Bioequivalency of Samagra tablet formulations of SDI in healthy volunteers using HPLC.

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(Received on 27/ 7 /2005) (Accepted for publication on 19/8/2006)

Abstract

A simple and reliable assay method in clinical laboratory was designed for determination of sildenafil citrate, in a plasma samples by using Solid_ Phase extraction (SPE, C-18) method and high_ performance liquid chromatography. Solid Phase extraction (SPE) was an efficient sample for extraction with a recovery of about 91%, sildenafil have found to have linear dynamic range of 5.0 _ 1000 ng /ml. Twenty healthy male volunteers with average age of 32±12 years old received 25 mg of each of the two sildenafil formulations; (SDI, Samagra) and Kamagra (India), there was a one week wash out period between doses. The plasma were purified on SPE mini column, then the drugs molecules were separated on reversed phase (250 X 4.6 mm i.d) C-18 column , using Acetonitrle : 50 μM formic acid buffer pH (4.5). (15 : 85 v/v) . The eluted drug were monitor on UV set at 230 nm. With a detection limit of 5.0 ng / ml. Plasma concentration-time curve were monitored by HPLC over a period of 18 hours after administration of both drugs. Maximum plasma sildenafil concentration C_{max} for Samagra was (150±7.15 ng/ml) and C_{max} for(Kamagra) India (160.5 ± 6.80 ng / ml) respectively both reach maximum concentrations of sildenafil at about 1 hour obtained from plasma concentration _ time curve data.The results indicate no significant difference between the two formulations,

therefore both medication of sildenafil are bioequivalent.

Keyword: Samagra , bioequivalency, HPLC separation

الخلاصة

صممت طريقة تحليلية بسيطة ومعتمدة في المختبرات الطبية لتقدير في نماذج البلازما بواسطة استخدام تقنية كروماتوغرافيا الطور العكوس ذات الأداء العالي .طريقة الاستخلاص بالطور العكوس للنماذج كانت كفوءه مع نسبة استرجاع 91% . عشرون متطوع من الذكور الأصحاء بأعمار تتراوح 32± 12 سنة استلموا25 ملغم من كل تركيبية . وبعد أسبوع واحد فقط ،استرجع المتطوعون حالة الدم الطبيعة .تم تنقية البلازما على عمود الطور العكوس ذو أبعاد (250 -4.6) وباستخدام اسيتونترايل وحامض الفورميك وبنسبة (15 :85) والدالة الحامضية عند (4.5) .الدواء الخارج من العمود تم تشخيصه باستخدام جهاز الفوق بنفسجي عند الطول الموجي 230 نانومتر مع حدود كشف 5.0 نانوغرام / مل .منحني تغير الزمن مع تركيز البلازما شخص باستخدام تقنية كروماتوغرافيا السائل ذات الأداء العالي بعد مرور 18 ساعة على إعطاء كلا الدوائيين.التركيز الأعظم للدوائيين في البلازما كان في حدود150(±7.15 نانوغرام/مل) و (160.5 ±6.8نانو غرام/مل) علي التوالي . من خلال النتائج يتضح بأنه لا يوجد اختلاف معنوي بين تركيبي الدوائيين لذلك سيكون هناك تكافوء حيوي بين الدوائيين.

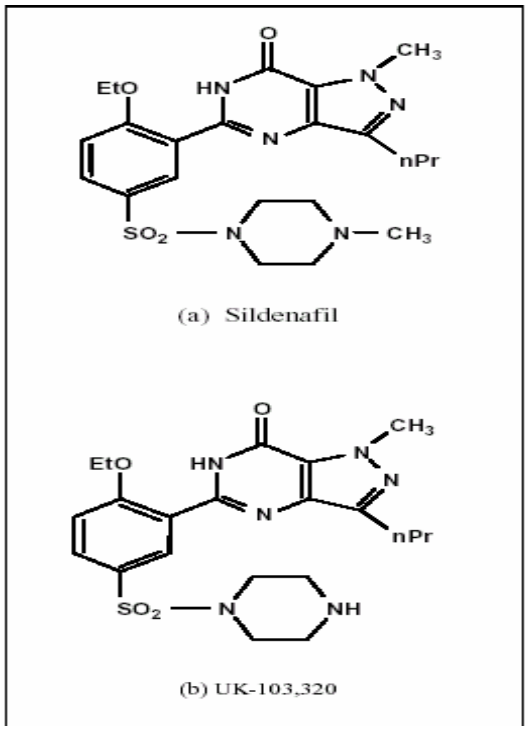
Introduction

Sildenafil citrate (Samagra) are used for treatment of ereticle dysfunction (ED),Sildenafil citrate is designed chemically as 1-[4-ethoxy-3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo-[4,3-d]pyrimidin-5-yl)phenylsulphonyl]-4-methylpiperazine citrate,(see Figure 1 a), it is a potent inhibitor of the cGMP-specific phosphodiesterase type 5 enzyme (PDE5) found predominantly In the penile corpus cavernosum (1). Cyclic guanosine monophosphate (cGMP), which is broken down by PDE5, is directly responsible for producing smooth muscle relaxation in the corpus cavernosum and allowing the inflow of blood. Thus, by inhibiting PDE5, sildenafil has the potential to improve male erectile function (2). After oral administration, sildenafil is rapidly absorbed, reaching peak plasma concentrations in 30- 120 minutes (3). It is metabolized in the liver predominantly to the active desmethyl metabolite, as shown in fig 1 (b)

Fig (1) represent sildenafil and their metabolite called desmethyl metabolite, (1-[4-ethoxy-3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-5-yl)phenyl-sulphonylpiperazine, see Figure (1) exhibits approximately the

same terminal half-life of about 4 hours.Sildenafil, when used properly, is relatively safe. There are, however, certain side effects that could create potential hazards. For example, Sildenafil has been shown to potentiate the hypotensive effects of nitrates commonly employed in the treatment of certain heart conditions (3). Moreover, while sildenafil inhibits PDE5, it also has a high affinity for phosphodiesterase type 6 (PDE6), which is a retinal enzyme involved in phototransduction (4,5). The inhibition of PDE6 can result in the inability to discriminate between blue and green colors, resulting in a condition known as _blue tinge_ (6). Although only about 3% of patients report visual disturbances, this blue-green impairment could cause problems in the execution of certain tasks.

There are several liquid chromatographic methods whichwere simultaneous determination of sildenafil citrate and metabolites in human plasma using column switching technique (7,8) and positive chemical ionization mass spectrometry (9).In this work accurate sensitive and reliable reversed phase methods were adopted for analysis of sildenafil citrate after preconcentration using solid phase extraction cartridge mini column type C-18, the methods are suitable for pharmacokinetics studies and pharmaceutical analysis.



Materials and Methods

Reagents, Standards and Supplies.

All solvents were of HPLC-grade obtained from Fisher Scientific (Fischer Scientific Co.,). Double distilled water was prepared using a Millipore model Milli-QT bench-top purification device (Millipore, Continental Water Systems) and was used for all reagent preparations. Formic acid was obtained from Fisher Scientific Co. . Sildenafil -was obtained from Samara pharmaceutical company as pure standard purchased from Sigma (Sigma Co.,). A standard of sildenafil was prepared at 1 mg/mL in acetonitrile. The HPLC buffer was 50 µM formic acid adjusted to pH 4.50 with ammonium hydroxide.

Instrumentation .

Analyte separation was achieved using Shimadzu 10AVTp HPLC (Japan,Koyot Co.) equipped with a LC-18 guard column (4.0 mm x 3.0 mm i.d., 40 µM particles) followed by a Supelcosil LC-18 column (150 mm x 4.6 mm i.d., 5 µM particles) from Supelco (Bellefonte, PA). Samples were injected using a Rheodyne 7125 U.S.A sample injector with 750 ul loop. Identification and quantitation were

accomplished using UV-Visible detector model SPD-6A set at 230 nm .Control of HPLC system, integration of the chromatographic peaks and concentration was carried out using CR-8A data processor .The binary gradient HPLC system was run at 1 mL/min and a mobile phase composition of acetonitrile/ formic acid buffer (15::85,v/v). The sample injection volume was 750 µL. Retention time for sildenafil was approximately 5.5 minutes.

Calibrators and controls .

Calibration curves were prepared in plasma at concentrations ranging from 5-1000 ng/ml. A minimum of 8 calibrator values were used to construct the linear calibration curve as shown in fig 2. Controls used for the determination of accuracy, precision, and stability were prepared in plasma at 50 and 200 ng/ml using drug standards prepared separately from those used for the calibrators. Controls were prepared in pools large enough to provide samples for the entire study.

Analyte concentrations were determined by comparison peaks area of sample with that of the authentic standard under the same optimal separation condition .

Sample Extraction .

The blood samples were collected from 20 healthy male volunteers on a rotary extractor for 15 minutes. Centrifugation at 900 x g for 5 minutes provided removal of proteins. The supernatant was transferred to 15 ml vials and evaporated in a water bath at 40°C under for each sildenafil for mula in different times between (0.5-18 hr) after oral administration of 25 mg of sildenafil citrate tablets.Calibrators, and plasma specimens were prepared and extracted in the following manner., liquids sample were diluted with water (1:1 v/v)The samples were vortexes and allowed standing for 10 minutes. To these were added 9 ml ice cold acetonitrile, and the combinations were mixed a stream of dry nitrogen to a volume less than 1 ml. To this was added 4 ml 0.1 M phosphate buffer, pH 6.0. The extracts were transferred to solid phase extraction (SPE) columns, which were preconditioned with 2 ml

methanol, followed by 3 ml 0.1 M phosphate buffer, pH 6.0. The SPE columns were Bond Elute columns obtained from Varian (Varian Co.,.). Care was taken not to dry the column prior to extract addition. Column flow rates of 1mL/min were maintained in each step using a Varian 24 port pressure manifold with a nitrogen pressure of 3 pound per square (psi). Once the samples had passed through the columns, the columns were washed with 1 ml of 1 M acetic acid, followed by 6 ml methanol, then dried completely between each wash with 25 psi nitrogen for 5 minutes. The analytes were eluted off the columns with 4 ml of 2% ammonium hydroxide in ethyl acetate, which was prepared daily. Eluents were evaporated to dryness in a water bath at 40°C under a stream of dry nitrogen, brought up in 500 µL acetonitrile and transferred to sample vials for analysis.

Recovery:

The recovery of Sildenafil was determined by spiked the plasma of volunteers with 50–200ng/ml of drug prior to extraction, after extraction the concentration was calculated with a recovery of 91% for both concentrations.

Results and Discussion

The procedure described herein provides a fast, reproducible, and accurate method for the determination of sildenafil, using solid-phase extraction and HPLC using Uv detector set at 230 nm. The use of solid phase extraction provided a cleaner sample and required less organic solvent than did an alternative liquid-liquid extraction procedure. The extraction efficiency for the SPE was also notably superior to that of the liquid -liquid extraction. The average recovery of sildenafil at a concentration of 50 ng/ml were 92.2 ± 7.3 % and 91.2 for concentration of 200 ng/ml. Sildenafil, peak was completely resolved and experienced no interference from endogenous sample matrix components. All analytes were eluted from the column in less than 10 minutes and had theoretical plates ranging from 4000 to 8000. Figure 2 shows a typical chromatogram of sildenafil , LC retention times were additionally used as analyte acceptability criteria and were required to be within 2% of the average calibrator retention time. A typical retention time was 5.5 minutes.

The detection limit was 5 ng/ml when the injector loop were changed

from 20 µl to 750 µl ,therefore 10 ng/ml show a reasonable peak as shown in fig 2.0 The plasma concentration-time curve for both drug were represented in fig 3 and 4, while the comparison of plasma concentration-time curve were shown in fig 5.

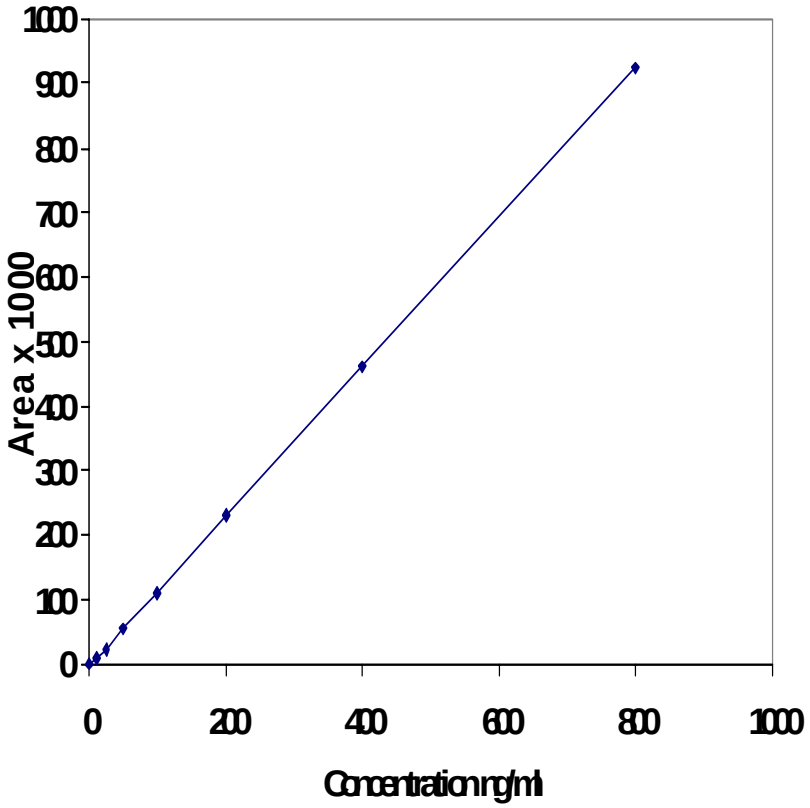
The linear dynamic range (LDR), limit of detection and lower limit of quantitation were determined by analysis of blood spiked with the analytes. The LDR of the calibration curves were 10- 1000 ng/ml for sildenafil .The correlation coefficients were exceeded 0.998. A representative curve is shown in Figure 2.

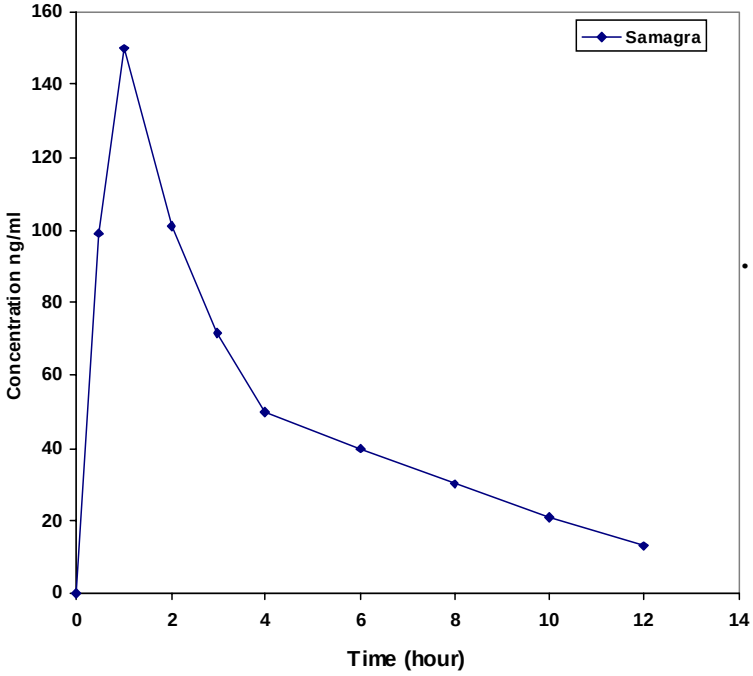
The concentration of sildenafil were measured in serum of 20 healthy volunteers for both formula in interval time range from 0.5-18 hrs, then pharmacokinetic parameters (K_{ab}, K_{elemin}, $t_{1/2}$, and T_{max}) were tabulated in table 1.

The accuracy and precision for sildenafil citrate were determined by measuring the relative standard error between the experimentally determined and prepared concentration of the sample,. The stability of sildenafil in blood serum were evaluated by measure the control value after 1 week , the measured concentration show no apparent decrease in the concentration after 1 week at 4 ºC.

Table 1. Pharmacokinetic parameters of samagra and kamagra tablets (25 mg) administrated to 20 healthy volunteers.

Compound	Ka	Ka 0.5t	Kelm.	Kelem 0.5t	Cmax	Tmax	AUC
Mean samagra	0.816	0.848	0.1608	4.385	150.0	1	810.1
±SD	0.053	0.055	0.0223	0.579	7.159		53.09
Mean kamagra	0.789	0.881	0.148	4.7704	160.5	1	905.4
±SD	0.046	0.052	0.015	0.5022	6.817	0	56.36


Area x 1000
Concentration ng/ml
0
100
200
300
400
500
600
700
800
900
1000
0
200
400
600
800
1000


Samagra
Concentration ng/ml
Time (hour)
0
20
40
60
80
100
120
140
160
0
2
4
6
8
10
12
14

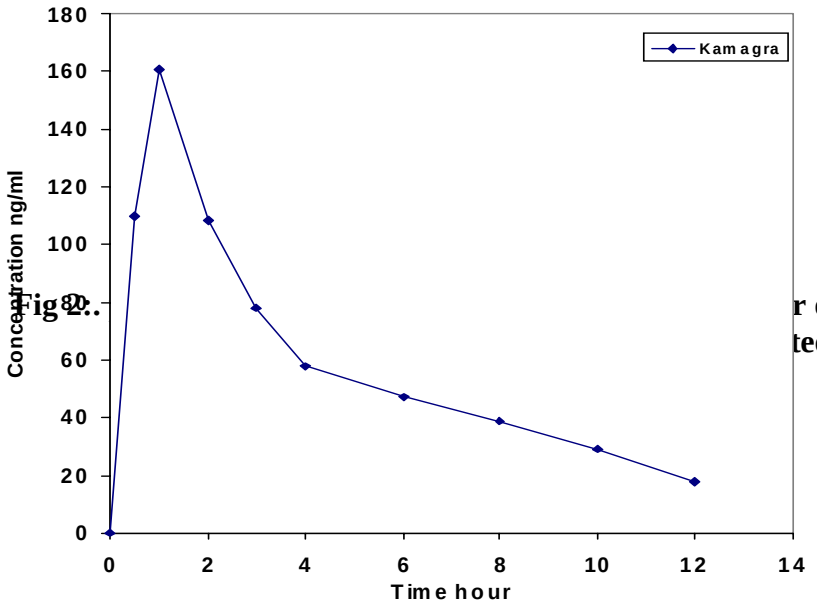


Fig 2.. r oral
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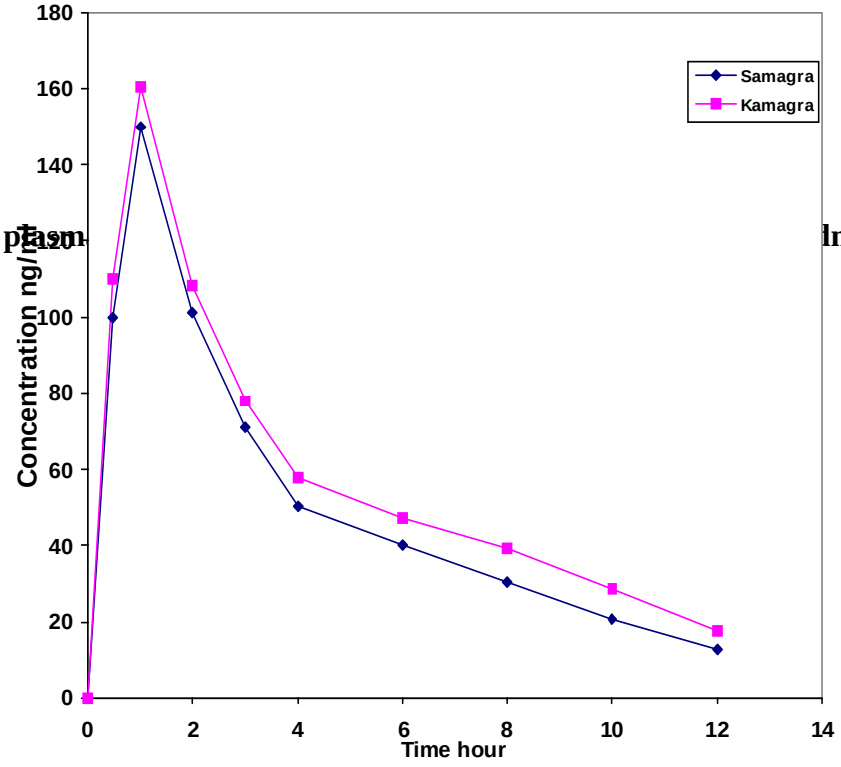

Fig 3: plasm- lministration

Fig 4: Comparison of mean plasma concentration _ time profile of reference drug kamagra test drug Samagra .

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