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Development and Validation of Analytical Method for Simultaneous Estimation of Quetiapine and Quetiapine Fumarate in Human Plasma and Evaluation of Pharmacokinetic Profile and Quetiapine

Monika Kaushik [1*], Lav Kaushik [2], Arun Garg [3], Rajiv Kumar Arora [4], Minakshi Gupta [4]

[1] *Hindu College of Pharmacy, Sonepat*

[2] *Crompton Greaves, Malanpur, Gwalior*

[3] *Faculty of Pharmaceutical Sciences, K R Mangalam University, Gurgaon*

[4] *Faculty of Pharmaceutical Sciences, Baba Mastnath University, Asthal Bohar, Rohtak*

Address for Correspondance
Monika Kaushik,
Monikakaushik28@gmail.com

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ABSTRACT: There are some shortages for those methods described in literature for the simultaneous determination of quetiapine and its metabolite quetiapine fumarate in human plasma. So, the purpose of experiment was to develop and validate the analytical method for simultaneous determination of Quetiapine and its metabolite, Quetiapine fumarate and to determine the concentration level of Quetiapine and Quetiapine fumarate in human plasma samples and their pharmacokinetics evaluation in human volunteers after single dosing of two formulations of Quetiapine 25mg in fasting conditions. Sample preparation process was accomplished by solid phase extraction with methanol. The dried and reconstituted solution was subjected to chromatography on Atlantis dC18 column (100mm×3.0 mm,3µm) at 40±5◦C using a mobile phase mixture (acetonitrile–methanol-ammonium acetate). The sample was injected at a flow rate of 0.4ml/ min. and the eluent detected by MS/MS system by optimizing m/z ratio. The total precision (% Coefficient of variation) for the Quetiapine and Quetiapine fumarate ranged from 1.1 % to 5.7 % respectively and within batch accuracy ranged from 91.5 % to 109.6% and 96.86% to 102.77 % respectively. The mean recovery of Quetiapine and Quetiapine fumarate were found to be 72.96% & 69.3% respectively. The concentration data was pharmacokinetically evaluated using ANOVA (Analysis of variance) and evaluation of bioequivalence was based on pharmacokinetic parameters. Both objectives of the study were successfully met. From the result of safety evaluation, it was concluded that both the test and reference formulations were well tolerated.

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Keywords Quetiapine; Analytical; Method; Disorder; Plasma.

INTRODUCTION

A first generation of antipsychotics, known as typical antipsychotics, was discovered in the 1950s. Most of the drugs in the second generation, known as atypical antipsychotics, have been developed more recently. Although the first atypical antipsychotic, clozapine, was discovered in the 1950s and introduced clinically in the 1970s. Both generations of medication tend to block receptors in the brain's dopamine pathways, but antipsychotic drugs encompass a wide range of receptor targets.

The original antipsychotic drugs were happened upon largely by chance and then tested for their effectiveness. The first, chlorpromazine, was developed as a surgical anesthetic. It was first used on psychiatric patients because of its powerful calming effect; at the time it was regarded as a non-permanent

"pharmacological lobotomy". Lobotomy at the time was used to treat many behavioral disorders, including psychosis, although its effect was to markedly reduce behavior and mental functioning of all types. However, chlorpromazine proved to reduce the effects of psychosis in a more effective and specific manner than lobotomy, even though it was known to be capable of causing severe sedation. The underlying neurochemistry involved has since been studied in detail, and subsequent antipsychotic drugs have been discovered by an approach that incorporates this sort of information.

Quetiapine

Quetiapine (Seroquel) was approved by the USFDA (United States Food and Drug Administration) in 1997 and is an atypical antipsychotic with established efficacy in the treatment of schizophrenia [1]. Quetiapine has negligible affinity for cholinergic muscarinic receptors, thereby contributing to its low risk for anticholinergic side effects. There is also evidence from animal models of low potential for extra pyramidal side effects.

The preclinical profile of Quetiapine is similar to the first atypical antipsychotic, clozapine, but with a reduced tendency to cause motor disturbances [2]. Quetiapine is a dibenzothiazepine derivative for which the mechanism of action is unknown. It acts as an antagonist at serotonin 5-HT_{1A} (Hydroxytryptamine) and 5-HT_{2A}, D_1 (Dopamine) and D_2, histamine H_1 and adrenergic α_1 and α_2 receptors [3].

Of note, it has a much higher level of occupancy of 5-HT_{2A} receptors compared to D_2 receptors , a factor generally considered to be predictive of an atypical antipsychotic [4].

In terms of pharmacokinetics, the absorption of Quetiapine are rapid, with the median time to maximum observed plasma concentration ranging from 1 to 2 hours [5].

Uses [6]

Quetiapine is used to treat the symptoms of schizophrenia (a mental illness that causes disturbed or unusual thinking, loss of interest in life, and strong or inappropriate emotions). Quetiapine, a dibenzothiazepine derivative, is an atypical prescription medication antipsychotic, multireceptor antagonist that has a preclinical profile similar to clozapine. Randomized studies have demonstrated the efficacy of quetiapine relative to placebo in the treatment of acute relapse and the long-term management of schizophrenia. Quetiapine is generally well tolerated relative to other antipsychotic medications. It is also used to treat episodes of mania (frenzied, abnormally excited or irritated mood) or mixed episodes (symptoms of mania and depression that happen together) in patients with bipolar I disorder (manic depressive disorder; a disease that causes episodes of depression, episodes of mania, and other abnormal moods).

Mechanism of Action of Quetiapine [7]

Quetiapine has the following mechanism of action:

- D_1, D_2, D_3 and D_4 receptor antagonist
- 5-HT_{1a}, 5-HT_{2a}, 5-HT_{2c}, 5-HT_7 receptor antagonist
- α_1 adrenergic and α_2 -adrenergic receptor antagonist
- H_1 receptor antagonist
- mACh receptor antagonist

It inhibits communications between nerves of the brain. It does this by blocking the receptors on the nerves for several neurotransmitters, the chemicals that nerves use to communicate with other. It is thought that its beneficial effect is due to blocking of the dopamine type 2 (D_2) and serotonin type 2 (5-HT_2) receptors.

This means Quetiapine is a dopamine, serotonin, and adrenergic antagonist, and a potent antihistamine with clinically negligible anticholinergic properties. Quetiapine binds strongly with serotonin receptors. Serial PET (Positron Emission Tomography) scans evaluating the D_2 receptor occupancy of Quetiapine have demonstrated that Quetiapine very rapidly dissociates from the D_2 receptor. Theoretically, this allows for normal physiological surges of dopamine to elicit normal effects in areas such as the nigrostriatal and tubroinfundibular pathways, thus minimizing the risk of side effects such as pseudo-parkinsonism as well as elevations in prolactin. Some of the antagonized receptors (serotonin, norepinephrine) are actually auto receptors whose blockade tends to increase the release of neurotransmitters.

Method Development and Validation [8]

Bioanalytical method validation includes all of the procedures required to demonstrate that a particular bioanalytical method for the quantitative determination of the concentration of an analyte (or series of analytes) in a particular biological matrix is reliable for the intended application. The most widely employed bioanalytical techniques include, but are not limited to, conventional chromatographic-based methods such as GC (Gas Chromatography) and HPLC (High Performance Liquid Chromatography), mass spectrometry-based methods such as GC-MS (Gas Chromatography- Mass Spectrometry) and LC-MS (Liquid Chromatography- Mass Spectrometry), and ligand-based assays such as RIA (Radioimmunoassay) and ELISA (Enzyme-linked immunosorbent assay). Many of the principles, procedures, and requirements for quantitative bioanalytical method validation are common to all types of

analytical methodologies. BMV (Bioanalytical method validation) employed for the quantitative determination of drugs and their metabolites in biological fluids plays a significant role in the evaluation and interpretation of bioavailability, bioequivalence, pharmacokinetic, and toxicokinetic study data. These studies generally support regulatory filings. The quality of these studies is directly related to the quality of the underlying bioanalytical data. It is therefore important that guiding principles for the validation of these analytical methods be established and disseminated to the pharmaceutical community.

Validation of Bioanalytical Method

It is accepted that during the course of a typical drug development program, a defined bioanalytical method will undergo many modifications. These evolutionary changes (eg, addition of a metabolite, lowering of the LLOQ (Lower Limit of Quantification)) require different levels of validation to demonstrate continuity of the validity of an assay's performance.

Method validation is the process that provides evidence that a given analytical method, when correctly applied, produces results that are fit for purpose. No matter how well a method performs elsewhere, analysts need to confirm that the method is valid when applied in their laboratory. There is now much greater emphasis on method validation in the ISO/IEC 17025 (International Organization for Standardization/ International Electrotechnical Commission) accreditation standard.

Different levels/types of method validations

- Full Validation
- Partial Validation
- Cross-validation
- Revalidation

Full Validation

Full Validation is necessary when developing and implementing a bioanalytical method for the first time for a new drug entity. If metabolites are added to an existing assay for quantification, then Full Validation of the revised assay is necessary for all analytes measured.

Partial Validation

Partial Validations are modifications of validated bioanalytical methods that do not necessarily require full revalidations. Partial Validation can range from as little as 1 assay accuracy and precision determination to a "nearly" Full Validation. Typical bioanalytical method changes that fall into this category include, but are not limited to, bioanalytical method transfers between laboratories or analysts, instrument and/or software platform changes, change in species within matrix (e.g.: rat plasma to mouse plasma), changes in matrix within a species (e.g., human plasma to human urine), change in analytical methodology (e.g., change in detection systems), and change in sample processing procedures.

Cross-validation

Cross-validation is a comparison of 2 bioanalytical methods. Cross-validations are necessary when 2 or more bioanalytical methods are used to generate data within the same study. For example, an original validated bioanalytical method serves as the "reference" and the revised bioanalytical method is the "comparator." The comparisons should be done both ways. Cross-validation with spiked matrix and subject samples should be conducted at each site or laboratory to establish inter-laboratory reliability when sample analyses within a single study are conducted at more than 1 site, or more than 1 laboratory, and should be considered when data generated using different analytical techniques (e.g., LC-MS-MS vs ELISA) in different studies are included in a regulatory submission.

Revalidation

A revalidation is necessary whenever a method is changed, and the new parameter lies outside the operating range. If, for example, the operating range of the column temperature has been specified to be between 30 and 40°C, the method should be revalidated if, for whatever reason, the new operating parameter is 41°C.

Revalidation is also required if the scope of the method has been changed or extended, for example, if the sample matrix changes or if operating conditions change. Furthermore, revalidation is necessary if the intention is to use instruments with different characteristics, and these new characteristics have not been covered by the initial validation. For example, an HPLC method may have been developed and validated on a pump with a delay volume of 5 mL, but the new pump has a delay volume of only 0.5 mL.

Parameters of Validation

The parameters for method validation have been defined in different working groups of national and international committees and are described as:

- Specificity
- Selectivity
- Accuracy
- Precision

- Recovery
- Reproducibility
- Linearity
- Range
- Limit of detection
- Limit of quantitation
- Robustness
- Ruggedness

Bioequivalence Studies in human based on Pharmacokinetic measures [9]

Bioequivalence studies are designed to compare the in vivo performance of a multisource product with that of a comparator product. Pharmacokinetic bioequivalence studies on products designed to deliver the API (Active Pharmaceutical Ingredient) for systemic exposure serve two purposes:

- as a surrogate for clinical proof of equivalence; and
- They provide an *in vivo* statics measure of pharmaceutical quality.

The design of the study should minimize the variability that is not caused by formulation effects and eliminate bias as far as possible. Test conditions should reduce variability within and between subjects. In general, for a pharmacokinetic bioequivalence study involving a multisource and a comparator product, a two-period, single-dose, cross-over study in healthy volunteers will suffice. However, in certain circumstances, an alternative, well-established and statistically appropriate study design may be adopted. A Randomized, open label, two-treatment, two-sequence, two-period, single-dose, and crossover oral bioequivalence study is the first choice for pharmacokinetic bioequivalence studies. Each subject is given the multisource and the comparator product in randomized order. An adequate wash-out period should follow the administration of each product. The interval (wash-out period) between doses of each formulation should be long enough to permit the elimination of essentially the entire previous dose from the body. The wash-out period should be the same for all subjects and should normally be more than five times the terminal half-life of the API. Consideration will need to be given to extending this period if active metabolites with longer half-lives are produced and under some other circumstances.

For example, if the elimination rate of the product has high variability between subjects, the wash-out period may be longer to allow for the slower elimination in subjects with lower elimination rates. Just prior to administration of treatment during the second study period, blood samples are collected and assayed to determine the concentration of the API or metabolites. The minimum wash-out period should be at least seven days.

The adequacy of the wash-out period can be estimated from the pre-dose concentration of the API and should be less than 5% of Cmax.

It is currently not foreseen that there would be a need for blood samples to be collected for more than 72 hours.

Pharmacokinetic parameters [10]

In studies to determine bioequivalence after a single dose, AUCt, AUC∞, Cmax and tmax should be determined. Additional parameters that may be reported include the terminal rate constant, λz, and t1/2. For products where rapid absorption is of importance, partial AUCs can be used as a measure of early exposure. The partial area can in most cases be truncated at the population median of tmax values for the reference formulation. However, an alternative time point for truncating the partial AUC can be used when clinically relevant. The time point for truncating the partial AUC should be pre-specified and justified in the study protocol. In studies to determine bioequivalence at steady state, AUCτ, Cmax, ss, Cmin, ss, tmax, ss and fluctuation should be determined.

PHARMACOKINETIC TERMS [10]

C_{max}

This is the maximum drug concentration achieved in systemic circulation following drug administration.

C_{min}

This is the minimum drug concentration achieved in systemic circulation following multiple dosing at steady state.

T_{max}

It is the time required to achieve maximum drug concentration in systemic circulation.

AUC_t

Area under the plasma concentration curve from administration to last observed concentration at time t.

AUC_∞

Area under the plasma concentration curve extrapolated to infinite time.

AUC_τ

AUC during a dosage interval at steady state.

Partial AUC

AUC truncated at the population median of t_{max} values for the reference formulation;

AUC_{0-t}

Areas under the plasma concentration - time curve from 0 h to the last quantifiable concentration to be calculated using the trapezoidal rule.

K_{el}

Apparent first-order terminal elimination rate constant calculated from a semi-log plot of the plasma concentration versus time curve.

$T_{1/2}$

Elimination half life of a drug is the time necessary to reduce the drug concentration in the blood, plasma, or serum to one-half of its initial concentration.

Bioavailability

The rate and extent to which the active moiety is absorbed from a pharmaceutical dosage form and becomes available at the site(s) of action.

Bioequivalence

Bioequivalence of a drug is achieved if its rate and extent of absorption is not statistically significantly different from those of reference product when administered at same molar dose.

SupraBioavailability

It is the term used when the test product shows appreciable large bioavailability than the reference product.

Steady State

Steady state is the state when the plasma concentration of drug at any time point during any dosing interval should be identical to the concentration at the same time during any other dosing interval.

MATERIALS AND METHODS [11,12]

Reagents Used

Triethylamine, Human EDTA (Ethylene diamine tetra acetic acid) Plasma, Acetonitrile, Milli Q water, Orthophosphoric acid, Cartridges (Hyper Sep Retain PEP 30mg, 1ml), Container and Utensils (polypropylene and borosilicate glass), Quetiapine (Working Standard), Quetiapine Fumarate (Working Standard), Clozapine (Internal Standard), Blank human plasma was obtained of healthy volunteers.

Software

Empower Software

Preparation of Reagent Solutions [11]

Mobile phase

The mobile phase was a mixture of acetonitrile–methanol–0.01M ammonium acetate (31:19:50, v/v/v); pH was adjusted with acetic acid (pH 3.5). Before use, the mobile phase was degassed by vacuum filtration through a 0.45 µm filter.

Diluent solution

Milli Q water and Acetonitrile was mixed in the ratio 80:20 (v/v) and filter through 0.2 µm nylon membrane filter and degas for about 10 minutes.

Rinsing solution

The diluents solution was used as rinsing solution.

Preparation of stock solution [13]

- **Preparation of Internal Standard (Clozapine) Stock Solution**

The stock internal standard solution was prepared by accurate weighing of CLO (Cloazapine) (0.0080 g), 8 mg of drug of which was dissolved in 10 mL MeOH/H_2O (Methanol/Water) (70:30, v/v), into a volumetric flask so that final concentration is 0.8 mg/1 mL. The WIS (working internal standard) was prepared by accurate dilution of stock internal standard with MeOH/H_2O (70:30, v/v) to get a final concentration of 4000.0 ng/mL. Stock of internal standard was stored at 4^0C for 5 days. Volume of 50µL WIS was added to 0.50 mL plasma samples.

- **Preparation of Quetiapine (QUE) Standard Stock Solution**

The stock standard solutions of QUE were prepared by dissolving accurately weighed 10 mg of drug of which was dissolved in 10 mL MeOH/H_2O (70:30, v/v), so that final concentration is 1 mg/1 mL. The prepared stock solution is stored in 4°C protected from light. The stock standard solution was then diluted with MeOH/H_2O (70:30, v/v) to achieve a working standard solution at the concentration of 38218 ng/mL.

- **Preparation of Quetiapine (QUE) Fumarate Standard Stock Solution**

The stock standard solutions of QUE Fumarate were prepared by dissolving accurately weighed 10 mg of drug of which was dissolved in 10 mL MeOH/H_2O (70:30, v/v), so that final concentration is 1 mg/1 mL. The prepared stock solution is stored in 4°C protected from light. The stock standard solution was then diluted with MeOH/H_2O (70:30, v/v) to achieve a

working standard solution at the concentration of 38218 ng/mL.

Calibration Curve (CC) Standards

Preparation of Dilution of Quetiapine and Quetiapine fumarate Stock Solution

Just prior to spiking, stock dilutions of Quetiapine and Quetiapine fumarate was prepared by using diluents solution in stopper glass test tubes as described below:

Table 2: Preparation of dilution of Quetiapine Standard Stock Solution

Stock ID	Stock Concentration (ng/ml)	Stock Aliquot (ml)	Final Volume (ml)	Final Concentration of Quetiapine (ng/ml)	Dilution ID
CCST-1	100000.000	0.880	25	29401.000	CCDIL-1-01
CCDIL-1-01	29401.000	8.500	10	20521.000	CCDIL-1-02
CCDIL-1-02	20521.000	7.000	10	10944.000	CCDIL-1-03
CCDIL-1-03	10944.000	5.000	10	5472.000	CCDIL-1-04
CCDIL-1-04	5472.000	4.000	10	1047.200	CCDIL-1-05
CCDIL-1-05	1047.000	1.500	10	527.880	CCDIL-1-06
CCDIL-1-06	527.000	1.600	10	92.832	CCDIL-1-07
CCDIL-1-07	92.832	5.000	10	10.000	CCDIL-1-08

Table 3: Preparation of dilution of Quetiapine fumarate Standard Stock Solution

Stock ID	Stock Concentration (ng/ml)	Stock Aliquot (ml)	Final Volume (ml)	Final Concentration of Quetiapine fumarate (ng/ml)	Dilution ID
CCST-2	100000.000	0.875	25	29200.000	CCDIL-2-01
CCDIL-2-01	29200.000	8.500	10	25297.000	CCDIL-2-02
CCDIL-2-02	25297.000	7.500	10	10328.000	CCDIL-2-03
CCDIL-2-03	10328.000	6.000	10	5975.250	CCDIL-2-04
CCDIL-2-04	5975.250	4.600	10	1379.825	CCDIL-2-05
CCDIL-2-05	1379.825	2.575	10	748.25	CCDIL-2-06
CCDIL-2-06	748.25	1.900	10	114.575	CCDIL-2-07
CCDIL-2-07	114.575	5.000	10	15.095	CCDIL-2-08

Table 4: Dilution of Quetiapine Standard Stock Solution for QC

Stock ID	Stock Concentration (ng/ml)	Stock Volume Used (ml)	Total Volume made upto (ml)	Final Concentration of Quetiapine (ng/ml)	Prepared Stock ID
QC STOCK-1	100000.000	0.800	25.000	30401.000	AQ-HQC1-01
AQ-HQC-1	30401.000	6.000	10.000	304.000	AQ-MQC1-01
AQ-M1QC-1	304.000	0.200	10.000	152.000	AQ-LQC1-01
AQ-LQC-1	152.000	3.800	10.000	2.000	AQ-LOQQC1-01

Table 5: Dilution of Quetiapine fumarate Standard Stock Solution for QC

Stock ID	Stock Concentration (ng/ml)	Stock Volume Used (ml)	Total Volume made upto (ml)	Final Concentration of Quetiapine fumarate (ng/ml)	Prepared Stock ID
QC STOCK-2	100000.000	0.800	25.000	30401.000	AQ-HQC2-01
AQ-HQC-2	30401.000	6.000	10.000	304.000	AQ-MQC2-01
AQ-M1QC-2	304.000	0.500	10.000	152.000	AQ-LQC2-01
AQ-LQC-2	152.000	3.800	10.000	12.000	AQ-LOQQC-01

Quality Control (QC) Samples [14]

Preparation of Dilution of Quetiapine and Quetiapine fumarate Standard Stock Solution for QC

Quality control (QC) working standard solution was prepared from QUE and QUE fumarate stock quality control solution at the concentration of 30401 ng/mL. Similarly the remaining quality control samples (QC3 and QC2) were prepared from the most concentrated quality control sample QC1 by sequential dilution with blank plasma to get the final concentrations of QCs.

Spiking of Plasma for Quality Control Samples (Quality control of commercial tablets, 2003)

Blank plasma samples (9.9 mL) were spiked by working solutions (100µL) to gain either the most concentrated quality control sample (QC1). All plasma samples were stored at -25 ± 5^{0}C. 0.99 ml of each of above described stock dilutions of Quetiapine and Quetiapine fumarate into 10ml volumetric flask was transferred and volume was made upto mark with plasma to achieve the following Quality Control Samples and labeled them as HQC-(1A,1B,1C,1D) respectively as described in the table below.

Table 6- Spiking of Plasma for Quality Control Samples of Quetiapine

Stock ID	Final Stock Concentration of Quetiapine (ng/ml)	Final Concentration in Plasma of Quetiapine (ng/ml)	Prepared Spiked QC ID
AQ-HQC1-01	30401.000	600.000	HQC-1
AQ-MQC1-01	304.000	60.000	MQC-1
AQ-LQC1-01	152.000	30.000	LQC-1
AQ-LOQQC1-01	2.000	0.400	LOQQC-1

Table 7- Spiking of Plasma for Quality Control Samples of Quetiapine fumarate

Stock ID	Final Stock Concentration of Quetiapine fumarate (ng/ml)	Final Concentration in Plasma of Quetiapine fumarate (ng/ml)	Prepared Spiked QC ID
AQ-HQC2-01	30401.000	600.000	HQC-2
AQ-MQC2-01	304.000	60.000	MQC-2
AQ-LQC2-01	152.000	30.000	LQC-2
AQ-LOQQC2-01	12.000	0.240	LOQQC-2

Sample Processing [13]

The required number of calibration curve standards, quality control samples and subject plasma samples from deep freezer were drawn from the sample storage device and they were allowed to thaw at room temperature. The thawed samples were vortexed to ensure complete mixing of contents. 50 µL of internal standard dilution (25ng/ml of CLO) was aliquoted in micro centrifuge tube and 400 µL of each sample was added. The sample was vortexed to mix well. 0.2 ml of 0.01M ammonium acetate (31:19:50, v/v/v) was added and vortexed. The contents are transferred into a stoppered flask and shaken for 20 mins. to extract the drug. Contents are carefully transferred into a centrifuge tube and centrifuged for 4000 rpm for 20 mins. The supernatant liquid is taken and diluted with diluents, to obtain approximately final concentration of 25 × 10^{-3} g/Lt. Solid phase extraction (SPE) was used for sample pretreatment. Oasis HLB (hydrophilic-lipophilic balance) cartridges (30 mg, 1 mL) from Waters (USA) were activated with 2mL of MeOH and conditioned with 3mL H_2O. The plasma sample (0.5 mL) was spiked with 50µL of WIS, alkalized with 200ml of 0.4M NaOH, and vortex-mixed. The mixture was loaded on the prepared cartridges. The cartridge was washed with 3mL H_2O, and the analyte was eluted with 200 µL of mobile phase. A 20-µL aliquot was then injected onto the HPLC system with MS/MS detection.

Note: 1. Blank and blank IS samples were processed with or without internal standard.

2. Samples processing were carried out under low light conditions.

3. The centrifuge was run at temperature 4◦C.

RESULTS AND DISCUSSION

Method Development Results

A liquid chromatographic mass spectroscopic method for the simultaneous estimation of Quetiapine and its metabolite Quetiapine fumarate was developed successfully and validated. Sample preparation process was accomplished by solid phase extraction with methanol. The dried and reconstituted solution was subjected to chromatography on Atlantis dC18 column (100mm×3.0 mm,3µm) at 40±5^{0}C using a mobile phase which is a mixture of acetonitrile–methanol–0.01M ammonium acetate (31:19:50, v/v/v). The sample was injected at a flow rate of 0.4ml/min and the eluent detected by MS/MS system by optimizing m/z ratio of analyte and internal standards as Quetiapine 523.29 (Q1 mass) and 346.30 (Q3) mass and Clozapine (internal standards) 533.33 (Q1 mass) and 349.30 (Q3 mass), Quetiapine fumarate 486.28(Q1 mass) and 309.20 (Q3 mass) respectively.

Chromatographic and Mass Spectroscopic conditions [15]

Table 8: Chromatographic Conditions

Particulars	Values
Column	Atlantis dC18 column (100mm×3.0 mm,3µm)
Column oven temperature	40±5◦C
Mobile Phase	mixture of acetonitrile–methanol–0.01M ammonium acetate (31:19:50, v/v/v)
Flow Rate	0.4 mL/min.
Rinsing Solution	MeOH/H_2O (70:30, v/v)
Rinsing Volume	500µL
Sample Cooler temperature	10±5◦C
Injection Volume	10 µL
Retention Time	0.70min. approx. for Quetiapine (QUE)
	0.85min. approx. for Quetiapine (QUE) fumarate
	0.70min. approx. for CLO
Total Run Time	2.5min.

Table 9: Mass Spectroscopic Conditions

Parameters	Quetiapine	Clozapine	Quetiapine fumarate	Clozapine
MRM Conditions	417.29/234.30	422.33/239.40	389.28/207.20	395.25/213.40
Declustering Potential	67.00	73.00	54.00	67.00
Collision Energy	21.00	30.00	30.00	30.00
CCE Potential	15.00	15.00	13.00	13.00

A linear response between concentration and peak area ratio of the drug in human plasma was found over a concentration range of 0.240-600ng/ml and 0.400-600ng/ml for Quetiapine and Quetiapine fumarate respectively.

Method Validation Results

Blank Screening and Selectivity

The coefficient of variation in 66 batches of human plasma spiked with 2% LOQQC of analyte and IS for blank screening and selectivity of

- Quetiapine and Clozapine (IS)-2.8 and 1.3 respectively.
- Quetiapine fumarate and Clozapine (IS)-1.1 and 1.5 respectively.

Linearity (The back calculated concentrations of the calibration standards)

Quetiapine- A Standard calibration curve ranging from 0.1ng/mL to 70.040ng/mL was run and the coefficient of correlation was found to be 0.9954-0.9994.The back calculated % nominal value of calibration curve concentration and % CV for Quetiapine of standard A, Standard B, Standard C, Standard D, Standard E, Standard F, Standard G, Standard H was found to be 103, 99.2, 103.8, 104.8, 101.1, 98.5, 98.5, 97.2 respectively and 2.9, 5.4, 0.2, 1.8, 2.4, 1.1, 0.8, 1.2 respectively.

Quetiapine Fumarate - A Standard calibration curve ranging from 0.301ng/mL to 70.038ng/mL was run and the coefficient of correlation was found to be 0.9972-0.9988.The back calculated % nominal value of calibration curve concentration and % CV for Quetiapine of standard A, Standard B, Standard C, Standard D, Standard E, Standard F, Standard G, Standard H was found to be 99.6, 99.94, 101.55, 99.01, 99.46, 100.24, 99.83, 99.97respectively and 4.2, 2.5, 4.4, 1.3, 1.16, 0.6, 0.2, 0.08 respectively.

Accuracy and Precision:-

a) With-in batch accuracy and precision of the quality control samples

- **Quetiapine:-**%CV was found to be 1.1-5.7 and % nominal was 91.5-109.6.
- **Quetiapine Fumarate:-**%CV was found to be 0.032-2.7 and % nominal was 97.42-102.26.

b) Between batch accuracy and precision of the quality control samples

- **Quetiapine:-**%CV was found to be 1.6-9.2 and % nominal was 92.7-102.6.
- **Quetiapine Fumarate:-**%CV was found to be 0.033-3.8 and % nominal was 96.86-102.77.

Recovery

Quetiapine: - The percent mean recovery for low, middle and high concentration was found to be 76, 69.5 and 73.4 respectively .

Quetiapine Fumarate:-The percent mean recovery for low, middle and high concentration was found to be 79.4, 65.5 and 63 respectively.

Stability:-

a) Freeze thaw Stability (After 3 cycles):

Quetiapine: The percentage stability of freeze thaw stability for low and high concentration was found to be 97.8 and 98.4 respectively and % CV for low and high concentration was found to be 2.9 and 2.5 respectively.

Quetiapine Fumarate:-The percentage stability of freeze thaw stability for low and high concentration was found to be 93.36 and 99.82 respectively and %CV for low and high concentration was found to be 0.90 and 0.007 respectively.

b) Bench Top Stability (7.52 hours):-

Quetiapine: The percentage stability for low and high concentration was found to be 99.0 and 102.0 respectively and %CV for low and high concentration was found to be 1.7 and 5.4 respectively.

Quetiapine Fumarate:-The percentage stability of bench top stability for low and high concentration was found to be 93.91 and 99.77 respectively and %CV for low and high concentration was found to be 1.3 and 0.04 respectively.

c) Bench Top Extraction Stability (12 hours):-

Quetiapine:-The percentage stability for low and high concentration was found to be 98.62 and 99.61 respectively and %CV for low and high concentration was found to be 1.3 and 0.10 respectively.

Quetiapine fumarate:-The percentage stability for low and high concentration was found to be 95.84 and 99.80 respectively and %CV for low and high concentration was found to be 1.2 and 0.05 respectively.

d) In-injector stability (42.27 hours)

Quetiapine:-The percentage stability for low and high concentration was found to be 104.6 and 102.0 respectively and %CV for low and high concentration was found to be 1.8 and 1.4 respectively.

Quetiapine fumarate:-The percentage stability for low and high concentration was found to be 94.02 and 99.8 respectively and %CV for low and high concentration was found to be 1.6 and 0.015 respectively.

e) Long term stability (using K3EDTA below -15◦C for 11days)

Quetiapine:-The percentage stability of long term stability for low and high concentration was found to be 98.87 and 99.49 respectively and %CV for low and high concentration was found to 2.8 and 0.05 respectively.

Quetiapine fumarate:-The percentage stability of long term stability for low and high concentration was found to be 93.30 and 99.80 respectively and %CV for low and high concentration was found to 2.24 and 0.05 respectively.

Evaluation of Pharmacokinetic profile

The test quetiapine 25mg tablet was compared with Seroquel tablets 25mg of Medichem pharmaceuticals with a sample size of 12 subjects who completed the present study.

1. The back calculated concentration for Quetiapine

A standard calibration curve ranging from 0.1ng/ml. to70.040 ng/ml was run and the coefficient of correlation was found to be 0.9982- 0.999 for 12 subjects. The back calculated % nominal value of calibration curve concentration and %CV for Quetiapine of Standard A, Standard B, Standard C, Standard D, Standard E, Standard F, Standard G, Standard H was found to be 98.9, 100.2, 105.3, 101.2, 99.6, 100.1, 98.8, 97.2 respectively and 2.3, 4.8, 1.6, 1.5, 0.7, 1.1, 0.9, 3.1 respectively.

2. The back calculated concentrations for Quetiapine Fumarate

A standard calibration curve ranging from 0.1ng/ml. to70.040 ng/ml was run and the coefficient of correlation was found to be 0.9982- 0.999 for 12 subjects. The back calculated % nominal value of calibration curve concentration and %CV for Quetiapine of Standard A, Standard B, Standard C, Standard D, Standard E, Standard F, Standard G, Standard H was found to be 99.7, 101.2, 97.4, 100.4, 99.9, 100.4, 100.5, 100.1 respectively and 2.7, 2.5, 5.1, 0.6, 0.2, 0.2, 0.1 respectively.

3. Precision and Accuracy for Quality Control Samples for Quetiapine

- **LQC:-** %CV was 7.32 and nominal was 100.2
- **M1QC:-** %CV was 5.67 and nominal was 99.43
- **M2QC:-** %CV was 2.20 and nominal was 94.80
- **HQC:-** %CV was 3.03 and nominal was 96.85

4. Precision and Accuracy for Quality Control Samples for Quetiapine Fumarate

- **LQC:-** %CV was 1.90 and nominal was 101.0
- **M1QC:-** %CV was 0.90 and nominal was 99.81
- **M2QC:-** %CV was 1.0 and nominal was 99.97
- **HQC:-** %CV was 0.40 and nominal was 100.0

5. The concentration obtained at various time intervals for test and reference formulations of Quetiapine are tabulated in table-1.25 for test formulation and table-1.26 for reference formulation and for Quetiapine Fumarate are tabulated in table-1.27 for test formulation and table-1.28 for reference formulation.

6. Pharmacokinetic Evaluation of Quetiapine

- **T_{max}:-** %CV for reference formulation was 41.293 and test was 33.31
- **C_{max}:-** %CV for reference formulation was 46.564 and test was 50.377
- **AUC_{last}:-** %CV for reference formulation was 63.687 and test was 28.049
- **AUC_{inf_obs}:-** %CV for reference formulation was 63.066 and test was 27.785
- **AUC % $_{Extrap\_obs}$:-** %CV for reference formulation was 80.317 and test was 67.166

7. Pharmacokinetic Evaluation of Quetiapine Fumarate

- **T_{max}:-** %CV for reference formulation was 50.052 and test was 41.583
- **C_{max}:-** %CV for reference formulation was 32.183 and test was 39.779
- **AUC_{last}:-** %CV for reference formulation was 37.064 and test was 35.405
- **AUC_{inf_obs}:-** %CV for reference formulation was 31.209 and test was 41.7
- **AUC % $_{Extrap\_obs}$:-** %CV for reference formulation was 65.725 and test was 52.63

8. Summary Statistics of Pharmacokinetic Parameters of Quetiapine and Quetiapine Fumarate

a) Reference Product:-

- **For Quetiapine:-**C_{max} was 10.32033, AUC_{0-t} was 15.389 and AUC_{0-inf} was 16.689

- **For Quetiapine Fumarate:-** C_{max} was 12.867, AUC0-t was 26.416 and $AUC_{0\text{-}inf}$ was 20.691

b) Test Product:-

- **For Quetiapine:-**C_{max} was 10.654, AUC0-t was 13.954 and $AUC_{0\text{-}inf}$ was 15.408

- **For Quetiapine Fumarate:-** C_{max} was 11.196, AUC0-t was 26.058 and $AUC_{0\text{-}inf}$ was 18.405

c) 90% Confidence Interval:-

- **For Quetiapine:-**C_{max} was 81.16-113.24%, $AUC_{0\text{-}t}$ was 73.78-112% and $AUC_{0\text{-}inf}$ was 71.86-107.86%

- **For Quetiapine Fumarate:-** C_{max} was 72.14109.43%, $AUC_{0\text{-}t}$ was 75.30-114.54% and $AUC_{0\text{-}inf}$ was 71.86-117.34%

d) % Power:-

- **For Quetiapine:-**C_{max} was 92.51%, $AUC_{0\text{-}t}$ was 50.22% and $AUC_{0\text{-}inf}$ was 57.41%

- **For Quetiapine Fumarate:-** C_{max} was 80.21%, $AUC_{0\text{-}t}$ was 47.63% and $AUC_{0\text{-}inf}$ was 60.25%

e) Intra subject CV%:-

- **For Quetiapine:-**C_{max} was 12.5%, $AUC_{0\text{-}t}$ was 23.2% and $AUC_{0\text{-}inf}$ was 21.1%

- **For Quetiapine Fumarate:-** C_{max} was 15.63%, $AUC_{0\text{-}t}$ was 28.41% and $AUC_{0\text{-}inf}$ was 17.532%

f) Inter subject CV%:-

- **For Quetiapine:-**C_{max} was 47.8%, $AUC_{0\text{-}t}$ was 41.7% and $AUC_{0\text{-}inf}$ was 39.5%

- **For Quetiapine Fumarate:-** C_{max} was 42.53%, $AUC_{0\text{-}t}$ was 52.64% and $AUC_{0\text{-}inf}$ was 65.425%

CONCLUSION

The primary objective of the study is to develop and validate the analytical method for simultaneous determination of Quetiapine and its metabolite Quetiapine fumarate in human plasma and the second objective of the study is to determine the concentration level of Quetiapine and Quetiapine fumarate in human plasma samples.

The analytical method was developed over an analytical range of 0.2-600 ng/ml for Quetiapine and 0.4-600 ng/ml for Quetiapine fumarate respectively. The method was validated for various parameters including Selectivity, linearity, precision, accuracy, stability and recovery. The various stabilities for which this method was validated including Freeze thaw stability, Bench top stability, Bench top extraction stability, In-injector and Long-term stability. The result for these validation parameters were found to within acceptance limits as devised by US FDA guidelines. Therefore this method was found to be selective, accurate and showed the considerable recovery of more than 65% for both Quetiapine and Quetiapine fumarate .The validated method showed the samples to be stable after three cycles of freeze thaw and also they were proven to be stable within 7.52 hours if kept on Bench-top without processing. The stability of samples was also proved for 12 hours during extraction process and they were also found to be stable for 47.27 hours when kept in injector of LC-MS instrument. The long term stability was proven for 11 days below -15^0 C in order to account for time period between the first day of sample collection and sample analysis.

Therefore, the first objective of the study was successfully met by development and validation of analytical method for both Quetiapine and Quetiapine fumarate over an analytical range 0.2-600ng/ml and 0.4-600ng/ml respectively with all validation parameters meeting the acceptance criteria.

The study was based on single dose, open label, randomized, two way, crossover design, under fasting period with 11days wash out period on quetiapine 25mg in healthy volunteers was performed. Subjects were randomized to one of the two sequences to receive the formulations acc. to randomization scheme. The test preparation was 25mg of Quetiapine and the reference formulation was 25mg Seroquel, Medichem, Barcellona, Spain.

The concentration of Quetiapine and Quetiapine fumarate was estimated in human plasma using the above validated method. The concentration data was pharmacokinetically evaluated using ANOVA and the evaluation of bioequivalence was based on following pharmacokinetic parameters: the area under the plasma concentration-time curve from zero to last quantifiable concentration ($AUC_{0\text{-}t}$) and that extrapolated to infinity $AUC_{0\text{-}\infty}$ and the maximum observed concentration (C_{max}).

In the study the last sampling time for Quetiapine was 10 hours. Quetiapine was rapidly absorbed and the elimination was fast and 8 hours after administration only 2 subjects showed detectable Quetiapine concentration in two periods. Quetiapine in literature is found to display triphasic elimination kinetics with half lives of 2 to 4 hours, 9 to 18 hours and greater than 59 hours. The triphasic elimination is due to all tissues (initial half life), clearance of free Quetiapine

fumarate from plasma (intermediate half life), and dissociation of Quetiapine fumarate from tissues (terminal half life).
From the result of safety evaluation, it was concluded that both the test and reference formulations were well tolerated. A clinically relevant difference to adverse events stated in literature was not detected.

The 90% confidence interval of the ratios (test/reference) of Quetiapine and Quetiapine fumarate after statically evaluation was found to be 81.16-113.24% and 72.14-109.43% for C_{max}, 73.78-112% and 75.30-114.54% for AUC $_{(0\text{-}t),}$ 71.86-107.86% and 71.86-117.34% for AUC $_{(0\text{-}inf.)}$ respectively. Products are considered to bioequivalent, if the 90% confidence interval of the difference in the average values of AUC and C_{max} between test and reference product within the acceptable range of 80-125%. Therefore the result of statically evaluation of C_{max}, $AUC_{(0\text{-}t),}$ $AUC_{(0\text{-}inf.)}$ for Quetiapine and Quetiapine fumarate were entirely out of bioequivalence range of 80-125%.

The method for the determination of quetiapine in human Na_2EDTA plasma covering the concentration range 1.0–382.2 ng/mL, using 0.5mLof plasma was proposed and validated. No interferences from endogenous plasma components or other sources were found and no "cross-talk" was observed in plasma samples. The assay showed good precision and accuracy.

A simple preparation procedure and short retention time could allow determination of more than 250 samples per day. The analytical method presented here has been proved useful for the investigation of the characteristics of QUE in human plasma in pharmacokinetic studies.

In conclusion, the two Quetiapine formulations were not found to be bioequivalent in terms of rate and extent of absorption and therefore, cannot be assumed therapeutically equivalent and use exchangeable in therapeutic practice.

SUMMARY

A liquid chromatographic mass spectroscopic method for the simultaneous estimation of antipsychotic drug Quetiapine and its metabolite Quetiapine fumarate was developed successfully and validated. Sample preparation process was accomplished by solid phase extraction with methanol. The dried and reconstituted solution was subjected to chromatography on Atlantis dC18 column (100mm×3.0 mm,3µm) at 40±5◦C using a mobile phase which is a mixture of acetonitrile–methanol–0.01M ammonium acetate (31:19:50, v/v/v). The sample was injected at a flow rate 0f 0.4ml/ min. and the eluent detected by MS/MS system by optimizing m/z ratio of analyte and internal standards as Quetiapine 523.29(Q1 mass) and 346.30(Q3) mass and Clozapine (internal standards) 533.33(Q1 mass) and 349.30(Q3 mass), Quetiapine fumarate 486.28(Q1 mass) and 309.20(Q3 mass) respectively.

A linear response between concentration and peak area ratio of the drug in human plasma was found over a concentration range of 0.2-600ng/ml and 0.4-600ng/ml for Quetiapine and Quetiapine fumarate respectively. The accuracy and precision of the method was evaluated by peak area ratio of the drug and internal standard.

The total precision (%CV) for the Quetiapine and Quetiapine fumarate ranged from 1.1 % (HQC-1A) to 5.7 % (HQC-1D) respectively and within batch accuracy ranged from 91.5 % (HQC-1A) to 109.6% (HQC-1D) and 96.86% (HQC-1A) to 102.77 % (HQC-1D) respectively. The mean recovery of Quetiapine was found to be 72.96% and Quetiapine fumarate was found to be 69.3%. The %CV for Quetiapine and Quetiapine fumarate in various stabilities was found to be 97.8-98.4% and 93.36-99.82% for Freeze thaw stability, 99.0-102% and 93.91-99.77% for Bench-top stability, 98.61-99.60% and 94.86-99.60% for Bench-top extraction stability, 104.6-102.0% and 94.02-99.8% for In-injector stability 98.87-99.49% and 93.30-99.80% for Long Term stability for HQC-1C – HQC-1A respectively.

The study was carried out in order to assess the bioequivalence between Reference formulation (Seroquel 25mg tablet Medichem (Barcelona, Spain)) and generic formulation of Quetiapine in 12 healthy human volunteers who had participated in this study after giving their informed consent under fasting conditions. The concentration of Quetiapine was estimated in human plasma using the above validated method after administration of test and reference (25mg tablets) to volunteers as per randomization schedule after withdrawal of blood from volunteers at specified time intervals.

The concentration data was pharmacokinetically evaluated using ANOVA and evaluation of bioequivalence was based on the following pharmacokinetic parameters: the area under the plasma concentration-time curve from zero to last quantifiable concentration ($AUC_{0\text{-}t}$) and that extrapolated to infinity ($AUC_{0\text{-}\infty}$), and the plasma observed concentration (Cmax). The %CV for reference and test formulations was found to be 41.29% and 33.31% for Tmax 46.56% and 50.38% for Cmax, 63.69% and 50.38% for AUC_{last}, 63.07% and 23.79% for $AUC_{INF\text{-}obs}$, 80.32% and 67.17% for $AUC_{Extrap\text{-}obs}$ respectively for Quetiapine and for Quetiapine fumarate ,they were found to be 50.05% and 41.58% for T_{max}, 32.18% and 39.78% for

C_{max}, 37.06% and 35.41% for AUC_{last}, 31.21% and 41.70% for $AUC_{INF-obs}$,65.73%and 52.53% Extrap-obs respectively.

The intrasubject variability of C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, for Quetiapine and Quetiapine fumarate was found to be 12.5, 23.2, 21.1 and 15.63, 28.41, 17.53, respectively. The intersubject variability of C_{max}, AUC_{0-t}, $AUC_{0-\infty}$ for Quetiapine and Quetiapine fumarate was found to be 47.8, 41.7, 39.5 and 42.53, 52.64, 65.42 respectively. The %Power for Quetiapine and Quetiapine fumarate was found to be 92.51, 50.22, 57.41 and 80.21, 47.63, 60.25 for C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ respectively. The 90% confidence interval of the ratios (test/reference) of Quetiapine and Quetiapine fumarate were 81.16 to113.24 and 72.14-109.43 for C_{max}, 73.78-112 and 75.20-114.53 for AUC_{0-t},71.86-107.86 and 71.86-117.34 for $AUC_{(0-inf.)}$ respectively.

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ABBREVIATIONS

SR. NO.	ABBREVIATED TERM	MEANING
1.	**ICH**	International Conference on Harmonisation
2.	**PET**	Positron Emission Tomography
3.	**GC**	Gas Chromatography
4.	**HPLC**	High Performance Liquid Chromatography
5.	**GC-MS**	Gas chromatography–mass spectrometry
6.	**LC-MS**	Liquid chromatography–mass spectrometry
7.	**LLOQ**	Lower limit of quantification
8.	**ISO/IEC**	International Organization for Standardization/ International Electrotechnical Commission
9.	**LC-MS-MS**	Liquid chromatography– Tandem mass spectrometry
10.	**API**	Active Pharmaceutical Ingredient
11.	**AUC**	Area under the curve
12.	**C_{max}**	Maximum (or peak) serum concentration
13.	**C_{min}**	Minimum blood plasma concentration
14.	**T_{max}**	The amount of time that a drug is present at the maximum concentration in serum
15.	**AUC_t**	Area under the plasma concentration-time curve from time zero to time t
16.	**AUC_∞**	Area under the plasma concentration-time curve from time zero to infinity
17.	**AUC_τ**	Area under the plasma concentration-time curve during a dosage interval (τ)
18.	**AUC_{0-t}**	Area under the concentration time-curves from time zero to time t
19.	**K_{el}**	Elimination rate constant from the central compartment
20.	**$T_{1/2}$**	Half Life
21.	**EDTA**	Ethylene diamine tetra acetic acid
22.	**PEP**	Polar Enhanced Polymer
23.	**CLOI**	Clozapine Internal Standard
24.	**IS CDIL**	Internal standard calibration dilution
25.	**MeOH**	Methanol
26.	**CCST**	Calibration Curve Standard
27.	**CCDIL**	Calibration Curve Dilution
28.	**QC STOCK**	Quality Control Standard stock solution
29.	**AQ-HQC**	Aqueous High Quality Control
30.	**AQ-M1QC**	Aqueous Middle-1 Quality Control
31.	**AQ-LQC**	Aqueous Low Quality Control
32.	**AQ-LOQQC**	Aqueous Limit of quantification Quality Control
33.	**LOQQC**	Limit of quantification Quality Control
34.	**WIS**	Working Internal Standard
35.	**NaOH**	Sodium Hydroxide
36.	**USA**	United States of America
37.	**CLO**	Clozapine
38.	**MRM**	Multiple Reaction Monitoring
39.	**CCE**	Controlled Current electrochemistry
40.	**Q1**	First Mass Analyzer
41.	**Q3**	Third Mass Analyzer
42.	**CV**	Coefficient of Variation
43.	**K3EDTA**	Tri-potassium EDTA
44.	**M2QC**	Middle-2 Quality Control
45.	**AUC_{last}**	Area under the concentration time curve upto the last measureable concentration
46.	**AUC_{inf_obs}**	AUC estimated from the first sampled data extrapolated to infinity
47.	**AUC % $_{Extrap\_obs}$**	Percentage of the AUCinf_obs that is contributed by the extrapolation from the last sampling time to infinity
48.	**US FDA**	United States Food & Drug Administration
49.	**ANOVA**	Analysis of Variance
50.	**Na_2EDTA**	Disodium EDTA
51.	**HQC**	High Quality Control
52.	**$AUC_{0-\infty}$**	Area under the concentration time-curves from time zero to infinity

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