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Simple and sensitive LC-ESI-MS method for estimation of sildenafil in plasma samples

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Abstract

*The quantity of sildenafil in human plasma was determined using a simple and incredibly accurate LC-ESI-MS approach. Thermo, Hypurity Cl8, 100*4.6 mm, 5µm, was injected with sildenafil and sildenafil d3 (internal standard), which were extracted from the plasma using the solid phase extraction technique. Acetonitrile: 0.1% Formic Acid (70:30, v/v) made up the mobile phase, which was supplied isocratically at a flow rate of 0.6 mL/min. The run time was 3 minutes, and the MRM mode for sildenafil and internal standard sildenafil d3 was found at m/z 475.2→283.2 and 478.2→283.2. The calibration curve for sildenafil was linear between 25.0 and 2000 ng/mL, and the detection limit was 25.0 ng/mL. A validated LC-MS/MS method was used to determine drug concentrations.*

Keywords: LC-MS/MS, Plasma, Validated method, Sildenafil, SAS, Statistical analysis.

Introduction

[4,3d]pyrimidin-5-yl)phenyl-sulfonyl,1-[4-ethoxy-3-(6,7-dihydro-1-methyl-7-oxo-3-propyl1H-pyrazolo] A common treatment for male erectile dysfunction of several causes is 4-methyl piperazine, a strong and selective inhibitor of the phosphodiesterase type 5 enzyme. Throughout the suggested dosage range, it exhibits dose-proportionate behavior. After receiving 100 mg orally, healthy male volunteers' mean maximal sildenafil plasma concentration is 450ng/mL. With a half-life of roughly four hours, it is primarily removed by hepatic metabolism (cytochrome P450 (CYP) enzymes, CYP3A4, the major pathway, and CYP2C9, the minor route). CYP3A4 inhibitors can prevent sildenafil from being metabolized by the liver. Cimetidine, erythromycin, clarithromycin, ketoconazole, itraconazole, ritonavir, and saquinavir are a few examples of them. As a result, sildenafil combined with one of these inhibitors may significantly raise[1-5].

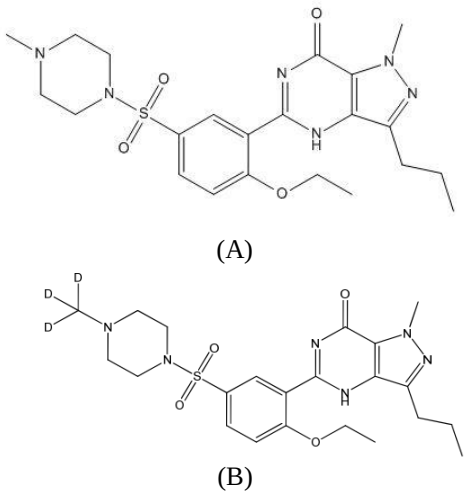
Reliable data must be made available in order to enable an effective risk assessment and provide the necessary level of protection. Stated differently, it is necessary to build advanced and reliable analytical methods. Many techniques have been published for identifying sildanafil in plasma, urine, post-mortem fluids, and tissues. These techniques often involve the use of HPLC combined with UV detection, electrochemical detection, or HPLC coupled with MS/MS. Owing to its cost-effective adaptability, LC in conjunction with ESI mass spectrometry (LC-MS) has emerged as a preferred analytical method for high-throughput drug concentration assessments in biological samples derived from toxicological or pharmacokinetic research. The literature only contains one article on the use of gradient elution and LC-MS with API to measure[6-11].

Alkalinizing the sample, combining extraction solvents, and using a large amount of plasma (3 mL) were all part of the process. According to Pistos and colleagues, most methods that were considered accurate and sensitive in the literature turn out to be problematic when applied to human blood. Due to the fact that human plasma is often the only biological fluid available or the preferred biological fluid for analyzing drug dispositions, an ESI-based, user-friendly, fast, precise, and sensitive LC-MS method was developed to identify sildenafil in human plasma. This method allows the use of an isocratic mobile phase and covers a wide range of sildenafil concentrations for different analytical applications, such as pharmacokinetic and toxicological research. 90% ACN plus 10% ammonium acetate (20mM) with 0.02% formic acid was the mobile phase used, and it was supplied isocratically at a flow rate of 0.2mL/min. Prior to use, 0.22mm filters were used to filter and degas the mobile phase. The ESI operating in the positive mode was provided with optimized MS conditions, which included a capillary voltage of 3.8kV, a cone voltage of 50 V, a source temperature of 1001 C, and a desolvation temperature of 3001 C. Cone gas was set at 50L/h, and nitrogen, the desolvation gas, was set at 250 L/h[12-14].

Materials and Methods

Reagents and chemicals: Sildenafil was from Clearsynth labs ltd and the internal standard (IS, Sildenafil d3) was obtained from Clearsynth labs ltd. Acetonitrile and methanol of HPLC grade were obtained from J.T. Baker Chemical Company and Fisher ltd respectively. HPLC grade water was purified by means of a Milli-Q, HPLC & Spectroscopy water was manufactured by Finar. HPLC grade formic acid was obtained from Biosolve. Dimethyl Sulphoxide grade formic acid was obtained from Biosolve.

Analytical system: Sildenafil levels in plasma were measured using the LC-MS/MS TRIPLE QUAD 4500 (Sciex) and HPLC (Exion LC TM) equipped with a quadrupole mass analyzer and an ESI probe. Data collection and LC-MS system control were conducted using Sciexanalyzers 1.7.1 and 1.7.2. Hypurity Cl8 compounds measuring 100x4.6mm and 5µm were separated on a Thermo. The mobile phase, which was supplied isocratically at a flow rate of 0.6 mL/min, was acetonitrile: 0.1% formic acid (70:30, v/v). The mobile phase was filtered and degassed using 0.22 mm filters before use. The MRM mode of sildenafil and internal standard sildenafil D3 were found at m/z 475.2 → 283.2 and 478.2 → 283.2. The ideal MS conditions for positive mode ESI.



(A)

(B)

Figure-1: Chemical structures of sildenafil (A) and sildenafil D3 (B).

Preparation of Calibration Standards and Quality Control Samples: A stock solution containing 5 mg/ml of sildenafil was prepared by mixing methanol and dimethyl sulfoxide. The stock solution was serially diluted with diluent solution (methanol: water: 50:50v/v) to produce standard sildenafil solutions (1.0, 2.0, 10.0, 20.0, 40.0, 60.0, 80.0, and 100.0ng/ml). Sildenafil (QC) solutions (4.0, 10.0, 40.0, and 80.0 ng/ml) were prepared in a similar manner for low, medium-1, medium, and high concentration quality control. The IS sildenafil d3 stock solution (1.0mg/ml) was likewise made in methanol and dimethyl sulfoxide. It was subsequently diluted to a final concentration of 2000.0ng/ml using a diluent solution (methanol: water: 50:50, v/v). Until used, all solutions were kept between 2 and 8°C.

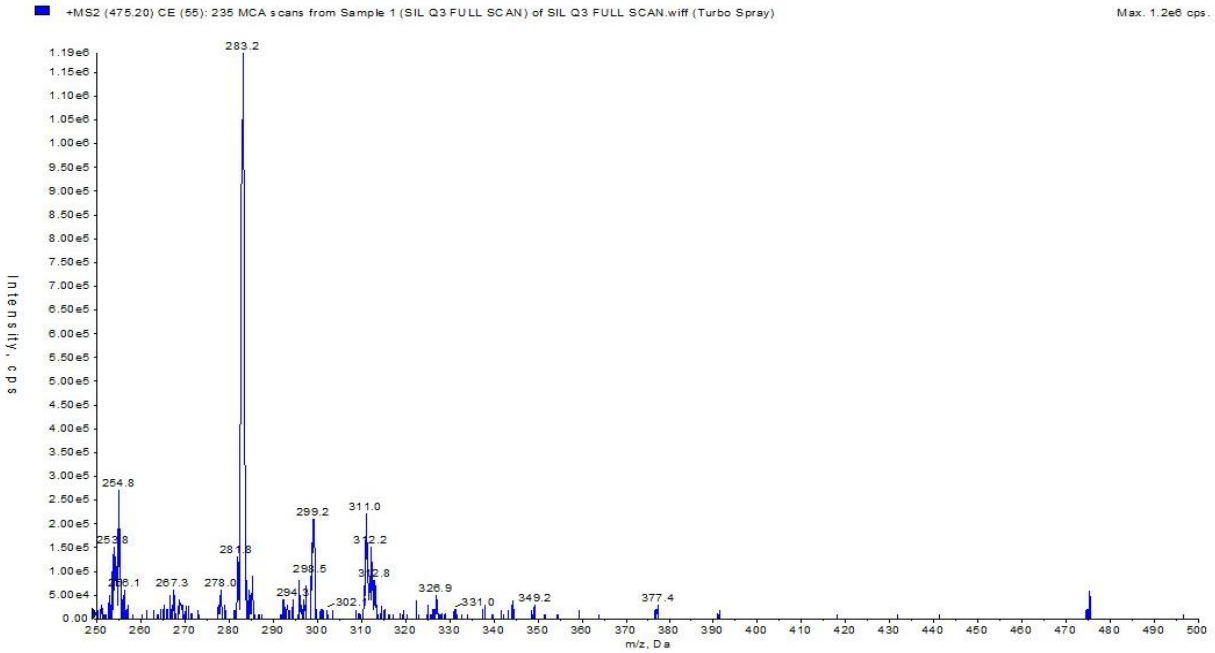
Sample preparation: Take out the necessary set of CC and QC samples from the deep freezer, along with the subject samples (if the analysis is for a project sample), let them thaw at room temperature, and then arrange the samples according to batch sequence. To guarantee thorough mixing, vortex the defrosted samples. Place 200µl of the plasma sample into a Ria vial that has already been labeled. To each pre-labelled Ria vial, add 50 µl of the internal standard dilution solution (1950.800 ng/mL for Sildenafil D3), excluding the blank sample, then, vortex the blank sample for a short while with 50µl of the diluent solution. Pour 200 µl of a 0.1% formic acid solution into each sample, then give them a brief vortex. Utilize Orochem Panther Deluxe Cartridges and the SPE Approach. Conditioning: 1 ml of methanol. 1 ml of water for equilibration. Fill cartridges with all of the samples. 1 ml of water for Wash-I. Wash-II: 1 ml of water containing 5% methanol. Use 1 ml of mobile phase to elute the samples, then vortex for a few seconds. Place the samples in auto sampler vials that have been previously labeled. Note: All activity shall be carried out at room temperature.

Analysis of Patient Samples: The previously developed bioanalytical method was utilized to measure the concentrations of sildenafil in plasma after oral administration to healthy human subjects. Synergen Bio Pvt. was the company that hired these volunteers. Ltd. Pune, India. A 100 mg dose (one 100mg tablet) was given orally to each of the 32 healthy volunteers along with 240ml of drinking water. The test Sildenafil Citrate Tablets T (formulation) and the product Viagra tablets R (Pfizer, Netherlands) 100mg were utilized. Every subject will have twenty (20) blood samples taken during each period. In every period, a blood sample of 05 mL for the pre-dose (0.00 hours) will be drawn no later than one hour before the dose. Further samples of 05mL each will be collected and at 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.5, 3.00, 3.50, 4.00, 5.00, 6.00, 8.00, 10.00, 12.00, 16.00 and 24.00 post-dose m each period. The subjects will leave the facility after the 24.00-hour blood sample has been taken. The post-dose samples will be collected within 02 minutes of the scheduled sampling time.

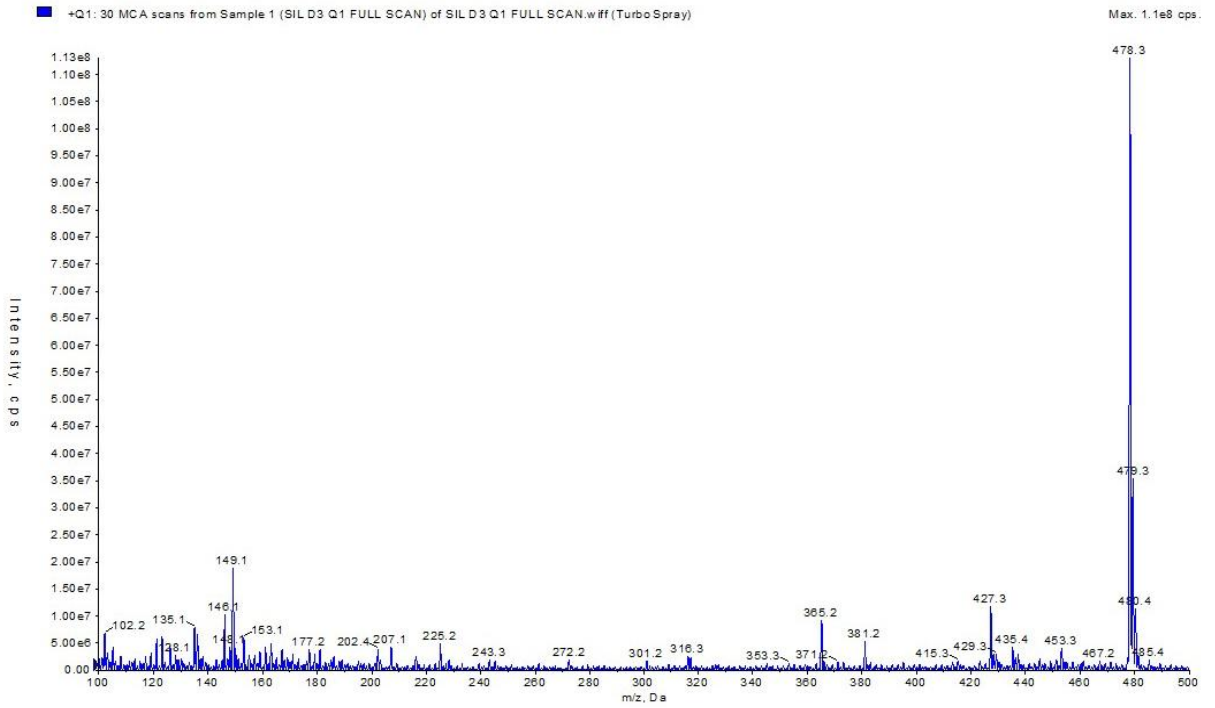
To reduce blood clotting in the indwelling cannula, a standard saline solution (0.5mL) will be injected into the cannula following each sample collection. After that, they were frozen at -70±10°C until the sample was analyzed. The identical human volunteers underwent separate fasting periods and appropriate washing intervals in accordance with protocol to receive the test and reference.

Pharmacokinetics (PK) and Statistical Analysis: SAS (SAS Institute Inc.) was used to calculate the pharmacokinetics (PK) parameters from human plasma samples. U. s. An. ( ). Visual inspection of the plasma sildenafil concentration-time profiles was done in order to determine the Cmax and Tmax values. For sildenafil, the primary pharmacokinetic parameters were Cmax and AUC0-t. Using the linear trapezoidal method, the AUC0–t was determined. The AUC0-∞ was determined up to the final concentration that could be measured. Based on this concentration and the terminal elimination rate constant (Kel), which was estimated using the linear regression method on the slope of the plasma concentration-time curve of sildenafil, extrapolations were obtained. Next, 0.693/Kel was determined to be the terminal elimination half-life, or t1/2.

Statistical analysis of the pharmacokinetic parameters will be performed using SAS (SAS Institute Inc., U.S.A.). For the pharmacokinetic parameters, descriptive statistics were calculated and presented. The natural log-transformed Primary Pharmacokinetic parameters Cmax and AUC0-t forsildenafil will be subjected to an ANOVA (Analysis of Variance) in accordance with the two-sided test procedure for bioequivalence.



(I)



(II)

Figure-2: (I) sildenafil and (II) sildenafil D3 MSMS Positive Scan.

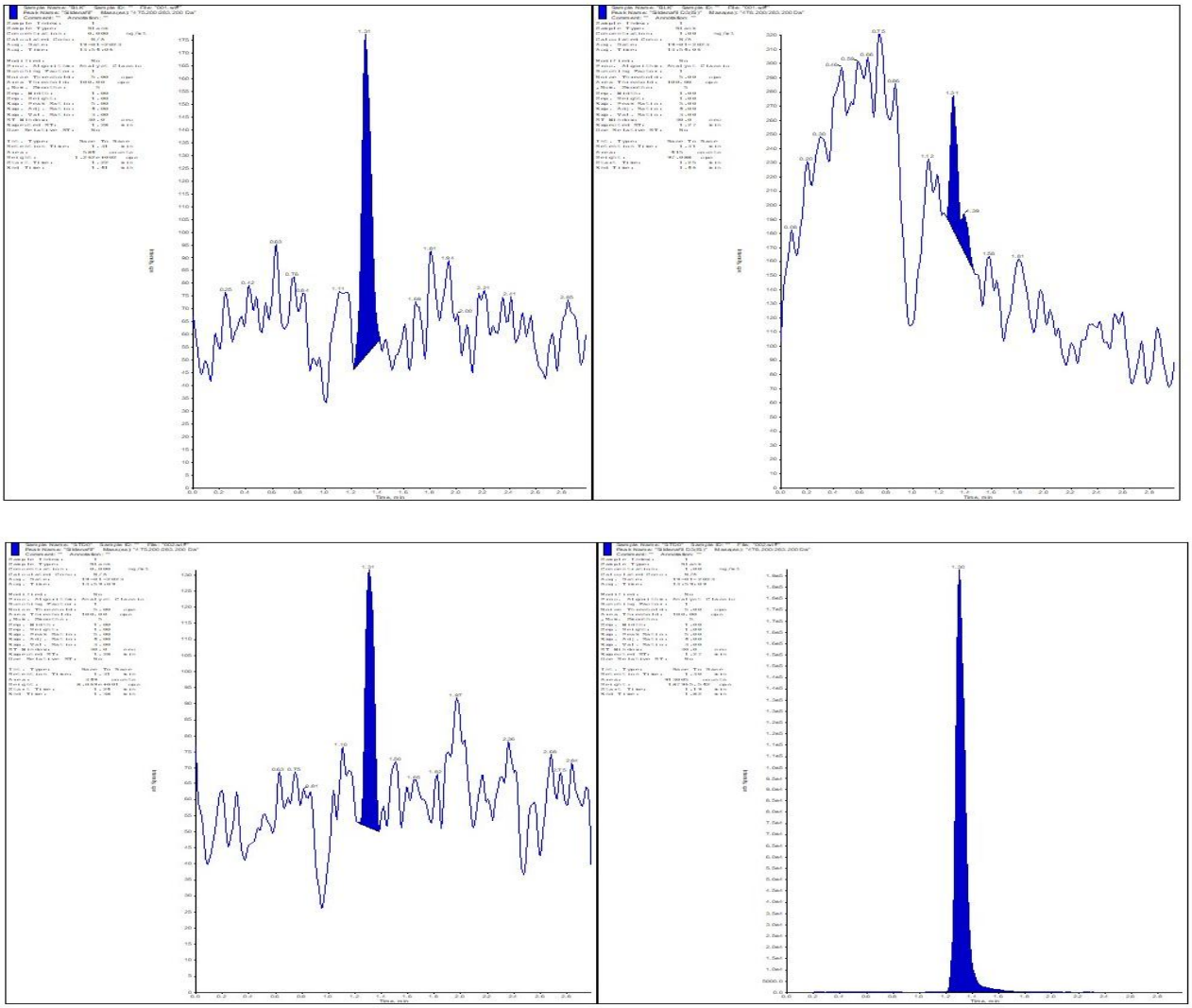


Figure-3: Chromatograms of (I) Blank plasma and (II) IS Sildenafil D3.

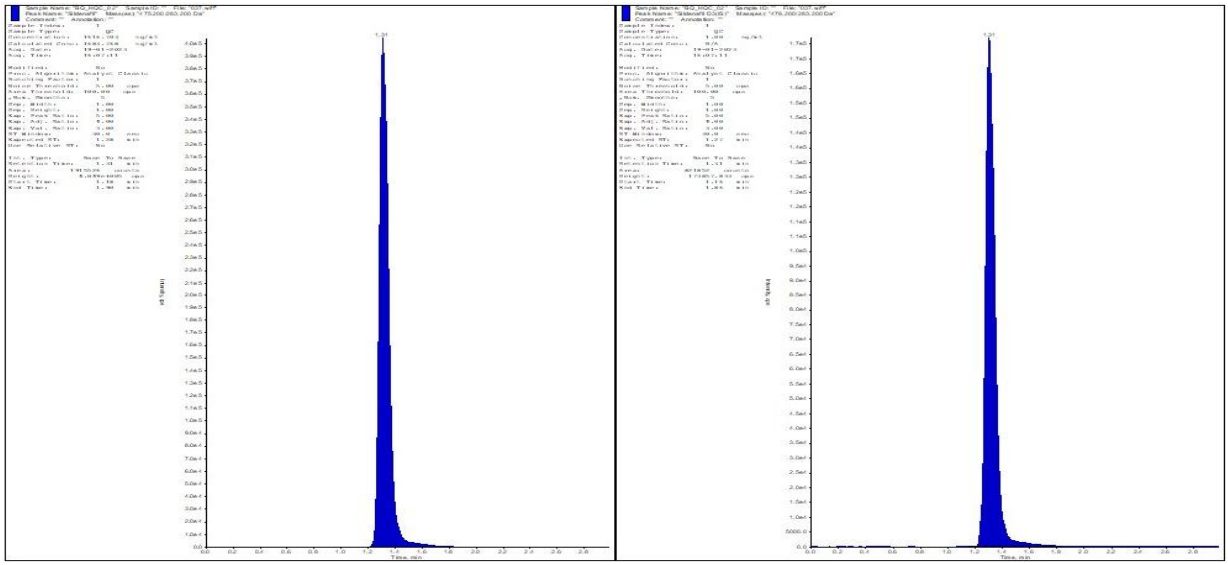


Figure-4: Chromatograms of LLOQ Sildenafil and it IS Sildenafil D3.

Results and Discussion

Because of its selectivity, sensitivity, and reproducibility, LC-MS/MS validation has proven to be one of the most helpful analytical tools in clinical pharmacokinetics. This study aims to measure the amount of the medication sildenafil in human plasma. To maximize the signal of the analytes and internal standard and achieve the best resolution, a number of experiments were conducted to optimize the composition and nature of the mobile phase. This procedure combines solid phase extraction (LCMS/MS) with ESI triple quad 4500 MS chromatographic conditions. Data collected from 32 participants who finished the study were subjected to pharmacokinetics (PK) and statistical analysis using SAS.

Graphical representation of Mean Plasma Concentrations versus Time in gives following two figures:

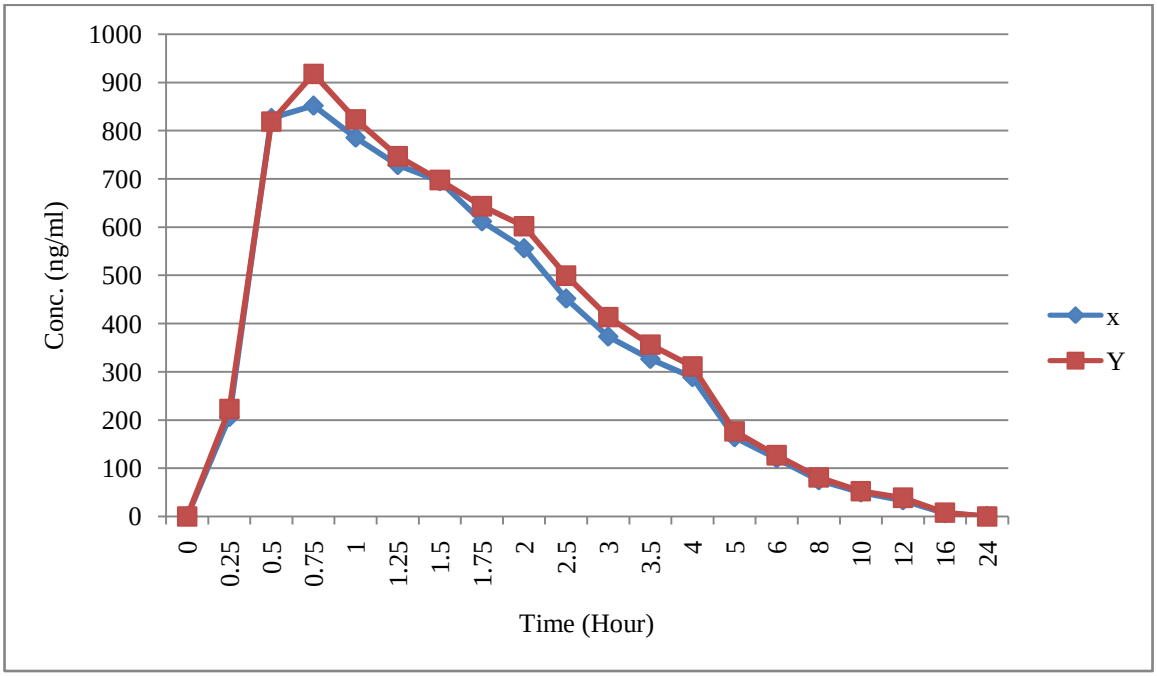


Figure-5: Mean Linear Plot of Mean Plasma Concentrations (ng/mL) of Sildenafil vs. Time (hr).

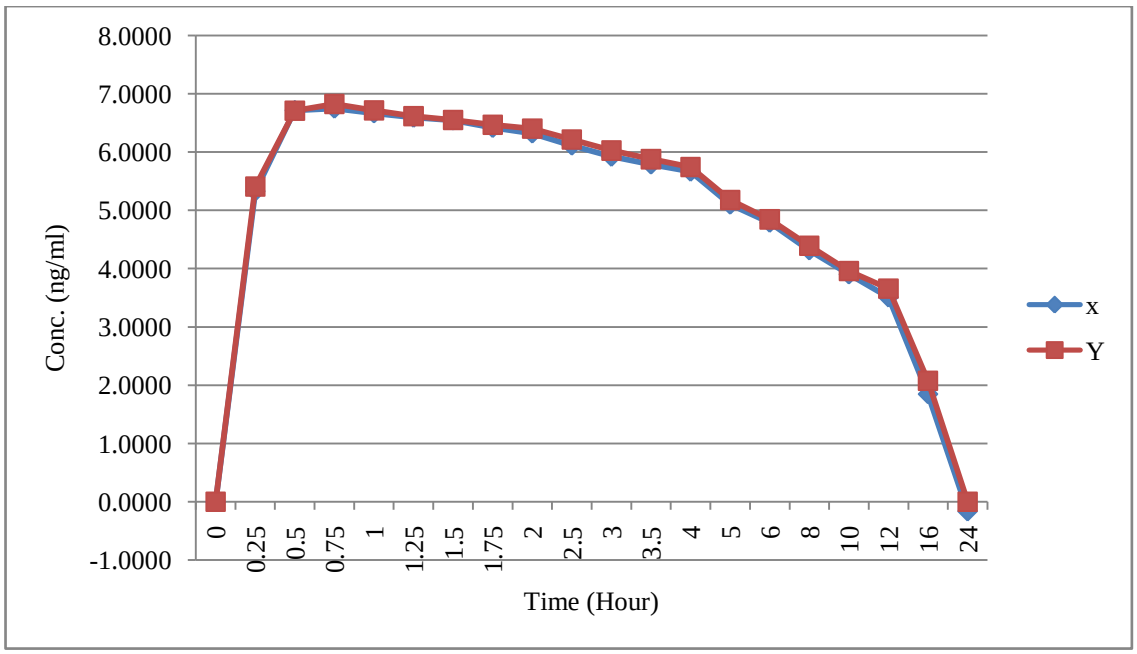


Figure-6: Mean Log-Linear Plot of Mean Plasma Concentrations (ng/mL) of Sildenafil vs. Time(hr).

Table-1: Pharmacokinetic parameters for sildenafil: Descriptive Statistics of Pharmacokinetic (PK) Parameters of Test Product (T) and Reference Product (R) for Sildenafil.

Form	PK Parameters	Mean	SD	CV%	Minimum	Median	Maximum
R	C_{max} (ng/mL)	1089.2416	399.0547	36.6360	451.7080	1037.2905	1877.6800
	T_{max} (hr)	0.8600	0.4751	55.2477	0.2500	0.7500	2.0000
	AUC_{0-t} (ng.hr/mL)	2840.5418	1070.8198	37.6977	791.1810	2670.5820	5439.3080
	$AUC_{0-\infty}$ (ng.hr/mL)	3011.6470	1103.1163	36.6283	919.4450	2843.9260	5662.6280
	K_{el} (hr^{-1})	0.2452	0.0921	37.5701	0.1220	0.2125	0.4410
	$t_{1/2}$ (hr)	3.2088	1.1178	34.8354	1.5700	3.2650	5.6800
T	C_{max} (ng/mL)	1131.1568	490.2674	43.3421	353.9030	1025.9110	2430.8340
	T_{max} (hr)	0.9694	0.5926	61.1293	0.5000	0.7500	2.5000
	AUC_{0-t} (ng.hr/mL)	3022.9201	1135.9058	37.5764	1162.2170	2898.6175	5724.9260
	$AUC_{0-\infty}$ (ng.hr/mL)	3210.2974	1175.5049	36.6167	1268.5130	3036.8090	6013.1480
	K_{el} (hr^{-1})	0.2227	0.0720	32.3412	0.1260	0.2040	0.3970
	$t_{1/2}$ (hr)	3.4331	1.0724	31.2355	1.7500	3.4000	5.5000

Statistical Result of analyzed formulations: For the Ln-transformed pharmacokinetic parameters Cmax and AUC0-t of sildenafil, the ratios (T/R) of geometric least squares means (and confidence intervals) of the test product (T) and reference product (R) were determined to be 101.44 (91.63% - 112.29%) and 106.28 (97.70% - 115.62%), respectively.

The test product (T) and reference product (R) had Power (& Intra-subject CV%) of 94.87 (24.30%) and 98.99 (20.05%), respectively, for the Ln-transformed pharmacokinetic parameters Cmax and AUC0-t of sildenafil.

Bioequivalence Conclusion: The bioequivalence acceptance limits of 80.00–125.00 percent are met by the 90 percent confidence intervals of the ratio of geometric least squares mean for the Ln-transformed pharmacokinetic parameters Cmax and AUC0-t of sildenafil. Thus, it can be concluded that the test product (T) and reference product (R) are bioequivalent in terms of absorption rate and extent.

Application to Biological Samples: The validated method was utilized to analyze sildenafil levels in plasma samples from 32 healthy volunteers who received a single 100 mg dose. The pharmacokinetics parameters were determined, and plasma concentration over time profiles are displayed in Figure-3 and Figure-4. All sildenafil plasma concentrations fell within the standard curve range and remained above the lower limit of quantitation (LLOQ) throughout the sampling period. The results are presented in Table-4, indicating that the reference and test formulations are bioequivalent.

Conclusion

This paper describes the use of LCMS/MS for the precise and reliable quantification of sildenafil concentrations in human plasma after 100 mg is administered orally to healthy volunteers. The method presented here is accurate, dependable, and fast. Each sample takes less than three minutes to complete. The assay is sensitive enough to monitor sildenafil's pharmacokinetics after oral administration with accuracy.

Acknowledgement

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