Short communication

DETERMINATION OF PHARMACOKINETIC PARAMETERS OF SILDENAFIL IN IRANIAN VOLUNTEERS BY AN HPLC METHOD.

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Abstract

Pharmacokinetic parameters of sildenafil in Iranian volunteers were determined by using a simple, rapid and sensitive HPLC-UV method. The assay procedure involves extraction of sildenafil in basic pH with dichloromethane. After evaporation of dichloromethane under a steam of nitrogen; the residue was dissolved in 0.2 mL of mobile phase. The mobile phase consists of acetonitrile - KH_2PO_4 (30 mM) (56:44, v/v). The column was RPC18 and flow rate was 1.0 mL/min. at ambient temperature (25°C). Peaks were monitored by UV detector set at 290 nm. Chlordiazepoxide was used as internal standard (IS). The peak areas of sildenafil/IS ratio versus drug concentrations relationship was linear (r>0.992). The extraction efficiency was 92%. The minimum quantifiable concentration was found to be 10 ng/mL. The method was used to determination of plasma concentrations. 2x50 mg of Viagra tablets were administered to11 healthy male volunteers and sildenafil was determined in the plasma and pharmacokinetic parameters of sildenafil (Ke, Tmax, $T_{1/2}$, C_{max} and AUC) were calculated.

Key words: *Sildenafil, HPLC, Pharmacokinetics*

İranlı Gönüllülerde Sildenafil İçin Farmakokinetik Prametrelerin Bir HPLC Yöntemiyle Tayini

İranlı gönüllülerde sildenafil için farmakokinetik prametreler basit, hızlı ve duyarlı bir HPLC-UV yöntemiyle tayin edilmiştir. Analiz yöntemi, bazik pH da sildenafilin diklorometan ile ekstraksiyonunu içerir. Azot akımı altında diklorometanın uçurulmasından sonra kalıntı 0.2 mL mobil faz içerisinde çözülmüştür. Mobil faz asetonitril-KH_2PO_4 (30 mM) (56:44, v/v) den oluşmaktadır. Kolon RPC18 ve akış hızı oda sıcaklığında (25°C) 1.0 mL/dk dır. Pikler 290 nm de UV dedektör ile izlenmiştir. Klordiazepoksit iç standart (IS) olarak kullanılmıştır. Sildenafil/IS pik alanları oranı– konsantrasyon ilişkisi doğrusaldır (r>0.992). Ekstraksiyon etkinliği % 92 dır. Tayin edilebilen minimum değer 10 ng/mL olarak bulunmuştur. Yöntem plazma konsantrasyonlarının tayininde kullanılmıştır. 11 sağlıklı erkek gönüllüye 2x50 mg Viagra tableti verilmiş ve sildenafil plazmada tayin edilmiş ve sildenafil in kinetik parametreleri ((Ke, Tmax, $T_{1/2}$, C_{max} and AUC) hesaplanmıştır.

Anahtar kelimeler: *Sildenafil, HPLC, Farmakokinetik*

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INTRODUCTION

Sildenafil (1- [[3 - (6,7 - dihydro - 1- methyl - 7 oxo - 3- propyl - 1H- pyrazolo [4,3-d] pyrimidine - 5 -yl) - 4 - ethoxyphenyl] sulfonyl] - 4- methyl piperazine (fig. 1) is a potent and selective inhibitor of cGMP - specific phosphodiesterase capable of enhencing the relaxation of the penile corpus covernosum and therefore having the potential improve penile erectile function. Quantification of sildenafil is essential during the evaluation of drug. Several HPLC methods have been developed for determination of sildenafil in various samples, such as dietary supplements (1-3), pharmaceutical preparation (4), and mouse skin (5), human plasma and urine (6, 7, 8). For a review see "Sildenafil determination in various matrices" (9) Analyses were based on liquid-liquid extraction at basic pH (6, 7). Here we decided to evaluate the pharmacokinetic parameters of sildenafil in Iranian healthy male volunteers. For this purpose we used this simple, rapid and accurate HPLC method.
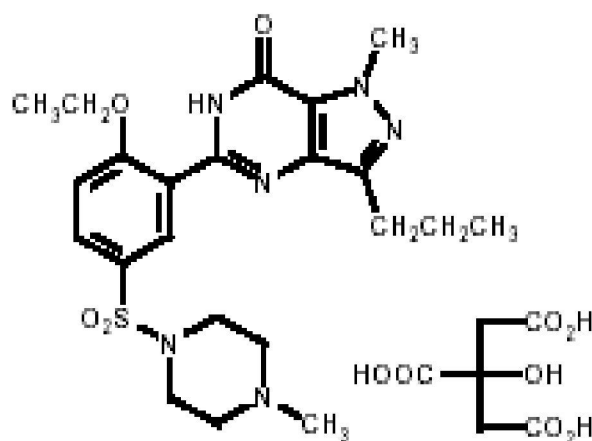

Figure 1. The chemical structure of sildenafil.

MATERIALS AND METHOD

Sildenafil working standard was obtained from Razak Lab. Co. All chemicals were of analytical grade.

A Cecil series 1100 liquid chromatograph system equipped with an UV detector (Cecil 1100) set at 290 nm, vacuum degasser (Cecil) and data control computer program was used. The mobile phase consists of acetonitrile-KH_2PO_4 30 mM (56:44, pH=4.7). The mobile phase was pumped through the system using a Cecil series 1100 HPLC pump at a flow rate of 1.0 mL/min. A 25 cm x 4.6 mm i.d. C18 column packed with 5 μm reversed phase particles (Dr. Maisch) was employed and the procedures were performed at room temperature (25°C).

To the test tubes containing 0.5 mL human plasma were added 100 μL of 1μg/ml chlordiazepoxide (as IS), 50 μL 1N NaOH and 4 mL dichloromethane. The samples were vortexed for 2 min. and centrifuged 10 min at 15000 rpm. 3 mL of lower layer was transferred to another tube and evaporated under a steam of dry nitrogen until dryness. The residue was dissolved in 250 μL of mobile phase and an aliquot of 100 μL was injected into the HPLC.

Aliquots of 0.5 mL blank human plasma were spiked with 100 µL of 1µg/ml of IS and various quantities of sildenafil to yield 10, 50, 100, 500 and 1000 ng/mL. Standard samples were extracted and analyzed as described above for test plasma samples.

Accuracy was expressed as the recovery [(mean measured concentration)/ (expected concentration)] x 100.

Precision was calculated as intra-day coefficient of variation [CV = (SD/ mean) x 100].

2x50 mg Viagra tablets (Pfizer Co.) were orally administered to 11 healthy male volunteers. The proposal of this study was approved by ethical committee of Razi Ins. of Drug Res. (IUMS). Written informed consent was obtained before administering the tablets. Venous blood samples were collected at 0, 0.33, 0.66, 1, 1.5, 2, 2.5, 4, 6, 8 and 24h. after administration. Plasma was harvested and kept at -20 °C until analyzed.

All pharmacokinetic parameters were calculated using a computer software package called DKNT (10).

The non- compartmental model contained in the program was used.

RESULTS

Typical chromatograms of blank plasma, spiked plasma and an actual sample obtained from the pharmacokinetic study are shown in Fig. 2.

Retention times of sildenafil (SIL) and chlordiazepoxide (IS) were 6 and 8 min, respectively. The total HPLC run time was 10 min. There were no interfering peaks in the blank plasma samples. Standard curves prepared for sildenafil in human plasma were linear over 10 to 1000 ng/mL. The mean calibration curve for sildenafil was y = (221.05 x) + 2.75037 (R=0.9988) where y and x are peak area ratio (SIL/IS) and concentration (ng/mL) respectively.

Sildenafil concentration greater than 10 ng/mL could be quantified. The sensitivity of assay was sufficient for pharmacokinetic study. Interday accuracy of assay was >90 % and CV did not exceed 20 % (Table 1).
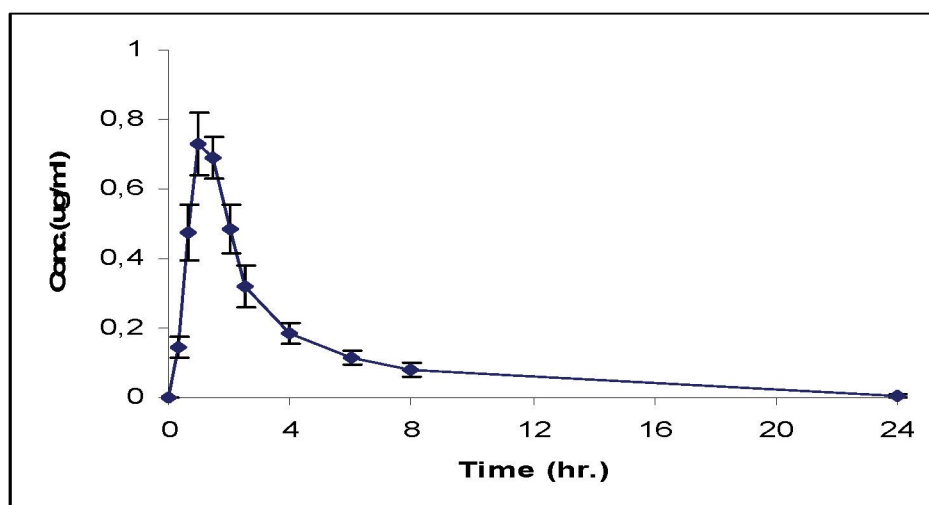
The mean plasma concentration time curve is shown in Fig.3 while corresponding pharmacokinetic parameters are reported in Table 2.

Table 1. Accuracy and precision of sildenafil assay in human plasma. N is the number of repetitions in the assay.

Prepared Conc. (ng/mL)	Mean Measured Conc. ± SE (ng/mL)	N	CV (%)	Accuracy
50	47.10 ± 2.37	5	11.23	-5.80
100	107.08 ± 8.17	5	17.05	7.08
500	473.66 ± 7.51	5	3.55	-5.27
1000	1010.81 ± 3.15	5	0.70	1.08

A

B

C
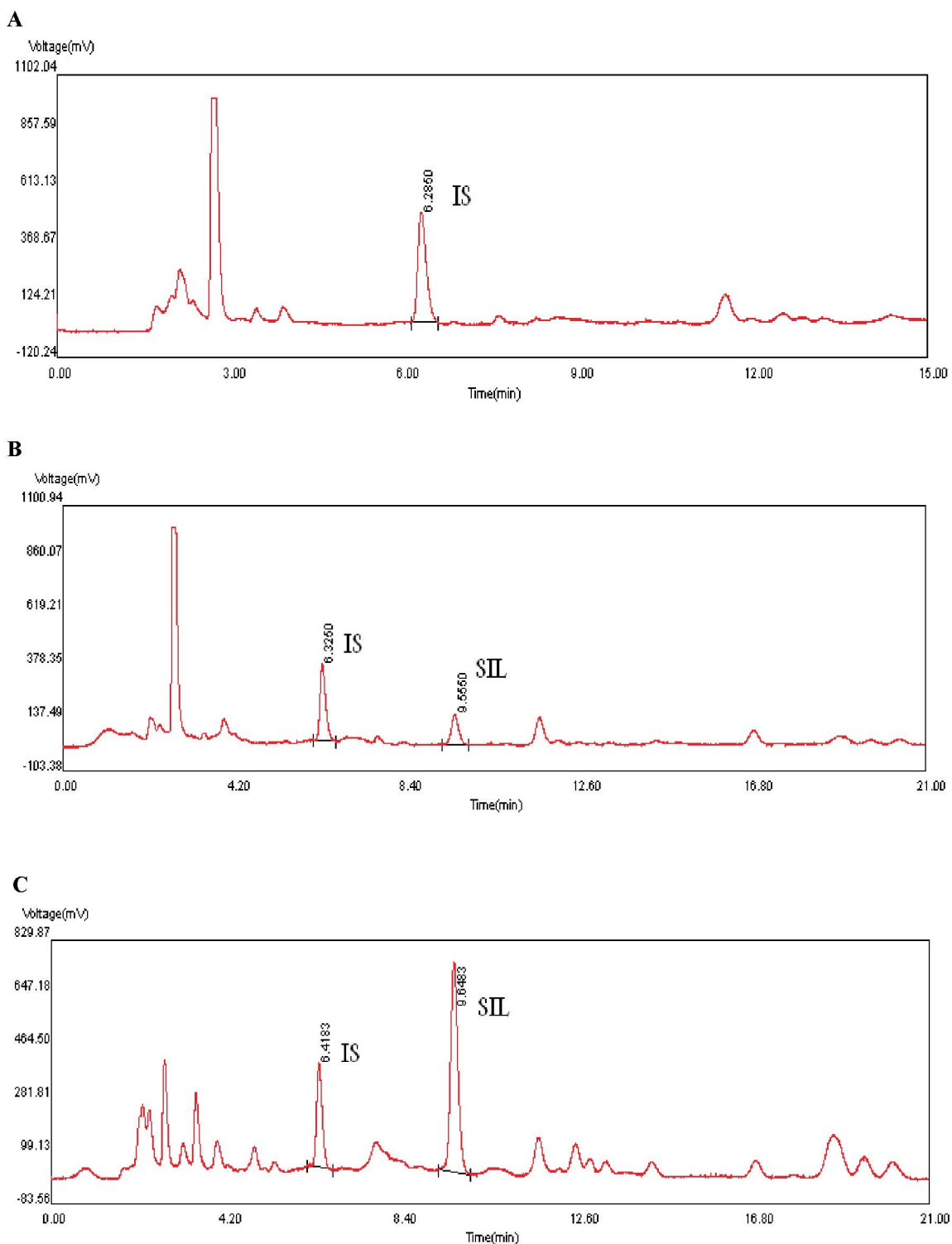
Figure 2.A: typical chromatogram of blank plasma spiked with 10 ng/mL of chlordiazepoxide as internal Standard (IS) - **B**: Chromatogram of blank plasma spiked with 10 ng/mL of chlordiazepoxide and 100 ng/mL of sildenafil (SIL) – **C**: chromatogram of plasma sample of a male volunteer 1 hour after administration of 100 mg sildenafil

Figure 3. Mean sildenafil plasma concentration - time profile in 11 human volunteers after oral administration of 2 x 50 mg Viagra tablets (each point represents the mean ± SE).

Table 2. Pharmacokinetic parameters of sildenafil following oral administration of 2 x 50mg Viagra in 11 human volunteers.

Subject	Ke	T$_{1/2}$ (hr.)	T$_{max}$ (hr.)	C$_{max}$ (µg/mL)	AUC $_{(0-24)}$ (µg/mL.hr)	AUC $_{(0-\infty)}$ (µg/mL.hr)
Mean±S.E.	0.32 ± 0.04	2.90 ± 0.71	1.13 ± 0.11	0.840 ± 0.05	2.79 ± 0.28	2.87 ± 0.29

DISCUSSION

We had applied another extraction method for determination of sildenafil in human plasma which was based on protein precipitation by adding methanol, high speed centrifugation and direct injection of supernatant to HPLC. Some difficulties, like lower sensitivity in result of sample dilution, poor separation of interfering components and lack of reliability, especially at low levels of drug, conducted us to use a method based on liquid-liquid extraction at basic pH and concentration of sample before injection. This method was the same which was used by Sheu et al (11) with some modifications. They were used butylparaben as internal standard (IS) and ethyl acetate for extraction of sildenafil from alkalinized plasma, followed by solvent evaporation. We used chlordiazpoxide (CDZ) as IS. The extractive behavior of CDZ resembled to both sildenafil and butylparaben but its chromatographic behavior was oppositely different from butylparaben. For faster evaporation of organic layer and taking cleaner samples, we replaced dichloromethane instead of ethyl acetate in extraction step. The method was reliable at the levels of 10 – 1000 ng/mL. The percentage of coefficient of variation was between 0.697 – 17.054 %. Recovery percentages in that concentration range were 94.2 – 107.08 % with an average of 100.64 %. Because of the low quantification limit level, this modified method was applicable in pharmacokinetic studies of sildenafil.

ACKNOWLEDGEMENT

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