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Determination and quantitation of benzofuran, Indole and piperazine containing selective serotonin reuptake inhibitor vilazodone hydrochloride in human plasma by LC-ESI-MS/MS with an application to pharmacokinetic study under the frame work of bioequivalence study

Abstract

Aim and objectives: Among the various SSRI and 5-HT-1A, partial agonist vilazodone is one of them. It has antidepressant and anti-anxiety activities. This method's main aim and the objective was to develop and validate a bio-analytical method of Vilazodone in human plasma by LC-MS/MS (API-4000) and its application to estimate pharmacokinetics.

Method: For reporting this investigation, Analyst software, 1.6.3 used. The mobile phase was acetonitrile with 0.1% formic acid as an organic solvent and Milli Q water with 10Mm ammonium acetate and 0.1% formic acid using the gradation method 7.0min run time. The calibration standard concentrations were 1.0 to 64 ng/ml. Plasma precipitation was by protein precipitation technique.

Result: The accuracy of calibration concentrations of Vilazodone was 93.5-104.39% and stability study showed 96.41-106.71%, 94.77-96.36%, 92.22-101.38%, 94.15-98.47%, 93.95-95.75% remaining for freeze-thaw, short term, long term, benchtop and autosampler stability respectively. Recovery was to be 98.10-98.99%; the matrix factor was 0.94-0.96. The maximum plasma concentration of reference preparation was 13.445±2.842ng/ml (C_{max}) at a time 6.792±0.846hr. (T_{max}). The maximum plasma concentration of test preparation was 13.218±3.231ng/ml (C_{max}) at a time 6.958±0.793hr (T_{max}) The relative bioavailability of the test preparation was to be 94.66 % of that of the reference preparation.

Conclusion: The present investigation was highly selective, sensitive, reproducible, low matrix effect, high recovery and low time-consuming method. It was validated as per USFDA and EMA guideline and successfully used in comparative pharmacokinetics.

Keywords: vilazodone, LC-MS/MS-ESI, Human volunteers, BA/BE study

Volume 10 Issue 1 - 2021

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Received: September 15, 2019 | **Published:** Febrauary 08, 2021

Abbreviations: SSRI, selective serotonin reuptake inhibitor; SERT, serotonin transporter; SPARI, serotonin partial agonist reuptake inhibitor

Introduction

SSRIs are commonly an antidepressant drug and used to treat moderate to severe depression by increasing serotonin concentration in the brain.[1] Serotonin is neurotransmitters which carry signals between different brain cells. The reuptake of serotonin in the brain is blocked, resulting in more serotonin available. SSRIs are selective because they seem to affect serotonin, not other neurotransmitters[2] primarily. Vilazodone serotonergic antidepressant drug. Its action mechanism is to combine selective serotonin reuptake inhibitors like activity (SERT inhibition) and 5HT1AR partial agonism like an anxiolytic drug.[3] Vilazodone is chemically 5-(4-[4-(5-cyano-1H-indol-3-yl) butyl] piperazin-1-yl) benzofuran-2-carboxamide (CAS № 163521-12-8), and chemical formula $C_{26}H_{27}N_5O_2$ and molar mass[4] 441.5. Vilazodone is a small molecule containing benzofuran, indole, and piperazine moiety.[5] Benzofuran has varieties of pharmacological effect namely, anticancer, angiotensin II blocker, pyrogenic (P2Y1) receptor antagonist, human GPR 119 agonist, anti hyperlipidaemic, protein tyrosine phosphatase1B inhibitor, anti-inflammatory, analgesic, antipyretic, antimicrobial,[6] endothelium receptor antagonist, fibril formation inhibitor[8] and oxytocin antagonist[7] activity. Indole is a parent substance that forms important compounds in nature like acetamide to form tryptamine when added to the 3β position of the indole ring,[9] hydroxyl radical attaches to 5 positions of indole ring to form 5-hydroxytryptamine or serotonin.[12] Piperazine consists of a six-membered ring containing two opposite nitrogen atoms.[13] It was introduced into medicine as a solvent for uric acid; it has a remarkable power to dissolve uric acid and produce a soluble urate. It exists as a small alkaline deliquescent crystal with a saline taste.[14] It is partly oxidized and partly eliminated unchanged;[15] it has anthelmintic action by paralyzing the parasite and efficiently removing from the body.[16]

Vilazodone is used to treat depression; SSRI and serotonin partial agonist reuptake inhibitor (SPARI). It inhibits central nervous

system neuron serotonin reuptake with minimal or no effect on the reuptake of norepinephrine or dopamine.[17,36] Serotonergic activity and antidepressant action occur at presynaptic somatodendritic autoreceptors.[18] This effect may theoretically diminish sexual dysfunction caused by serotonin reuptake inhibition.[19,37] The approved indication is a major depressive disorder. Long-time exposure of Vilazodone causes common adverse effects namely, diarrhoea, headache, vomiting, dry mouth and some uncommon side effects like acute pancreatitis, somnolence, paraesthesia, tremor, and abdominal pain dreams, akathisia, restlessness, myoclonus, muscle rigidity.[20] Vilazodone has negligible affinity for other serotonin receptors. Vilazodone, primarily eliminated by hepatic metabolism, only 2% via faeces and 1% stopped unchanged urine.[21] Pharmacokinetic study of Vilazodone did not show a significant difference between mild to moderate depression patient and healthy subjects due to its increased systemic exposure.[22] The absolute bioavailability of Vilazodone is 72% under the fed condition, and maximum concentration reached at a median of 4-5 hours after drug administration.[23,38] The protein binding was approximately 96-99%.[24] Vilazodone, widely distributed, and distribution volume is 605 ltr after 5mg IV infusion for 4hours. CYP 3A4 is the primary isoenzyme for vilazodone metabolism among CYP pathways with a minor contribution from CYP2C19 and CYP2D6.[26]

It was reported an LC-MS/MS method for quantification of Vilazodone in rat plasma having a narrow calibration range (1.0 to 100ng/mL) with a total run time of 2.2min,[28] another reported LC-MS/MS assay of Vilazodone in dog plasma where full validation was not done. Real run time was 6min.[29] A UPLC-MS/MS method for quantifying Vilazodone in rat and human plasma using protein precipitation technique expressed, giving low recovery results (53.6%).[30] Recently another UPLC-MS/MS method using liquid-liquid extraction method was reported, which is a very time-consuming and tedious extraction process, again recovery was low (79-83%) and run time was longer.[31] The efforts to develop and validate a bioanalytical method estimate vilazodone in healthy human plasma by LC-MS/MS with a clinical application. Comparative pharmacokinetics' objective of the present investigation evaluated the pharmacokinetic parameters and compared the bioequivalence of film Coated tablet containing Vilazodone Hydrochloride 20 mg in 24 healthy human volunteers in a randomized, two-way complete crossover design with the help of this validated method. Hence, the scope of present work was to develop a fast, rapid, sensitive and specific LC-MS/MS method for analysis of human plasma samples from healthy Indian volunteers with short time-consuming protein precipitation with better sensitivity and significantly higher throughput than liquid-liquid extraction method. This bio-analytical method was applied successfully to a comparative pharmacokinetic study.

Material and methods

Chemical and reagents

Acetonitrile purchased from Merck (MERCK India Ltd., Mumbai), isopropyl alcohol, formic acid was A.R. grade and HPLC grade solvents. Milli-Q water was procured from Millipore (Elix, Milli-Q A10 Academic, Bedford, MA, USA) until a resistivity of 18.2MΩ achieved. EDTA-K3 anticoagulant containing blank human plasma was collected from TAAB Biostudy Services (CPU), Kolkata, and stored at −20°C until analysis.

Ethical clearance, volunteer consenting and study design details

The HURIP Independent Bio-ethics committee, Kolkata, India [Central Drugs Standard Control Organization (CDSCO) registration: ECR/103/Indt/W.B./2013/RR-19 which is valid up to 21-Nov.-2024] all documents related to this investigation were submitted. The ethical clearance obtained before the initiation of the research.[10,11] After giving the written informed consent, twenty-six volunteers screened, and twenty-four volunteers included in the investigation. The ethics committee and study team ensured that volunteers' rights and health protection during the study. The volunteers were randomized and blinded. As the review was a comparative pharmacokinetic study,[27] the volunteers exposed in both the test and reference drugs after completing two periods. A washout period of 14 days maintained between two study periods.

Study design

This comparative pharmacokinetic study was randomized, single-dose, two treatments and two –way cross over, open-label, bio-equivalence study. Vilodon 20mg containing vilazodone hydrochloride was the reference product compared with the film-coated tablet containing vilazodone hydrochloride 20 mg as a test product. The volunteers have received either test or reference product based on the randomization code in each clinical period specified in the Table 1. Drugs were taken with 240ml of drinking water on an empty stomach with at least 8-10hrs fasting condition in single-dose without chewing. Total blood sampling time point were 0 hr. (before drug administration) and 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 6.5, 7.0, 7.5, 8.0, 10.0, 24.0, 48.0, 72.0, 96.0 and 120.0 hrs, after blood sample collection plasma was separated and stored frozen at –20^{0}C with appropriate labeling of volunteer code no., study date and collection time. Blood sampling was done by cubital vein puncture. The body weight of each volunteer as specified in Table 2.

Table 1 Randomization schedule

Vol. No.	Sex	Age (Yr)	Height (cm)	Weight (kg.)	BMI(kg/m^2)
1	M	40	156	55	22.6
2	M	42	189	73	20.44
3	M	43	162	65	24.77
4	M	35	150	52	23.11
5	M	32	164	65	24.17
6	M	34	158	60	24.03
7	M	42	152	55	23.81

Table Continues...

Vol. No.	Sex	Age (Yr)	Height (cm)	Weight (kg.)	BMI(kg/m^2)
8	M	34	160	55	21.48
9	M	38	169	65	22.76
10	M	40	166	57	20.69
11	M	40	158	55	22.03
12	M	42	164	61	22.68
13	M	39	167	55	19.72
14	M	42	158	60	24.03
15	M	38	157	50	20.28
16	M	38	158	51	20.43
17	M	34	157	59	23.94
18	M	34	169	66	23.11
19	M	29	166	60	21.77
20	M	28	164	60	22.31
21	M	30	155	51	21.23
22	M	36	158	51	20.43
23	M	25	145	50	23.78
24	M	35	157	52	21.1
Mean	-	36.25	160.79	57.63	22.28
S.D.	-	4.95	8.44	6.08	1.5

Table 2 Demographic data

Subject No.	Period I	Period II
1	A2	A1
2	A1	A2
3	A1	A2
4	A2	A1
5	A2	A1
6	A1	A2
7	A1	A2
8	A2	A1
9	A1	A2
10	A2	A1
11	A2	A1
12	A1	A2
13	A2	A1
14	A1	A2
15	A1	A2
16	A2	A1
17	A1	A2
18	A2	A1
19	A1	A2
20	A2	A1
21	A1	A2

Table Continues...

Subject No.	Period I	Period II
22	A2	A1
23	A2	A1
24	A1	A2

Bioanalytical method development by Liquid chromatography Tandem Mass Spectrometry

Chemical name of Vilazodone is 5-[4-[4-(5-cyano-1H-indol-3-yl) butyl] piperazin-1-yl]-1-benzofuran-2-carboxamide, and chemical formula is $C_{26}H_{28}ClN_5O_2$.[35] Vilazodone has shallow plasma exposure; thus, a susceptible and selective method is desired. The exact mass of Vilazodone is 441.2165 (molecular wt. 441.54), hydrogen bond donor count=2 and hydrogen bond acceptor count=5. According to Lipinski's rule, an orally active drug should maintain this rule or rule of 5(RO5) and increase the activity and selectivity of the compound because it ensured that physicochemical properties of the drug are maintained and confirm the RO5 rule tend to have lower failure rate during preclinical or clinical development and hence have a higher possibility of reaching the market. Considering all the parameters following Lipinski's rule, Vilazodone has hydrogen bond acceptor count 7(not more than 10 for HBA). Hydrogen bond donor count 3(not more than 5 for HBD), molecular mass 441.2165 (less than 500daltons), octanol/water partition coefficient (logP) value 2.59, AlogP value 4.03 (not greater than 5), rotatable bond count=7 (According to Veber's rule 10 or fewer) and polar surface area=102.29 (According to Veber's rule not greater than 140Å^2), therefore it is predictable for Vilazodone to have good oral bioavailability. A positive value of logP and log Ko_W value 3.59 denotes a higher concentration of drug molecule in the lipid phase, and the compound is lipophilic, it is soluble in DMSO, methanol and ethanol. Still, aqueous solubility is very low at 0.123mg/mL. Protein binding of Vilazodone is 96-99%, pKa_1 values of Vilazodone are 8.60 due to Piperazine, pKa_2 value is 14.19 due to ester, so one side is elementary (weak acid), and another side is weak basic but the whole molecule is neutral. Due to multiple pKa values, it was imperative to set an optimum condition for plasma extraction, chromatography and mass detection for simultaneous separation and detection.

Propranolol used as internal standard (IS) its chemical name is 1-naphthalen-1-yloxy-3-(propan-2-ylamino) propan-2-ol. The molecular weight of propranolol is 259.35, and monoisotropic mass is 259.1572. H-bond donor count=2 and H-bond acceptor count=3. Octanol/water partition coefficient (logP) value is 2.58, AlogP value is 2.90 (not greater than 5), log Ko_W value 0.45, rotatable bond count=6(According to Veber's rule 10 or fewer) and polar surface area 41.49(According to Veber's rule not greater than 140Å^2). It is soluble in DMSO, methanol and ethanol, but its aqueous solubility is low 0.0617 mg/mL. The pKa value of propranolol is 9.42 due to naphthalene and 13.84 due to propan-2-ol, so propranolol is basic. Mass spec analysis used positive polarity to achieve adequate response for their simultaneous analysis. The precursor ions $[M+H]^+$, which protonated at m/z 442.2 (highest peak), 442.7(2nd peak), were observed in Q1 MS scan for Vilazodone. Product ions (MS2 scan) found to be at m/z 155.4, 197.5, 203.5, 243.6. However, the most stable and consistent selected product ion was m/z 155.4 for [4-[4-(5-cyano-1H-indol-3-yl) butyl] piperazin-1-methyl] that is $[M+H\text{-}C_{17}H_{27}N_4]^+$. The protonated precursor ions were $[M+H]^+$ at m/z 260.4(highest peak),260.9(2nd peak), for propranolol and characteristic product ions were found to be 116.4, 155.1, 183.3, 157.2. The m/z was 116.4 for [1-yloxy-3-(propan-2-ylamino) propan-2-ol.] that is $[M+H\text{-}C_7H_{14}N_1O_2]^+$ which is most stable and consistent fragment ion. Precursor (Q1) and product ion (MS2) scanning for Vilazodone and Propranolol showed in Figure 1(A, B) and Figure 2(A, B) respectively.

Figure 1 (A) Parent Ion (Q1) Scan of Vilazodone.

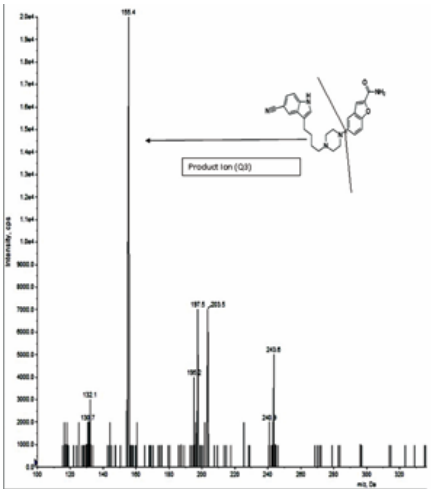

Figure 1 (B) Product Ion(Q3) Scan of Vilazodone.

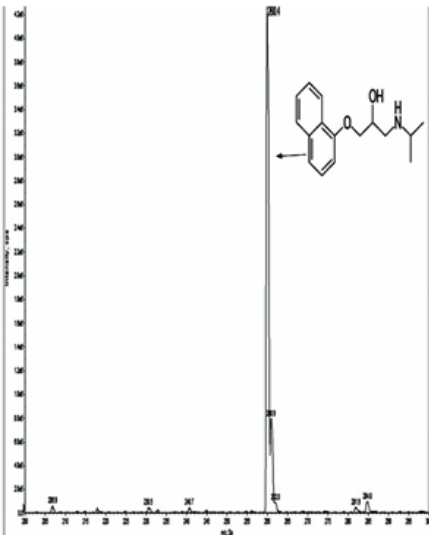

Figure 2 (A) Parent Ion (Q1) Scan of Propranolol (I.S.).

Citation: Ghosh B, Mandal P, Chakraborty S, et al. Determination and quantitation of benzofuran, Indole and piperazine containing selective serotonin reuptake inhibitor vilazodone hydrochloride in human plasma by LC-ESI-MS/MS with an application to pharmacokinetic study under the frame work of bioequivalence study. *J Anal Pharm Res.* 2021;10(1):1–12. DOI: 10.15406/japlr.2021.10.00361

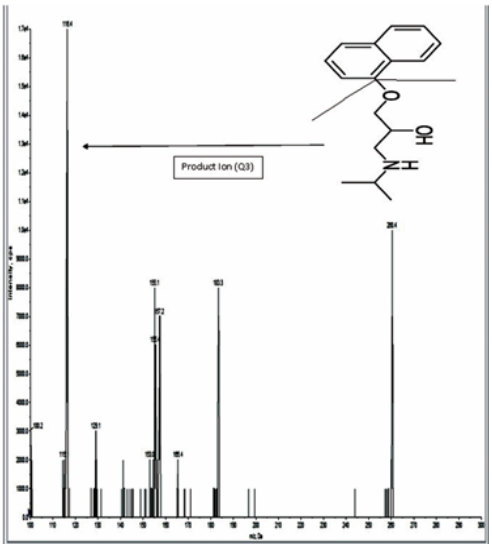

Figure 2 (B) Product Ion (Q3) Scan of Propranolol2 (I.S.).

Shimadzu's HPLC system is equipped with LC-20AD binary pump, SIL-20A autosampler, CTO-10ASvp Oven, and CBM-20A lite for chromatographic analysis system control compartment was utilized. Mass spectrometric detection executed on an API 4000 triple, quadruple mass spectrometer (Applied Biosystems/MDS SCIEX, Toronto, ON, Canada) equipped with a Turbo electrospray ionization (ESI) interface and represented in Table 3. The elution of Vilazodone done by LC-MS/MS column 5µ C18 100Å 50*3mm of Phenomenex Kinetex initiated as a rapid, sensitive and rugged analytical method covering the dynamic linear range. The mobile phase selection was crucial for the synchronized determination of the drug having two pKa values. Thus, the mobile phase's pH, buffer concentration, choice and proportion of diluents were very delicate for chromatographic resolution, useful feedback to accomplish the coveted sensitivity. Initially, acetonitrile/methanol with five mM ammonium acetate buffer (pH 6.5) responded to Vilazodone and propranolol. However, the response was not reproducible. The buffer concentration was changed from 5 mM to 10 mM for the better response of LLOQ sample. The chromatographic response was better and higher using an acetonitrile--buffer as compared to a methanol-buffer combination. Subsequent efforts directed to optimize the mobile phase's pH and the buffer solution's concentration as they had a significant impact on analyte retention, peak shape, and resolution., The resolution of propranolol was affected at pH above 5.0, which further deteriorated with an increase in pH; thus, low pH buffers used. Better peak shape and reproducibility were observed 0.1% Formic acid in acetonitrile, but at the LLOQ level, the signal to noise ratio was not adequate. Finally, better signal to noise ratio (≥22) and resolution obtained for the analyte by replacing 10 Mm ammonium acetate buffer with 0.1% (v/v) formic acid in Milli-Q water with a flow rate of 0.5000 mL/min having apparent pH 3.50. The chromatographic elution time for Vilazodone & I.S. (propranolol) were 2.62 & 2.61 min, respectively, with an experimental time of 7.0 min. The analysis was done by gradient elution method where the solvent gradient was 0.01 to 1.00 min for organic solvent 10%, 1.00 to 4.00 min for organic solvent 90% and then 4.00 to 7.00 min for aqueous solvent 90% was observed for washing purpose—the gradient curve of method development shown in Figure 3. The MRM chromatogram Blank, Blank with I.S. and LLOQ, LQC, MQC, HQC of Vilazodone, and propranolol represented Figure 4(A–F)**.**

Table 3 Optimized instrumental (mass) parameters for analytes and IS

Parameter(s)	Value
Ionization mode	MRM (+ve)
Source temperature (°C)	400
Dwell time per transition (msec)	100
Curtain gas (psi)	30
CAD gas (psi)	8
Ion spray voltage (V)	5500
Ion source gas 1 (psi)	55
Ion source gas 2 (psi)	45
Focusing potential (V)	400
Declustering potential (V)	83 (Vilazodone) and 61 (I.S.)
Entrance potential (V)	11
Collision energy (V)	56 (Vilazodone) and 32 (I.S.)
Collision cell exit potential (V)	15 (analytes and I.S.)
Transition pair of Vilazodone (analyte)	442.2/155.4
Transition pair of Propranolol (I.S.)	260.4/116.4

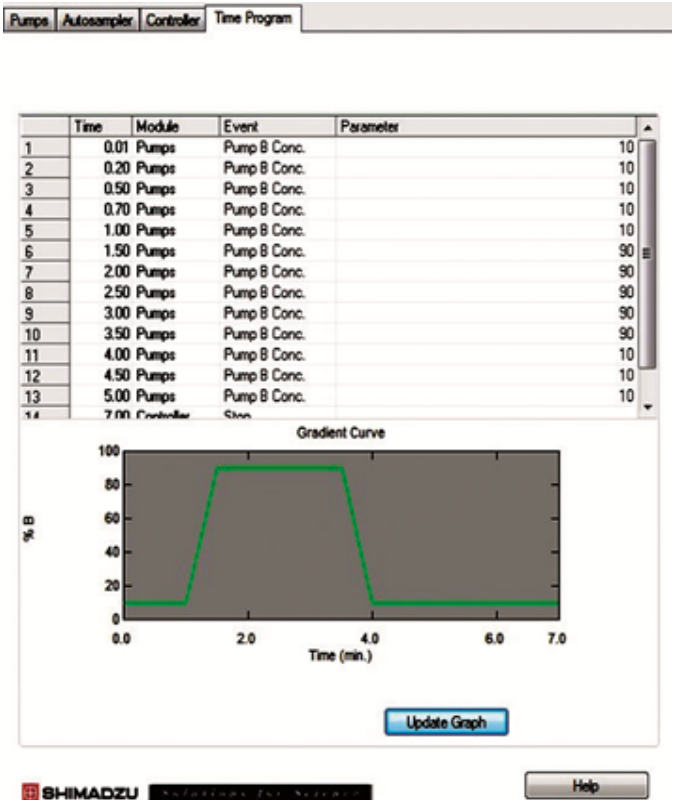

Figure 3 Gradient Curve Vilazodone.

Citation: Ghosh B, Mandal P, Chakraborty S, et al. Determination and quantitation of benzofuran, Indole and piperazine containing selective serotonin reuptake inhibitor vilazodone hydrochloride in human plasma by LC-ESI-MS/MS with an application to pharmacokinetic study under the frame work of bioequivalence study. *J Anal Pharm Res.* 2021;10(1):1–12. DOI: 10.15406/japlr.2021.10.00361

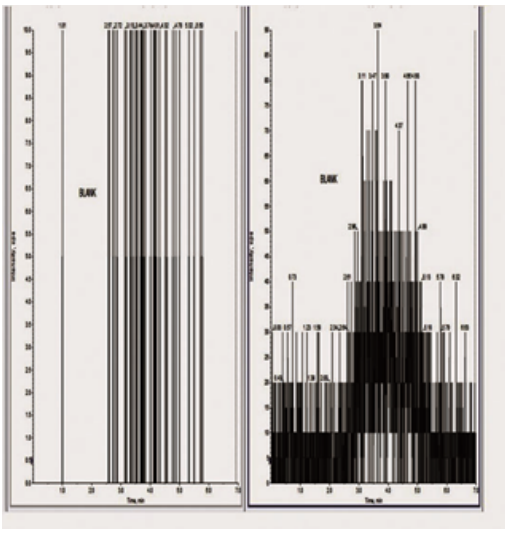

Figure 4 (A) Blank Sample Chromatogram.

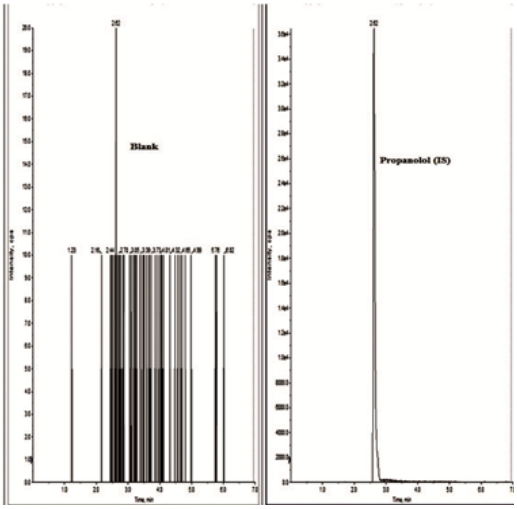

Figure 4 (B) Blank with Internal Standard Sample Chromatogram.

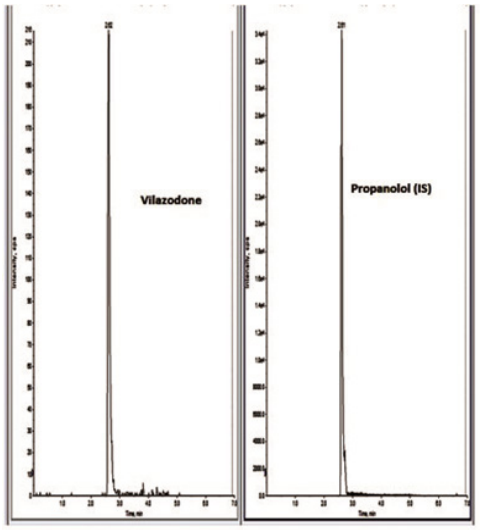

Figure 4 (C) Chromatogram of LLOQ Sample.

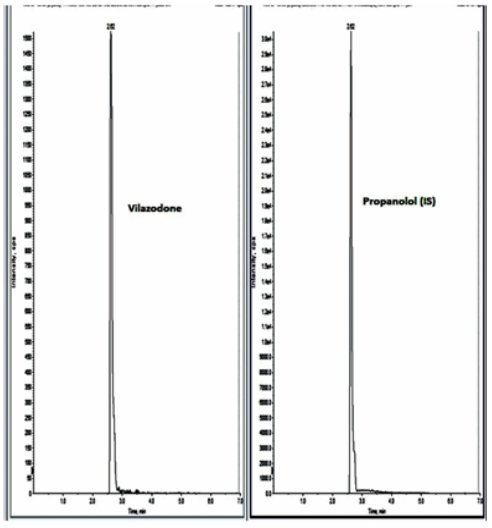

Figure 4 (D) Chromatogram of LQC Sample.

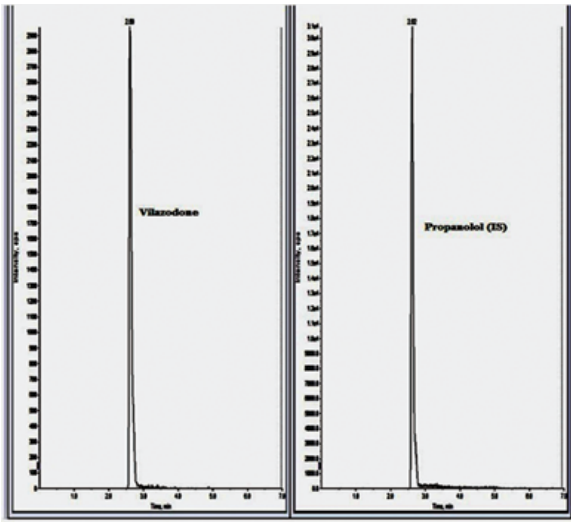

Figure 4 (E) Chromatogram of MQC Sample.

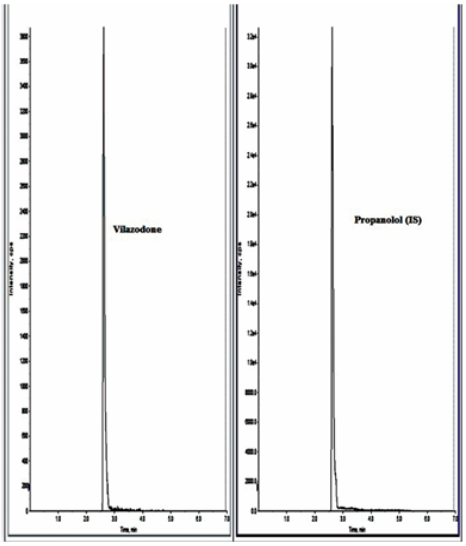

Figure 4 (F) Chromatogram of HQC Sample.

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Extraction of plasma and sample preparation

Plasma extraction performed by Protein precipitation technique, 100 µl of plasma was taken and precipitated with 400 µl of MeCN containing 500ng/ml Propranolol (I.S.) vortexed for 10 min, followed by Centrifugation for 10 mins at 12,000 rpm at 4^0C. For injection, 300µl supernatant was taken and transferred to autosampler vials.

Stock solution and calibration standards preparation

Accurately stock solutions of Vilazodone and I.S. (Propranolol) were prepared by dissolving weighed samples in the DMSO to obtain 1mg/ml concentrations. The stock solutions gradually diluted with acetonitrile: water: 50: 50 (v/v) to get calibration samples of 1.00, 2.00, 4.00, 8.00, 16.00, 32.00, and 64.00 ng/ml for Vilazodone.

Method validation

For selectivity, sensitivity, stability, linearity, precision, accuracy, and recovery following US-FDA and EMA guidelines, the method validated.[39,40]

Specificity, selectivity and linearity

The double blank samples' chromatograms illustrated the assay's specificity and selectivity; double blank piece means plasma samples processed without adding analyte or internal standard. Six such double blank samples prepared, and execution performed. Then six blank matrix samples which contain internal standard but not analyte were run. Absence of any interfering peak at analyte or standard internal R.T. indicates high selectivity of the method. An unweighted least square regression analysis determined the linearity of the calibration curve. Representative calibration curves of Vilazodone from human plasma depicted in the linearity graph.

Precision and accuracy

Inter-day precision and accuracy investigated by LQC, MQC and HQC. Intraday precision and accuracy determined from 5 replicates of each LQC, MQC and HQC. The (LQC, MQC and HQC) analyzed on day 2. The QC samples concentrations of Vilazodone determined from calibration curve LIN2. Precision was expressed as per cent variation (%CV), while accuracy measured as the per cent nominal.[39]

Stability

In the present study, the freeze-thaw, short term (S.T.), long term (L.T.) and autosampler (AS) and freeze-thaw (F.T.) stability study performed. As per USFDA guideline, the freeze-thaw stability percentage should be within 80–120%. The short term and long term stability percentage should be within 90–110%, and autosampler stability percentage should be within 85–115%.[40]

Matrix effect and recovery

In the present investigation, the matrix effect for the analyte and internal standard executed. Matrix effect and recovery determined at three different concentrations (HQC, MQC and LQC) in triplicate. The matrix effect percentage should be within 85–115% as per US-FDA guidelines.[40] Matrix effect (M.E.) determined by comparing the analyte area of drug spiked after extraction from plasma and 1:1 MeCN/Water solution containing the same analyte and I.S. concentration. The percentage improvement determined by comparing the analyte area of drug spiked into plasma before extraction and analyte area of medicine spiked into 1:1 MeCN/Water mixture.[41] Recovery at different concentration should be within ±15% variation as per USFDA guideline.

Results and discussion

Method validation

Specificity, selectivity and linearity

Plasma calibration standards prepared at following concentrations 1.00, 2.00, 4.00, 8.00, 16.00, 32.00, and 64.00 ng/ml for vilazodone. The proposed assay was found linear. The representative calibration curves were showed as linearity graphs in Figure 5. Back calculated concentration of the calibration samples for Vilazodone represented in Table 4. The lower limit of detection (LLOD) and lower limit of quantification (LLOQ) was 0.85ng/ml and 1.0ng/ml respectively for Vilazodone. The detailed elaborated in Table 4 and Figure 5**.**

Table 4 Pre-study linearity of detector response (n=3)

Linearity	Concentration (ng/ml)							Statistics		
	1	2	4	8	16	32	64	Slope (m)	Intercept	R square
LIN 1	1.02	2.03	3.67	7.28	17.29	31.97	67.81	0.00249	0.00008	0.9968
LIN 2	0.96	2.18	3.87	8.07	16.68	30.91	61.76	0.00268	0.00008	0.9982
LIN 3	0.99	2.04	4.22	7.1	16.14	32.75	65.06	0.00271	0.00032	0.9979
Mean±S.D	0.990±0.030	2.083±0.084	3.920±0.278	7.483±0.516	16.703±0.575	31.877±0.924	64.877±3.029	0.00263±0.00012		0.99763±0.00074
% C.V.	3.03	4.026	7.102	6.895	3.445	2.897	4.669	4.54201	-	0.07389
% NOMINAL	99	104.167	98	93.542	104.396	99.615	101.37	-		

Citation: Ghosh B, Mandal P, Chakraborty S, et al. Determination and quantitation of benzofuran, Indole and piperazine containing selective serotonin reuptake inhibitor vilazodone hydrochloride in human plasma by LC-ESI-MS/MS with an application to pharmacokinetic study under the frame work of bioequivalence study. *J Anal Pharm Res.* 2021;10(1):1–12. DOI: 10.15406/japlr.2021.10.00361

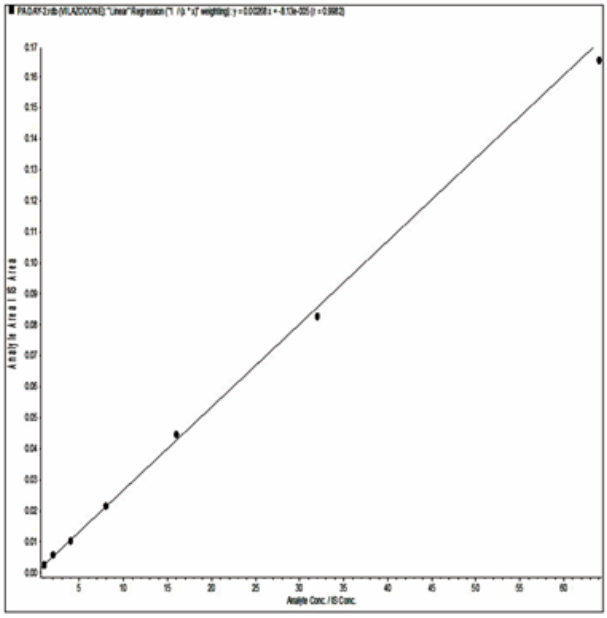

Figure 5 Calibration Curve of Vilazodone.

Precision and accuracy

Between run precision values (%CV) ranged from 4.186% to 5.548% for Vilazodone. Between run accuracy values (% nominal) were 101.467% for LLOQ, 100.622% for low Q.C. (LQC), 97.786% for medium Q.C. (MQC) and 96.593% for high Q.C. (HQC) samples. Within-run precision values (%CV) ranged from 1.385% to 5.802% for Vilazodone. Intraday accuracy values (% nominal) were 102.400% LLOQ, 99.667% for low QC (LQC), 91.983% for medium QC (MQC) and 90.442% for high QC (HQC) samples. The betwixt run and within run precision results represented in Table 5.

Stability

The stability study data elaborated in Table 5, QC samples were kept for 24 hrs at room temperature and then processed and analyzed, % benchtop stability was within 94.15% to 98.47% for Vilazodone. The autosampler stability of Vilazodone ranges between 93.95% to 95.75%. Freeze-thaw strength of the low, medium and high-quality control samples determined after three freeze-thaw cycles comparing against freshly prepared and extracted samples of the same concentration. The stability of Vilazodone ranges between 96.41% -106.71% after three cycles. Short term stability was within 94.77% to 96.51%; Long term stability found to be within 92.22% to 101.38% for Vilazodone. The detailed elaborated in Table 6.

Table 5 Precision and accuracy (n=5).

	Between Run			**Within run**		
	Mean ± S.D.	**C.V.%**	**Absolute bias (%)**	**Mean ± S.D.**	**C.V.%**	**Fundamental bias (%)**
LLOQ (1ng/ml)	1.015± 0.056	5.548	101.467	1.024±0.059	5.802	102.4
LQC(3ng/ml)	3.019± 0.126	4.186	100.622	2.990±0.109	3.648	99.667
MQC (24ng/ml)	23.469±1.245	5.305	97.786	22.076±0.350	1.586	91.983
HQC (48 ng/ml)	46.365±2.071	4.467	96.593	43.412±0.601	1.385	90.442

Table 6 Stability study data

		Inj No.	**LQC 3 ng/ml**	**MQC 24 ng/ml**	**HQC 48 ng/ml**
	Freshly thawed sample (PA Batch)	1	3.07	23.6	48.79
		2	3.18	23.91	48.14
		3	3.24	24.86	48.79
		4	3.23	25.01	48.45
		5	2.96	24.51	49.26
		Mean	3.14	24.38	48.69
Freeze-thaw stability	After Three Freeze-Thaw Cycle	1	3.15	22.87	52.94
		2	3.26	23.29	52.34
		3	3.19	22.68	51.92
		4	2.97	23.7	51.5
		5	3.11	24.97	51.07
		Mean	3.14	23.5	51.95
	% Stability		100	96.41	106.71
Short Term Stability	After 24 hours of freezing	1	3.07	23.11	48.74
		2	3.16	23.23	46.67
		3	2.93	23.59	46.1
		4	2.83	24.16	48.56

Table Continues...

		Inj No.	LQC 3 ng/ml	MQC 24 ng/ml	HQC 48 ng/ml
		5	2.87	23.36	44.87
		Mean	2.97	23.49	46.99
	% Stability		94.77	96.36	96.51
Long Term Stability	Long Term stability after 15 days of Freezing	1	3.01	22.91	46.46
		2	3.17	22.51	50.39
		3	3.21	22.28	50.49
		4	3.18	22.34	49.14
		5	2.79	22.37	50.32
		Mean	3.07	22.48	49.36
	% Stability		97.96	92.22	101.38
Bench Top Stability	Bench Top Stability after 24 hours	1	41.86	309.49	653.15
		2	40.54	320.04	670.5
		3	41.85	347.99	618.18
		4	40.35	307.99	635.64
		5	41.64	327.71	639.33
		Mean	41.25	322.64	643.36
	% Stability		99.26	104.73	105.26
Autosampler stability	Auto-sampler after	1	3.11	23.26	46.92
	24 hours	2	2.77	22.61	46.33
		3	2.99	22.45	46.25
		4	3.06	22.85	46.66
		5	3.04	23.35	46.92
		Mean	2.99	22.9	46.62
	% Stability		95.47	93.95	95.75

Matrix effect and recovery

The matrix effect of internal standard (propranolol) ranged between 93.64%-98.18%, and the same found between 94.47 % - 96.56 % in case of Vilazodone presented in Table 7. The values acknowledged and within the limit as on guidelines The peak area of the samples calculated the recovery samples of LQC, MQC, HQC. The peak regions of the low, medium and high-quality control plasma samples compared to the total peak regions of the unextracted standards containing the same concentrations of the Vilazodone. Recovery after extraction was found 98.10% - 98.99% for Vilazodone and 92.61% - 99.07% for IS and represented in Table 8.

Table 7 Matrix effect (area) (N=5)

		Internal Standard (Propanolol) AREA			
Sample	**Statistical Evaluation**	**Extracted Blank Plasma**	**Aqueous**	**Matrix Effect %**	**Matrix Factor**
LQC	Mean ± SD	146494.72±8907.94	151011.98±7236.15	97.02±4.11	0.97±0.04
(3 ng/ml)	C.V.%	6.08	4.79	4.24	4.24
MQC	Mean ± SD	155059.00±2949.47	165603.63±2364.87	93.64±1.74	0.94±0.02
24 ng/ml	C.V.%	1.9	1.43	1.85	1.85
HQC	Mean ± SD	152022.15±4605.37	154841.55±4111.52	98.18±1.07	0.98±0.01
48 ng/ml	C.V.%	3.03	2.66	1.09	1.09

Table Continues...

Analyte (Vilazodone) AREA					
Sample	**Statistical Evaluation**	**Extracted Blank Plasma**	**Aqueous**	**Matrix Effect %**	**Matrix Factor**
LQC	Mean ± SD	1492.95±58.46	1547.24±86.00	96.56±1.92	0.97±0.02
3 ng/ml	C.V.%	3.92	5.56	1.99	1.99
MQC	Mean ± SD	12588.96±482.10	13130.69±407.68	95.86±1.38	0.96±0.01
24 ng/ml	C.V.%	3.83	3.1	1.44	1.44
HQC	Mean ± SD	17776.33±938.35	18821.28±992.93	94.47±2.27	0.94±0.02
48 ng/ml	C.V.%	5.28	5.28	2.4	2.4

Table 8 Absolute recovery of analytes from plasma samples (peak area) (n=5)

Inj No.	Aqueous (AREA)			In Plasma (AREA)		
	LQC	**MQC**	**HQC**	**LQC**	**MQC**	**HQC**
	3 ng/ml	**24 ng/ml**	**48 ng/ml**	**3 ng/ml**	**24 ng/ml**	**48 ng/ml**
1	1056.02	8562.21	16638.86	1094.85	8602.55	17231.16
2	1119.87	8411.3	18719.81	1014.24	8892.52	17791.38
3	1059.14	8699.78	15488.17	1038.09	8236.31	17530.33
4	1117.18	8994.8	17912.9	1043.28	8484.98	18039.62
5	1046.78	9019.45	18512.34	1132.35	8639.42	15798.21
Mean	1079.8	8737.51	17454.42	1064.56	8571.16	17278.14
	% Recovery			98.59	98.1	98.99

Comparative pharmacokinetic study in human volunteers

The comparative pharmacokinetic study investigated in 24 healthy human Indian volunteers under fasting condition with single-dose administration. The volunteers have received both test and reference samples. Administration of the reference preparation, vilodon 20 mg tablet containing vilazodone hydrochloride 20mg. produced the maximum plasma concentration of 13.445±2.842ng/ml (C_{max}) at the time 6.792± 0.846hr. (t_{max}) for Vilazodone. The test preparation of film-coated tablet containing vilazodone hydrochloride 20mg, produced the C_{max} of 13.218±3.231ng/ml at 6.958± 0.793hr. (t_{max}) for Vilazodone. The AUC 0-t of reference préparation was 189.626±119.727 ng. hr./ml. and in test préparation was 179.504± 130.613 ng. hr./ml., and the value of the area under curve zero to-infinitives of reference and test preparation préparation was 200.366±122.664 ng. hr./ml and 185.071±132.133 ng. hr./ml respectively. The elimination constant k_{el} value of reference préparation was 0.032±0.006 $hr.^{-1}$ and in test preparation was 0.040±0.009 hr^{-1}. The elimination half-life $t_{1/2}$ of reference préparation was 22.581±3.948 hr. In test preparation, it was 18.354±4.279 hr. The pharmacokinetic parameters elaborated in the Table-9. Representative chromatograms of the volunteer plasma samples analysis and the mean plasma concentration against time profile for both the formulations were represented in Figure 6 and Figure 7, respectively.

Table 9 Pharmacokinetic parameters in human volunteers for the test and reference preparation (n=24)

Pharmacokinetic parameters	Reference Preparation (A1)	Test Preparation (A2)
Cmax (ng/ml.)	13.446±2.842	13.218±3.231
tmax (hr.)	6.792±0.846	6.958±0.793
AUC 0-t (ng. hr./ml.)	189.626±119.727	179.504±130.613
AUC 0-¥ (ng. hr./ml.)	200.366±122.664	185.071±132.133
kel (hr.-1)	0.032±0.006	0.040±0.009
t1/2 (hr.)	22.581±3.948	18.354±4.279
Relative Bioavailability (%)	100%	94.66%

Citation: Ghosh B, Mandal P, Chakraborty S, et al. Determination and quantitation of benzofuran, Indole and piperazine containing selective serotonin reuptake inhibitor vilazodone hydrochloride in human plasma by LC-ESI-MS/MS with an application to pharmacokinetic study under the frame work of bioequivalence study. *J Anal Pharm Res.* 2021;10(1):1–12. DOI: 10.15406/japlr.2021.10.00361

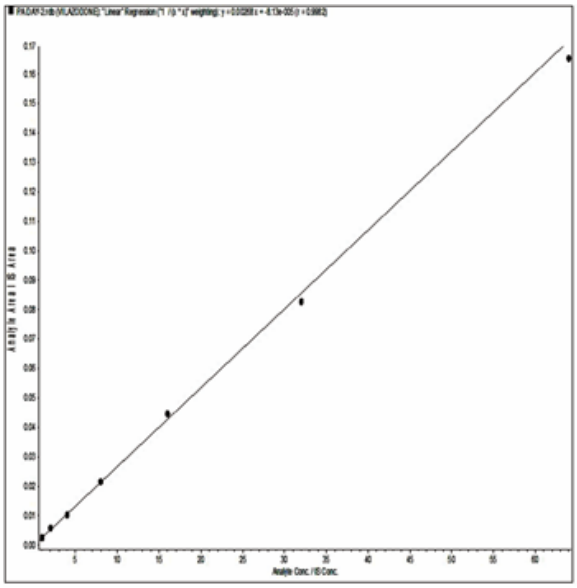

Figure 6 Volunteer Chromatogram.

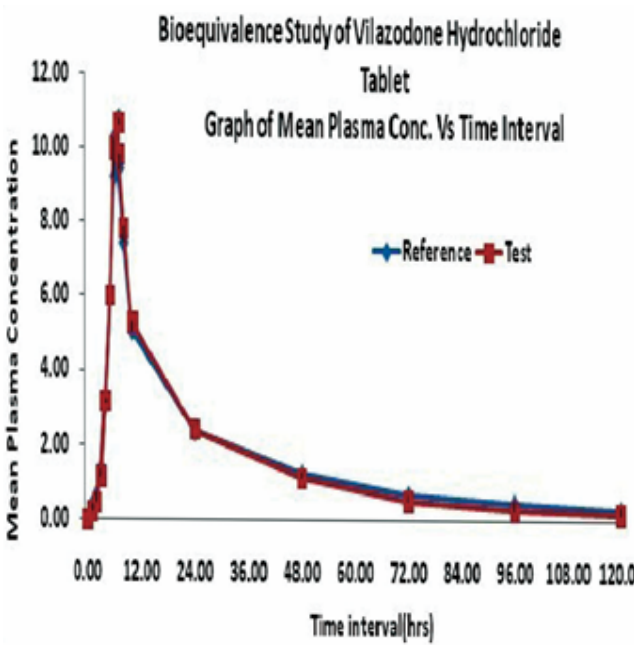

Figure 7 Mean Plasma Concentration of Test Vs Reference Preparation of Vilazodone-1.

Overall conclusion

The literature survey reveals that there were already some published articles describing the methods for determining the Vilazodone in human plasma. The developed method and validation for determining the drug vilazodone in human plasma are still not available. The present investigation deals with the advancement of an LC-MS/MS method using gradient elution technique. The developed method for determining and quantifying Vilazodone in human plasma validated as per the US-FDA guidelines.[12] The validation parameters found to be within the specified regulatory limit, hence acceptable. The present method also has a short run time (7.00 min) and easy extraction process. The developed way was simple, specific, highly selective, sensitive and reproducible. They are applied in analysing the plasma samples from volunteers obtained from the comparative pharmacokinetics study. The comparative pharmacokinetic data concluded that maximum plasma concentration of Vilazodone in the test sample was more than the reference sample but time to reach maximum plasma concentration of the same composition of two products is almost the same. The pharmacological activity of Vilazodone is due to benzofuran heterocyclic structure in the drug, so test preparation is more active than reference preparation. Moreover, the elimination constant of test preparation is more than reference preparation. As a result, the rate of a fraction of vilazodone removal from the system per hour is more in test preparation than the reference preparation. Uric acid cannot be deposited in the body because in the test preparation piperazine is more rapidly solubilized in uric acid as urate and excreted more quickly than the reference preparation.

The relative bioavailability test preparation of Vilazodone found 94.66 % of the reference preparation based on the comparison of the AUC_{0-t}. Statistical ANOVA test (subject, period, treatment) applied to the C_{max}, Ln C_{max}, AUC_{0-t} and Ln AUC_{0-t} values. No statistically symbolic discrepancy for the treatment values of C_{max}, Ln C_{max}, AUC_{0-t}, and Ln AUC_{0-t}.90% confidence interval for C_{max}, Ln C_{max}, AUC_{0-t}, and Ln AUC_{0-t} values of test preparation were within the acceptable limit of that of the reference preparation (*i.e.* 0.8–1.2). The comparable pharmacokinetic parameters depending on the chemical structure of vilazodone drug, it is proved that test preparation of Vilazodone is more active than the reference preparation of Vilazodone formulated drug. The comparative pharmacokinetics study's clinical phase under the framework of bioequivalence carried out according to the Ethics Committee's supervisions and all other pertinent ICH requirements [Step 6] 'Guidance on Good Clinical Practice'.[14] Total sixteen healthy human volunteers of 33.5±8.5 years (average age) and 22.245±2.525 kg/m^2 (average BMI) exposed to the drugs in a crossover manner represented in Table 1. Any adverse reaction during the entire clinical study period observed.

Acknowledgements

Authors are grateful to M/S, TAAB Biostudy Services, Kolkata-700032, and India to conduct clinical studies in healthy human volunteers and to the Department of Pharmaceutical Technology, Jadavpur University and Calcutta School of Tropical Medicine, Kolkata 700073, India and National Institute of Technology, Tiruchirappalli, Government of India, India.

Conflict of interest

The author declares there is no conflict of interest.

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