Article

UPLC–MS-MS Method for the Determination of Vilazodone in Human Plasma: Application to a Pharmacokinetic Study

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Abstract

A sensitive, rapid and simple liquid chromatographic–electrospray ionization tandem mass spectrometric (LC–ESI–MS-MS) method was developed for the quantitative determination of vilazodone in human plasma and for the study of the pharmacokinetic behavior of vilazodone in healthy Egyptian volunteers. With escitalopram as internal standard (IS), liquid–liquid extraction was used for the purification and preconcentration of analytes from human plasma matrix using diethyl ether. The separation was performed on an Acquity UPLC BEH shield RP C18 column (1.7 µm, 2.1 × 150 mm). Isocratic elution was applied using methanol–0.2% formic acid (90:10, v/v). Detection was performed on a triple-quadrupole tandem mass spectrometer with multiple reaction monitoring mode via an electrospray ionization source at m/z 442.21 → 155.23 for vilazodone and m/z 325.14 → 109.2 for escitalopram. Linear calibration curves were obtained over the range of 1–200 ng/mL with the lower limit of quantification at 1 ng/mL. The intra- and inter-day precision showed relative standard deviation ≤3.3%. The total run time was 1.5 min. This method was successfully applied for clinical pharmacokinetic investigation, and a preliminary metabolic study was also carried out.

Introduction

Vilazodone, 5-[4-[4-(5-cyano-1H-indol-3-yl)butyl]piperazin-1-yl]benzofuran-2-carboxamide (Figure 1A), is an indolepiperazine that functions as a potent serotonin reuptake inhibitor and 5-HT1A receptor partial agonist. It is approved for the treatment of major depressive disorder (1, 2). It produces the antidepressant effect by selective serotonin-reuptake inhibition (SSRI) together with partial serotonin (5-HT1A)-receptor agonist activity (3, 4).

Peak plasma concentration of orally administered vilazodone is 21 ng/mL (Cmax) (5). The absolute bioavailability of vilazodone was reported to be 72% under fed conditions (6, 7). It was also found that food increases the drug bioavailability (both Cmax and AUC), so it is recommended to take it under fed conditions. Vilazodone is widely distributed and is highly protein bound (96–99%). It is extensively metabolized in liver, and only 1–2% of the ingested dose is recovered in the urine and feces in unchanged form (6, 7). Cytochrome P450 (CYP3A4) is the major group of enzymes that are responsible for the metabolism and thus co-administration of CYP3A4 inhibitor or inducer may alter the metabolism of vilazodone (7). On the other hand, the pharmacokinetic profile of vilazodone is not altered in the presence of mild-to-moderate renal impairment, and no dose adjustment is recommended (7, 8).

A literature review revealed that till now there are only three approaches for the determination of vilazodone in biological matrices. Sui et al. reported a LC–MS-MS method for the quantification of vilazodone in rat plasma having a narrow calibration range (1.0–100 ng/mL) with a total run time of 2.2 min (9). Another reported LC–MS-MS assay of vilazodone in dog plasma did not describe full validation results with a run time of 6 min (5). Iqbal et al. reported a UPLC–MS-MS method for the quantification of vilazodone in rat and human plasma using protein precipitation technique as the sample preparation procedure for the extraction of vilazodone from plasma, which gave low recovery results (53.6%) (10). Considering vilazodone as an approved antidepressant drug, a simple, sensitive and reliable method is needed for the determination of vilazodone in human plasma. In the present study, a sensitive, rapid, and simple liquid chromatographic–electrospray ionization tandem mass spectrometric (LC–ESI–MS-MS) method was developed for the quantitative determination of vilazodone in human plasma.
bioanalytical assay is required for pharmacokinetic, therapeutic drug monitoring and forensic toxicology studies. Hence, the scope of the present investigation is to develop a fast, sensitive and specific UPLC–MS-MS method for the rapid quantitation of vilazodone in human plasma with a small sample volume and short run time. Moreover, it employs a cheaper and more effective plasma deproteinization, liquid–liquid extraction, as it avoids matrix effect and gives better sensitivity than the protein precipitation method that was used in the reported method (10). Furthermore, it was successfully applied to a pharmacokinetic study. Also, a preliminary metabolic study in vitro and in vivo was carried out.

Experimental

Chemicals and reagents
Vilazodone HCl standard (certified to contain 99.1%) and Viibryd tablets, nominally containing 40 mg of vilazodone HCl per tablet (batch no. MTCT17451), were supplied by Forest Pharmaceuticals, Inc., USA. Bi-distilled water was produced in-house (Aquatron Water Still, A4000D, UK). Methanol of HPLC grade was purchased from Sigma-Aldrich, Germany. All other chemicals and reagents used were of analytical grade. Blank ethylene diamine tetra acetic acid human plasma samples were obtained from Kasr El-Aini Teaching Hospital, Cairo, Egypt.

Chromatographic conditions
Chromatographic elution was performed on a Waters TQD LC–MS-MS system with a mobile phase consisting of methanol–0.2% formic acid in water (90:10, v/v), which was degassed ultrasonically for 5 min and then pumped through an Acquity UPLC BEH shield RP C18 column (1.7 µm, 2.1 x 150 mm) at a flow rate of 0.3 mL/min.

The LC–UV separation was carried out on a Supelco Discovery HS [C18 (25 cm, 4.6 mm, 5 µm)] column as the stationary phase using a mobile phase consisting of methanol–water (75:25 v/v). The flow rate was adjusted to 1 mL/min. Twenty microliters of each solution were injected (20 μL). Vilazodone and its metabolites were monitored with UV detection at 242 nm.

Mass spectrometry conditions
Waters TQD LC–MS-MS system was operated in the positive ion mode for the detection of vilazodone and the IS. The desolvation gas flow was set at 600 L/h, and desolvation temperature was 400°C. The source temperature was set at 120°C. Multiple reaction monitoring (MRM) transitions were measured at positive mode at m/z 442.21 → 155.23 for vilazodone and m/z 325.14 → 109.2 for IS with a 0.146 s dwell time of all drugs. The cone voltage was set at +30 V for vilazodone and +35 V for IS. The collision energy was set at 35 V for vilazodone and 50 V for IS. Quantitation of the analytes in human plasma was based on the peak area ratios of the cited drug versus the IS. Data acquisition and processing were performed using Masslynx Workstation software (4.1 SCN 805).

Preparation of stock and working solutions
Vilazodone stock solution of 100 µg/mL was prepared in methanol. Working solution of vilazodone was prepared at 2 µg/mL by diluting 2 mL of stock solution to 100 mL methanol. Escitalopram (IS) was prepared in methanol at a concentration of 100 ng/mL. Calibration standards ranging from 1 to 200 ng/mL were prepared by adding 50 µL of known serial dilutions from the working solution of vilazodone, 50 µL of conc. ammonia and 50 µL of IS solution to 450 µL of drug-free human plasma. The quality control (QC) samples were prepared in a manner similar to the calibration standard at three concentration levels: low, medium and high (3, 80 and 160 ng/mL). All samples were vortexed to ensure complete mixing. During each run, six replicates of QC samples were used along with the calibration standards to verify the reproducibility, repeatability and integrity of the method.

Figure 1. Chemical structure of (A) vilazodone, (B) escitalopram, (C) hydroxy and (D) sulfonated metabolites of vilazodone.
Extraction procedure
The liquid–liquid extraction method was used for extraction of vilazodone from plasma matrix. To 500 µL of each spiked calibration plasma standards or QC samples, 5 mL of diethyl ether was added. The solution was vortexed for 1 min and then centrifuged at 5,000 rpm at 4°C for 5 min. Then, 3.5 mL of the supernatant were collected and evaporated to dryness at 45°C. The residue was reconstituted in 100 µL of the mobile phase and then transferred into a glass vial for LC–MS-MS analysis.

Method validation
The method validation is performed as per FDA guidelines (11).

Linearity
A series of calibration standards (1–200 ng/mL) were prepared, and three linearity curves containing nine non-zero concentrations were analyzed. A good correlation of more than 0.99 was obtained.

Specificity
Six randomly selected drug-free human plasma samples were processed by the same liquid–liquid extraction procedure and analyzed to determine the extent to which endogenous plasma components may contribute to the interference at the retention time of analyte and IS.

Recovery (extraction efficiency) from plasma matrix
Recovery of vilazodone was evaluated by comparing the mean peak responses of six extracted QC samples of low, medium and high concentrations to mean peak responses of six plain standards of equivalent concentrations. Similarly, the recovery of IS was also evaluated at the concentration of 100 ng/mL.

Accuracy and precision (inter- and intraday)
Intraday accuracy and precision were evaluated by six-replicate analysis of vilazodone at concentrations of LLOQ, low, medium and high QC samples in human plasma. The interday accuracy and precision were assessed by analysis of low, medium and high QC samples for vilazodone on three consecutive days. The precision of the method was determined by calculating the percent coefficient of variation (% CV) for the concentrations obtained for different determinations.

Stability
To evaluate the stability of vilazodone under different storage conditions, three aliquots of each low, medium and high QC samples were stored in a deep freezer at −80 ± 2°C for 6 days. After 6 days, the samples were processed along with precision and accuracy batches. Concentrations obtained were compared with nominal concentrations to determine the long-term stability of vilazodone in human plasma. The short-term stability was determined by keeping three aliquots of unprocessed QC samples at ambient temperature for 6.0 h. After 6.0 h, the samples were processed, analyzed and compared with nominal concentrations. In addition, autosampler stability was determined by analyzing three aliquots of QC samples that were processed and reconstituted before storing for 24 h. At the end of 24 h, samples were reanalyzed and concentrations were compared with the freshly prepared control samples for the cited drug, and the area of IS obtained from analysis of control samples after 24 h was compared with the area of IS obtained from freshly prepared control sample analysis. The stability of QC samples was also studied after three freeze and thaw cycles. Three aliquots of unprocessed QC samples were stored at −80 ± 2°C and subjected to three freeze and thaw cycles. After the completion of the third cycle, the samples were processed, analyzed and the results were compared with nominal values.

Table I. Recovery Results of Vilazodone from Plasma by the Proposed LC–MS-MS Method

<table>
<thead>
<tr>
<th>QC sample</th>
<th>Mean recovery ± SD</th>
<th>CV %</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>83.1 ± 3.2</td>
<td>3.8</td>
<td>6</td>
</tr>
<tr>
<td>Medium</td>
<td>79.1 ± 3.0</td>
<td>3.8</td>
<td>6</td>
</tr>
<tr>
<td>High</td>
<td>81.0 ± 2.1</td>
<td>2.6</td>
<td>6</td>
</tr>
</tbody>
</table>

Figure 2. (A) Chromatogram of the extracted blank plasma sample. (B) Chromatogram of vilazodone in the plasma taken from volunteers after 5 h of administration and spiked with IS.
Table II. Intraday and Interday Results of Vilazodone in Human Plasma by the Proposed LC–MS-MS Method

<table>
<thead>
<tr>
<th>Accuracy and precision</th>
<th>QC samples</th>
<th>Concentration of vilazodone in plasma (ng/mL)</th>
<th>Mean found conc. of vilazodone (ng/mL)</th>
<th>±SD</th>
<th>CV%</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraday</td>
<td>LLOQ</td>
<td>1</td>
<td>0.97</td>
<td>0.1</td>
<td>6.8</td>
<td>97.1</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>3</td>
<td>2.9</td>
<td>0.2</td>
<td>3.3</td>
<td>99.3</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>80</td>
<td>82.6</td>
<td>1.1</td>
<td>0.3</td>
<td>101.7</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>160</td>
<td>161.3</td>
<td>1.6</td>
<td>1.0</td>
<td>100.8</td>
</tr>
<tr>
<td>Interday</td>
<td>LLOQ</td>
<td>1</td>
<td>0.99</td>
<td>0.1</td>
<td>4.7</td>
<td>98.7</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>3</td>
<td>2.9</td>
<td>0.1</td>
<td>1.1</td>
<td>99.1</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>80</td>
<td>82.0</td>
<td>1.9</td>
<td>0.5</td>
<td>101.1</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>160</td>
<td>161.1</td>
<td>1.7</td>
<td>1.0</td>
<td>100.7</td>
</tr>
</tbody>
</table>

Pharmacokinetic study

Samples for a pharmacokinetic study were collected from six healthy male volunteers after administration of a single dose of a Viibryd tablet (containing 40 mg vilazodone HCl). The study protocol was reviewed and approved (PC (1236), approved on November 2014) by the Institutional Review Board (REC-FOPCU; Research Ethics Committee-Faculty of Pharmacy, Cairo University) in Egypt. The nature and the purpose of the study were fully explained, and an informed written consent was obtained from each volunteer.

The volunteers were provided with an informed written consent. Blood samples were collected into heparinized tubes before (0 h) and at 0.5, 1.0, 2.0, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 7.0, 8.0, 10, 24 and 48 h after administration. The tubes were centrifuged at 4,250×g for 5 min. Then, the plasma was separated and stored at −80°C until further analysis. An aliquot of 500 µL of thawed plasma samples was spiked with 50 µL of IS, and 50 µL of ammonia then was processed and analyzed.

Pharmacokinetic parameters of vilazodone were calculated by a noncompartmental method using Kineta2000 version 3.0 program. The maximum plasma concentration (C_{max}) and the corresponding peak time (T_{max}) were observed directly from the individual drug plasma concentration–time profile. The terminal elimination rate constant (ke) was estimated by log-linear regression of concentrations observed during the terminal phase of elimination. The area under the plasma concentration–time curve was calculated by the linear trapezoidal rule.

Preliminary metabolic study

In vitro study

Five male Wistar rats (220 ± 10 g body weight) were maintained in compliance with the policy of animal care expressed in the National Research Council guidelines (NRC 1985). Liver homogenate and liver subcellular fraction (9,000x g S9 fraction) were prepared. For liver homogenate, the animals were killed by cervical dislocation; the livers were immediately perfused with cold NaCl (0.9%, w/v), excised, minced in saline and homogenized. For liver Homogenate Fraction “S-9”, we used the procedure of Garner et al. (12). All steps were performed at 0–4°C with cold and sterile solutions and glassware. The liver (rat livers were 10–25 g each) was washed in an equal volume of 0.15 M KCl, minced with sterile scissors in three volumes of 0.15 M KCl (3 mL/g of wet liver) and homogenized with a Heidolph silent crusher M homogenizer with a Teflon pestle. The homogenate was centrifuged for 10 min at 9,000 × g, and the supernatant, (S-9) fraction, was decanted and saved.

Vilazodone (1 mL) solution (0.01195 g Vilazodone HCl) was dissolved in 25 mL dimethylsulfoxide (DMSO) to prepare a solution 1 mM of Vilazodone HCl) was incubated with 3 mL of the supernatant (S9 fraction) or with 3 mL of the whole liver homogenate. After

Table III. Summary of Stability of Vilazodone in Human Plasma

<table>
<thead>
<tr>
<th>Stability term</th>
<th>Concentration of vilazodone (ng/mL)</th>
<th>Mean found conc. ± SD (%)</th>
<th>Accuracy (%)</th>
<th>Precision (CV %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long-term (6 days)</td>
<td>3</td>
<td>2.9 ± 0.1</td>
<td>98.3</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>81.2 ± 0.6</td>
<td>101.5</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>160</td>
<td>161.0 ± 1.2</td>
<td>100.6</td>
<td>0.7</td>
</tr>
<tr>
<td>Short-term (6 h)</td>
<td>3</td>
<td>3.1 ± 0.1</td>
<td>101.8</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>80.6 ± 1.8</td>
<td>100.7</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>160</td>
<td>159.7 ± 1.3</td>
<td>99.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Autosampler (24 h)</td>
<td>3</td>
<td>3.0 ± 0.5</td>
<td>100.6</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>79.1 ± 1.1</td>
<td>98.8</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>160</td>
<td>159.1 ± 0.4</td>
<td>99.5</td>
<td>0.3</td>
</tr>
<tr>
<td>Freeze and thaw</td>
<td>3</td>
<td>3.1 ± 0.01</td>
<td>103.1</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>80.7 ± 1.7</td>
<td>100.9</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>160</td>
<td>160.2 ± 0.7</td>
<td>100.1</td>
<td>0.4</td>
</tr>
</tbody>
</table>

different time intervals (30 min, 1, 2, 3, 4, 6 h), protein precipitation was done by methanol (2 × vol). The samples were vortexed for 2 min, centrifuged for 5 min, and the supernatant was injected into LC–UV and LC–MS-MS.

In vivo study

Blood samples withdrawn from volunteers after 8 h of a single dose administration of Viibryd tablet were used for the detection of vilazodone metabolites. The tubes were centrifuged at 4,250×g for 5 min. Then, the plasma was separated and stored at −70°C until further analysis.

Results

Linearity and limit of quantitation

The calibration curve for vilazodone was constructed by plotting the peak area ratio of the drug to the IS versus the concentration of the drug. The constructed calibration curve was found to be linear and precise over the linearity range of 1–200 ng/mL. The lower limit of quantitation was 1 ng/mL (lowest standard level) with accuracy of 97.1%.

The regression equation was also computed and found to be peak area ratio (PAR = 0.0213 C (ng/mL) + 0.0073, and the correlation coefficient was found to be 0.998. The regression equation for the overall calibration curve was computed for calculating the concentration of vilazodone in human plasma. Back calculations were made from the calibration curve to determine accuracy of each calibration standard.

Specificity

There was no significant interference at the retention times of vilazodone or IS from the six different batches of drug-free human plasma
Figure 3. Mass spectra of (A) vilazodone and (B) escitalopram.
used for analysis (Figure 2) and from a human plasma sample taken from volunteers after 5 h post-dose.

Recovery (extraction efficiency) from plasma and matrix effect
The mean recoveries of vilazodone extracted from human plasma were 83.1% ± 3.2, 79.1 ± 3.0 and 81.0 ± 2.1 at concentrations of 3, 80 and 160 ng/mL (Table I), respectively. IS recovery from plasma was found to be 81.6 ± 0.8 (mean ± SD, %). As per the acceptance criteria (US DHHS, FDA, CDER, 2001), the recovery of the analyte do not need to be 100%, but the extent of recovery of an analyte should be consistent and within the accepted values for precision and reproducibility.

The matrix effect on vilazodone from six different lots of human plasma was in the range of 96.7–98.9% with RSD values below 4.2% at concentrations of 3, 80 and 160 ng/mL. The matrix effect on IS was 97.7%, and the RSD was 5.4%. These results suggested that the effect of matrix on the quantification of vilazodone was insignificant.

Accuracy and precision (inter- and intraday)
For the evaluation of precision, the deviation of each concentration level from the nominal concentration was expected to be within ±15.0%. Similarly, the mean accuracy should not deviate by ±15.0% of the nominal concentration (11). Coefficient of variation for intraday precision was between 0.3 and 6.8%, and the accuracy values were found to be between 97.1 and 101.7%. Interday accuracy was between 98.7 and 101.1% with a coefficient of variation of 0.5–4.7%. The results are presented in Table II.

Stability
Vilazodone was stable at −80 ± 2°C in a deep freezer for 6 days (long-term stability) in human plasma. Also, it was found to be stable over 6 h in human plasma at room temperature (23–30°C) (short-term stability). In the autosampler, reconstituted samples of vilazodone were stable for 24 h after sample processing.

Frozen plasma samples were found to be stable even after subjecting to three freeze-thaw cycles at concentrations of low, medium and high QC samples (Table III). All the stability samples were considered stable if the deviation from the nominal concentration was within ±15%.

Pharmacokinetic study
The suitability of the developed method for clinical use was demonstrated by the determination of vilazodone levels in plasma from six healthy male volunteers after administration of a single dose of Viibryd tablet (containing 40 mg vilazodone HCl). The MRM chromatogram of vilazodone in plasma taken from a volunteer after 5 h of tablet administration is shown in Figure 2B, where no interfering peaks were observed. The plasma concentration–time profile of vilazodone in volunteers’ plasma was calculated after a single oral dose of 40 mg. The maximum plasma concentration (C_max) was 22.85 ± 17.9 ng/mL attained at 7 ± 1.41 h (T_max). The AUC0–48h value was 275.99 ± 136.13 ng h/mL. The data obtained from this study was comparable with the previous FDA reports (11). Thus, our method could be useful for clinical pharmacokinetic analysis.

Discussion
Chromatography and mass spectrometry
During method optimization, several chromatographic conditions were attempted using various mobile phase compositions of water with methanol and 0.2% formic acid with methanol or acetonitrile in different proportions in an isotropic mode. It was found that a mobile phase consisting of methanol–0.2% formic acid (90:10, v/v), at a flow rate of 0.3 mL/min, using an isocratic mode gave good elution of the drug and IS. The use of methanol allowed a better elution of analytes with sensitive response. Then, 0.2% formic acid was used to obtain higher detection response through assisting the ionization of the cited drug molecules. Different ISs (montelukast, aripiprazole and escitalopram) were tried. It was found that escitalopram is the IS of choice as it showed no interference with the cited drug and gave optimum response. The use of UPLC BEH shield the RP C18 column with a particle size of 1.7 µm packing material achieved the desirable peak shape and elution of vilazodone as well as the IS. Also, the use of shorter column gave the advantage of shorter run time, allowing faster batch analysis of human plasma samples.

MS conditions were optimized to obtain sensitive and stable signal response of mass transition of the analyte and IS for the
quantification of the analyte. Vilazodone was easily ionized to form protonated ion of \([M + 2]^+\) at \(m/z\) 442.4 in positive ionization mode. In product-ion full scan spectrum of vilazodone, the most abundant fragment ion was \(m/z\) 155.3 (Figure 3A). Similarly, mass transition of \(m/z\) 325.1 → 109.0 was used for the detection of escitalopram (IS) (Figure 3B).
Preparation of plasma samples
Liquid–liquid extraction is a more effective method than protein precipitation as it avoids matrix effect and gives better sensitivity, and thus it was adopted in the present method for the preparation of samples. Different organic solvents [ethyl acetate, dichloromethane, diethyl ether and mixture of dichloromethane–diethyl ether (30:70 v/v)] were tried to find the suitable solvent for extraction of vilazodone from plasma. It was found that diethyl ether was the optimum solvent for extraction as it gave the highest response with optimum peak shape in short time (1.8–2.0 min).

Preliminary metabolic study
In vitro study
Investigation of the results obtained from the samples incubated with S9 fraction and with whole liver homogenate for 1 h showed that S9 fraction is a more complete drug metabolizing enzyme system than the whole liver homogenate. This appears in HPLC–UV chromatograms (Figure 4), where the vilazodone peak almost disappeared after incubation with S9 fraction for 1 h. The total ion current (TIC) of S9 fraction (Figure 5) showed the presence of vilazodone, in addition to two peaks at $t_r$ (3.8–4.5, 3–3.6, 1.8–1.98 min, respectively). The MS$_2$ scan (quadrupole product-ion scan) of the peak at 3–3.6 min showed M$^+$ at 454.3 (Figure 6A). This peak could be the hydroxy metabolite of vilazodone, which is the major metabolite previously reported by FDA (13) (Figure 1C). Moreover, the small polar peak at 1.8–1.98 min showed a molecular ion M$^+$ at 539.2 in (MS$_2$ scan) spectrum (Figure 6B), which is suggested to be the sulfonate conjugate of the hydroxyl metabolite (Figure 1D).

In vivo study
The total ion current (TIC) chromatogram of plasma sample, withdrawn from volunteers after 8 h of single-dose administration of Viibryd tablet, was used for the detection of vilazodone metabolites. The MS$_2$ (quadrupole product-ion scan) (Figure 7) showed small peaks of hydroxy and acid metabolites of vilazodone at M$^+$ 458 and 443, respectively.

Conclusion
The described bioanalytical method for the determination of vilazodone in human plasma is specific, sensitive, accurate and precise to be successfully applied to human clinical pharmacokinetic studies. Sample preparation was performed by using liquid–liquid extraction with adequate recovery, followed by isocratic UPLC coupled with tandem mass spectrometric detection (LC–MS–MS). The LC–MS–MS method was sensitive and capable of estimating vilazodone down to 1 ng/mL in human plasma with good accuracy and precision. Preliminary metabolic investigation of vilazodone showed good correlation between in vitro and in vivo with published FDA data.

References