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# BIOEQUIVALENCY OF SILDENAFIL CITRATE TABLET FORMULATIONS OF SAMAGRA AND KAMAGRA USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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## ABSTRACT

This study was designed to establish a simple and reliable assay method in clinical laboratory for determination of sildenafil citrate, in a plasma samples by using solid– phase extraction (SPE, C-18) method and high– performance liquid chromatography (HPLC). Solid phase extraction (SPE) was an efficient sample extraction with a recovery of about 91%. Sildenafil was found to have linear dynamic range of 0.0 – 500 ng/mL. Twenty healthy male volunteers with average age of 32 ± 12 years old received 50 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 and 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 drugs were monitored 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 12 hours after administration of both the drugs. Maximum plasma sildenafil concentration $C_{max}$ for Samagra was (232+17.29 ng/mL) and $C_{max}$ for (Kamagra) India (228 ± 11.53 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 and therefore, both medication of sildenafil are bioequivalent.

**Key words**: Samagra, Kamagra, Sildenafil, Bioequivalency, HPLC.

## INTRODUCTION

Sildenafil citrate (Samagra) is used for treatment of ereticle dysfunction (ED). Sildenafil citrate is chemically. 1-[4-ethoxy-3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-

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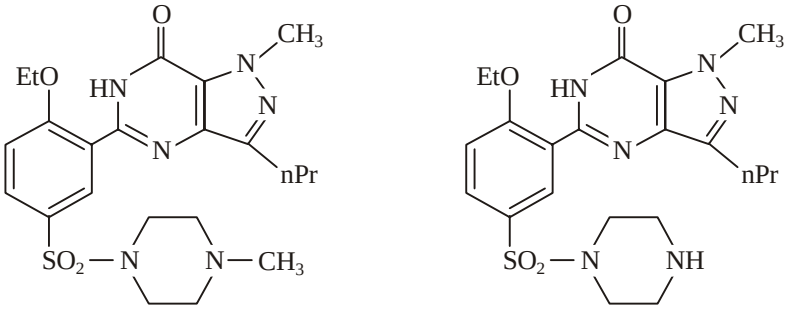
pyrazolo-[4,3-d]pyrimidin-5-yl) phenylsulphonyl]-4-methylpiperazine citrate, (shown in Figure 1 a). It is a potent inhibitor of the cGMP Cyclic guanosine monophosphate -specific phosphodiesterase type 5 enzyme (PDE5) found predominantly in the penile corpus cavernosum[1,2]. (cGMP), which is broken down 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[3,4]. After oral administration, sildenafil is rapidly absorbed, reaching peak plasma concentrations in 30-120 minutes (median 1 hr)[5]. It is metabolized in the liver predominantly to the active desmethyl metabolite,[6] Fig. 1(b).




**(a) Sildenafil** **(b) UK - 103, 320**

**Fig. 1: (a) Standard sample and (b) Extracted sample**

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[5]. Moreover, while sildenafil inhibits PDE5, it also has a high affinity for phosphodiesterase type 6 (PDE6), which is a retinal enzyme involved in phototransduction[7,8]. The inhibition of PDE6 can result in the inability to discriminate between blue and green colors, resulting in a condition known as “blue tinge”[9]. 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 for simultaneous determination of sildenafil citrate and metabolites in human plasma using column switching technique[10,11] and positive chemical ionization mass spectrometry[12,13].

In this work, an accurate, sensitive and reliable reversed phase method has been adopted for analysis of sildenafil citrate after preconcentration using solid phase extraction

cartridge mini column type C-18. This method is suitable for pharmacokinetics studies and pharmaceutical analysis.

## EXPERIMENTAL

### Materials and method

### Reagents, standards and supplies

All solvents were of HPLC-grade and were 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. Sildenafil was obtained from Sammara 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, Kyoto 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 µL 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 0-500 ng/mL. A minimum of 7 calibrator values was used to construct the linear calibration curve (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 of 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 for each sildenafil formula in different times between (0.0-12 hr) after oral administration of 50 mg of sildenafil citrate tablets (Tables 1 and 2).

**Table 1: Average plasma concentration of Samagra (SDI) (ng/mL) with time after oral administration of 50 mg Samagra tablets to 20 healthy volunteers**

| Subj. No. | Time (hr) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **0** | **0.5** | **1** | **2** | **3** | **4** | **6** | **8** | **10** | **12** |
| **Mean** | 0 | 164.9 | 232.4 | 170.25 | 119.95 | 90.4 | 45.1 | 32 | 24.6 | 18.35 |
| **± SD** | 0 | 15.49 | 17.29 | 8.44 | 8.66 | 6.90 | 4.31 | 3.32 | 4.95 | 2.47 |

**Table 2: Average plasma concentrations (ng/mL) with time after oral administration of 50 mg (Kamagra, India) tablet to 20 healthy volunteers**

| Subj. No. | Time (hr) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **0** | **0.5** | **1** | **2** | **3** | **4** | **6** | **8** | **10** | **12** |
| **Mean** | 0 | 178.2 | 228.05 | 173.5 | 123.05 | 88.85 | 43.77 | 33.2 | 26.4 | 17.35 |
| **± SD** | 0 | 12.35 | 11.53 | 10.75 | 9.00 | 8.07 | 6.95 | 5.14 | 5.18 | 3.49 |

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, 9 mL ice cold acetonitrile, and the combinations was added were mixed 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 a stream of dry nitrogen to a volume less than 1 mL. To this 4 mL 0.1 M phosphate buffer, pH 6.0 was added. 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 column obtained from Varian (Varian Co.). Care was taken not to dry the column prior to extract addition. Column flow rates of 1 mL/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, and 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 spiking the plasma of volunteers with 50 and 200 ng/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 ± 6.5 at 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. Fig. 2 shows a typical chromatogram of sildenafil for both; standard and sample, 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 (Fig. 2). The average plasma concentration-time curve for both drug indicated no significance between the two drugs. 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 0-500 ng/mL for sildenafil. The correlation coefficients exceeded 0.998.

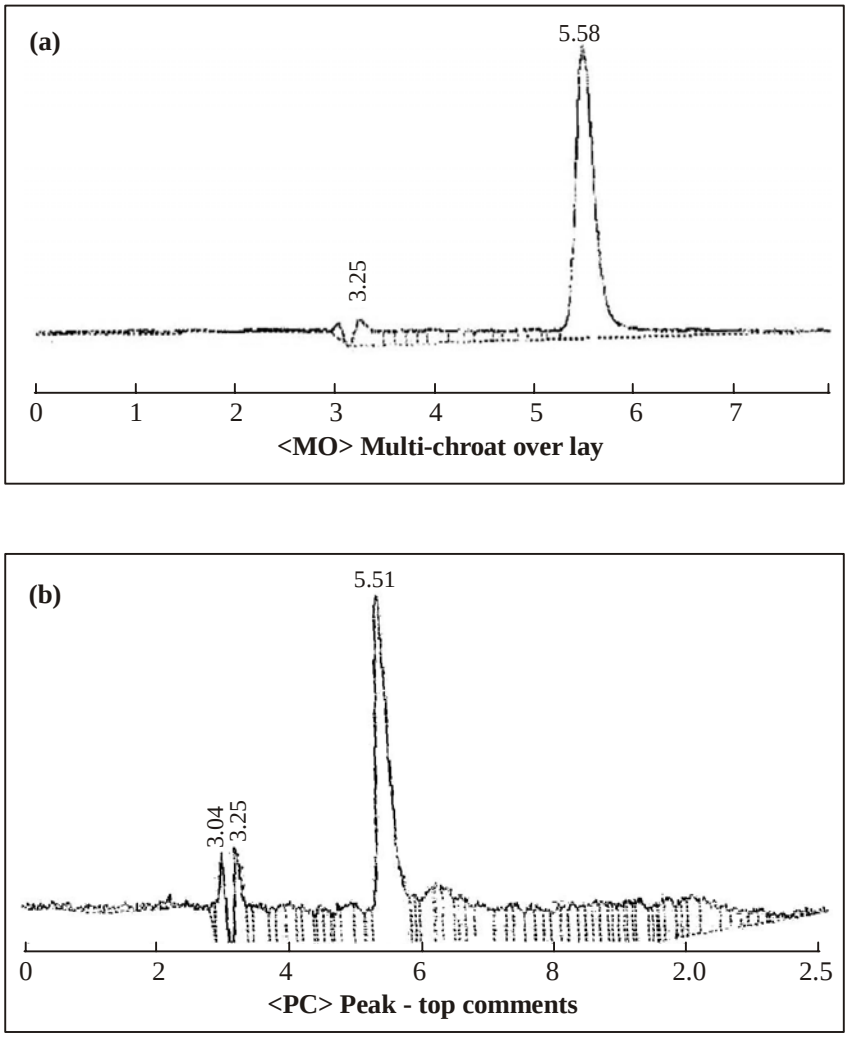



**Fig. 2: Comparison of mean concentration-time profile of (a) Standard drug Samagra and (b) reference drug Kamagra**

The concentration of sildenafil were measured in serum of 20 healthy volunteers for both formula in interval time range from 0.0-12 hrs and the pharmacokinetic parameters ($K_{ab}$, $K_{ele.}$, $t_{1/2}$, and $T_{max}$) are shown in Table 3.

The stability of sildenafil in blood serum were evaluated by measuring the control value after 1 week. The measured concentration shows no apparent decrease in the concentration after 1 week at 4ºC.

**Table 3: Pharmacokinetics data of Samagra (SDI) and Kamagra 50 mg tablets after oral administration for twenty healthy volunteers**

| No. | Kele. | Half life (hr) | $C_{max}$ | $T_{max}$ (hr) | AUC |
|---|---|---|---|---|---|
| **Samagra mean** | **0.138** | **5.02** | **232.4** | **1.0** | **562** |
| **± SD** | | | **± 17.29** | | **± 47.8** |
| **Kamagra mean** | **0.137** | **5.1** | **228** | **1.0** | **572** |
| **± SD** | | | **± 11.53** | | **± 52.4** |

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