Determination of Quetiapine in Pharmaceutical Dosage Form

Overview

The present chapter deals with the determination of quetiapine in solid oral dosage form using the developed and validated, stability indicating, RP-UPLC method.

4.1 LITERATURE REVIEW

The advantages of the therapeutic profile of quetiapine have led to increasing use in the clinical practice, which encourages the development of new analytical method to provide driving force in today’s pharmaceutical industry. Higher sample throughput with more information per sample may decrease the time to market, an important driving force in today's pharmaceutical industry [1-3].

UPLC is a new category of separation technique based upon well-established principles of liquid chromatography, which utilizes sub-2 µm particles for stationary phase. These particles operate at elevated mobile phase linear velocities to affect dramatic increase in resolution, sensitivity and speed of analysis. Owing to its speed and sensitivity, this technique is gaining considerable attention in recent years for pharmaceuticals and biomedical analysis.

The parent drug stability test guideline Q1A (R2) issued by the International Conference on Harmonization (ICH) [4] suggests that stress studies should be carried out on a drug to establish its inherent stability characteristics, leading to identification of degradation products and, hence, supporting the suitability of the present analytical procedures. It also requires that analytical procedures for testing the stability of samples should be stability-indicating and should be fully validated. Chemical structures, IUPAC names and UV spectra of quetiapine / impurities / degradation products are presented in Figure 4.1.
A detailed literature survey for quetiapine reveals that few analytical methods are available using HPLC where; S. Radha Krishna et al. [5], describe stability indicating method for related substances of quetiapine drug substance; F. Belal et al. [6], demonstrated separation of two impurity from quetiapine; V. Pucci et al. [7], determination of quetiapine is described with non stability indicating method. Polarographic method is also reported for the analysis of quetiapine in pharmaceuticals [8]. HPTLC method is also reported by S.R. Dhaneshwar et al. [9]. Determination of quetiapine by Capillary zone electrophoretic method is reported by S. Hillaert et al. [10]. A Voltammetric analysis of quetiapine in human serum and urine is also reported [11]. GC/MSD [12], LC-MS [13-21], and LC-MS-MS [22] are reported for determination of quetiapine in drug substance, pharmaceutical formulations and biological matrices. Identification and characterization of quetiapine impurities is also reported [23, 24]. Quetiapine pharmaceutical formulation is also not official in any pharmacopoeia yet.

A) Quetiapine (QUE)

2-(2-(4-(dibenzo[b,f][1,4]thiazepin-11-yl)piperazin-1-yl)ethoxy)ethanol

![Chemical structure of Quetiapine](image)
B) Quetiapine-N-Oxide (N-Oxide)
11-(-4-[2-(2-Hydroxyethoxy)ethyl]piperazin-1-yl)-dibenzo[b,f][1,4]thiazepine-N-oxide

C) Quetiapine-S-Oxide (S-Oxide)
11-(-4-[2-(2-Hydroxyethoxy)ethyl]piperazin-1-yl)dibenzo[b,f][1,4]thiazepine-sulphoxide
D) Quetiapine-IV (Que-IV)

11-(piperazin-1-yl)dibenzo[b,f][1,4]thiazepine

![Chemical structure of Quetiapine-IV](image)

Que-IV, RT-1.872

E) Des-Ethanol (Des-E), O-Dealkylated Quetiapine

2-(4-(dibenzo[b,f][1,4]thiazepin-11-yl)piperazin-1-yl)ethanol

![Chemical structure of Des-Ethanol](image)

Des-Ethanol, RT-2.015
Determinaton of Quetiapine in Pharmaceutical Dosage Form

F) Quetiapine Dimer (Dimer)

11-[[4-dibenzo[bf][1,4]thiazepine-11-yl]piperazinyl]dibenzo[bf][1,4]thiazepine

[Figure 4.1 Chemical structures, IUPAC names and UV spectra of quetiapine and its impurities]

4.2 THE SCOPE AND OBJECTIVES OF PRESENT STUDY

None of the analytical methods can separate all the known related compounds and degradation impurities of quetiapine dosage form. Quetiapine pharmaceutical formulation is also not official in any pharmacopoeia yet. Furthermore, there is no less time-consuming and stability-indicating RP-UPLC method reported in the literature that can adequately separate all the substance and accurately quantify quetiapine in solid oral dosage form. Also the cost of the analysis using LC-MS, GC/MSD and LC-MS-MS is very high and also very delicate instrument are needed compared to UPLC with respect to routine quality control analysis. Hence, the research work on developing a selective, fast, cost-effective and stability-indicating method using this advance technique (UPLC) for the assay determination of quetiapine in solid pharmaceutical dosage forms is under taken.
Hence a reproducible stability-indicating RP-UPLC method is developed which is less time-consuming and more selective compared to the all present methods, which takes only about 5 min for a single run. Thereafter, this method is successfully validated according to the ICH guideline [25].

The objectives of the present work are as follow:

- Development of rapid, stability indicating RP-UPLC method for determination of QUE in solid oral dosage form.
- Forced degradation study.
- To separates QUE from its all five (N-Oxide, S-Oxide, Que-IV, Des-E and Dimer) known impurities/ degradation products and any unknown degradation product if generated during forced degradation study.
- Perform analytical method validation for the proposed method as per ICH guideline.
- Application of developed and validated method on (Dr. Reddy’s lab) pharmaceutical dosage form.

4.3 QUETIAPINE (QUETIAPINE FUMARATE)

Quetiapine, 2-[(4-dibenzo[b,f][1,4]thiazepin-11-yl-1-piperazinyl)ethoxy]ethanol, is an atypical antipsychotic drug with a unique receptor-binding profile belonging to a new chemical class, the dibenzothiazepine derivatives [26-29] [Figure 4.2]. Quetiapine is an antagonist at a broad range of neurotransmitter receptors [28, 29]. Quetiapine is used in the treatment of schizophrenia or manic episodes associated with bipolar disorder. These antipsychotics have a low incidence of extrapyramidal side effects and tardive dyskinesias compared to older antipsychotics. Quetiapine (Quetiapine Fumarate, Seroquel™) is approved by the US Food and Drug Administration (FDA) in 1997. Molecular formula of quetiapine fumarate [Figure 4.3] is \( (C_{21}H_{25}N_3O_5S)_2 \cdot C_4H_4O_4 \) with molecular weight 883.1 g/mole [30]. Its melting point is 172°C to 173°C [31, 32]. Quetiapine fumarate is a weak acid with dissociation constant (pKa) 3.3 and 6.8 with moderate pH dependent solubility, 94.3 mg/mL to 2.37 mg/mL at pH values from 1 to 9 reported [33].
Strengths

Quetiapine tablets are available in seven strengths equivalent to 25 mg, 50 mg, 100 mg, 150 mg, 200 mg, 300 mg and 400 mg of Quetiapine in form of quetiapine fumarate as an active ingredient for oral administration.

Innovator

Seroquel tablets and Seroquel XR tablets, AstraZeneca.

Pharmacology

Pharmacodynamics

Quetiapine is an atypical antipsychotic agent. Quetiapine and the human plasma metabolite, N-desalkyl quetiapine, interact with a broad range of neurotransmitter receptors. Quetiapine and N-desalkyl quetiapine exhibit affinity for brain serotonin (5HT2) and dopamine D1 and D2 receptors. It is this combination of receptor antagonism with a higher selectivity for 5HT2 relative to D2
receptors which is believed to contribute to the clinical antipsychotic properties and low extrapyramidal side effects (EPS) liability of SEROQUEL. Additionally, N-desalkyl quetiapine has high affinity at serotonin 5HT1 receptors. Quetiapine and Ndesalkyl quetiapine also have high affinity at histaminergic and adrenergic $\alpha_1$ receptors, with a lower affinity at adrenergic $\alpha_2$ receptors.

Quetiapine has no appreciable affinity at cholinergic muscarinic or benzodiazepine receptors. Quetiapine is active in tests for antipsychotic activity, such as conditioned avoidance. It also reverses the action of dopamine agonists, measured either behaviourally or lectrophysiologically, and elevates dopamine metabolite concentrations, a neurochemical index of D2 receptor blockade. The extent to which the N-desalkyl quetiapine metabolite contributes to the pharmacological activity of SEROQUEL in humans is not known. In pre-clinical tests predictive of EPS, quetiapine is unlike standard antipsychotics and has an atypical profile. Quetiapine does not produce dopamine D2 receptor supersensitivity after chronic administration. Quetiapine produces only weak catalepsy at effective dopamine D2 receptor blocking doses. Quetiapine demonstrates selectivity for the limbic system by producing depolarization blockade of the mesolimbic but not the nigrostriatal dopamine-containing neurons following chronic administration. Quetiapine exhibits minimal dystonic liability in haloperidol-sensitised or drug-naive Cebus monkeys after acute and chronic administration. The results of these tests predict that SEROQUEL should have minimal EPS liability, and it has been hypothesised that agents with a lower EPS liability may also have a lower liability to produce tardive dyskinesia.

**Pharmacokinetics**

**Absorption**

Quetiapine is well absorbed and the bioavailability of quetiapine is not significantly affected by administration with food [34].
**Distribution**

The elimination half-lives of quetiapine and N-desalkyl quetiapine are approximately 7 and 12 hours respectively. Quetiapine is approximately 83% bound to plasma proteins. Steady state peak molar concentrations of the active metabolite N-desalkyl quetiapine are 35% of that observed for quetiapine. The pharmacokinetics of quetiapine and N-desalkyl quetiapine are linear across the approved dosage range. The kinetics of quetiapine do not differ between men and women.

**Metabolism**

Quetiapine is extensively metabolised by the liver following oral administration, with parent compound accounting for less than 5% of unchanged drug related material in the urine or faeces, following the administration of radiolabelled quetiapine. The average molar dose fraction of free quetiapine and the active human plasma metabolite N-desalkyl quetiapine is <5% excreted in the urine. In vitro investigations established that CYP3A4 is likely to be the primary enzyme responsible for cytochrome P450 mediated metabolism of quetiapine. N-desalkyl quetiapine is primarily formed and eliminated via CYP3A4, CYP2D6 and CYP2C9 are also involved in quetiapine metabolism.

Quetiapine and several of its metabolites (including N-desalkyl quetiapine) are found to be weak to modest inhibitors of human cytochrome P450 3A4, 2C19, 2D6, 1A2 and 2C9 activities in vitro. In vitro CYP inhibition is observed only at concentrations approximately 5 to 50-fold higher than those observed at a dose range of 300 to 800 mg/day in humans. Based on these in vitro results, it is unlikely that coadministration of quetiapine with other drugs will result in clinically significant drug inhibition of cytochrome P450 mediated metabolism of the other drug. From animal studies it appears that quetiapine can induce cytochrome P450 enzymes. In a specific interaction study in psychotic patients, however, no increase in the cytochrome P450 activity is found after administration of quetiapine. Following a single oral dose of $^{14}$C-quetiapine, less than 1% of the administered dose is excreted as unchanged drug, indicating that quetiapine is highly metabolized. Approximately 73% and 20% of the dose is recovered in the urine and faeces, respectively.
Use in renal impairment

The mean plasma clearance of quetiapine was reduced by approximately 25% in subjects with severe renal impairment (creatinine clearance less than 30 mL/min/1.73m²), but the individual clearance values are within the range for normal subjects.

Sustained-release

AstraZeneca submitted a new drug application for a sustained-release version of quetiapine in the United States, Canada, and the European Union in the second half of 2006 for treatment of schizophrenia [35]. AstraZeneca will retain the exclusive right to market sustained release quetiapine until 2017. The sustained-release quetiapine is marketed mainly as Seroquel XR. Other marketing names are Seroquel Prolong and Seroquel Depot.

On May 18, 2007, AstraZeneca announced that the FDA approved Seroquel XR for acute treatment of schizophrenia [35]. During its 2007 Q2 earnings conference, AstraZeneca announced plans to launch Seroquel XR in the U.S. during August 2007. However, Seroquel XR has only become available in U.S. pharmacies after the FDA approved Seroquel XR for use as maintenance treatment for schizophrenia, in addition to acute treatment of the illness, on November 16, 2007 [35]. The company has not provided a reason for the delay of Seroquel XR's launch. Health Canada approved sale of Seroquel XR on September 27, 2007. The FDA approved Seroquel XR for the treatment of bipolar depression and bipolar mania in early October, 2008. According to AstraZeneca, Seroquel XR is "the first medication approved by the FDA for the once-daily acute treatment of both depressive and manic episodes associated with bipolar."

On July 31, 2008, Handa Pharmaceuticals, based in Fremont, California, announced that its abbreviated new drug application ("ANDA") for quetiapine fumarate extended-release tablets, the generic version of AstraZeneca’s SEROQUEL XR, has been accepted by the FDA. On December 1, 2008, Biovail announced that the FDA had accepted the company's ANDA to market its own version of sustained-release quetiapine [35]. Biovail's sustained-release tablets will compete with AstraZeneca's Seroquel XR.
On December 24, 2008, AstraZeneca notified shareholders that the FDA had asked for additional information on the company's application to expand the use of sustained-release quetiapine for treatment of depression [35].

**Contraindications**

Seroquel™ tablets and Seroquel XR™ are contraindicated in patients who are hypersensitive to any component of this product.

**Synthesis**

![Chemical structure](“image”)


### 4.4 EXPERIMENTAL

#### 4.4.1 Materials and reagents

Tablets (Batch No.-F017), Placebo and standard of Quetiapine Fumarate (99.5 %) and its five impurities namely N-oxide (98.1 %), S-oxide (98.5 %), Que-IV (99.2 %), Des-ethanol (96.8 %) and Dimer (98.4 %) are provided by Dr. Reddy’s laboratories Ltd., Hyderabad, India. HPLC grade acetonitrile and methanol are obtained from J.T.Baker (NJ, USA). GR grade orthophosphoric acid, perchloric acid and GR grade triethylamine are obtained from Merck Ltd. (Mumbai, India). 0.2 µm PVDF filter and PVDF syringe filters are purchased from Millipore (India). 0.2 µm nylon syringe filter is purchased from Pall life science limited (India). High purity water is generated by using Milli-Q Plus water purification system (Millipore, Milford, MA, USA).
4.4.2 Equipments

Acquity UPLC™ system (Waters, Milford, USA), consisting of a binary solvent manager, sample manager and PDA (photo diode array) detector. System control, data collection and data processing are accomplished using Waters Empower™-2 chromatography data software. Cintex digital water bath is used for specificity study. Photo stability studies are carried out in a photo-stability chamber (SUNTEST XLS+, ATLAS, Germany). Thermal stability studies are performed in a dry air oven (Cintex, Mumbai, India).

4.4.3 Preparation of mobile phase and its gradient program

Mobile Phase-A (MP-A): 0.1% aqueous triethylamine (adjusted to pH 7.2 with orthophosphoric acid).

Mobile phase-B (MP-B): Mixture of acetonitrile and methanol in the ratio of 80:20 (v/v).

Table 4.1 Gradients program for elution

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Flow rate (mL/min)</th>
<th>% MP-A</th>
<th>% MP-B</th>
<th>Gradient curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>0.5</td>
<td>70</td>
<td>30</td>
<td>Isocratic</td>
</tr>
<tr>
<td>0.5</td>
<td>0.5</td>
<td>70</td>
<td>30</td>
<td>Isocratic</td>
</tr>
<tr>
<td>3.0</td>
<td>0.5</td>
<td>5</td>
<td>95</td>
<td>Linear</td>
</tr>
<tr>
<td>4.0</td>
<td>0.5</td>
<td>5</td>
<td>95</td>
<td>Isocratic</td>
</tr>
<tr>
<td>4.1</td>
<td>0.5</td>
<td>70</td>
<td>30</td>
<td>Isocratic</td>
</tr>
<tr>
<td>5.0</td>
<td>0.5</td>
<td>70</td>
<td>30</td>
<td>Equilibration</td>
</tr>
</tbody>
</table>

MP-A and MP-B is filtered through 0.22 µm PVDF filter and degassed under vacuum prior to use.

4.4.4 Diluent preparation

Mixture of water, acetonitrile and perchloric acid in the ratio of 200:800:0.13 (v/v/v) respectively.

4.4.5 System suitability solution preparation

System suitability solution is prepared by dissolving standard substance and impurity in diluent to obtain solution containing 3 µg/mL of Des-E and 125 µg/mL of QUE.
4.4.6 **Standard solution preparation**

Standard solution is prepared by dissolving standard substance in diluent to obtain solution containing 125 µg/mL of QUE.

4.4.7 **Sample solution preparation**

Twenty tablets are crushed to fine powder. An accurately weighed portion of the powder equivalent to 25 mg of QUE is taken into 200 mL volumetric flask. About 150 mL of diluent is added to this volumetric flask and sonicated in an ultrasonic bath for 10 min. This solution is then diluted up to the mark with diluent and mixed well. It is then filtered through 0.2 µm PVDF syringe filter and the filtrate is collected after discarding first few milliliters.

4.4.8 **Placebo solution preparation**

Twenty tablets of placebo are crushed to fine powder. An accurately weighed portion of the placebo powder equivalent to 25 mg of QUE is taken into 200 mL volumetric flask. About 150 mL of diluent is added to this volumetric flask and sonicated in an ultrasonic bath for 10 min. This solution is then diluted up to the mark with diluent and mixed well. It is then filtered through 0.2 µm PVDF syringe filter and the filtrate is collected after discarding first few milliliters.

4.4.9 **Sample solution preparation (stability study of in house formulation)**

Twenty tablets are crushed to fine powder. An accurately weighed portion of the powder equivalent to 25 mg of QUE is taken into 200 mL volumetric flask. About 150 mL of diluent is added to this volumetric flask and sonicated in an ultrasonic bath for 10 min. This solution is then diluted up to the mark with diluent and mixed well. It is then filtered through 0.2 µm PVDF syringe filter and the filtrate is collected after discarding first few milliliters.

4.4.10 **Chromatographic conditions**

The chromatographic condition is optimized using Agilent Eclipse Plus C18, RRHD 1.8 µm (50 mm x 2.1 mm) column. The finally selected and optimized conditions are as follows: injection
volume 1 µL, gradient elution [Table 4.1], at a flow rate of 0.5 mL/min at 40°C (column oven) temperature, detection wavelength 252 nm. Under these conditions, the backpressure in the system is about 6,000 psi. The stress degraded samples and the solution stability samples are analyzed using a PDA detector covering the range of 200-400nm.

4.4.11 Method Validation

The RP-UPLC method described herein has been validated for Assay determination of QUE.

4.4.11.1 Specificity

Forced degradation studies are performed to demonstrate selectivity and stability-indicating the capability of the proposed method. The sample are exposed to acid hydrolysis [1N HCl (5 ml), 60°C, 1h], base hydrolysis [1N NaOH (5 mL), 60°C, 1h], oxidative [30% H₂O₂ (5 mL), 60°C, 1h], water hydrolysis [60°C, 24h] thermal [60°C, 6h], and photolytic degradation [1.2 million Lux hours]. All exposed samples are than analysed by the proposed method.

4.4.11.2 System Suitability

System suitability parameters are measured so as to verify the system, method and column performance. System precision is determined on six replicate injections of standard preparation. All important characteristics including % RSD, resolution (between Des-E and QUE), tailing factor and theoretical plate number and capacity factor for QUE are measured.

4.4.11.3 Precision

The precision of the system is determined using the sample preparation procedure described above for six real samples of solid oral (tablets) formulation and analysis using the same proposed method. Intermediate precision is studied by other scientist, using different columns, different UPLC, and is performed on different days.
4.4.11.4  **Accuracy**

To confirm the accuracy of the proposed method, recovery experiments are carried out by the standard addition technique. Five levels (50 %, 75 %, 100 %, 125 % and 150 %) of standards are added to pre-analyzed placebo samples in triplicate. The percentage recoveries of QUE at each level and each replicate are determined. The mean of percentage recoveries (n=15) and the relative standard deviation are also calculated.

4.4.11.5  **Linearity**

Linearity is demonstrated from 50 to 150 % of standard concentration using a minimum of five calibration levels (50 %, 75 %, 100 %, 125 % and 150 %) for QUE. The method of linear regression is used for data evaluation. The peak area of the standard compound is plotted against the respective QUE concentration. Linearity is described by the linearity equation, correlation coefficient and Y-intercept bias is also determined.

4.4.11.6  **Robustness**

The robustness is a measure of the capacity of a method to remain unaffected by small but deliberate changes in flow rate (± 0.05 mL/min), change in column oven temperature (± 3°C) and change in MP-B (± 10% of methanol composition).

4.4.11.7  **Solution stability**

The stability of the sample solution is established by storage of the sample solution at ambient temperature for 24h. The sample solution is re-analyzed after 6, 12 and 24h, and the results of the analysis are compared with the results of the fresh sample. The stability of standard solution is established by the storage of the standard solution at ambient temperature for 24h. The standard solution is re-injected after 6, 12 and 24h, and % RSD are calculated.
4.4.11.8 Filter compatibility

Filter compatibility is performed for nylon 0.22 μm syringe filter (Pall Life sciences) and PVDF 0.22 μm syringe filter (Millipore). To confirm the filter compatibility in proposed analytical method, filtration recovery experiment is carried out by sample filtration technique. Sample is filtered through both syringe filters and percentage assay is determined and compared against centrifuged sample.

4.4.12 Application of the Method to Dosage Forms (Formulation Stability Study)

The present method is applied for the estimation of QUE in pharmaceutical dosage form, for its stability study.

4.5 RESULTS AND DISCUSSION

4.5.1 Method Development and Optimization

The main criteria for development of successful UPLC method for determination of QUE in tablets is: the method should be able to determine assay of drug in single run and should be accurate, reproducible, robust, stability indicating, free of interference from blank / placebo / impurities / degradation products and straightforward enough for routine use in quality control laboratory.

The spiked solution of related compounds (N-Oxide, S-Oxide, Que-IV, Des-E, Dimer) and QUE are subjected to separation by RP-UPLC. The Rasayan Journal of Chemistry [5] literature for QUE states a RP-HPLC method using C18 column for its related substances. The mobile phase is containing a gradient mixture of MP-A (5mM of ammonium acetate) and MP-B (acetonitrile) [5]. The separation of all compounds form QUE is studied using this composition on UPLC column (Acquity BEH C18 50 x 2.1 mm, 1.7μm) and Waters (UPLC) system with the linear gradient program [Table 4.1]. The flow rate of 0.5 mL/min is selected with regards to the backpressure and analysis time as well. When study is performed with above condition we observed that N-Oxide
and S-Oxide is co-eluting and Des-E and QUE are found co-eluting next to them. Que-IV is well separated from QUE and Dimer is relatively retained for longer time and it is well separated from peak of QUE. Based on this experiment Des-E and QUE is selected as a critical pair for separation. During this study column oven temperature is capped 40°C. Various types of MP-A and B are studied to optimize the method, which are summarized in Table 4.2 with the observation. Based on above solvent selection experimental study optimized UPLC parameters are; flow rate 0.5mL/min; column oven temperature 40°C; gradient solvent program as per Table 4.1; as a MP-A 0.1 % aqueous triethylamine (adjusted to pH 7.2 with orthophosphoric acid) and as MP-B mixture of acetonitrile and methanol in the ratio of 80:20 (v/v) respectively.

**Table 4.2  Summary of solvent used to optimize the method**

<table>
<thead>
<tr>
<th>MP-A</th>
<th>MP-B</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 mM Ammonium acetate solution</td>
<td>Acetonitrile</td>
<td>Co-eluting peak of Des-E and QUE is observed</td>
</tr>
<tr>
<td>0.1 % aqueous triethylamine</td>
<td>Acetonitrile</td>
<td>Co-eluting peak of Des-E and QUE is observed</td>
</tr>
<tr>
<td>0.1 % aqueous triethylamine (adjusted to pH 7.2 with H₃PO₄)</td>
<td>Mixture of acetonitrile and water in the ratio of 90:10 (v/v)</td>
<td>Poor resolution between Des-E and QUE, and higher peak tailing for the peak of QUE are observed.</td>
</tr>
<tr>
<td>0.1 % aqueous triethylamine (adjusted to pH 7.2 with H₃PO₄)</td>
<td>Mixture of acetonitrile and methanol in the ratio of 80:20 (v/v)</td>
<td>1.3 USP resolution is observed between Des-E and QUE</td>
</tr>
</tbody>
</table>

In order to achieve symmetrical peak of QUE and more resolution between Des-E and QUE, various stationary phases like C8 (different dimension), C18 (different brand), phenyl are also studied. Summary of stationary phases are presented in Table 4.3. Based on this summary it is concluded that Eclipse Plus C18; RRHD (50 x 2.1mm, 1.8µm) giving a better resolution (Des-E and QUE) and symmetrical peaks of all component with respect to other stationary phases and other equivalent columns. Column oven temperature is also studied (at room temperature and 40°C) and found that 40°C temperature is more appropriate with respect to resolution and peak shape. The wavelength of QUE maximum (252 nm) is used to have good detection of QUE and impurities (at
2.5% of standard concentration). QUE and their impurities are well resolved in reasonable time of 5 minutes which is presented in Figure 4.4 (spiked chromatogram of QUE along with impurities). There is no any interference of excipients (placebo) and blank (diluent) at the retention time of QUE peak. Overlaid specimen chromatograms of blank, placebo and standard solution is presented in Figure 4.5. Specimen chromatogram of resolution solution is presented in Figure 4.6.

### Table 4.3  Summary of stationary phase used to optimize the method

<table>
<thead>
<tr>
<th>Stationary phase</th>
<th>Dimension</th>
<th>Observation/Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acquity BEH C8</td>
<td>(50 x 2.1) mm, 1.7 µm</td>
<td>Poor resolution between Des-E and QUE</td>
</tr>
<tr>
<td>Acquity BEH C8</td>
<td>(100 x 2.1) mm, 1.7 µm</td>
<td>Poor resolution between Des-E and QUE</td>
</tr>
<tr>
<td>Acquity BEH Phenyl</td>
<td>(50 x 2.1) mm, 1.7 µm</td>
<td>Peak merging of Des-E and QUE</td>
</tr>
<tr>
<td>Acquity BEH C18</td>
<td>(50 x 2.1) mm, 1.7 µm</td>
<td>Poor resolution between Des-E and QUE</td>
</tr>
<tr>
<td>Eclipse Plus C18, RRHD</td>
<td>(50 x 2.1) mm, 1.8 µm</td>
<td>Satisfactory resolution between Des-E and QUE</td>
</tr>
</tbody>
</table>

### 4.5.2  Analytical Parameters and Validation

After satisfactory development of method it is subjected to method validation as per ICH guideline [25]. The method is validated to demonstrate that it is suitable for its intended purpose by the standard procedure to evaluate adequate validation characteristics (system suitability, accuracy, precision, linearity, robustness, solution stability, filter compatibility and stability indicating capability).

#### 4.5.2.1  Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities [36]. Forced degradation studies are performed to demonstrate selectivity and stability indicating capability of the proposed RP-UPLC method. Figure 4.4 and 4.5 are show that there is no any interferences at the RT (retention time) of QUE due to blank, placebo, impurities and degradation products. Significant degradation is not observed when QUE is subjected to acid [Figure 4.7], alkali [Figure 4.8], thermal [Figure 4.9], hydrolytic [Figure 4.10] and photolytic.
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[Figure 4.11] conditions. Significant degradation is observed when the drug product is subjected to oxidative hydrolysis (30% H$_2$O$_2$, 60°C, 1h) leading to the formation of N-Oxide and S-Oxide. Overlay chromatograms (blank, placebo and sample) of peroxide degradation study are presented in Figure 4.12. Peak due to QUE is investigated for spectral purity in the chromatogram of all exposed samples and found spectrally pure [Figure 4.13-4.18]. The purity and assay of QUE is unaffected by the presence of its impurities and degradation products and thus confirms the stability-indicating power of the developed method. Results from forced degradation study are given in Table 4.4.

Table 4.4 Summary of forced degradation results

<table>
<thead>
<tr>
<th>Degradation condition</th>
<th>Assay (% w/w)</th>
<th>Purity Flag</th>
<th>Major degradation products$^@$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control sample</td>
<td>99.9</td>
<td>No</td>
<td>N.A.</td>
</tr>
<tr>
<td>Acidic hydrolysis (1N HCl, 60°C, 1h)</td>
<td>99.2</td>
<td>No</td>
<td>No significant degradation observed</td>
</tr>
<tr>
<td>Alkaline hydrolysis (1N NaOH, 60°C, 1h)</td>
<td>98.8</td>
<td>No</td>
<td>No significant degradation observed</td>
</tr>
<tr>
<td>Oxidation (30% H$_2$O$_2$, 60°C, 1h)</td>
<td>90.0</td>
<td>No</td>
<td>N-Oxide (4.25 %)$^@$ and S-Oxide (4.68 %)$^@$</td>
</tr>
<tr>
<td>Water hydrolysis (60°C, 24h)</td>
<td>99.5</td>
<td>No</td>
<td>No degradation observed</td>
</tr>
<tr>
<td>Thermal (60°C, 6h) solid</td>
<td>100.1</td>
<td>No</td>
<td>No degradation observed</td>
</tr>
<tr>
<td>Photolytic [1.2 million Lux hours]</td>
<td>100.3</td>
<td>No</td>
<td>No degradation observed</td>
</tr>
</tbody>
</table>

$^@$ Percentage area against quetiapine; N.A. Not applicable.
[Figure 4.5  Specimen chromatograms of blank, placebo and standard preparation]

[Figure 4.6  Specimen chromatogram of resolution (system suitability) solution]

[Figure 4.7  Overlaid chromatograms of acid degradation study]
Determination of Quetiapine in Pharmaceutical Dosage Form

**Figure 4.8** Overlaid chromatograms of base degradation study

**Figure 4.9** Overlaid chromatograms of heat degradation study

**Figure 4.10** Overlaid chromatograms of water hydrolysis degradation study
[Figure 4.11  Overlaid chromatograms of photolytic degradation study]

[Figure 4.12  Overlaid chromatograms of peroxide degradation study]

[Figure 4.13  Acid degraded sample, purity plot]
Determination of Quetiapine in Pharmaceutical Dosage Form

[Figure 4.14  Base degraded sample, purity plot]

[Figure 4.15  Thermal degraded sample, purity plot]

[Figure 4.16  Water hydrolyzed sample, purity plot]
4.5.2.2 System suitability

System suitability parameters are measured so as to verify the system, method and column performance. The % RSD (relative standard deviation) of QUE area count of six replicate injections (standard preparation) is below 0.4%. Low values of % RSD of replicate injections indicate that the system is precise. Results of other system suitability parameters such as resolution, theoretical plates, tailing factor, capacity factor and similarity factor (between two standard preparations) are presented in Table 4.5. As seen from this data, the acceptable system suitability parameters would be: resolution between Des-E and QUE is not less than 1.5, theoretical plates for QUE is not less than 30000, tailing factor for QUE is not more than 2.0, similarity factor (between two standard preparations) is not less than 0.98 and not more than 1.02 and % RSD of replicate injections is not
more than 2.0 %. Results of system suitability parameters from different studies are also presented in Table 4.5. System suitability chromatogram is presented in Figure 4.6. Overlaid chromatograms of six replicate standard injections are presented in Figure 4.19.

Table 4.5  System suitability results (precision, intermediate precision and robustness)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Resolution between Des-E and QUE</th>
<th>Theoretical plates for QUE</th>
<th>Tailing factor for QUE</th>
<th>Capacity factor for QUE</th>
<th>Standard Similarity factor</th>
<th>% RSD* of Standard Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precision</td>
<td>1.61</td>
<td>38771</td>
<td>1.50</td>
<td>6.95</td>
<td>1.01</td>
<td>0.31</td>
</tr>
<tr>
<td>Intermediate Precision</td>
<td>1.64</td>
<td>38213</td>
<td>1.48</td>
<td>6.97</td>
<td>0.99</td>
<td>0.27</td>
</tr>
<tr>
<td>At 0.45 mL/min flow rate</td>
<td>1.52</td>
<td>35191</td>
<td>1.55</td>
<td>7.27</td>
<td>0.99</td>
<td>0.35</td>
</tr>
<tr>
<td>At 0.55 mL/min flow rate</td>
<td>1.72</td>
<td>40663</td>
<td>1.44</td>
<td>6.65</td>
<td>1.00</td>
<td>0.21</td>
</tr>
<tr>
<td>At 37°C Column temp.</td>
<td>1.61</td>
<td>37611</td>
<td>1.50</td>
<td>6.94</td>
<td>1.01</td>
<td>0.28</td>
</tr>
<tr>
<td>At 43°C Column temp.</td>
<td>1.62</td>
<td>38781</td>
<td>1.47</td>
<td>6.91</td>
<td>1.00</td>
<td>0.20</td>
</tr>
<tr>
<td>MP-B [-10 % methanol]</td>
<td>1.61</td>
<td>37807</td>
<td>1.47</td>
<td>6.92</td>
<td>0.99</td>
<td>0.34</td>
</tr>
<tr>
<td>MP-B [+10 % methanol]</td>
<td>1.60</td>
<td>37972</td>
<td>1.48</td>
<td>6.90</td>
<td>1.01</td>
<td>0.22</td>
</tr>
</tbody>
</table>

* Determined on six values.

[Figure 4.19  Overlaid chromatograms of six replicate standard injections]
4.5.2.3 Precision

The precision of the assay method is verified by repeatability and by intermediate precision. Precision is investigated using sample preparation procedure for six real samples of tablets and analyzing by proposed method. Intermediate precision is studied using different column, and performing the analysis on different day. The average % assay (n=6) of QUE is 99.9 with RSD of 0.38 %. Results are presented in Table 4.6 along with intermediate precision data. Low values of % RSD, indicates that the method is precise. Overlaid chromatograms of precision and intermediate study are presented in Figure 4.20 and 4.21 respectively.

Table 4.6  Precision and intermediate precision results

<table>
<thead>
<tr>
<th>Quetiapine</th>
<th>Precision at 100 %</th>
<th>Intermediate precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Assay</td>
<td>% RSD*</td>
</tr>
<tr>
<td>Quetiapine</td>
<td>99.9</td>
<td>0.38</td>
</tr>
</tbody>
</table>

* Average of six determinations; * Determined on six values

[Figure 4.20 Overlaid chromatograms of precision study]
4.5.2.4 Accuracy

To confirm the accuracy of the developed method, recovery experiments are carried out by standard addition technique. Five different levels (50 %, 75 %, 100 %, 125 % and 150 %) of standards are added to pre-analyzed placebo samples in triplicate. The percentage recoveries of QUE at each level and each replicate are determined. The mean of percentage recoveries (n =15) and the % RSD is calculated. The amount recovered is within ±1 % of amount added, which indicates that the method is accurate and also there is no interference due to excipients present in tablets. The results of recoveries for assay are shown in Table 4.7. Overlaid specimen chromatograms are presented in Figure 4.22.

Table 4.7 Accuracy results

<table>
<thead>
<tr>
<th>Quetiapine</th>
<th>At 50 %</th>
<th>At 75 %</th>
<th>At 100 %</th>
<th>At 125 %</th>
<th>At 150 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Recovery</td>
<td>99.4</td>
<td>99.8</td>
<td>99.7</td>
<td>100.0</td>
<td>99.3</td>
</tr>
<tr>
<td>% R.S.D.*</td>
<td>0.40</td>
<td>0.35</td>
<td>0.25</td>
<td>0.26</td>
<td>0.20</td>
</tr>
</tbody>
</table>

* Determined on three values; # Mean of three determinations
4.5.2.5  Linearity

Linearity is demonstrated from 50 % to 150 % of standard concentration using minimum five calibration levels (50 %, 75 %, 100 %, 125 % and 150 %) for the QUE compound, which gives us a good confidence on analytical method with respect to linear range. The response is found linear for QUE from 50 % to 150 % of standard concentration and correlation coefficient is also found greater than 0.9999. Y-intercept bias is also found within ± 2. The result of Correlation coefficients, Y-intercept bias and linearity equations for QUE are presented in Table 4.8. Overlaid specimen chromatograms of linearity study are presented in Figure 4.23. Linearity curve is presented in Figure 4.24.

Table 4.8  Linearity results

<table>
<thead>
<tr>
<th>Linearity range (µg/mL)</th>
<th>Correlation Coefficient ($r^2$)</th>
<th>Linearity (Equation)</th>
<th>Y- intercept bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>62.5 to 187.5</td>
<td>0.99993</td>
<td>y =3955.2943(x) - 4182.2</td>
<td>-1.865</td>
</tr>
</tbody>
</table>
4.5.2.6 Robustness

The robustness is a measure of method capacity to remain unaffected by small, but deliberate changes in chromatographic conditions is studies by testing influence of small changes in flow rate (± 0.05 mL/min), change in column oven temperature (± 3°C) and change in MP-B (± 10 % of methanol composition). No significant effect is observed on system suitability parameters such as
resolution, theoretical plates, tailing factor, capacity factor, similarity factor and % RSD of QUE, when small but deliberate changes are made to chromatographic conditions. The results are presented in Table 4.5 along with system suitability parameters of precision and intermediate precision study. Thus, the method is found to be robust with respect to variability in above conditions.

### 4.5.2.7 Solution stability

Drug stability in pharmaceutical formulations is a function of storage conditions and chemical properties of the drug, preservative and its impurities. Condition used in stability experiments should reflect situations likely to be encountered during actual sample handling and analysis. Stability data is required to show that the concentration and purity of analyte in the sample at the time of analysis corresponds to the concentration and purity of analyte at the time of sampling. Stability of sample solution is established by storage of sample solution at ambient temperature for 24h. QUE sample solution is re-analyzed after 6, 12 and 24h time intervals and assay is determined and compared against fresh sample. Sample solution does not show any appreciable change in assay value when stored at ambient temperature up to 24h, which are presented in Table 4.9. The results from solution stability experiments confirmed that sample solution is stable for up to 24h during assay determination.

Standard solution is re-injected after 6, 12 and 24h time intervals and % RSD of all injected standard injections are calculated. Standard solution did not show any appreciable change in % RSD (RSD for QUE is less than 1.0%) value when stored at ambient temperature up to 24h. Overlaid specimen chromatograms for standard and sample solution stability are presented in Figure 4.25 and 4.26 respectively.

#### Table 4.9 Solution stability results

<table>
<thead>
<tr>
<th></th>
<th>Initial</th>
<th>After 6h</th>
<th>After 12h</th>
<th>After 24h</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Assay</td>
<td>100.1</td>
<td>100.2</td>
<td>99.9</td>
<td>99.8</td>
</tr>
</tbody>
</table>
Filter compatibility

Filter compatibility is performed for nylon 0.2 µm syringe filter (Pall Life sciences) and PVDF 0.2 µm syringe filter (Millipore). To confirm the filter compatibility in proposed method, filtration recovery experiment is carried out by sample filtration technique. Sample is filtered through both syringe filter and percentage assay is determined and compared against centrifuged sample. Sample solution does not show any significant changes in assay percentage with respect to centrifuged sample. Percentage assay results are presented in Table 4.10. In displayed result difference in %
assay is not observed more than ±0.2, which indicates that both syringe filters having a good compatibility with sample solution.

**Table 4.10 Filter compatibility results**

<table>
<thead>
<tr>
<th></th>
<th>Centrifuged 99.9</th>
<th>PVDF Syringe filter 0.2µm (Millipore) 100.1</th>
<th>Nylon Syringe filter 0.2µm (Pall Life Sciences) 99.8</th>
</tr>
</thead>
</table>

**4.5.3 Application of the Method to Dosage Forms**

The present method is applied for the estimation of drugs in the available in-house dosage form. The results (stability study) obtained are as shown in Table 4.11. Based on obtained results, it can be concluded that developed method is suitable for the determination of quetiapine in solid oral dosage form.

**Table 4.11 Data of drug product stability study**

<table>
<thead>
<tr>
<th>Drug Product (B. No.-F017) Labeled claim (400mg/Tab)</th>
<th>% Assay of QUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>99.3</td>
</tr>
<tr>
<td>1 Month (40°C/ 75% RH)</td>
<td>99.1</td>
</tr>
<tr>
<td>2 Month (40°C/ 75% RH)</td>
<td>98.9</td>
</tr>
<tr>
<td>3 Month (40°C/ 75% RH)</td>
<td>99.1</td>
</tr>
</tbody>
</table>

**4.6 CALCULATION FORMULA**

**4.6.1 Assay (% w/w)**

Calculated the quantity, in mg, of QUE in the portion of solid oral pharmaceutical formulation using the following formula:

\[
\text{Assay} \ (% \ w/w) = \frac{C_{\text{std}} \times R_s \times 10,000}{C_s \times R_{\text{std}}}
\]

Where,

- \( C_{\text{std}} \) = Concentration of standard solution in mg/mL
- \( C_s \) = Concentration of sample solution in mg/mL
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\[ R_s = \text{Compound peak response obtained from the sample preparation} \]

\[ R_{\text{std}} = \text{Compound peak response (mean peak area) obtained from the standard preparation} \]

### 4.6.2 Relative standard deviation (% RSD)

It is expressed by the following formula and calculated using Microsoft excel program in a computer.

\[
\text{Related Standard Deviation} \% = \frac{SD \times 100}{\bar{X}}
\]

Where,

- SD = Standard deviation of measurements
- \( \bar{X} \) = Mean value of measurements

### 4.6.3 Accuracy (% Recovery)

It is calculated using the following equation:

\[
\% \text{ Recovery} = \frac{\text{Amount of substance found (mg) \times 100}}{\text{Amount of substance added (mg)}}
\]

### 4.7 CONCLUSION

A gradient RP-UPLC method is successfully developed for the estimation of quetiapine in pharmaceutical dosage form. The method validation results have proved that the method is selective, precise, accurate, linear, robust, filter compatible and stability indicating. The run time (5.0 min) enables for rapid determination of drug. Moreover, it may be applied for determination of QUE in the study of blend uniformity, tablet content uniformity and \textit{in-vitro} dissolution profiling of QUE dosage forms, where sample load is higher and high throughput is essential for faster delivery of results.

\textbf{Note: Intellectual Property Management (IPM) clearance number for the present research work is: PUB-00047-10.}
4.7 REFERENCES


[34] Seroquel® Product Information CNS.000-149-416.5.0