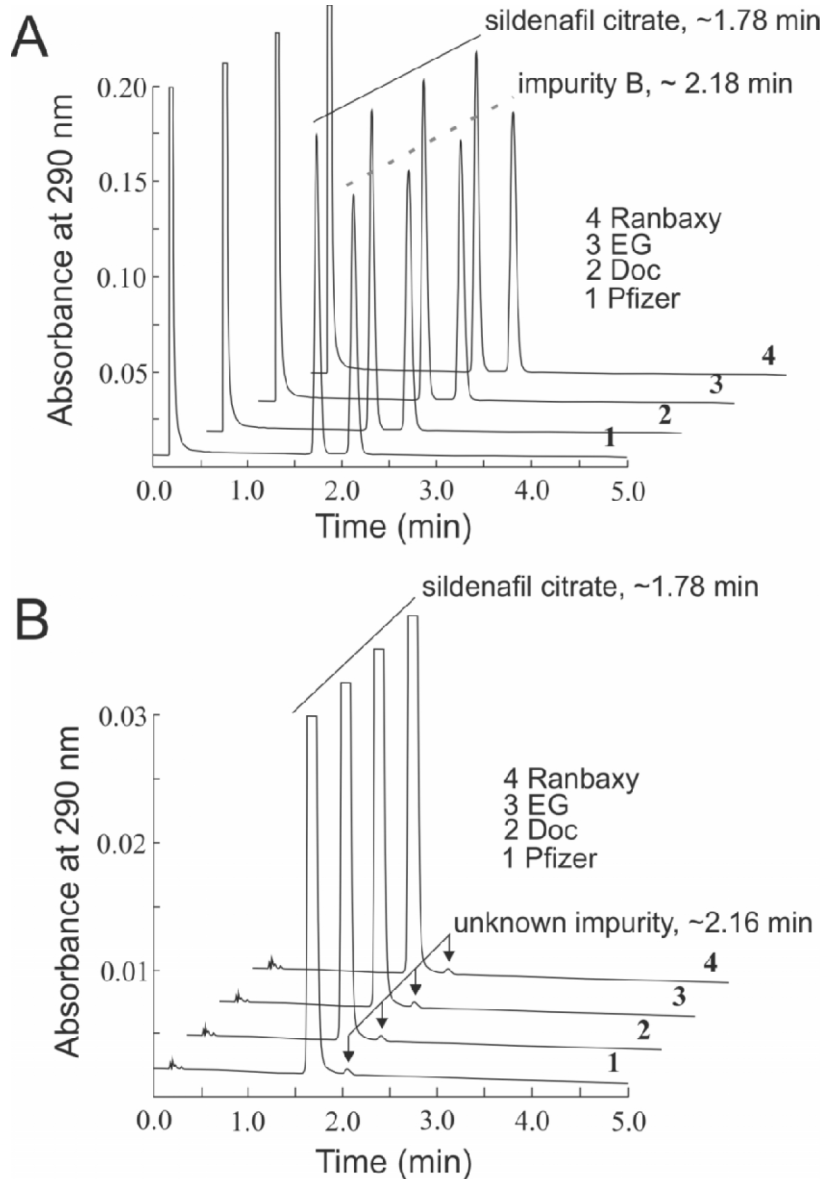

Fig. 3. Elution profiles of Italian market formulation of sildenafil citrate samples (50 mg tablets from Pfizer®, DOC generic, EG SpA, Ranbaxy Italia SpA) after chemical treatment with hydrogen peroxide (A) or heating (B) conditions

4. CONCLUSION

In the last decade, several works have been published reporting the identification of Sildenafil citrate in pure form or in pharmaceutical dosages [12-14]. However, they are poor in information when compared to a single step quantification method such as the analytical tool UPLC-diode array detector (or UPLC–DAD) [15]. In the present work, the qualitative and quantitative chemical profile of both Viagra Italian Pfizer® and 3 Italian commercial Sildenafil citrate tables (generic pharmaceutical manufacturers) has been investigated using UPLC-DAD. After the identification of Sildenafil citrate in the samples, features such as purity of products, quantity of Sildenafil citrate comparable with the corresponding labelled amounts, presence of other active pharmaceutical ingredients and the specificity after chemical treatments were demonstrated. In the proposed study, the collected data shows that all the Italian formulations of Sildenafil citrate tablets analysed by the UPLC did not present another unknown pharmaceutical ingredients and the commercial samples have the same quantity of Sildenafil citrate as labelled. Furthermore, the analysis carried out after chemical-physical treatment confirm that each formulation was very resistant to acid and basic treatments and only in the presence of hydrogen peroxide there is a partially degradation of the Sildenafil citrate. Finally, the proposed UPLC-DAD method gives good resolution and short analysis time (less than 5 min).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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