A Rapid and Selective LC-MS/MS Method for Quantification of Quetiapine in Human Plasma and its Application to Pharmacokinetic Study on Indian Schizophrenia Patients

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Abstract: A rapid, robust and selective high pressure liquid chromatography–positive electrospray ionization tandem mass spectrometry method has been developed and validated for the quantification of quetiapine (QUE) in human plasma with K2EDTA using oxcarbazepine (IS) as an internal standard. Analyte and internal standard were extracted from human plasma by solid-phase extraction using acetonitrile. The eluted samples were chromatographed on a C18 column by using a 10:75:15v/v mixture of ammonium formate buffer (5 mM, pH 4.50) and acetonitrile and methanol as an isocratic mobile phase at a flow rate of 0.4 mL/min and analyzed by mass spectrometry in the multiple reaction monitoring mode using the respective [M+H]+ ions, m/z 384.3/253.2 for Quetiapine and m/z 253.1/208.1 for the internal standard. The assay exhibited a linear dynamic range of 5.01 - 2501.04 ng/mL for quetiapine in human plasma. Acceptable precision and accuracy were obtained for concentrations over the standard curve range. A run time of 2.5 min for each sample made it possible to analyze 300 patient plasma samples per day. The validated method has been successfully used for the estimation of quetiapine in real time schizophrenia patient’s plasma samples for pharmacokinetic study.

Keywords: Quetiapine, LC-MS/MS, Schizophrenia patients Plasma, Pharmacokinetics

Introduction

Quetiapine (Figure 1) is an atypical antipsychotic and is a dibenzothiazepine derivative with greater in vitro binding affinity for serotonin 5-HT2A receptors than for dopamine D2 receptors1.
This property is widely considered to predict atypicality, which is defined clinically as minimal or absent extrapyramidal symptoms (EPS)\(^2\) at clinically relevant doses. Quetiapine is a widely used second-generation antipsychotic that is effective in the treatment of schizophrenia and bipolar mania\(^3\). Quetiapine is an antagonist at a broad range of neurotransmitter receptors\(^1,4\). The advantages of the therapeutic profile of quetiapine have led to increasing use in the clinical practice, which encourages the development of new pharmaceutical preparations\(^5\). Quetiapine is primarily and extensively eliminated by hepatic metabolism, mainly through sulphoxidation by cytochrome P450 (CYP) 3A4\(^5,6\). The activity of quetiapine is mainly due to the parent drug, whose elimination half-life is 5.8 hours in men and 6.6 hours in women. Quetiapine is an antagonist at a broad range of neurotransmitter receptors\(^1,4\). The activity of quetiapine is mainly due to the parent drug, whose elimination half-life is 5.8 hours in men and 6.6 hours in women.

![Structural formula of quetiapine fumarate](image)

**Figure 1.** Chemical structures of QUE and IS

Many methods have been used to determine quetiapine in human in biological materials including HPLC with UV\(^7,14\). Electrospray ionization MS\(^15,18\) tandem MS/MS\(^4,19,20\). UPLC with tandem MS\(^21,22\). GC\(^23,24\).

Although chromatographic methods offer high degree of specificity, yet, sample clean up, sample pretreatment and the instrumental limitations preclude their use in routine analysis. The above mentioned methods are having some advantages and some disadvantages in terms of sensitivity, sample preparation either time consuming and/or require complex pretreatment or long run time, sample volume, instrumental advantages, and extraction techniques. The goal of our work was to develop and validate HPLC-MS/MS method for determination of QUE in plasma and to use the method for evaluating pharmacokinetic parameters in schizophrenia patients under stable regimen of dosage. It was therefore necessary to develop a simple and selective analytical method, with a low sample volume for extraction and a short run time for quantification of quetiapine in schizophrenia patient’s plasma. We reported a new validated LC–MS–MS method that includes a simple solid phase extraction (SPE) technique with 5.0 ng/mL as LOQ.

The following are the advantages of the proposed method over those reported earlier: (1) Because of wide linearity range, quetiapine can be estimated at any given strength of dosage regimen (2). The Method uses less plasma and also employees a less time consuming sample preparation process (3) because of the wide linearity it can be compressed for the estimation of the lower strength up to 25 mg (4) the method is well suited for schizophrenic
patients (5) the rapid sample turnaround time of 2.5 min makes it an attractive procedure in high-
throughput for commercial estimation of the drug in human plasma. The application of this assay in a clinical pharmacokinetic study following oral administration of quetiapine is described.

**Experimental**

Quetiapine fumarate working standard (Potency w/w 99.6%), was procured from the Aurobindo Pharma Limited, India. Oxcarbazepine working standard (IS) (Potency w/w is 99.6%) was procured from Amoli Organics Ltd, India. HPLC grade methanol and acetonitrile manufactured by Merck Ltd.; Formic acid AR grade and Ammonium formate (Pure grade) manufactured by Merck Ltd., were purchased from Merck Specialties Ltd., (Mumbai, India).

High purity water was prepared in-house using a Milli-Q water purification system obtained from Millipore Pvt Limited (Bangalore, India). Oasis® HLB 1 cc (30 mg) SPE cartridges were procured from waters corporation, Ireland. Blank human plasma was obtained from Cauvery Diagnostics and Blood Bank, Secunderabad, India and stored at -20 °C prior to use.

**HPLC operating conditions**

An HPLC system (Shimadzu Corporation, Kyoto, Japan) consisting of Shimadzu LC-10 AD HPLC pump, Shimadzu series DGU-20A5 Degasser and a Shimadzu SIL-HTC autosampler, was used to inject 5 µL aliquots of the processed samples on an Inertsil ODS-3, an RP C18 column (4.6x33 mm, 3µ from GL Sciences, Inc., USA), which was kept at a temperature of 30±1 °C. The isocratic mobile phase, a 10:75:15 (v/v) mixture of ammonium formate buffer solution (5 mM, pH 4.50) and acetonitrile and methanol was filtered through a 0.45 µm membrane filter (X15522050) (Millipore, USA or equivalent) and then degassed ultrasonically for 10 min was delivered at a flow rate of 0.400 mL/min (with out splitter) into the mass spectrometer electro spray ionization chamber. The retention times of QUE and IS are 1.40±0.3 min and 1.10±0.3 min, respectively.

**Mass spectrometry operating conditions**

Quantitation was achieved by MS/MS detection in positive ion mode for analyte and IS using a MDS Sciex (Foster City, CA, USA) API-3000 mass spectrometer, equipped with a Turboionspray™ interface at 400 °C. The ion voltage spray was set at 3500 V. The ion source parameters viz., curtain gas, nebulizer gas (GS1) and CAD gas were set at 8, 10 and 8 psi, respectively. Heater gas (GS2) was set at 8000 cc/min. The compound parameters viz., declustering potential (DP), collision energy (CE) and collision exit potential (CXP) for the analyte and IS were shown in Table 1. Detection of the ions was carried out in the multiple reaction monitoring mode (MRM), by monitoring the transition pairs of m/z 384.1 precursor ion to the m/z 253.2 for QUE, and m/z 253.0 precursor ion to the m/z 208.1 product ion for the IS. Quadrupoles Q1 and Q3 were set on unit resolution. The analysis data obtained were processed using Analyst software™ (version 1.4.2).

**Preparation of stock solutions of analyte and IS.**

Primary stock solutions of QUE for preparation of standard and quality control (QC) samples were prepared from separate weighing. Primary and stock solutions of QUE (1 mg/mL) were prepared in methanol. The primary stock solution of IS (1 mg/mL) was prepared in methanol. The stock solutions of QUE and IS were stored at 2-8 °C, which were found to be stable for 15 days. One set of working stock solutions of QUE made in methanol (from primary CC stock) was successively diluted with methanol to prepare appropriate working solutions to prepare calibration curve (CC) standards. Another set of working stock
solutions of QUE made in methanol (from primary QC stock) was successively diluted with methanol to prepare appropriate dilutions for preparation of QC samples. A working IS solution (50.00 µg/mL) was prepared in methanol: water (80:20, v/v).

**Preparation of calibration curve standards and quality control samples**

Calibration samples and quality control samples were prepared by spiking 20 µL of appropriate working solution of QUE into 980 µL of control human plasma. Calibration curve (CC) consists of a set of eight non-zero standard concentrations ranging from 5.01 to 2501.04 ng/mL. The quality control samples were prepared at concentrations of 5.17 (LLOQ), 15.01 (LQC), 1128.77 (MQC) and 1905.42 ng/mL (HQC). Samples for the determination of precision and accuracy were prepared by spiking control human plasma in bulk with QUE at appropriate concentrations and 300 µL plasma aliquots were distributed into different tubes. All the spiked samples were stored at -20±5 °C.

**Sample preparation**

A simple solid phase extraction method was followed for extraction of QUE from human plasma. To an aliquot of 200 µL plasma, IS solution (50 µL of 50 µg/mL) was added and mixed for 15 sec on a cyclomixer (Spinx Instruments, Mumbai, India). Prior to sample extraction, HLB SPE cartridges (1 cc, 30 mg, Waters Oasis®) were conditioned with 1 mL of methanol followed by 1 mL of water. Plasma samples were loaded on to SPE cartridges and samples were eluted completely under slow vacuum. Then SPE cartridges are washed with 1 mL of water for 2 times (1 mL x 2) and dried for 2 minutes. The analyte and IS were eluted from SPE cartridge with 2.0 mL of Acetonitrile (1 mL x 2) and collected the elution sample into prelabeled ria vial tube and transferred in to auto sampler vials and 5 µL was injected on to LC-MS/MS system.

**Validation parameters**

A thorough validation of analytical method for the assay in human plasma was carried out according to the USFDA guidelines

**Specificity and selectivity**

The specificity of the method was evaluated by analyzing human plasma samples from six different lots to investigate the potential interferences at retention times of analytes (QUE) and IS. The responses of the interfering substances or background noises at the retention time of the QUE are acceptable if they are less than 20% of the response of the lowest standard curve point or LLOQ. The responses of the interfering substances or background noise at the retention time of the internal standard are acceptable if they are less than 5% of the mean response of internal standard in LLOQ samples.

**Calibration curve**

Linearity was assessed in the concentration range of 5.01-2501.04 ng/mL by weighted linear regression \((1/X^2)\) of analyte-IS peak area ratios based on four independent calibration curves prepared on two different days using eight-point calibration curve. The calibration curve had to have a correlation coefficient \((r)\) of 0.99 or better. The acceptance limit of accuracy for each of the back-calculated concentrations was ±15% except for LLOQ, where it was ±20% (US DHHS, FDA, CDER, 2001). The calibrators used for analyte were 5.01, 10.02, 25.06, 50.12, 250.60, 750.31, 1500.63 and 2501.04 ng/mL. The samples were run in the order from low to high concentration. In addition, blank plasma samples were also analyzed to confirm the absence of direct interferences, but these data were not used to construct the calibration curve. For a calibration run to be accepted at least 75% of the standards, including the LLOQ and ULOQ, were required to meet the acceptance criterion, otherwise the calibration curve was rejected.
**Precision and accuracy**

The intra-assay precision and accuracy were estimated by analyzing six replicates containing QUE at four different QC levels viz., LLOQ, LQC, MQC and HQC in human plasma. The inter-assay precision and accuracy was determined by analyzing six replicates at four different QC levels on four different runs. The acceptance criteria included accuracy within ±15% deviation from the nominal values, except LLOQ QC, where it should be ±20% and a precision of ±15% relative standard deviation (RSD), except for LLOQ QC, where it should be ±20% (US DHHS, FDA, CDER, 2001).

**Recovery**

The extraction efficiency of QUE and IS extraction from human plasma was determined by comparing the responses of the analyte extracted from replicate QC samples (n = 6) with the response of analyte from post extracted plasma QC samples at equivalent concentrations. Recoveries of analyte was determined at low QC, medium QC and high QC concentrations (QUE: 15.01, 1128.77 and 1905.42 ng/mL), whereas the recovery of the IS was determined at a single concentration of 12.50 µg/mL. The mean overall recovery of the analytes and IS were determined by comparing the peak areas of extracted plasma QC Samples to the peak areas of post extraction plasma samples spiked at corresponding concentration.

**Matrix effect**

Matrix effect was investigated to ensure that precision, selectivity and sensitivity are not compromised by the matrix screened. Matrix Effect was performed by analyzing six replicates of post extracted QC samples at low and high concentrations (prepared by spiking aqueous solutions into extracted, processed blank plasma samples from 6 different volunteer matrix lots containing EDTA as anticoagulant) along with equivalent, similarly prepared aqueous samples (neat samples) at low and high concentrations. The percent matrix effect was determined by comparing the peak areas of post-extracted plasma QC Samples to the peak areas of neat samples spiked at corresponding concentration. The matrix effect was expressed as percentage ratio of mean peak area response of Post extracted and aqueous (neat) samples. Matrix effect was not considered significant if % responses were within 85%-115% and precision (≤15% RSD or CV %).

**Dilution integrity**

The dilution integrity exercise was performed with the aim of validating the dilution test to be carried out on higher analyte concentrations above the ULOQ during real-time analysis of subject samples. Dilution integrity experiment was carried out at 2.0 times the ULOQ concentration for the analyte. Six replicates each of half and quarter concentrations were prepared by 2 times and 4 times dilution with blank plasma and their concentrations were calculated by applying the dilution factors 2 and 4.

**Stability experiments**

The stability of QUE and IS in the injection solvent was determined periodically by injecting replicate preparations of processed plasma samples for up to 39.80 h (in the auto sampler at 10 °C) after the sample loading. Wet extract stability was successfully assessed by analyzing six replicates of wet extract stability samples stored at a temperature below 10 °C for 39.33 h at low and high concentrations. Stability of analyte (QUE) in plasma during 5.35 h (bench-top) was determined at ambient temperature (~25 °C) at two concentrations (LQC and HQC) in six replicates. The stability of QUE in human plasma following four freeze-thaw cycles
was assessed, where the samples were stored at -20±5 °C between freeze/thaw cycles and the samples were thawed by allowing them to stand (unassisted) at room temperature for ~1.5 h. The samples were then returned to the freezer. Freezer stability (Long-term) of analyte in human plasma was assessed by analyzing the LQC and HQC samples stored at -20°±5 °C. The samples were processed using the same procedure as described in the sample preparation section. Samples were considered stable if assay values were within the acceptable limits of accuracy (±15%) and precision (≤15% RSD or CV %). Refer Table 3 for stabilities.

Pharmacokinetic study
A pharmacokinetic study was conducted on steady state adult schizophrenic patients (n = 42) already receiving quetiapine under stable regimen of 300 mg tablet twice a day for atleast four days. And on fifth day following oral administration of quetiapine 300 mg, blood samples were collected at pre-dose (0.0) and at 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, 10.0 and 12.0 hours, using K<sub>2</sub>EDTA vacutainer collection tubes (BD, Franklin, NJ, USA). The tubes were centrifuged at 2500 RCF, 4 °C for 10 minutes and the plasma was collected. The collected plasma samples were stored at –20 °C until use. Plasma samples were spiked with IS and processed as per extraction procedure described above. Along with clinical samples, QC samples at low, middle and high concentrations were assayed in duplicate and were distributed among the unknown samples in the analytical run. The analytical runs were accepted; if not more than 33% of the QC samples were greater than ±15% of the nominal concentration. Plasma concentration-time profile of QUE was analyzed by non-compartmental method using WinNonlin Version 5.2 (Pharsight Corporation, Mountain View, CA).

Results and Discussion
Mass spectrometry
To obtain optimum sensitivity and selectivity, ESI technique operated in the positive ion mode was used for the LC-MS/MS multiple reaction monitoring (MRM) analysis. Protonated form of analyte and IS [M + H]<sup>+</sup> ions was the parent ion in the Q<sub>1</sub> spectrum and was used as the precursor ion to obtain Q<sub>3</sub> product ion spectra. The optimized compound parameters viz., declustering potential (DP), collision energy (CE) and collision cell exit potential (CXP) are presented in Table 1. The product ion mass spectrum of positively charged ion of quetiapine shows the formation of characteristic product ions at m/z 158.2, 221.4, 253.2 and 279.2 (Figure 2). The most sensitive mass transition was monitored from m/z 384.1 to 253.2.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>DP (Volts)</th>
<th>FP (Volts)</th>
<th>EP (Volts)</th>
<th>CE (Volts)</th>
<th>CXP (Volts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>QUE</td>
<td>51</td>
<td>280</td>
<td>10</td>
<td>33</td>
<td>19</td>
</tr>
<tr>
<td>IS</td>
<td>41</td>
<td>230</td>
<td>10</td>
<td>28</td>
<td>19</td>
</tr>
</tbody>
</table>

Selectivity and chromatography
The degree of interference by endogeneous plasma constituents with analyte and IS was assessed by inspection of chromatograms derived from processed blank plasma sample. As shown in Figure 3, no significant interferences in the blank human plasma traces were found from endogeneous components in drug-free human plasma at the retention times of the analyte and IS.
Figure 2. Product ion mass spectra of [M+H]$^+$ of QUE

Figure 2a. Product ion mass spectra of [M+H]$^+$ of IS
[A] \textit{m/Z}: 384.3/253.2

\textit{m/Z}: 253.0/208.1

[B] \textit{m/Z}; 384.3/253.2

\textit{m/Z}; 253.0/208.1
A Rapid and Selective LC-MS/MS Method for Quantification

**Figure 3.** Typical MRM chromatograms of QUE (left panel) and IS (right panel) in (A) human blank plasma (B) human plasma spiked with IS (C) LLOQ sample along with IS

**Sensitivity**

The lowest limit of reliable quantification for analyte was set as the concentration of the LLOQ. The precision and accuracy at LLOQ concentration were found to be 9.01% and 101.15%.

**Extraction efficiency**

A simple solid phase extraction with acetonitrile proved to be robust and provided cleanest samples. The recoveries of analytes and IS were good and reproducible. The mean overall recoveries (with the precision range) of QUE were 72.60% ±3.459 (0.51-7.71%). The recovery of IS was 72.24% with the precision range of 1.77-2.40%.
Matrix effect
No significant matrix effect was observed in all the six batches of human plasma for the analytes at LQC and HQC concentrations. The precision and %response for QUE at LQC were found to be 1.30 and 101.60% and at HQC were found to be 2.30 and 99.80%.

Linearity
After comparing the two weighting models \(1/X\) and \(1/X^2\), a regression equation with a weighting factor of \(1/X^2\) of analyte to IS concentration was found to produce the best fit for the concentration-detector response relationship for the analyte in human plasma. By using the recommended \(1/X^2\) model, values for correlation coefficient (r) were found \(\geq 0.99\) which indicate linearity over the whole calibration range for analyte. And also the mean value of r is greater than 0.99 in the concentration range of 5.01 to 2501.04 ng/mL.

Precision and accuracy
Accuracy and precision data for intra- and inter-day plasma samples for QUE are presented in Table 2. The assay values on both the occasions (intra- and inter-day) were found to be within the accepted variable limits.

<table>
<thead>
<tr>
<th>Quality control</th>
<th>Run</th>
<th>Measured concentration of QUE, ng/mL</th>
<th>Mean</th>
<th>SD</th>
<th>%CV</th>
<th>% Nominal</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Intra day variation (Six replicates at each concentration)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LLOQ*</td>
<td>1</td>
<td>5.48</td>
<td>0.219</td>
<td>4.00</td>
<td>106.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4.70</td>
<td>0.164</td>
<td>3.49</td>
<td>90.94</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4.86</td>
<td>0.103</td>
<td>2.12</td>
<td>93.97</td>
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</tr>
<tr>
<td></td>
<td>4</td>
<td>4.68</td>
<td>0.119</td>
<td>2.54</td>
<td>90.46</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>16.17</td>
<td>1.113</td>
<td>6.88</td>
<td>107.69</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>15.94</td>
<td>0.948</td>
<td>5.95</td>
<td>106.20</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>15.69</td>
<td>0.922</td>
<td>5.88</td>
<td>104.55</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>15.54</td>
<td>0.940</td>
<td>6.05</td>
<td>103.54</td>
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<tr>
<td></td>
<td></td>
<td>1203.99</td>
<td>14.732</td>
<td>1.22</td>
<td>106.66</td>
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<tr>
<td></td>
<td></td>
<td>1084.37</td>
<td>14.807</td>
<td>1.25</td>
<td>104.93</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>1183.64</td>
<td>25.116</td>
<td>2.12</td>
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<tr>
<td></td>
<td></td>
<td>1155.18</td>
<td>10.301</td>
<td>0.89</td>
<td>102.34</td>
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<tr>
<td>LQC#</td>
<td>1</td>
<td>2064.88</td>
<td>55.935</td>
<td>2.71</td>
<td>108.37</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2000.30</td>
<td>21.744</td>
<td>1.09</td>
<td>104.98</td>
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<td>3</td>
<td>2014.67</td>
<td>33.696</td>
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<td>105.73</td>
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<tr>
<td></td>
<td>4</td>
<td>1965.56</td>
<td>22.909</td>
<td>1.17</td>
<td>103.16</td>
<td></td>
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<tr>
<td>MQC1♦</td>
<td></td>
<td>Inter day variation (Twenty replicates at each concentration)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LLOQ*</td>
<td>4.93</td>
<td>0.364</td>
<td>7.38</td>
<td>95.34</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LQC#</td>
<td>15.84</td>
<td>0.949</td>
<td>5.99</td>
<td>105.50</td>
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<tr>
<td></td>
<td>MQC1*</td>
<td>1181.79</td>
<td>23.895</td>
<td>2.02</td>
<td>104.70</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HQC*</td>
<td>2011.35</td>
<td>49.730</td>
<td>2.47</td>
<td>105.56</td>
<td></td>
</tr>
</tbody>
</table>

*LLOQ is 5.17 ng/mL, LQC 15.01 ng/mL, MQC 1128.77 ng/mL, HQC 1905.42 ng/mL

Dilution integrity
The upper concentration limits can be extended to 4985.10 ng/mL for QUE; by a 1/2 or 1/4 dilution 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. The coefficient of variation (CV) for 1/2 and 1/4 dilution samples were 6.14% and 5.41% respectively and the %nominal for 1/2 and 1/4 dilution samples were 101.60% and 94.17% respectively.

**Stability studies**

The stability studies of analyte QUE in human plasma over four freeze thaw cycles indicate that the analytes are stable in human plasma, when stored at below -20 ± 5 °C and thawed at room temperature. Results of bench top (5.35 h), auto-sampler (39.80 h), wet extract stability (39.33 h), freeze-thaw stability (4 Cycles) were presented in Table 3. The long term stability of the analyte in human plasma stored for a period of 166.68 days at -20 ± 5 °C compared with zero day stability showed reliable stability behavior. The results of the tested samples were within the acceptance criteria.

**Table 3. Stability data of quality controls in human EDTA plasma quetiapine**

<table>
<thead>
<tr>
<th>QC ID</th>
<th>Name of the Stability</th>
<th>Mean</th>
<th>SD</th>
<th>% CV</th>
<th>% Nominal</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LQC</strong></td>
<td>Stability – bench top, 5.35 h</td>
<td>15.23</td>
<td>0.729</td>
<td>4.79</td>
<td>101.47</td>
</tr>
<tr>
<td></td>
<td>In – injector, 39.80 h</td>
<td>15.94</td>
<td>0.642</td>
<td>4.03</td>
<td>106.16</td>
</tr>
<tr>
<td></td>
<td>Wet extract, 39.33 h</td>
<td>15.87</td>
<td>0.685</td>
<td>4.32</td>
<td>105.72</td>
</tr>
<tr>
<td></td>
<td>Freeze &amp; thaw (4 Cycles)</td>
<td>15.28</td>
<td>0.121</td>
<td>0.79</td>
<td>101.79</td>
</tr>
<tr>
<td></td>
<td>LT stability in EDTA plasma, (166.68 days)</td>
<td>14.15</td>
<td>0.418</td>
<td>2.96</td>
<td>94.73</td>
</tr>
<tr>
<td><strong>HQC</strong></td>
<td>Stability – bench top, 5.35 h</td>
<td>1926.84</td>
<td>93.378</td>
<td>4.85</td>
<td>101.12</td>
</tr>
<tr>
<td></td>
<td>In – injector, 39.80 h</td>
<td>2028.14</td>
<td>91.380</td>
<td>4.51</td>
<td>106.44</td>
</tr>
<tr>
<td></td>
<td>Wet extract, 39.33 h</td>
<td>2027.68</td>
<td>76.123</td>
<td>3.75</td>
<td>106.42</td>
</tr>
<tr>
<td></td>
<td>Freeze &amp; thaw, 4 Cycles</td>
<td>1920.52</td>
<td>112.560</td>
<td>5.86</td>
<td>100.79</td>
</tr>
<tr>
<td></td>
<td>LT stability in EDTA plasma, (166.68 days)</td>
<td>1903.22</td>
<td>38.985</td>
<td>2.05</td>
<td>97.54</td>
</tr>
</tbody>
</table>

**Pharmacokinetic study**

In order to verify the sensitivity and selectivity of this method in a real-world situation, the present method was used to test for QUE in plasma samples collected from schizophrenia patients (n = 42). The mean plasma concentration vs. time profiles of QUE under study state on Day – 5 was shown in Figure 4. The Mean plot of Quetiapine plasma concentration Vs. time from day 1 to 5 showing study state is shown in Figure 4a. The arithmetic mean of minimum plasma Quetiapine concentrations (C_{min ss}) is 168.30 ng/mL. The arithmetic mean of maximum plasma Quetiapine concentrations (C_{max ss}) is 1135.00 ng/mL. The arithmetic mean of average plasma Quetiapine concentrations (C_{avg ss}) is 486.65 ng/mL. The time to reach C_{max ss} (T_{max}) is 2.50 h. The arithmetic mean of AUC_{0-τ} is 5839.82 h. ng/mL. The mean half-life is 3.97 hours.

Validated methods are essential for the estimation of QUE concentrations in human plasma for clinical pharmacokinetic studies in schizophrenia patients. The validated method is simple, rugged and rapid due to utilization of short run time of 2.5 min for each sample analysis. Here we developed and validated a method for the determination of QUE in human plasma with good / reasonable sensitivity (LLOQ 5.00 ng/mL) for quantification of QUE in schizophrenia patient plasma samples. The method used very simple sample preparation procedure using solid phase extraction (SPE) followed by direct injection.
Figure 4. Mean plasma concentration-time profile of QUE in human plasma following oral dosing of QUE tablet (Day-5) to Indian schizophrenia patients under study state

Figure 4a. The Mean plot of quetiapine plasma concentration Vs. time from day 1 to 5 showing study state

Conclusion
In summary, we have developed and validated a selective, reproducible and high-throughput LC-MS/MS method to quantify QUE using Oxcarbazepine as IS. To the best of knowledge, the cost-effectiveness, simplicity of the assay using Solid phase extraction and sample turnover rate of less than 2.5 min per sample, make it an attractive procedure in high-throughput bioanalysis of QUE. From the results of the validation parameters, we can conclude that the developed method can be useful for BA/BE studies and routine therapeutic drug monitoring (TDM) with desired precision and accuracy.

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A Rapid and Selective LC-MS/MS Method for Quantification

References
