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Original Research Article **Clinical Pharmacy**

Determination of Vardenafil in Human Plasma by LC/MS/MS and its Clinical Applications

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Abstract

Background: Sexual dysfunction as a result of the inability to achieve or maintain an erection is a common problem that increases with age. Vardenafil has shown results as an efficacious and safe therapeutic agent for treatment of erectile dysfunction. ***AIM*:** Development of a bio-analytical method for rapid quantification of vardenafil in biological fluids and its application in pharmacokinetics and bioavailability studies, clinical trials, and monitor its therapeutic levels to help attaining effective clinical results in treating erectile dysfunction. ***Methods:*** Vardenafil was extracted from plasma samples and chromatographed with eluting solvent consisting of 10mM Ammonium Acetate: Methanol (30:70) v/v at flow rate of 0.55ml/min, ESI positive mode, and m/z 489 → 151, 475 → 100 for vardenafil and sildenafil as internal standard respectively. As an application of the validated developed bioanalytical method, a comparative bioavailability study of vardenafil 20mg tablets generic product versus reference product was conducted in a randomized open label crossover design invovlving 24 volunteers. The criteria used to assess bioequivalence of the two products were C_{max}, AUC_{0-t}, AUC_{0-inf}, and T_{max}. ***Results:*** The average recovery of vardenafil from human plasma was 95.104 % with limit of quantitation of 0.05 ng/ml and the correlation coefficient (r2) obtained was 0.9998, moreover, statistical analysis (ANOVA) of the measured parameters showed that there was no significance between the two products. ***Conclusion:*** The developed bioanalytical LC/MS/MS method was valid for vardenafil quantification in human plasma and is suitable for application in pharmacokinetic studies and therapeutic monitoring of vardenafil in treating erectile dysfunction to ensure effective therapeutic drug levels and avoid potential undesired adverse events.

Key words: vardenafil, erectile dysfunction, LC/MS/MS, Validation, liquid-liquid extraction.

INTRODUCTION

Erectile dysfunction (ED) is a common condition affecting up to 30 million men of all ages in the USA. Several reports indicate that its prevalence increases with increasing age. e.g. in the Massachusetts Male Aging Study, a community-based observational study, the prevalence of severe ED increased from 5% to 15% and the prevalence of moderate ED from 17% to 34% between the ages of 40 and 70 years. Similarly, the National Health and Social Life Survey of 1410 men in the USA reported an increase in the prevalence of ED with age. In that survey, 11% of men aged 40–49 years and 18% aged 50–59 years had difficulty achieving or maintaining an erection [1].

Penile erection is triggered by the release of nitric oxide from autonomic nerves and vascular endothelial cells in the corpus cavernosum, activating guanylate cyclase to produce cyclic guanosine monophosphate (cGMP), the intracellular second messenger that mediates both cavernosal smooth muscle relaxation and increases arterial blood flow to the erectile tissue. Levels of cGMP are regulated by the rate of synthesis and rate of degradation via phosphodiesterase (PDE) enzymes, with PDE5 being the predominant PDE isozyme in the corpus cavernosum. PDE5 inhibitors act by increasing levels of cGMP and thus increase cavernosal tumescence and rigidity when there is a deficit of any kind in the release of nitric oxide within the erectile tissue in response to sexual stimulation [2].

Vardenafil, a potent, selective inhibitor of phosphodiesterase-5 (PDE-5), is effective and generally

well tolerated for treating ED, both in the general population with ED and in those who are difficult to treat [3, 4].

Single-dose oral vardenafil 10–40mg significantly increased penile rigidity and tumescence during visual sexual stimulation in men with erectile dysfunction (ED) in two double-blind, randomised, placebo-controlled crossover studies. The mean total duration of erections with >60% rigidity was significantly longer with vardenafil 10–40mg than with placebo, and the mean total duration of erections with >80% rigidity was significantly longer with vardenafil 20 or 40mg than with placebo. Rigidity activity units and tumescence activity units were significantly higher with vardenafil 10–40mg than with placebo. The mean time to onset of the first erection was 26.8, 26.2 and 34.9 minutes with vardenafil 20 or 40mg or placebo [5].

It was suggested that the pharmacokinetics of vardenafil might differ among different age groups. In healthy men aged 18–45 and ≥65 years (12 each) the area under the curve of vardenafil with time after dosing, and the maximum concentration were, respectively, 34% and 52% higher in the older men, although the differences with this small sample were not statistically significant. Times to peak concentration and half-life were similar in the two age groups [6].

As per reported in literature, after oral ingestion of vardenafil 20mg tablet, the (C_{max}) observed ranged from 15 to 17 ng/ml, the value of (T_{max}) ranged from 0.5 to 2 h, the mean ($AUC_{o\text{-}inf}$) observed was 70 ng.h/ml, and elimination half life ($T_{1/2}$) was 3.8 h [7, 8].

Few published methods exist for the analysis of vardenafil in pharmaceutical or biological samples. LC-MS/MS has been used to identify vardenafil and the other PDE-5 inhibitors sildenafil and tadalafil in dietary supplements and bulk herbal materials, while HPLC coupled with the use of conventional C18 and monolithic silica columns has been used to identify and quantify vardenafi l in pharmaceutical formulations. It was reported the use of LC-MS-MS for the analysis of the related PDE-5 inhibitor sildenafil in human plasma to allow the evaluation of concentration vs time profiles for that compound in healthy volunteers. Similarly, another LC-MS/MS method was developed and validated for the measurement of concentrations of tadalafil in plasma from participants in pharmacokinetic studies [9-13].

A sensitive high-performance liquid chromatography–tandem mass spectrometric (HPLC-MS/MS) assay has been developed for the quantitative analysis of vardenafil in human plasma. Vardenafil and the internal standard, sildenafil, were extracted from 0.2 mL aliquots of alkalinized plasma by a single solvent extraction into hexane: dichloromethane. Reversed phase chromatographic separation was affected by gradient elution with mobile phases consisting of 10 mM ammonium formate pH 7.0 (solvent A) and methanol (100%, solvent B), delivered at a flow rate of 0.4 mL/min. The analytes were detected by using an electrospray ion source on a 4000 QTrap triple quadrupole mass spectrometer operating in positive ionization mode. The mass transitions were m/z 489.3 → 312.2 for vardenafil and m/z 309.2 → 281.0 for alprazolam. The assay was linear over the concentration range of 0.2–100 ng/mL, with correlation coefficients ≥0.995. The intra- and inter-day precision was less than 5.4% in terms of relative standard deviation and the accuracy was within 12.7% in terms of relative error. The lower limit of quantitation was set at 0.2 ng/mL [14].

The objective of this study was to introduce a valid bioanalytical method for the determination of vardenafil in human plasma and its application in pharmacokinetic and bioavailability studies, clinical trials and therapeutic monitoring of vardenafil in patients to ensure safety and efficacy in treatment of erectile dysfunction.

To examine the efficiency of the developed bioanalytical method for the quantitation of vardenafil in biological fluids in this study, a comparative bioavailability study of vardenafil generic versus reference products was conducted according to international guidelines, where, the protocol called for 24 healthy volunteers with a washout period of seven days [15].

The developed validated LC/MS/MS method was in compliance with the international guidelines for the bioanalysis of plasma samples [16], and the pharmacokinetic calculations were done using WinNonLin program. Besides, statistical analysis (ANOVA) was performed using SAS software and the 90% C.I. limits for the ratio between generic and reference products for the obtained pharmacokinetic parameters of $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$ and C_{max} were calculated to investigate its compliance with range of 80 to 125% confidence limits [15].

METHODS

(I) LC/MS/MS Analytical Method

(a) Mass Parameters and Chromatography

The method was developed in-house as follow: eluting solvent composition is 10mM Ammonium Acetate :Methanol (30:70) v/v, the flow rate was set at 0.55ml/min with injection volume set at 20ul, the MS/MS 6410B detector was operated at ESI positive mode, m/z was 489→151, 475→100 for vardenafil and sildenafil as internal standard respectively. The fragmentor energy was set at 135 for vardenafil and sildenafil, and collision energy was set at 55 for vardenafil, 25 for sildenafil.

(b) Preparation of Solutions

1- Vardenafil Standard Solution

An accurately weighed 10.746mg of vardenafil hydrochloride standard (equivalent to 10mg vardenafil) was transfered into a 100 ml volumetric flask, 80 ml of methyl alcohol was added and sonicated for 10 minutes, then completed to volume with methyl alcohol, to obtain a solution with final concentration of 100ug/ml vardenafil *(Solution A)* of which 0.5ml was transfered to a 100ml volumetric flask and completed to volume with methyl alcohol to obtain final concentratrion of 500ng/ml vardenafil *(Solution B).*

2- Working Solutions

Master Solution used	Milliliters taken	Final concentration obtained (ng/ml)	Final volume (ml)
"Solution B"	0.01ml	0.5	10
"Solution B"	0.02ml	1	10
"Solution B"	0.1ml	5	10
"Solution B"	0.2ml	10	10
"Solution B"	0.5ml	25	10
"Solution B"	1ml	50	10
"Solution B"	2ml	100	10
"Solution B"	4ml	200	10
"Solution B"	6ml	300	10
"Solution B"	8ml	400	10

All dilutions are done with methanol

3- Sildenafil Standard Solution

An accurately weighed 10mg of sildenafil standard was transfered into a 100 ml volumetric flask and about 80ml of methanol was added and was sonicated for 10 minutes, then volume was completed with methanol to obtain final concentration solution of 100ug/ml sildenafil of which 0.7 ml was transfered to 100ml volumetric flask and completed to volume with methanol to obtain 700ng/ml sildenafil.

(a) Preparation of Serial Dilutions of Standard vardenafil in human plasma

Serial dilutions of standard vardenafil in human plasma were prepared by transfering 50ul aliquot of vardenafil of concentrations ranging from 0.5 to 400 ng/ml to a centrifuge tubes containing 500ul of blank plasma.

(b) Preparation of Plasma Samples

The collected plasma samples of subjects, 500 ul, were transferred into centrifuge test tubes and 50 ul of sildenafil working solution 700ng/ml was added, then vortex-mix were for 30 seconds followed by addition of 100ul of 0.25N (NaOH) and vortex-mix for 1 minute, followed by addition of 2 ml (Diethyl ether/Dichloromethane mixture 70/30 v/v) and vortex-mix for nearly 1 to 2 minutes. The samples were centrifuged at 4000rpm for 5 minutes, the clear organic supernatant layer was transfered to a clean test tube and evaporate till dryness, 100ul mobile phase were used to reconstitute dry residue and transfered to insert vial for analysis by LC/MS/MS.

(c) Quantitation

The unkown plasma sample concentration was calculated as per formula: $Y = aX + b$. Where **Y** is the response ratio, **X** is the unkown concentration of drug in plasma samples, **a** is the calibration slope, **b** is the Y-Intercept.

(II) Application on Bioequivalence Study

(a) Ethics

This study was conducted in accordance with ICH and GCP guidelines adopted by (EMEA) and after Ethics Committee approval on vardenafil 20mg tablet bioequivalence study protocol. All documents and records were archived according to drug research center internal procedures.

The participant, clinical investigator, and other responsible persons signed written informed consents. Before starting of screening step, all study aspects where discussed with participants. There were no any obligations on volunteers to continue the bioequivalence study if they didn't want to.

Principal investigator and clinical investigator were responsible for supervising all study procedures. Licensed physicians were responsible for physical examination and following-up of subjects for measurement of vital signs, including blood pressure, pulse rate, body temperature, respiratory rate, and monitoring appearance of any side or adverse events throughout the study. Blood sampling were performed by registered nurses.

(b) Inclusion Criteria

Subjects age within 18 to 55 years and calculated BMI within normal acceptable limits. Normal physiological examination and laboratory data were within normal limits. Subjects of no alcoholic or drug abusers and shouldn't have any known history for both, of no clinical study contribution history. Non-smoker subject was preferred over smoker subject and

if smoker, this should be not more than 8 cigarettes per day.

(c) Exclusion Criteria

Any vardenafil hypersensitivity, GIT problems, hematological abnormalities, kidney diseases, auto-immune diseases, CVS diseases, diabetics, hepatic disease, respiratory diseases, history of alcohol intake, and drug abuse, positive HIV, abnormal laboratory values, subject administered any medication less than two weeks of the study starting date, blood donation or participation in clinical studies that requires more than 500 ml of blood loss within month and half preceding starting date of the bioequivalence study.

(d) Subjects

Twenty-four healthy adult subjects participated in the bioequivalence study and were subjected to general physical examination, neurological examination, clinical urine tests and blood analysis. The selected subjects had no history of drug or alcohol abuse and have no acute or chronic gastrointestinal, cardiac, vascular, hepatic, or renal disease. Concurrent medication was not allowed during the time course of the study, meals, beverages drink, coffee or tea are not allowed for four hours after study dose administration. At 10:30 a.m. they received a standard breakfast meal followed by a lunch meal at 2:30 p.m.

(e) Study Design

The design of this study was a single-center open-label randomized single-dose two-way crossover design to compare the bioavailability of generic versus reference vardenafil 20mg tablet in 24 healthy male adults under fasting conditions with a washout period of seven days.

(f) Collection of Sample

The number and disposition of the blood collections as well as the wash out period were designed with respect to pharmacokinetic parameters of Vardenafil.

The number of blood collections for drug analysis was 16 samples in each period, 5ml per sample collected at the following intervals: 0 (directly prior to dosing), 10 min, 20 min, 30min, 45min, 1, 1.25, 1.5, 2, 4, 6, 8, 10, 12, 18 and 24 hours after dose administration in tubes containing anticoagulant EDTA disodium and centrifuged at approximately 4000 r.p.m. for 10 minutes and plasma samples were separated in a 5 ml-plastic wassermann tube. The collected samples were stored at a -80 ^{o}C until analysis. The study code, subject number, study period, time interval was recorded on the tubes. Total blood amount withdrawn during the whole study did not exceed 160 ml.

(g) Analysis of Samples

Determination of vardenafil in plasma samples of the participants was performed by LC-MS/MS using the developed bioanalytical method was validated according to the international guidelines.

(h) Calculation of the Pharmacokinetics Parameters

The following pharmacokinetic parameters of vardenafil were assessed; maximum plasma concentration (C_{max}), time point of maximum plasma concentration (t_{max}), half-life of drug elimination during the terminal phase ($t_{1/2e}$), terminal rate of elimination (K_e), area under plasma concentration-time curve from zero to time t (AUC_{0-t}), and K_{el}, area under plasma concentration-time curve from zero to infinity (AUC_{0-inf}).

(I) STATISTICAL ANALYSIS

Statistical analysis of the calculated pharmacokinetic data was performed using statistical computerized program SAS software for determination of analysis of variance (ANOVA). Bioequivalence could be demonstrated for vardenafil within the prescribed 90% confidence limit of 80 to 125% for AUC_{0-t}, AUC_{0-inf}, and C_{max} with respect to the parametric method on Ln-transformed data.

(j) Tolerability and safety

Subject medical histories, physical examination and laboratory reports, and all incidents of possible adverse reactions to the study formulations were reported.

(K) Measurement of Blood Pressure and Heart Rate

Blood pressure cyctolic / diastolic and pulse rate measurements before dosing and at regular intervals (at 2, 4, 6, and 10 hours) after drug administration were included in tolerability assessments. A 120/80 mmHg blood pressure reading and 50 to 100 beats per minute resting heart rate are considered normal.

RESULTS

Validation of the LC/MS/MS Analytical Procedure

(a) Chromatograms of Vardenafil

It is apparent from Figures (1), (2), and (3) that vardenafil and sildenafil were well separated and their retention time was 1.7, and 1.6 minute, sharp and symmetrical peaks showed a good baseline with minimum tailing thus facilitating the accurate measurement of the peak response. The in house developed chromatographic conditions was developed in accordance with litreature [14] showed a valid bioanalytical method for accurate determination of vardenafil in plasma with some modifications in extraction procedure and chromatographic conditions.

(b) Linearity, Precision and Accuracy

The peak area ratios of serial dilutions of vardenafil in human plasma of concentrations ranging from 0.05 to 40 ng/ml was highly linear with r^2 of 0.9998. The C.V.% of the average results of inter-day variation was 1.052% in accordance with FDA Guidelines [16] which strenghthen the possibility of its application in pharmacokinetics and bioavailability studies of vardenafil.

Accuracy and precision were assessed on within-day and between-day basis at three levels of drug concentrations at the expected range. Moreover, the results of intra-day inter-day accuracy showed an average recovery percentage of 94.849% and 94.654% respectively. The results of freeze-thaw, short term and long term stability in human plasma showed that the average recovery of vardenafil was greater than 95% providing that both targeting drug and internal standard were stable in the studied conditions.

Comparative Bioavailability Study

1-Clinical Observation (Safety and Tolerability)

The drug was to some extent tolerated by most of the participants. No treatment related adverse events or laboratory abnormalities were observed. Blood sampling during the whole study was performed at the proper time. No subjects withdrew from the study for any reason attributable to drug side effects.

2-Pharmacokinetic Data and Assessment of Bioequivelance

Results of pharmacokinetic parmeters presented in Tables (1) and (2) showed that the mean values for C_{max} was 16.997±3.806ng/ml and 16.431±4.182ng/ml, t_{max} was 1.302±0.338h and 1.344±0.337h, $t_{1/2e}$ 3.858±0.864 and 3.847±0.915h, AUC_{0-t} 47.702±12.462ng.h/ml and 49.340±16.198ng.h/ml, for generic and reference products respectively which were in accordance with those reported in the literature [7, 8].

3-Statistical Analysis

Two-way ANOVA was performed for C_{max}, AUC_{0-t}, AUC_{0-inf} of the two products, also, 90% confidence limit of 80 to 125% for C_{max}, AUC_{0-t}, AUC_{0-inf} with respect to the parametric method on Ln-transformed data should be fulfilled. In this bioequivalence study the point estimate (%) results for C_{max}, AUC_{0-t}, AUC_{0-inf} were 103.830, 98.054, 97.740 % respectively and the 90% confidence intervals of parametric means of C_{max}, AUC_{0-t}, AUC_{0-inf} were 97.735 to 110.306, 88.111 to 109.119, 88.096 to 108.439% respectively, (table 3) thus, providing a 90% confidence intervals limits within FDA acceptance limits [15].

4-Blood pressure and pulse rate

The reported measurements of blood pressure and pulse rate were all approaching normal levels and within the safe limits (Figures 5 and 6).

It is clear from the blood pressure results represented in Figure (7), for the generic product, that all approaches normal levels, as the reported mean values of systolic blood pressure were 116, 115, 111, 110, 109 mmHg and 75, 72, 70, 69, 69 mmHg for diastolic blood pressure at Zero (predose), 2, 4, 6, and 10 hours of drug administration respectively.

On the other hand, concerning the reference product, mean values of systolic blood pressure were 115, 113, 111, 107, 112 mmHg and 74, 71, 71, 69, 70 mmHg for diastolic blood pressure at Zero (predose), 2, 4, 6, and 10 hours of drug administration respectively. (Figure 8)

DISCUSSION

The developed bioanalytical method proved to be sensitive, specific, precise and accurate, showing linearity in the range of 0.05 to 40 ng/ml with r2 of 0.9998, and thus, in compliance with the FDA Guidelines [16], which could be applied in bioavailability and clinical studies, clinical trials, therapeutic monitoring of vardenafil to assure safety and efficacy in the erectile dysfunction treatment.

The in house developped chromatographic conditions was in accordance with published literature methods [14] with some modification in extraction procedure and chromatographic conditions and using sildenafil as a structurly related internal standard. It is worthy to mention that, the development of an accuratre and precise bioanalytical assay was important for ensuring accurate and precise therapeutic monitoring and testing the validity of generic products for commertical use with targeted clinical outcomes [17].

The clinical advantage that vardenafil has over sildenafil is that it does not inhibit phosphodiesterase-6 to alter colour perception, a rare side effect which sometimes occurs with sildenafil. Tadalafil has a longer duration of action than sildenafil and vardenafil. Tadalafil is similarly effective as sildenafil in the treatment of ED [18].

Vardenafil showed to provide an extended puration of action. This was proven from clinical data indicated that vardenafil is effective for at least 8 hours after dosage, which is roughly the length of a night, and may give couples with significantly greater flexibility in their sexual activities [19].

Oral PDE5 inhibitors have numerous well-known off-label beneficial effects, including anti-inflammatory, antioxidant, immune response modulation, and antiapoptotic effects. These features may justify the prospective adjuvant use of repurposed oral PDE5 inhibitors in COVID-19 treatment regimens [20].

Diabetes is a known risk factor for male sexual dysfunction, with diabetic men having a threefold higher risk of erectile dysfunction when compared to nondiabetic men [21]. Furthermore, diabetic individuals are more vulnerable to the severity and frequency of COVID-19 [22]. So, vardenafil can be added for covid-19 therapeutic protocol for its aforementioned advantages [19, 20].

It was reported that combining antibiotics with vardenafil resulted in considerably enhanced effectiveness in chronic bacterial prostatitis. Furthermore, some studies have suggested that NO may help to minimise lung damage, proinflammatory cytokine concentrations, and the amount of neutrophils entering the lungs. As a result, PDE5Is may be utilised to raise NO levels as part of the therapy of COVID-19 [23]. This could be an important contributing factor in reducing the antibiotics use duration, and hence reducing the progression of antibiotic resistance that may lead to therapeutice protocol changes in the next COVID-19 waves [24].

The reported results of the conducted comparative bioavailability study showed that the 90% confidence limit lied in the range of 80 to 125% for AUC_{0-t}, AUC_{0-inf}, and C_{max} with respect to the parametric method on Ln-transformed data and therefore conclusion of that both the generic and reference products of vardenafil were bioequivalent as per FDA acceptance limits (80 to 125%) [15].

CONCLUSION

The developed bioanalytical method for the quantitation of vardenafil in plasma is fully validated and can be applied for bioavailability studies, clinical trials, therapeutic drug monitoring. Moreover, the results of the comparative bioavailability study showed that both the generic and the reference products were as bioequivalent.

Vardenafil is an effective drug treatment for management of erectile dysfunction and its therapeutic monitoring is an important approach for meeting the required therapeutic goals as a consequence of monitoring patients' drug levels to avoid subtherapeutic or toxic drug levels.

Table-1: Pharmacokinetics Parameters of Vardenafil following administration of Reference Product

Subject	T_{max} (h)	C_{max}(ng/ml)	AUC_{0-t} (ng.h/ml)	AUC_{0-inf} (ng.h/ml)	K_{el} (h^{-1})	$T_{1/2}$ (h)
Mean	1.344	16.431	49.340	50.119	0.189	3.847
CV%	25.083	25.451	32.829	32.197	20.349	23.786
Range (Median)	1.00 - 2.00 (1.250)	12.336 - 25.900 (14.638)	28.324 - 91.622 (45.172)	30.786 - 92.232 (46.042)	0.110 - 0.280 (0.189)	2.477 - 6.285 (3.661)

Table-2: Pharmacokinetics Parameters of Vardenafil following administration of Generic Product

Subject	T_{max} (h)	C_{max}(ng/ml)	AUC_{0-t} (ng.h/ml)	AUC_{0-inf} (ng.h/ml)	K_{el} (h^{-1})	$T_{1/2}$ (h)
Mean	1.302	16.997	47.702	48.369	0.187	3.858
CV%	25.985	22.391	26.124	25.844	19.263	22.382
Range (Median)	0.50 - 2.00 (1.250)	11.751 - 24.249 (16.854)	25.968 - 75.188 (45.551)	26.424 - 75.534 (46.118)	0.104 - 0.263 (0.181)	2.636 - 6.690 (3.821)

Table-3: The 90 % Confidence Interval for Generic and Reference Products

Pharmacokinetic Parameter	90% Confidence intervals of parametric means		
	Point estimate (%)	Lower limit (%)	Upper limit (%)
C_{max}	103.830	97.735	110.306
AUC_{0-t}	98.054	88.111	109.119
AUC_{0-inf}	97.740	88.096	108.439

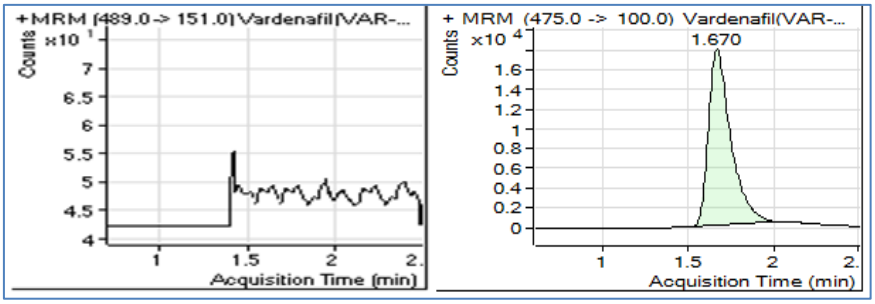

Fig-1: Chromatogram - an MRM Data of Blank Plasma Spiked with Internal Standard Sildenafil.

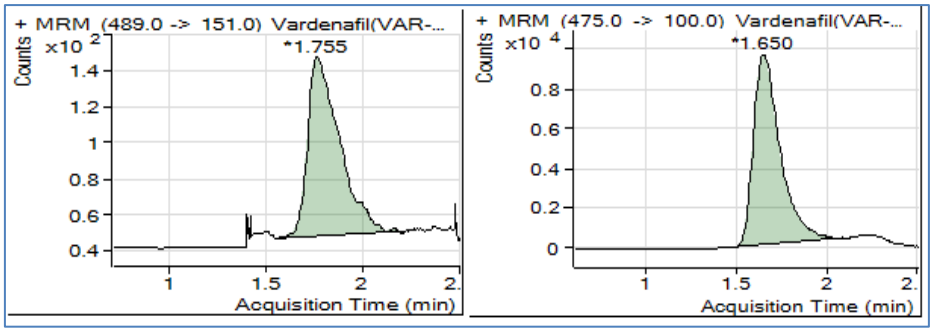

Fig-2: Chromatogram - an MRM Data of Blank Plasma Spiked with 0.05ng/ml Vardenafil and Internal Standard Sildenafil

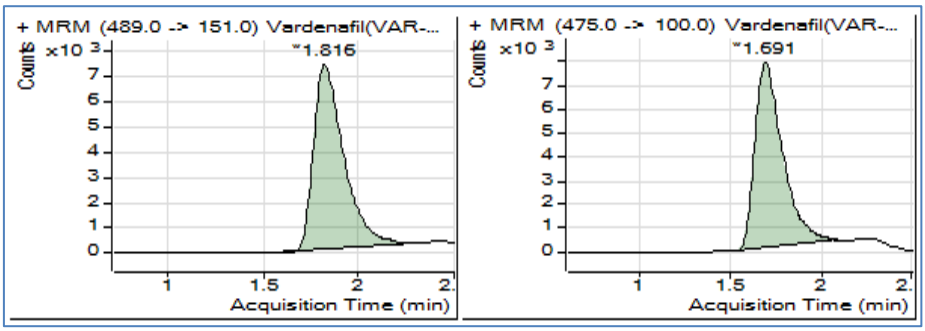

Fig-3: Chromatogram - an MRM Data of Blank Plasma Spiked with 20ng/ml Vardenafil and Internal Standard Sildenafil

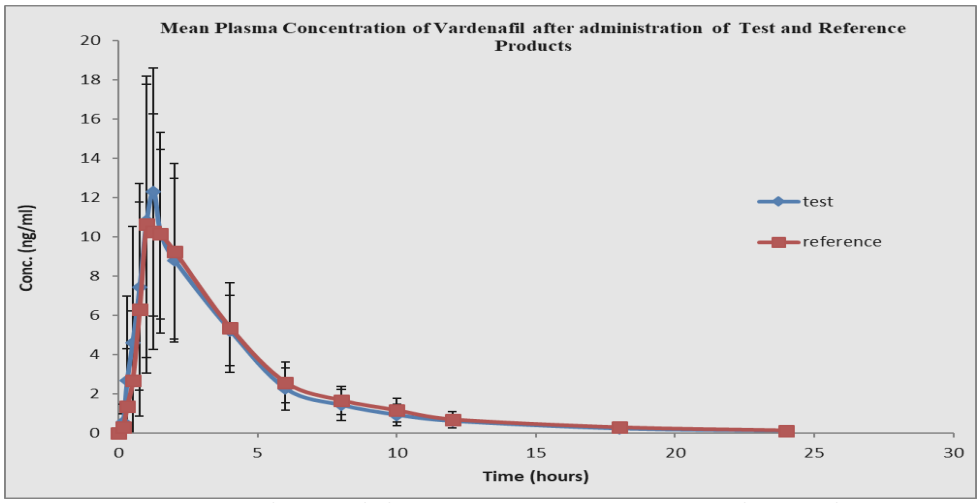

Fig-4: Plasma Concentration (Mean ± S.D.) of Vardenafil following Single Dose Administration of Vardenafil 20 mg Tablets of Generic and Reference Products

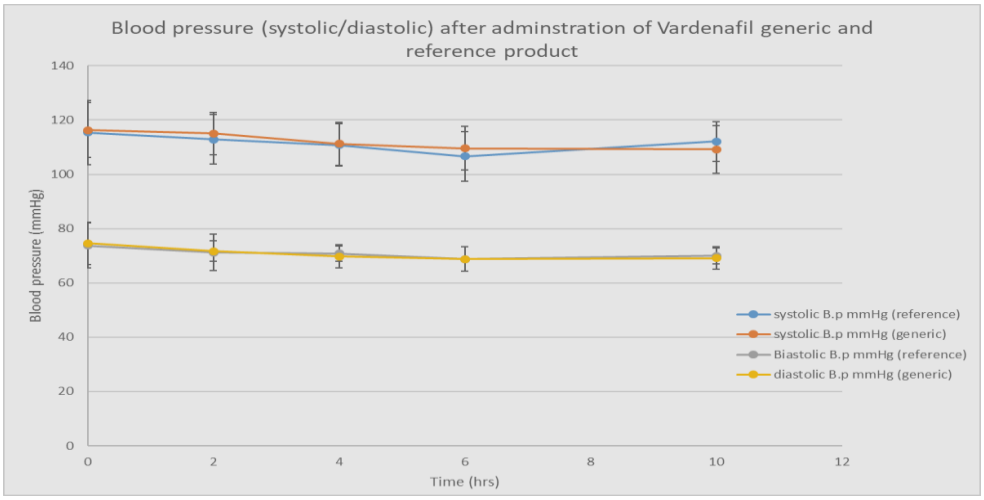

Fig-5: Blood pressure systolic / diastolic (Mean±SD) after single oral administration of Vardenafil generic and reference products

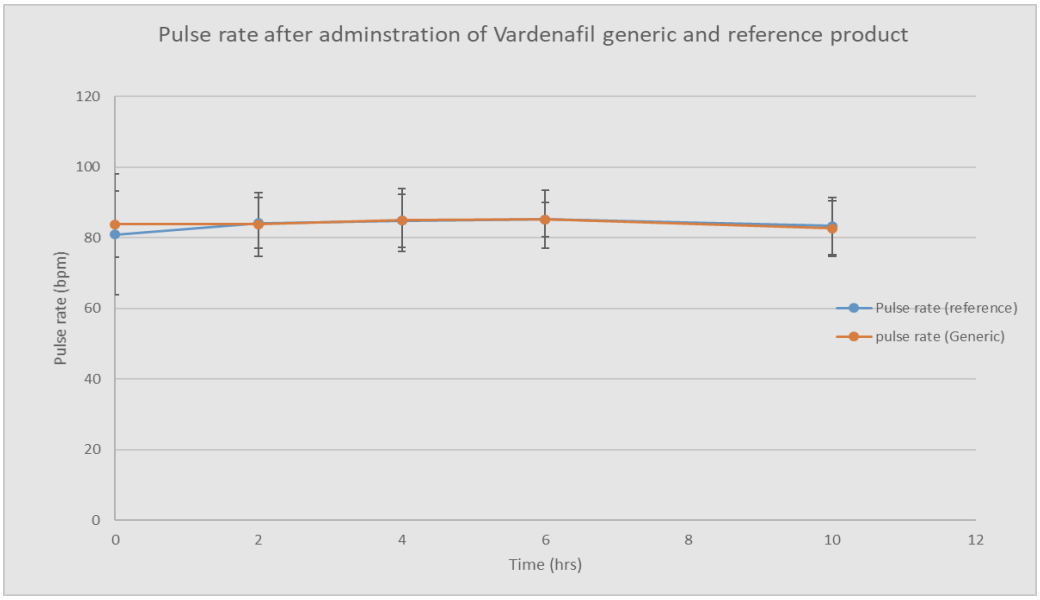

Fig-6: Pulse rate (Mean±SD) after single oral administration of Vardenafil generic and reference products

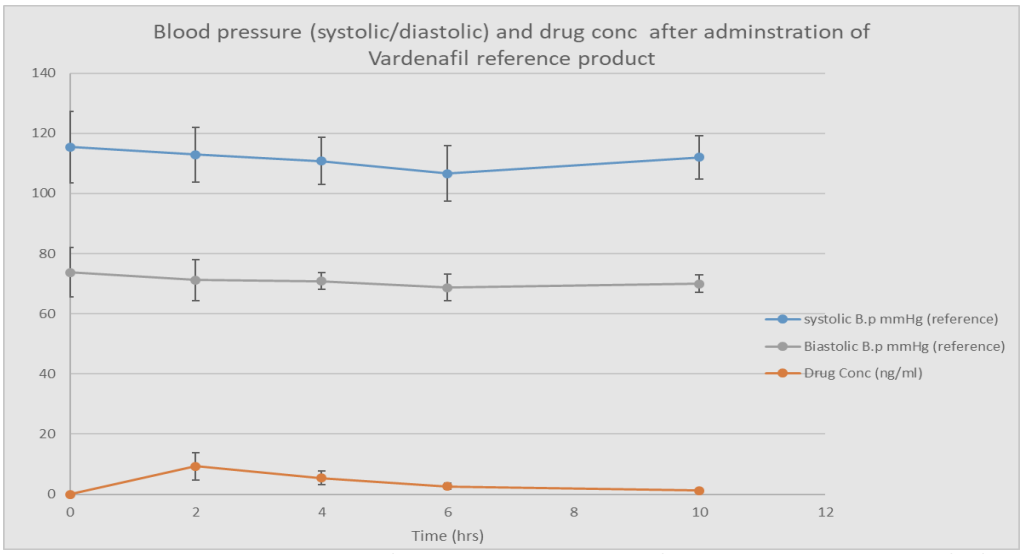

Fig-7: Blood pressure systolic / diastolic and Vardenafil plasma Conc (Mean±SD) after single oral administration of reference product

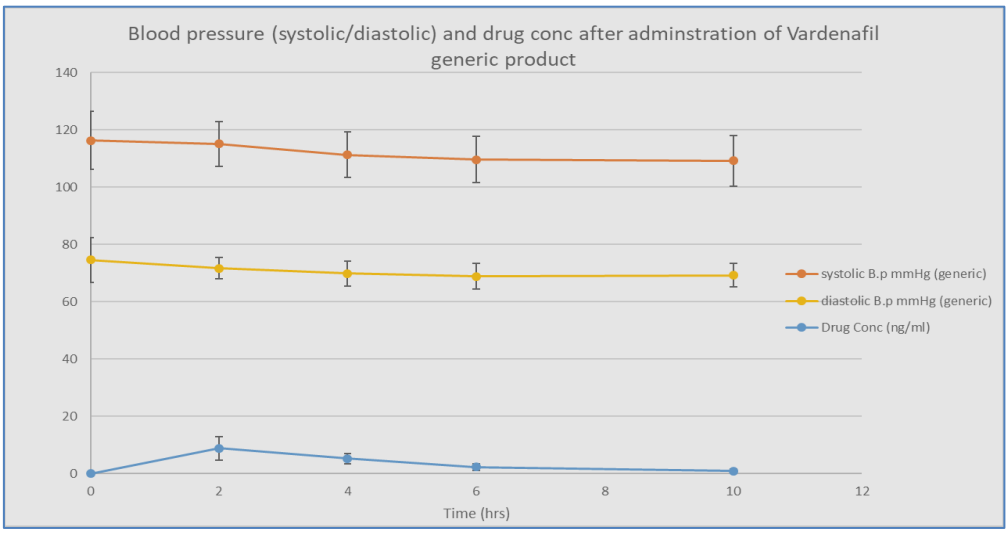

Fig-8: Blood pressure systolic / diastolic and Vardenafil plasma Conc (Mean±SD) after single oral administration of generic product

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