An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

A NOVEL LC-MS/MS METHOD FOR QUANTIFICATION OF VORTIOXETINE IN HUMAN PLASMA AND ITS APPLICATION TO PHARMACOKINETIC STUDIES

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ARTICLE INFO

Key Words

Human plasma, Vortioxetine, Solid–Phase extraction, LC/ESI–MS–MS, Pharmacokinetics,

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ABSTRACT

Background: Vortioxetine is an antidepressant which is useful in reducing major depressive disorder. It is useful in treatment of depression and cognitive disfunction in patients with major depressive disorder. After successful validation, the pharmacokinetic study was conducted by giving a dose of 20 mg of vortioxetine tablet. Objective: The aim of the current study was to develop simple, sensitive, reproducible and reliable liquid chromatography/electro spray ionization tandem mass spectrometry (LC-MS/MS) (Agilent Technologies) assay method for the determination of vortioxetine in human serum by using vortioxetine d_8 as internal standard (IS). Methods: A simple Solid-Phase Extraction (SPE) sample processing method was employed to for the extraction of vortioxetine from plasma and chromatographic method was developed with a short run time of 1.9 min. The linearity ranged from 0.05-80.0 ng/mL for vortioxetine. By applying the chromatographic method, method validation was carried by conducting different experiments such as carryover test, sensitivity, matrix effect, linearity, precision, accuracy, recovery, dilution integrity and stability. Under the fasting conditions the developed method was applied to pharmacokinetic study by giving a dose of 20mg tablet. Results: As per Food and Drug Administration (FDA) guidelines the results of validation proved that the developed method was accurate since results of validation parameters met the acceptance criteria. The validated method was successfully applied to the pharmacokinetic study of 20mg vortioxetine tablet in healthy male volunteers and estimated the amount of vortioxetine and IS. Conclusion: The developed method for vortioxetine in human plasma was validated and applied to pharmacokinetic studies.

INTRODUCTION:

Vortioxetine is an antidepressant drug using to treat depression which is made by LUNDBECK and TAKEDA pharmaceuticals companies. Vortioxetine is given orally once daily, to start with an initial dose of 5 mg or 10 mg and can be extended to a maximum of 20 mg, depending on the situation of the case under the fed condition or fasting conditions [1, 2]. Vortioxetine works in antidepressant as may agonist and

antagonist on serotonin receptors via blockade of serotonin reuptake [3-6]. Vortioxetine absorption will takes place through gastrointestinal route and reach maximum concentration (C_{max}) within 7 to 11 hrs (T_{max}) after administration [5, 7]. According to the literature, different studies have been reported on bioavailability and bioequivalence of vortioxetine [4, 8-14]. The analytical method applied for drugs quantification should be highly sensitive, quick and reproducible [15, 16]. The methods till reported for determination of vortioxetine in biological samples through UPLC/MS-MS [9, 10], HPLC/MS-MS have some drawbacks [17, 18, 19] like over range of concentration [20], methods of extraction [9, 11,14], type of biological sample [11], high run time [4,12,13] are not suitable for commercial purposes. Currently, usual chromatographic methods are not utilizing by the scientists for bioanalytical applications due to limitations in their rapidity, resolution and sensitivity. So, there is a need to develop new methods without compromising on the sensitivity and efficiency. Presently, LC–MS/MS methods are widely approved in bio-analytical applications due to its specificity and high sensitivity [17, 21].

With the above, to overcome the deficits and drawbacks the authors carried out current work to develop the quantification of vortioxetine in human plasma with a lower limit of quantification (LLOQ) 0.05 ng/mL using a high through put liquid chromatography/electro spray ionization tandem mass spectrometry LC/ESI–MS–MS using vortioxetine d_8 as internal standard (IS). The developed method solid phase extraction (SPE) technique found no variability in recoveries of analyte, IS and also free from potential matrix effect a higher sensitivity with a less run time of 1.9 min compared to other earlier reports and is applied to a clinical pharmacokinetic study in healthy male subjects successfully.

2. MATERIALS AND METHODS

2.1 Chemicals:

Vortioxetine (99.85%) was from MSN Pharmachem Private Limited (Hyderabad, India) and vortioxetine d_8 (99.57%) was obtained from DAICEL CHIRAL Technologies (Fig. 1). Milli Q water purification system procured from Millipore (Bangalore, India) was used as a source of water which is used for the LC–MS/MS analysis. HPLC grade methanol and acetonitrile was purchased from J.T. Baker (Phillipsburg, USA). Formic acid was purchased from Merck Ltd (Mumbai, India) analytical grade. The control K_2–human plasma sample was procured from Deccan's Pathological Lab's (Hyderabad, India).

2.2 LC–MS/MS instrument and conditions:

HPLC system (Shimadzu, Kyoto, Japan) consisting of a solvent degasser (DGU–20A_3), an auto sampler (SIL–HTc) and binary LC–20AD prominence pump with a peerless basic C_{18} column (100 mm × 4.6 mm, 5 µm; Chromatopak Analytical Instrumentation , INDIA) was used. An aliquot of 15 µL of the processed samples kept at ambient temperature were injected into the column. The separation of the analyte was achieved by applying a isocratic mobile phase consisting of a mixture of methanol, acetonitrile and 0.1 % formic acid (35:35:30, v/v) at flow rate 0.8 mL/min. MS–MS detection in positive ion mode for the analyte and IS using an MDS Sciex API–4000 mass spectrometer (Foster City, CA, USA) equipped with a Turboionspray ™ and the quantification was achieved with interface at 550°C and 5000 V ion spray voltage. The source parameters viz. nebulizer gas (GS1), auxiliary gas (GS2), curtain gas and collision gas were set at 35, 40, 40, and 6 psi, respectively. The compound parameters viz. the declustering potential (DP), collision energy (CE), entrance

potential (EP) and collision cell exit potential (CXP) were 90, 35, 10, 8 V for vortioxetine and 90, 35, 10, 8 V for the IS. By carefully observing the transition pairs of *m/z* 299.2 precursor ion to the *m/z* 150.1 for vortioxetine and *m/z* 307.2 precursor ion to the *m/z* 153.1 product ion for the IS, the detection of the ions was carried out in the multiple–reaction monitoring mode (MRM) and unit resolution for both the Quadrupoles Q1 and Q3 were set [22, 23, 24]. The analysis data obtained were processed by Analyst Software™ (version 1.4.2).

2.3 Preparation of plasma standards and quality controls:

In methanol standard stock solution of vortioxetine and IS (1 mg/mL) were prepared. In methanol and water (60:40, v/v; diluent) working solutions for calibration and controls were prepared by appropriate dilution. By diluting the stock solution with diluents, IS working solution (1000 ng/mL) was prepared. Stock solutions of vortioxetine and IS were found to be stable for 8 days at 2–8 °C. To obtain vortioxetine concentration levels of 0.05, 0.10, 0.51, 2.02, 12.03, 24.05, 36.01, 48.01, 60.01 and 80.01 ng/mL in plasma, 20 times of the above solutions at each concentration level in diluent was prepared and from that 50 µL was spiked into 950 µL of control K_2 EDTA human plasma. Similarly, quality control (QC) samples were also prepared as a bulk based on an independent weighing of standard drug, at concentrations levels of 0.05 (LLOQ), 0.15 (low), 12.99 (middle 1), 40.60 (middle 2) and 70.01 ng/mL (high). The aliquots of the calibration and control bulk samples were obtained by distributing into micro centrifuge tubes (Tarson, 2 mL) and stored in the freezer at –70 ± 10 °C until analyses.

2.4 Sample processing:

Before starting of the analysis calibration standards, quality control samples and all frozen subject samples, were thawed and allowed to equilibrate at room temperature. Prior to spiking the samples were vortexed to mix for 20 s. An aliquot of 300 µL of human plasma sample was mixed with 30 µL of the internal standard working solution (1000 ng/mL of vortioxetine d_8). To this, 500 µL of 1% formic acid in HPLC grade water was added and vortexed. The sample mixture will be loaded onto Copure™, 40µm, polymeric sorbent cartridges (30mg/1mL) that were pre-conditioned with 1.0 mL of HPLC grade methanol followed by 1.0 mL Milli Q/HPLC grade water. After applying the maximum pressure the extraction cartridge will be washed with 2mL of Milli Q/HPLC grade water (each time 1mL). Analyte and IS are eluted with 1.0 mL of mobile phase. Aliquot of 15 µL of the extract was injected into the LC– MS/MS system.

2.5 Bioanalytical method validation:

A complete and thorough method validation of vortioxetine in human plasma was carried out as per US FDA guidelines [25]. The method was validated for selectivity, sensitivity, specificity, carryover test, recovery, matrix effect, linearity, precision, accuracy, dilution integrity and stability. Selectivity of the method was assessed by comparing the chromatograms of six different batches of blank plasma obtained from six different sources including one lipemic and hemolyzed plasma. Carry over experiment was performed to verify any carryover of the analyte and IS are present, which may reflect in subsequent runs. The concomitant drugs such as acetaminophen, nicotine, pantoprazole, ibuprofen, caffeine, diphenhydramine, pseudoephedrine and dicyclomine were used to evaluate the potential interference. Sensitivity was determined by analyzing six replicates of plasma samples spiked with the lowest level of the calibration curve concentrations. Investigation of the Matrix effect was done to ensure that the precision, sensitivity and selectivity are not

comprised by the matrix. Matrix effect was determined by taking six different lots of K_2 EDTA plasma. Three replicate samples each of LQC and HQC were prepared from different lots of plasma (36 QC samples in total). The linearity of the vortioxetine was determined by analysis of standard plots associated with a ten–point (non–zero standards) standard calibration curve in the range of 0.05-80 ng/mL. In addition, blank plasma samples were also analyzed to confirm the absence of the direct interferences. The acceptance limit of accuracy for each of the back calculated concentration is 15% except LLOQ, where it is 20%. To determine intra–day accuracy and precision, a calibration curve and six replicates of LLOQ QC, LQC, MQC–1, MQC–2, and HQC were analyzed on the same day. Inter–day accuracy and precision were assessed by analyzing three batches of samples on two consecutive days. Recoveries of analyte and IS were determined by comparing the peak area of extracted analyte standard with the peak area of non–extracted standard. Recovery of vortioxetine was determined at a concentration of 0.15 (low), 40.60 (middle 2) and 70.01 (high) ng/mL, whereas for IS was determined at concentration of 1000 ng/mL. Dilution integrity was performed to extend the upper concentration limit with acceptable precision and accuracy. Six replicates each at a concentration of about 1.75 times and 3.5 times of the uppermost calibration standard were diluted two– and four–fold with blank plasma and there concentrations were calculated by applying the dilution factor 2 and 4. Under various conditions, stability tests were conducted to prove the analyte stability in stock solutions and in plasma samples. The stock solution stability at room temperature and refrigerated conditions (2–8°C) was performed by comparing the area response of the analyte (stability samples) with the response of the sample prepared from fresh stock solution. Bench top stability (14 h), processed samples stability (auto sampler stability for 70 h, wet extract stability for 65 h and reinjection stability for 94 h), freeze–thaw stability (4 cycles), long–term stability (108 days) were performed at LQC and HQC levels using six replicates at each level. Samples were considered to be stable if assay values were within the acceptable limits of accuracy (±15% SD) and precision (≤15% RSD).

2.6 Pharmacokinetic study design:

A single dose pharmacokinetic study was performed in healthy male subjects (*n* = 6). The Ethics Committee (Samkshema Independent Ethics Committee, Hyderabad, India) approved the protocol and the volunteers provided with written informed consent. Under the fasting condition blood samples were collected following oral administration of vortioxetine (20 mg tablet) from healthy Indian male subjects at pre–dose and 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 16, 20, 24, 36, 48 and 72 h, in K_2–EDTA vacutainer collection tubes (BD, Franklin, NJ, USA). The plasma was collected by centrifuging the tubes at 3200 rpm for 10 min and the collected plasma samples were stored at –70 ± 10 °C till their use. As described earlier the plasma samples were spiked with IS and processed as per the extraction procedure. Using WinNonlin Version 5.2 by non–compartmental model, the main pharmacokinetic parameters of vortioxetine were calculated. An incurred sample re–analysis was also conducted by selecting the 12 subject samples (2 samples from each subject) near C_{max} and the elimination phase. The percent change in the value should not be more than ±20% [26].

3. RESULTS AND DISCUSSION

3.1 Mass spectrometry:

During the process of method development, multiple reaction monitoring mode (MRM) was operated by using a standard analyte concentration of about

100 ng/mL which was injected in both the ionization modes positive and negative using electrospray as the ionization source into the mass spectrometer thereby mass parameters were optimized. The response obtained was superior in positive ionization mode for analyte and IS compared to the negative mode. Protonated form of analyte and IS, $[M+H]^+$ ion was the parent ion in the Q_1 spectrum and was used as the precursor ion to obtain Q_3 product ion spectra. The most sensitive mass transition was observed from m/z 299.2 to 150.1 for vortioxetine and from m/z 307.2 to 153.10 for the IS. The dwell time for each transition was 200 ms (Figs 2a, 2b).

3.2 Method development:

The method development incorporates selection of a suitable buffers, mobile phase, chromatography column, organic solvent, and to set a flow rate along with the injection volume. All the parameters should be carefully observed in order to produce the resolution required from endogenous components which in turn affect sensitivity and reproducibility of the analytical method by ion suppression. Once the buffers, organic solvent, mobile phase, pH, Chromatographic column, auto sampler temperatures all are set then column temperature, flow rate and buffer type and concentration can be changed for better response. Experiments were done by varying volume ratios of organic solvents such as methanol and acetonitrile, buffers like ammonium acetate (2–20 mM), ammonium formate as well as bases like ammonia, acids like formic acid (0.1–2.0%), phosphoric acid, acetic acid. The separation was achieved by changing the strength on different columns like C_{18} and C_8 of different makes (Grace, Kromasil, Zorbax, Inertsil, X bridge, develosil, phenyl, waters acquity, Hypurity advance, Hypersil etc) and also in order to obtain better chromatographic peak shapes, the effect of flow rate was varied from 0.3 to 1.0 mL/min. It was observed that combination of methanol, acetonitrile and 0.1 % formic acid (35:35:30, v/v) as the mobile phase with peerless basic C_{18} column (100 mm × 4.6 mm, 5 µm; Chromatopak Analytical Instrumentation , INDIA) column at 0.8 mL/min flow rate was more suitable and appropriate to give better peak shape, recoveries and sensitivity and also no interference and appropriate response even at a very low concentration (0.051 ng/mL) level for the analyte and chromatographic run time of 1.9 min was fixed with the retention time of analyte and the IS being very low enough (1.00 and 1.00 min).

By following the formerly reported procedures protein precipitation (PP) [8, 9,10,14] and SPE [9] to extract vortioxetine from human plasma, saliva and serum and rodents plasma [9,10,14] different sample pre–treatment methods like PP was carried out using different solvents like acetonitrile, methanol and ethanol solvents under normal, basic and acidic conditions using vortioxetine d_8 as IS. The response was varying and inconsistent particularly at the LLOQ level. In maximum cases all these solvents showed considerable ion suppression. Liquid–liquid extraction (LLE) was also tried using different solvents like hexane, dichloromethane, cyclo hexane, ethyl acetate, diethyl ether and methyl tert–butyl ether (MTBE), alone and also in combination by varying the compositions under all normal, basic and acidic conditions. LLE with various organic solvents and their combinations resulted in bad recoveries, poor chromatography and inconsistent results of the analyte and also interferences was obtained in blank plasma. But, as the aim was to develop a rapid, simple, selective and sensitive method without matrix effect, fine recoveries and good peak shapes, SPE was tried. There are two methods reported by employing HPLC–MS method using SPE with high run time i.e. about 4 min and 8 min [9,11] and also

not sensitive enough because its LLOQ is 0.200 ng/mL which is not suitable for present dosage. Thus, finally SPE was carried out using Copure™, 40µm, polymeric sorbent cartridges (30mg/1mL) in the presence of formic acid and clean samples were attained which are required for lessening of ion suppression and matrix effect on LC–MS/ MS. Consistent and quantitative recovery (~80%) was obtained at all QC levels for all the analyte. Therefore, a simple SPE technique was used for extraction of analyte and IS, mean recoveries of analyte and IS was superior and reproducible.

3.3. METHOD VALIDATION

3.3.1 Carryover effect:

To show that accuracy and precision of the specific method followed are not affected carryover was performed. There is a clear indication that no carry–over of the analyte in subsequent samples was obtained after injection of the highest concentration of analyte (upper limit of quantitation; ULQ) followed by the blank sample (data not shown).

3.3.2 Selectivity and chromatography:

The selectivity will be approved by checking the blank plasma. From the endogenous substances in drug–free human plasma at the retention time of the analyte and the IS there was no significant interference observed in the blank plasma and this was obtained by analyzing blank human plasma extract (Fig. 3a) and an extract spiked only with the IS (Fig. 3b). As shown in Fig. 3a. likewise, Fig. 3b shows the absence of direct interference from the IS to the MRM channel of the analyte. Fig. 3c shows a representative ion–chromatogram for the LLOQ (STD-A) sample (0.051 ng/mL. A representative chromatograms resulting from the analysis of subject blank plasma sample and 7.00 h subject plasma sample was obtained after the oral administration of the 20 mg single dose of vortioxetine (Fig. 4).

3.3.3 Matrix effect:

Matrix effect assessment was done with the aim to check the effect of different lots of plasma on the back calculated value of QC's nominal concentration. The results found were well within the acceptable limits (Table 1). There was no significant matrix effect was observed in all six batches of human plasma for the analyte at low and high quality control concentrations. Also, the extraction method was rugged enough and gave accurate and consistent results when applied to real subject samples.

3.3.4 Linearity, precision and accuracy:

The ten–point calibration curve was found to be linear over the concentration range of 0.05–80.01 ng/mL for the vortioxetine. After calculating, verifying and also comparing the two weighting methods ($1/x$ and $1/x^2$), a regression equation with a weighting factor of $1/x^2$ of the drug to the IS concentration are found to be the best fit for the concentration–detector response relationship.

The results obtained clearly indicate that during the method validation the mean correlation coefficient of the weighted calibration curves were was ≥ 0.99. All the results of intra–day and inter day precision deviation values were all within 15% of the relative standard deviation (RSD) at low, middle and high quality control level, whereas within 20% at LLOQ QCs level. Also the intra–day and inter–day accuracy deviation values were all within 100 ± 15% of the actual values at low, middle, and high quality control level, whereas within 100± 20% at LLOQ QCs level. The result indicates good precision and accuracy (Table 2).

Table No. 1: Matrix effect of vortioxetine in human plasma (*n* = 6)

Plasma lot	LQC (0.15 ng/mL)		HQC (70.01 ng/mL)	
	Concentration found (mean ± SD; ng/mL)	% Accuracy	Concentration found (mean ± SD; ng/mL)	% Accuracy
Lot 1	0.16± 0.00	103	70.60 ± 0.87	101
Lot 2	0.15± 0.00	101	70.41 ± 0.60	101
Lot 3	0.17± 0.01	110	70.60 ± 0.60	101
Lot 4	0.15± 0.01	101	69.64 ± 0.84	99.0
Lot 5	0.16± 0.00	108	69.93 ± 0.05	100
Lot 6	0.16± 0.00	104	70.65 ± 0.57	101

Table No. 2: Precision and accuracy data for Vortioxetine

Quality control	Run	Concentration found (mean ± SD; ng/mL)	Precision (%)	Accuracy (%)
Intra–day variations (Six replicates at each concentration)				
	1	0.05± 0.00	3.65	92.6
LLOQ	2	0.05± 0.00	3.22	98.1
	3	0.05± 0.00	5.75	104
	4	0.05± 0.00	5.11	103
	5	0.05± 0.00	4.26	104
	1	0.15± 0.00	0.89	102
LQC	2	0.16±0.00	1.68	106
	3	0.17±0.33	1.81	110
	4	0.16± 0.00	2.47	107
	5	0.16± 0.00	1.24	107
	1	13.3± 0.11	0.79	102
MQC1	2	12.3± 0.10	0.84	94.6
	3	13.7±0.16	1.13	106
	4	13.3± 0.06	0.49	103
	5	13.6± 0.08	0.60	105
	1	38.0 ± 0.31	0.80	93.6
MQC2	2	39.7 ± 0.13	0.32	97.8
	3	41.9± 0.14	0.34	103
	4	41.4± 0.30	0.74	102
	5	41.7± 0.23	0.55	103
	1	70.1± 0.49	0.70	100
HQC	2	74.1± 0.46	0.62	106
	3	73.7± 2.96	4.01	105
	4	67.1± 0.61	0.91	95.8
	5	66.4± 0.62	0.93	94.8
Inter–day variations (Thirty replicates at each concentration)				
LLOQ		0.05 ± 0.00	6.14	100
LQC		0.16 ± 0.00	3.03	106
MQC1		13.3 ± 0.53	4.00	102
MQC2		40.5± 1.52	3.74	99.8
HQC		70.3 ± 3.52	5.01	100

Spiked concentrations of LLOQ, LQC, MQC1, MQC2 and HQC are 0.05, 0.15, 13.0, 40.6 and 70.0 ng/mL, respectively.

Table No. 3: Stability data for vortioxetine in plasma (*n*=6)

Stability test	QC (spiked concentration (ng/mL)	Mean ± SD (ng/mL)	Precision (%)	Accuracy/ Stability (%)
Process[a]	0.15	0.16 ± 0.00	2.02	106
	70.0	67.3± 0.15	0.23	96.1
Wet extract[b]	0.15	0.16± 0.00	1.96	104
	70.0	67.5± 0.19	0.28	96.4
Bench top[c]	0.15	0.16± 0.00	1.20	105
	70.0	67.5± 0.52	0.77	96.4
FT[d]	0.15	0.16± 0.00	1.83	105
	70.0	67.6 ± 0.62	0.91	96.6
Reinjection[e]	0.15	0.15 ± 0.00	0.79	96.6
	70.0	68.2± 0.91	1.33	97.3
Long–term[f]	0.15	0.15 ± 0.00	1.01	101
	70.0	67.0± 0.70	1.05	96.4

[a] after 70 h in auto sampler at 10°C; [b] after 65 h at room temperature; [c] after 14 h at room temperature; [d] after 4 freeze and thaw cycles; [e] after 94 h of Reinjection;
[f] at –70°C for 108 days

Table No. 4: Pharmacokinetic parameters of vortioxetine after single oral administration of 20 mg to healthy South Indian male subjects (n=6, Mean ± SD)

PK parameter	Mean ± SD 20 mg
t_{max} (h)	8.17±1.33
C_{max} (ng/mL)	11.85±1.24
AUC_{0-t} (ng h/mL)	515.55±34.79
AUC_{0-inf} (ng h/mL)	946.71±166.57
$t_{1/2}$ (h)	59.32±16.99
Kel (h^{-1})	0.01±0.00

Table No. 5: Incurred samples re–analysis data of Vortioxetine

20 mg			
Sample	**Initial conc. (ng/mL)**	**Re–assay conc. (ng/mL)**	**Difference (%)**
1	6.185	6.221	0.58
2	3.679	3.790	2.97
3	6.278	6.311	0.52
4	4.334	4.488	3.49
5	12.309	12.436	1.03
6	5.732	5.819	1.51
7	4.040	4.091	1.25
8	2.497	2.463	1.37
9	9.458	9.436	0.23
10	5.393	5.328	1.21
11	9.699	9.627	0.75
12	5.844	5.652	3.34

[a] Expressed as [(initial conc.−re–assay conc.)/ average] × 100%.

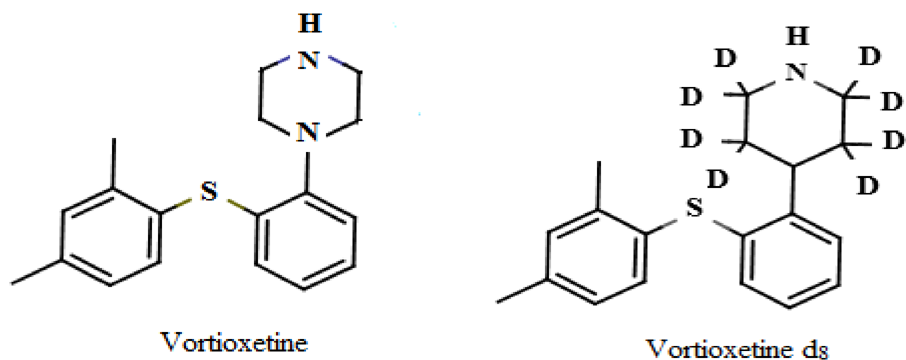

Fig 1: Chemical structures of Vortioxetine and Vortioxetine d_8 (IS)

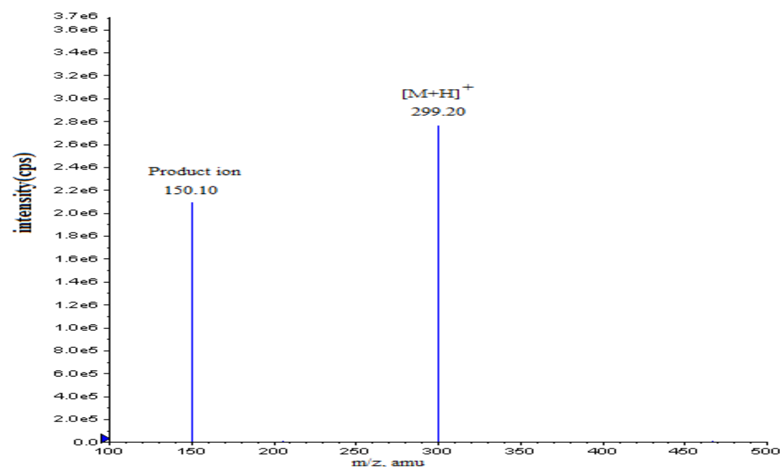

Fig no. 2a: Product ion mass spectra of $[M+H]^+$ of Vortioxetine

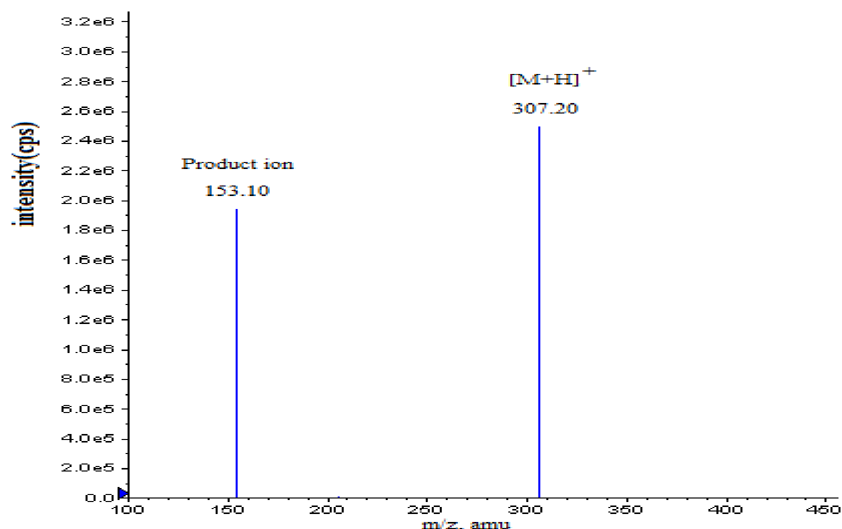

Fig no. 2b: Product ion mass spectra of $[M+H]^+$ of vortioxetine d_8 (IS)

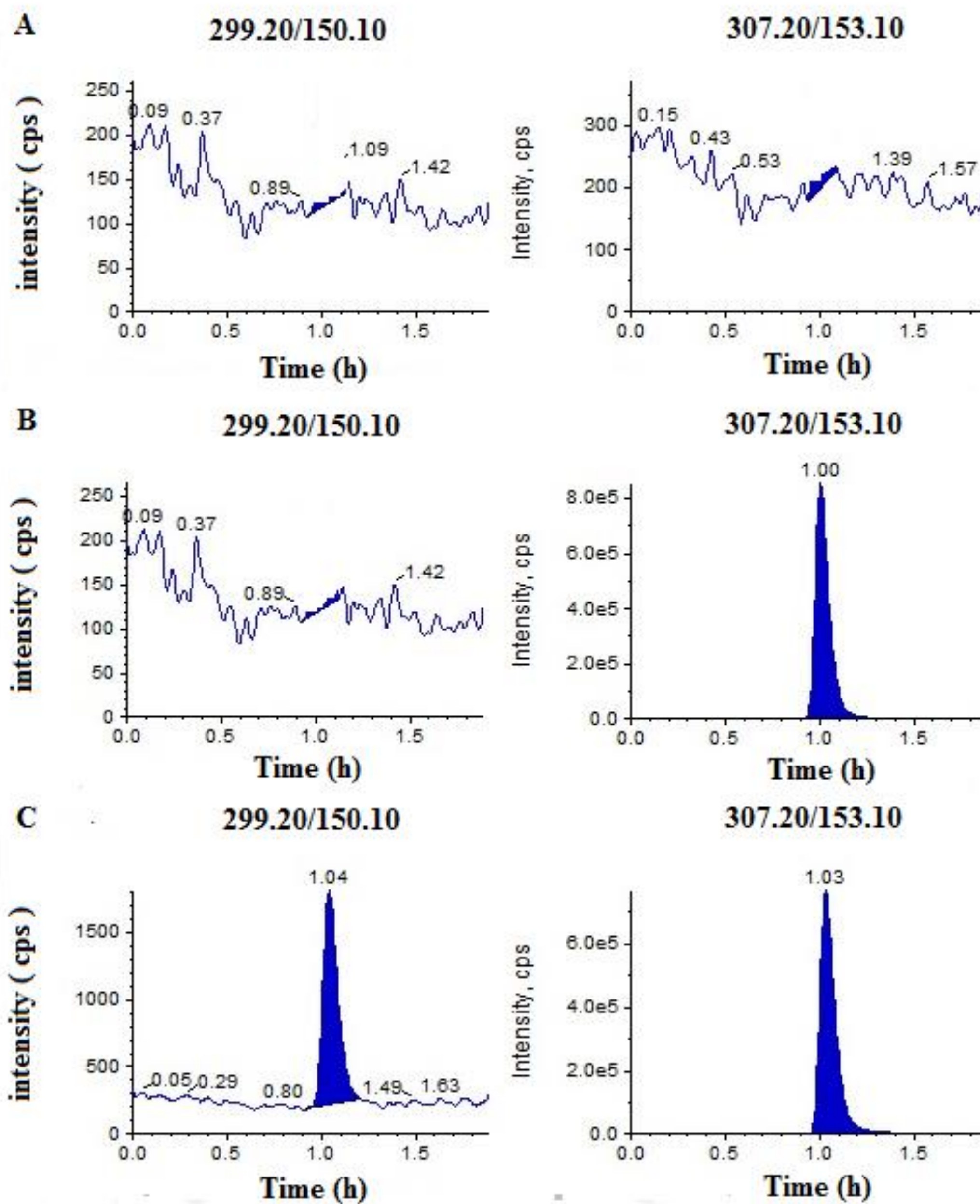

Fig no. 3: Typical MRM chromatograms of vortioxetine (left panel) and IS (right panel) in human blank plasma (A), and human plasma spiked with IS (B), a LLOQ sample along with IS (C)

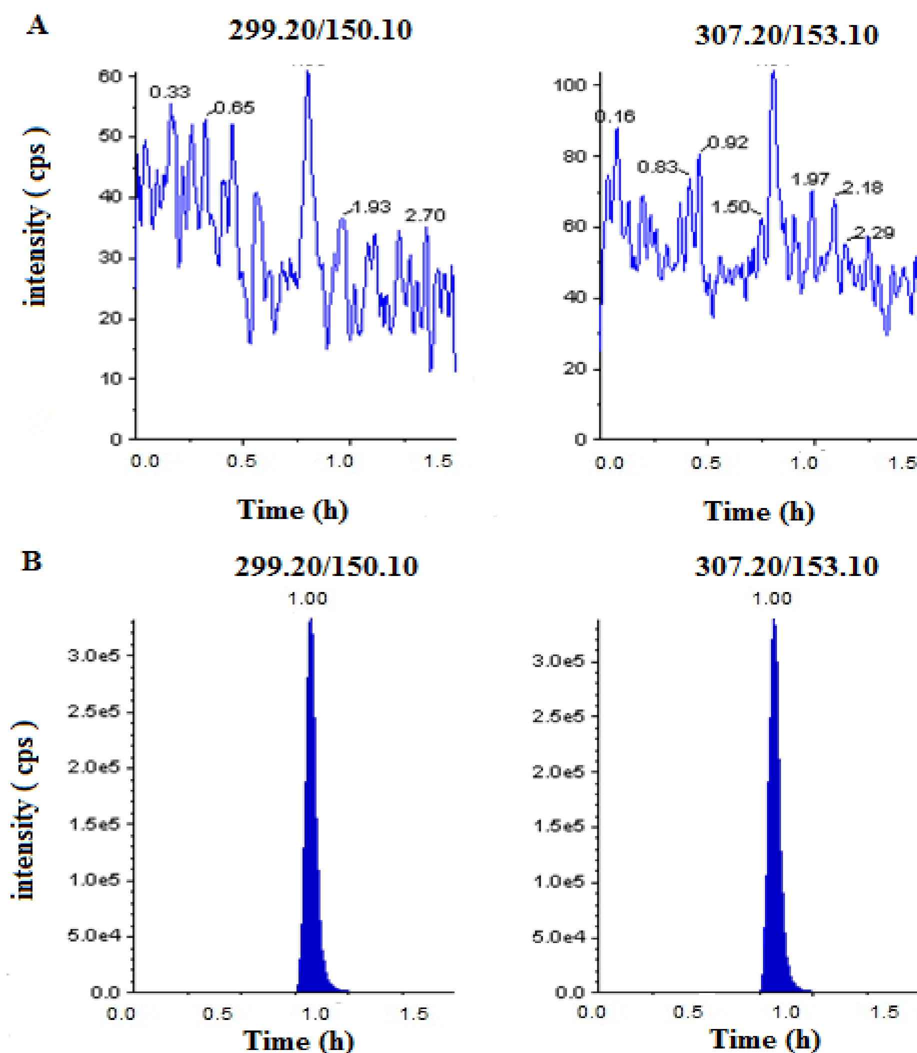

Fig no.4: MRM chromatograms resulting from the analysis of subject blank plasma sample (A) and 7.00 h subject plasma sample (B), after the administration of a 20 mg oral single dose of vortioxetine tablet. The sample concentration was determined to be 12.54 ng/mL

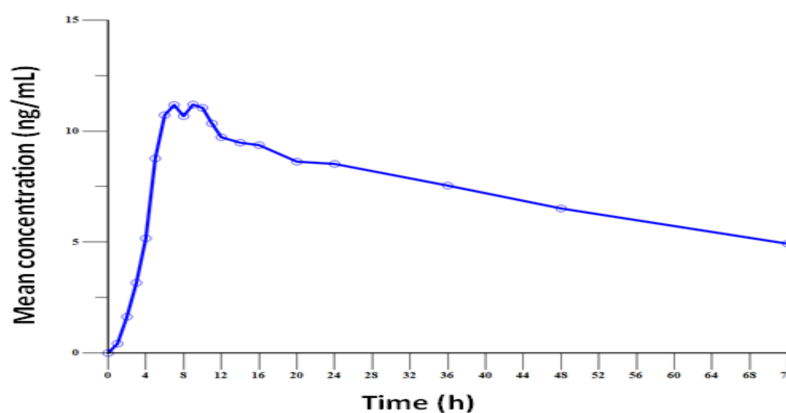

Fig no.5: Mean plasma concentration–time profile of vortioxetine in human plasma following oral administration of vortioxetine (20mg tablet) to healthy volunteers (*n*=6)

3.3.5 Extraction efficiency:

For recovery determination, six replicates at low, medium–2 and high quality control concentration for vortioxetine were prepared. The mean overall recovery of vortioxetine was 78.5±2.20% with the precision range of 0.36–3.25% and the recovery of IS was 76% with the precision range of 2.04–2.13%. The recoveries of analyte and IS were good and reproducible at all levels.

3.3.6 Stability studies and dilution integrity:

Of all the stability experiments carried out, the results achieved are bench top stability (14 h), auto sampler stability (70 h), wet extract stability (65 h), repeated freeze–thaw cycles (4 cycles), reinjection stability (94 h) and long term stability at –70 °C for 108 days the mean % nominal values of the analyte were found to be within ±15% of the predicted concentrations for the analyte at their LQC and HQC levels (Table 3). Thus, the results were found to be within the acceptable limits during the entire validation. Stock solutions of vortioxetine and IS were found to be even stable for 8 days at 2–8 °C. The percentage stability (with the precision range) of vortioxetine and IS was 104% (0.42–3.22%) and 103% (0.56–3.04%), respectively. The upper concentration limit of vortioxetine can be extended to 248 ng/mL for by 1/2 and 1/4 dilutions with screened human blank plasma. The mean back–calculated concentrations for 1/2 and 1/4 dilution samples were within 85–115% of their nominal value and the coefficients of variations (%CV) were in the range of 0.28- 1.07%.

3.4 Pharmacokinetic study results:

The validated method was successfully applied for a pharmacokinetic study of vortioxetine in 6 healthy South Indian adult male subjects who received 20 mg, respectively under the fasting condition. The mean plasma concentration–time profile of vortioxetine was presented in Fig. 5 and the corresponding pharmacokinetic parameters were listed in Table 4.

3.4.1 Incurred sample reanalysis

Incurred sample reanalysis determination and its significance was demonstrated by the FDA at the Crystal City III meeting [27], by using dosed subject samples and it is compulsory to prove assay reproducibility. Incurred sample reanalysis was analyzed by picking up two plasma samples from each subject and re–assayed in a separate run. By applying the present developed method, the results obtained were highly reproducibility and this was confirmed because the differences in concentrations between the ISR and the original initial values for all the tested samples results were less than 20% (Table 5).

CONCLUSIONS

As the present described paper was done following the present acceptable FDA guidelines, the results indicate the successful development and validation of a rapid, robost, simple and sensitive LC–MS/MS method for the determination of vortioxetine in human plasma samples. The method presented is highly sensitive (0.051 ng/mL) and usage of low plasma volume (300 µL) for analysis and interference in blank samples is nullified compared to other procedures and also additional points can be included since volume of the sample to be collected per time point is reduced from each subject during the study . Additionally, the total run time for the analysis is very low. Thus, a favorable outcome can be obtained by this method and relatively large number of samples can be analyzed in very short time. The simple SPE procedure simplifies the sample preparation procedure, reduces the chances of errors and saves much time.

The method is correctly suitability for pharmacokinetic studies in human beings. From the results of all the validation parameters, we can state that the prescribed method can be very much useful for bioavailability and bioequivalence (BA/BE) studies and routine therapeutic drug monitoring with the desired precision and accuracy.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Ana Cipher Clinical Research (Hyderabad, India) for providing necessary facilities to carry out this work.

CONFLICT OF INTEREST

We do not have any conflict of interest.

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